Society For Biomaterials

Matrigel coatings for Parylene sheath neural probes

Curtis D. Lee,[1] Seth A. Hara,[1] Lawrence Yu,[1] Jonathan T. W. Kuo,[1] Brian J. Kim,[1] Tuan Hoang,[1] Victor Pikov,[2] Ellis Meng[1,3]

[1]Department of Biomedical Engineering, University of Southern California, Los Angeles, California 90089-1111
[2]Neural Engineering Program, Huntington Medical Research Institutes, Pasadena, California 91105-3104
[3]Ming Hsieh Department of Electrical Engineering, University of Southern California, Los Angeles, California 90089-2560

Received 8 September 2014; revised 26 December 2014; accepted 29 January 2015
Published online 23 March 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.33390

Abstract: The biologically derived hydrogel Matrigel (MG) was used to coat a Parylene-based sheath intracortical electrode to act as a mechanical and biological buffer as well as a matrix for delivering bioactive molecules to modulate the cellular response and improve recording quality. MG was loaded with dexamethasone to reduce the immune response together with nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) to maintain neuronal density and encourage neuronal ingrowth toward electrodes within the sheath. Coating the Parylene sheath electrode with the loaded MG significantly improved the signal-to-noise ratio for neural events recorded from the motor cortex in rat for more than 3 months. Electron microscopy showed even coverage of both the Parylene substrate and the platinum recording electrodes. Electrochemical impedance spectroscopy (EIS) of coated electrodes in 1× phosphate-buffered saline demonstrated low impedance required for recording neural signals. This result was confirmed by *in vivo* EIS data, showing significantly decreased impedance during the first week of recording. Dexamethasone, NGF, and BDNF loaded into MG were released within 1 day in 1× phosphate-buffered saline. Although previous studies showed that MG loaded with either the immunosuppressant or the neurotrophic factor cocktail provided modest improvement in recording quality in a 1-month *in vivo* study, the combination of these bioactive molecules did not improve the signal quality over coating probes with only MG in a 3-month *in vivo* study. The MG coating may further improve recording quality by optimizing the *in vivo* release profile for the bioactive molecules.
 J Biomed Mater Res Part B: Appl Biomater 104B: 357–368, 2016.

Key Words: intracortical electrode, Matrigel, dexamethasone, neurotrophic factors, Parylene, platinum

How to cite this article: Lee CD, Hara SA, Yu L, Kuo JTW, Kim BJ, Hoang T, Pikov V, Meng E. 2016. Matrigel coatings for Parylene sheath neural probes. J Biomed Mater Res Part B 2016:104B:357–368.

INTRODUCTION

Penetrating multielectrode intracortical probes provide a means to record both extracellular field potentials and individual action potentials, and hence enabling the study of neural networks. There is great interest in using these neural recordings to drive prostheses that restore lost functions to patients with spinal cord injuries, neurodegenerative diseases, or other neural deficits.[1,2] However, reliability of chronic recordings is plagued by gradual signal degradation which is in part attributed to glial scar formation around the probe, the associated increase in electrode impedance, and retraction of dendrites away from the probe. The initial probe insertion may disrupt the blood–brain barrier and further contribute to the chronic inflammation associated with the continued presence of foreign material in the brain.[3]

Although traditional neural probe formats take on the form of solid shanks with electrode sites at the tip or shank surface (such as arrays of metal microwires that are deinsulated at their tips and held together with an adhesive[4] or micromachined silicon shanks decorated with exposed metal electrode sites[5,6]), an alternate approach that fundamentally changes the format and may provide access to reliable chronic recordings has been proposed. The "neurotrophic cone electrode" concept introduced by Kennedy consists of a small glass cone filled with a sciatic nerve segment containing neurotrophins or Matrigel (MG) to attract neuronal growth into the cone where neural signals are measured using the deinsulated tips of microwires in the cone interior.[7] It was reported that the neurotrophins eluted from the cone encouraged differentiation and neurite outgrowth of neurons near the openings of the cone for repeatable ingrowth of myelinated axons and occasional cell bodies[8] from which action potentials were recorded for up to 4 years in human subjects.[9]

Correspondence to: E. Meng; e-mail: ellis.meng@usc.edu
Contract grant sponsor: The Defense Advanced Research Projects Agency (DARPA) MTO under the auspices of Dr. Jack Judy through the Space and Naval Warfare Systems Center, Pacific; contract grant number: N66001-11-1-4207
Contract grant sponsor: CL received support from NSF GK-12 Graduate Fellowship ("Body Engineering Los Angeles"); contract grant number: DGE-1045595

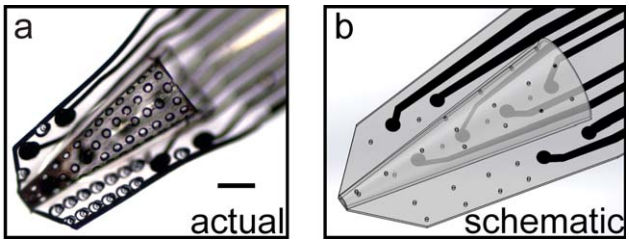

FIGURE 1. (a) Micrograph (scale bar = 100 μm) and (b) 3D illustration of PSE.

Despite the success of the neurotrophic cone electrode concept, the labor-intensive manual fabrication and assembly process has limited widespread use of the technology (low production yield and fragile construction) and the achievable electrode density. The cone is constructed from glass ($E = 100$ GPa) which is significantly stiffer than neural tissue (3.2×10^{-6} GPa [Ref. 10]) and may induce further damage during micromotion experienced during blood pulsing or head motion. To overcome these limitations while preserving the overall neurotrophic cone electrode concept, the Parylene sheath electrode (PSE) was introduced. The PSE consists of a Parylene sheath structure with four platinum (Pt) electrodes lining the inside of the sheath and four electrodes on two thin wings located on both sides of the sheath (Figure 1).[11] The Parylene C polymer ($E = 4$ GPa) is flexible and two orders of magnitude softer than glass, and hence partially alleviating the mechanical mismatch between the tissue and the probe substrate.

Parylene C can be structured using microfabrication, which permits flexibility in PSE design and precision PSE manufacture in large batches. The details of the microfabrication process were described previously[11,12] and summarized here for completeness. A 5-μm layer of Parylene C (Specialty Coating Systems, Indianapolis, IN) was first deposited on a bare silicon wafer. Next, Pt metal (200 nm) deposited using e-beam evaporation was patterned using a lift-off process. An overlying Parylene insulation layer (2 μm) was applied and etched with O_2 plasma to expose the electrodes [Figure 2(a)]. Following this, a thick photoresist layer (~10 μm) was spun on and patterned to form the microchannel that defined the inner lumen of the of the 3D Parylene sheath [Figure 2(b)]. Another final layer of Parylene (5 μm) was deposited on top of the photoresist structures to complete the sheath structure, and O_2 plasma was used to etch open both ends of the microchannel, expose the surface of the electrodes and contact pads, create perforations through the sheath, and cut out the device [Figure 2(c)]. After the devices were gently removed from the wafer, the microchannel was thermoformed into a cone shape by inserting a tapered microwire mold into the microchannel and heating the device to 200° under vacuum [Figure 2(d,e)].

For both the cone electrode and the PSE, it is desirable to have extracellular membrane proteins and bioactive molecules, such as neurotrophins, to encourage neuronal integration. The initial source of these molecules in the cone electrode was an autologous section of sciatic nerve,[7] but owing to potential complications and the complexity of the surgery, the nerve segment was replaced by MG[8] and nerve growth factor (NGF).[13] MG is a biologically derived collection of basement membrane proteins and growth factors, which gives MG unique biological properties that have made it useful in a variety of applications, such as a surface treatment to promote differentiation in cell cultures, a thick gel to induce specific differentiation in tissue explants (such as aortic rings), and a plug injected *in vivo* to measure the effect of drugs and growth factors on the ingrowth of surrounding tissues.[14] When used as a filler in the neurotrophic cone electrode, MG supported neuronal growth into the cone next to the internal wire electrodes.[15] Additionally, MG has been used to support transplanted neuronal stem cells in the brains of rats[16] and mice.[17] MG was chosen as a coating material because its unique composition has been shown to promote neuronal cell attachment and differentiation *in vitro*[18] as well as to promote neuronal survival *in vivo*.[16,17]

In this study, the MG used was used either by itself or supplemented with an immunosuppressant and combination of neurotrophins to improve neuronal integration onto the surface of the probe as the inclusion of either an immunosuppressant or combination of neurotrophic factors to MG had been previously shown to moderately improve the signal recording quality.[12] The surfaces of neural probes may be modified to modulate the immune response and improve signal quality recorded by implanted electrodes. Dexamethasone (DEX) incorporated into a biodegradable coating has been shown to suppress inflammation and inhibit the

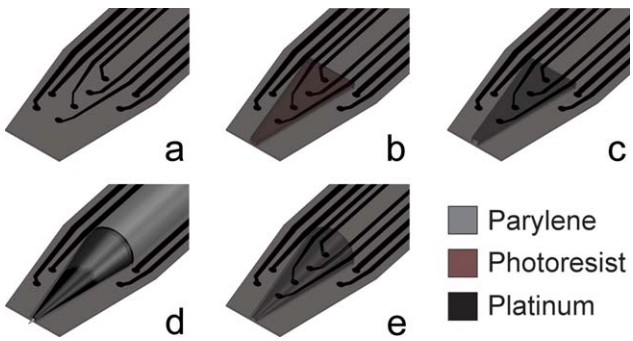

FIGURE 2. Simplified schematic representation of fabrication steps (modified from Ref. 12, © IOP Publishing. From Kim et al., J Neural Eng, 2013, 10, 045002, © IOP Publishing, reproduced by permission. All rights reserved). (*Note*: Fabrication steps are drawn on a single cut-off probe; however, probes are actually etched out during process step (c).) (a) Parylene (5 μm) was deposited onto a Si carrier wafer. Pt electrodes (200 nm) were defined using e-beam evaporation and lift-off. Parylene insulation (2 μm) was deposited and the electrode sites were exposed using O_2 plasma etching. (b) A sacrificial photoresist structure was used to form the microchannel structure. (c) An overlying Parylene (5 μm) layer completed the enclosed microchannel. The ends of the microchannel were etched open and, in the same etch step, the individual probes were cut out. Perforations (optional) were sequentially etched at each previous etch step and completely etched through during this final etch step. (d) After carefully removing each probe, a microwire mold was inserted into the sheath to form the 3D sheath structure. (e) The final mechanically stable sheath shape defined by the microwire was retained after a thermoforming process.

TABLE I. Composition of Coatings Used in this Study

Coating Name	MG (vol %)	PBS (vol %)	DEX (mg/mL)	NGF (μg/mL)	BDNF (μg/mL)
MG	75	25	x	x	x
MG/DEX	75	25	1.6	x	x
MG/NT	75	25	x	100	10
MG+	75	25	1.6	100	10

immune response.[19,20] Similar biodegradable coatings loaded with neurotrophins (including NGF,[21,22] brain-derived neurotrophic factor,[23] or neurotrophin-3[24]) have been shown to maintain neuronal density and encourage differentiation and outgrowth of neurons. Electrode surface modifications with anti-inflammatory peptides,[25] adhesion molecules such as laminin,[26] or conductive polymers[27] have also been shown to reduce chronic inflammation and enhance tissue integration.

Although the direct application of the coatings developed is for implantable multielectrode arrays, there are also implications on patterned cell cultures that use Parylene C (with or without electrodes) and other electronic devices that are coated with Parylene prior to implantation. Parylene C is a USP class VI biocompatible polymer that is inert, nonbiodegradable, and vapor deposited at room temperature to form pinhole-free, micromachinable, conformal coatings; these thin-film polymer coatings have been used extensively for insulating implantable biomedical devices[28] and have recently been used as a structural material as well.[29–31] Because of its biocompatibility and compatibility with microfabrication, there has also been interest in using Parylene C for cell cultures and other applications that require cell adhesion; however, cells will not adhere to hydrophobic Parylene C without first modifying the surface. Parylene C surface treatments include exposure to O_2 plasma to render the surface hydrophilic,[32] texturing the surface to create variable wettability,[33] or soaking in horse serum[34] or other proteins[32] to present natural binding sites to cells.

In this study, Parylene C coupons and PSEs were coated with MG to promote cell adhesion and encourage neurite ingrowth *in vivo* to improve neural recordings for neural probes such as the PSE. MG was chosen as a coating material because its unique composition has been shown to promote neuronal cell attachment and differentiation.[18] The modulus of MG (0.034–0.440 kPa[35–37]) is also much closer to the modulus of brain tissue (3.2 kPa [Ref. 10]), and thus could provide an additional mechanical buffer from the more rigid Parylene ($4.75 \times 10_3$ kPa [Ref. 38]). The MG-coated surfaces were characterized for wettability, uniformity of adhesion, thickness, and effect on impedance and stability over time. On a subset of probes, MG was loaded with DEX, NGF, and brain-derived neurotrophic factor (BDNF) and release rate was measured. The PSE was used to record neural events from rat motor cortex for 3 months and the signal-to-noise ratio (SNR) from signals obtained from coated (with either MG or MG supplemented with an immunosuppressant and neurotrophins) and uncoated probes were compared.

MATERIALS AND METHODS

Coatings

Coatings consisted of MG (BDbiosciences, San Jose, CA) with either phosphate-buffered saline (PBS) (EMD Millipore, Billerica, MA) or a combination PBS and the bioactive molecules: water-soluble DEX (D2915 Sigma Aldrich, St. Louis, MO), NGF (N2513 Sigma Aldrich, St. Louis, MO), and BDNF (amsBio, Abingdon, UK) mixed together using a vortex mixer at 4 °C. All coatings contained a final composition of 75 vol % MG and 25 vol % PBS, which was loaded with bioactive molecules. The concentration of MG was kept above 50% as per the manufacturer's guidance to retain its ability to gel. The composition of the different coatings used for the studies is summarized in Table I.

The concentrations of bioactive molecules were based on their solubility. DEX is soluble in PBS at ~20 mg/mL and NGF at 1 mg/mL. For the final coating, 100 μL of PBS filled with 16 mg/mL DEX and 1 mg/mL of NGF was added to MG to obtain the final concentrations of 1.6 mg/mL and 100 μg/mL, respectively. The concentration of BDNF was based on its availability of 10 μg, which was diluted into 50 μL of PBS and added to the coating mixture. The final concentration of DEX is similar to other slow-release DEX coatings that have reduced astrocytic responses.[39] Others reported using 1 mg/mL NGF solution added to hydrogel to form coatings[40] or reported a release of 8 ng of NGF from a hydrogel,[22] which would be available from the amount of coating deposited on our probes. A hydrogel coating containing 100 μg/mL of BDNF was used to support the growth of neurons; the eluate from the hydrogel was reported to have a similar affect as adding 100 ng/mL of solution directly to a cell culture.[23]

Parylene C test coupons were 5 × 10 mm pieces, cut from a silicon wafer coated with 8 μm of Parylene C (Specialty Coating Systems, Indianapolis, IN), which required pretreatment of the wafer with the adhesion promoter silane A-174 (Momentive Specialty Chemicals, Columbus, OH) to retain the deposited Parylene film. Additionally, 600 × 5000 × 10 μm Parylene C coupons were cut using a razor blade from 10-μm thick freestanding Parylene sheets. These smaller coupons were coated using the same methods used to coat the PSEs (described in the next section) and were used to measure the cross-sectional thickness of the MG coating.

Coating of PSE

The Parylene sheath on the PSE is in the shape of a truncated cone with a 94 μm base tapering down to a tip with a radius of 32 μm over a height of 652 μm (Figure 1). The sheath is designed not only as a space to carry

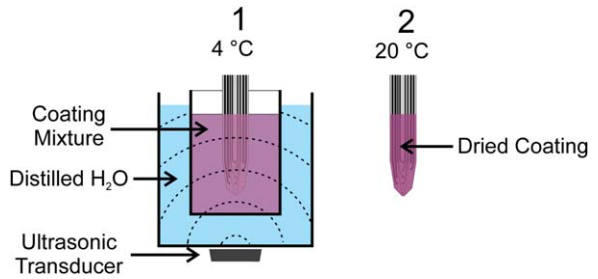

FIGURE 3. Schematic representation of the sonication method. (1) Probe is placed in coating mixture which is placed in sonication bath. (2) Coating is gelled and dehydrated at room temperature.

immunosuppressant and neurotrophic drugs to the insertion site to mitigate inflammation and scar tissue, but also to provide a surface that encourages the integration of surroundings neurons into the sheath.[11,12]

The amount of drug carried in the interior volume of the sheath is limited to 8.9 nL compared to the 3 mm^2 of surface area available to be coated on the wings, outside edges of the sheath, and the implanted portion of the cabling. To maximize drug loading and provide surfaces for neuronal integration, it was desirable that not only the lumen should be filled, but that the surrounding edges should be coated as well.

The hydrophobicity of the Parylene sheath requires strategies designed to incorporate water-soluble drugs into the lumen of the sheath.[41] Two different approaches were used to overcome the low-surface energy of Parylene. In the first method, the probe tip was immersed into coating solution contained in a centrifuge tube and then submersed in an ultrasonic bath (Branson Ultrasonics, Danbury, CT). After 5 min of sonication at 4 °C, the probe was removed from the tube and bath, after which the coating was allowed to gel for 5 min followed by air drying for at least 5 min to reduce the profile of the cone and prevent the coating from wicking onto other surfaces as it was prepared for implantation (Figure 3).

In the second method, the Parylene was first treated with the positively charged molecule poly-D-lysine (PDL) (P6407, Sigma Aldrich, St. Louis, MO) to increase the surface energy. Polylysine (a group of molecules that includes PDL) is a polycationic molecule that easily adsorbs to solid surfaces (including Parylene C[42]) and creates cationic binding sites.[43] Polylysine was chosen because it has been widely studied as a molecule used in biomaterials (mainly in the microencapsulation of exogenous cells[44]). The cationic binding sites increase the surface energy, which makes the surface hydrophilic. Once the PSE was coated with PDL, the probe was inserted into a 10-μL droplet of the coating mixture and incubated at 50 °C inside a polystyrene container for 5 min. After 5 min, the probe was removed from the container and held at 50 °C for an additional 5 min to dehydrate the coating (Figure 4).

Characterization of MG coatings

The coating was visualized to determine its structure and distribution on the Parylene substrate using optical and scanning electron microscopy (SEM; 7001, JEOL, Peabody, MA). Optical microscopy showed the structure of MG on a Parylene substrate, whereas SEM was used to determine the distribution of MG over the PSE, as well as the coverage of MG on the Pt and Parylene surfaces of the probe.

Electrochemical impedance spectroscopy (EIS) is widely used to assess the recording capability of microelectrodes.[45] Here, EIS was used here to determine the change in impedance at the electrode surface caused by coating the electrodes with MG and how this change varied over time as the coating was soaked in PBS. EIS was performed in 1× PBS at 37 °C with an amplitude of 10 mVrms and a frequency range of 1–100,000 Hz. A large area Pt plate was used as a counter electrode and an Ag/AgCl (3*M* NaCl) electrode was used as a reference. EIS data were taken once a day for 3 days. Between tests, probes were soaked in 1× PBS at 37 °C.

Drug loading and release from probe *in vitro*

To obtain a continuous measurement of drug release from MG, 12.5 μL of MG/DEX coating was placed on a bare microwell sidewall and, after heating, uncovered for 15 min at 55 °C (Sun Systems, Titusville, FL) to remove the liquid from the coating. The microplate was then filled with 100 μL of PBS, immediately covered with Parafilm to avoid evaporation, and was then placed into a spectrophotometric microplate reader (Epoch, Biotek, Winooski, VT) and scanned at various time intervals for several hours. The absorbance was compared to a calibration curve that was made by measuring the absorbance (242 nm) of known concentrations of DEX in PBS. In this manner, the mass of DEX released from the MG could be calculated.

To measure the effect of EtO sterilization on the release of DEX from MG deposited using the surface modification method, 5 μm of Parylene was deposited directly onto a 96-well plate. The wells were then coated with PDL by placing 100 μL of 100 μg/mL PDL solution into the wells and soaking for 1 h at 4 °C. Excess PDL was then removed by rinsing the wells three times with deionized water. A 12.5 μL droplet of MG loaded with DEX (1.6 mg/mL) was then deposited into the bottom of the microwell which was then covered with Parafilm and heated to 55 °C for 5 min. The Parafilm was removed and the well was held at 55 °C for an additional 5 min. The coated wells were then subjected to a 24-h EtO sterilization using a room-temperature sterilizing system (Anprolene, AN74i, Andersen Products, Haw River, NC).

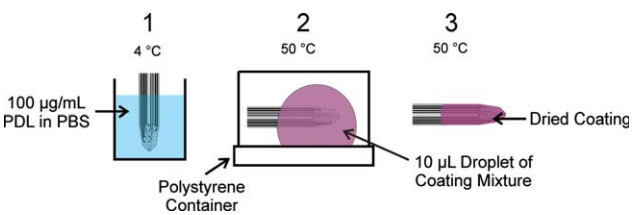

FIGURE 4. Schematic representation of the surface modification method. (1) Probe is soaked in PDL solution for 1 h. (2) Probe placed inside of 10 μL droplet for 5 min covered in a polystyrene container. (3) Probe is taken out of container and is dehydrated for 5 min.

After sterilization, the wells were filled with 100 μL of PBS at room temperature. At various time intervals, the PBS was removed and replaced with fresh PBS. The eluate was placed in a UV transparent microwell, scanned at 242 nm, and a calibration curve was used to determine the amount of eluted DEX. The results were compared to wells that had not been sterilized.

Parylene-coated microwells treated with PDL were also used to measure the amount of neurotrophic factors released from MG deposited onto Parylene using surface modification. For these tests, 1 μL droplet of MG/NT coating (100 μg/mL NGF and 10 μg/mL BDNF) was deposited into the bottom of 96-well plates previously coated with 5 μm of Parylene and treated with PDL. The droplet was then covered with Parafilm and heated to 55 °C for 5 min. The Parafilm was removed and the wells were held at 55° for an additional 5 min. The wells were then either filled with 100 μL of PBS at room temperature or sterilized for 24 h using a room-temperature EtO sterilizing system and then filled with 100 μL of PBS at room temperature. At various time intervals, the PBS was removed and replaced with fresh PBS. The eluate was then diluted and the concentration was determined using enzyme-linked immunosorbent assay (ELISA).

To determine the amount of drug that could be loaded on a probe and to confirm that drugs were released from the probe surface the same as the coupons, probes coated with MG/DEX (1.6 mg/mL DEX) using the surface modification method were submersed into 100 μL of PBS contained in a UV-transparent microwell (96-well microplate) and removed at various intervals to measure the absorbance of the PBS. The absorbance of the eluate was compared to a calibration curve again to calculate the mass of DEX released from the coated probe.

Immunohistochemical effect of drug release from probe *in vivo* from a 1-month study

Two male Sprague–Dawley rats (>320 g) were implanted with dual-probe arrays for 1 month. All procedures for the animal experiments were in accordance with the animal protocol approved by the Huntington Medical Research Institutes Institutional Animal Care and Use Committee (HMRI IACUC) and in compliance with the Animal Welfare Act. The implantation procedure is as described earlier.[12] Briefly, the probes were manually inserted in the motor cortex with a micromanipulator until reaching the required depth of 2 mm. At 1-month postimplantation, animals were transcardially perfused with PBS followed by phosphate-buffered 4% paraformaldehyde solution. The cerebral cortex was dissected and the cortical tissue block containing the probe tracks was embedded into paraffin. The tissue was sectioned perpendicular to the probe tracks. The sections were then immunostained for GFAP (marker for reactive astrocytic processes) and visualized using Vector nickel-DAB. The sections were subjected to the semi-automated image analysis using software, custom-written in Visual Basic 6.0 (Microsoft, Redmond, WA) using National Instruments Image ActiveX component (National Instruments, Austin, TX). Within the software, a rounded rectangle was placed on the image for use during analysis; its placement, width, and length were adjusted to match the perimeter of the probe track. Then, six larger concentric rounded rectangles were automatically drawn at 50 μm incremental distances from the inner one and the GFAP density was automatically calculated in these six areas and normalized to the average density in the outer-most area (270–300 μm).

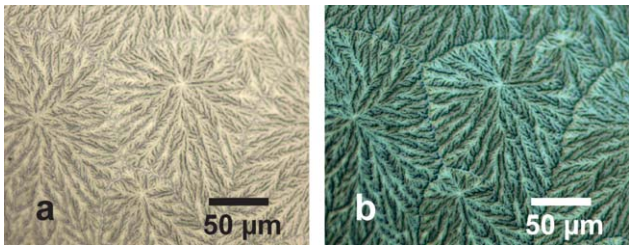

FIGURE 5. Optical images of fractal "trees" made from dried MG on a Parylene-coated silicon wafer under (a) unpolarized and (b) polarized light.

In vivo EIS and electrophysiological evaluation from a 3-month study

Three male Sprague–Dawley rats (>320 g) were implanted with four-probe arrays for 3 months (one array with uncoated probe, one with MG coating, and one with MG coating supplemented with DEX, NGF, and BDNF). To assess the functionality of the coated PSE, *in vivo* EIS and neural recordings were carried out weekly from week 1 to 14 postimplantation, and the collected data were used to calculate the EIS and SNR. For the EIS and electrophysiological measurements, rats were anesthetized with Ketamine/Xylazine (90/10 mg/kg, IP). The measurements of EIS were obtained using a PC4/300 potentiostat system (Gamry Instruments, Warminster, PA) in a two-electrode configuration, with the reference and counter connected to the titanium headplate and its six transcranial stainless steel screws. The data were collected with 10 mV_{rms} sinusoids at the frequencies from 1 Hz to 100 kHz, and 1 kHz values were selected for analysis. The electrophysiological data were acquired at 16 bit and 40 kHz per channel using a 64-channel data acquisition system (OmniPlex; Plexon, Dallas, TX) and high-pass filtered at 300 Hz to remove the low-frequency fluctuations from the baseline. In the 120-s data records, spike detection was performed using the Nonlinear Energy Operator algorithm. The neuronal noise was calculated as the standard deviation of the data after the removal of 0.8-ms-long segments containing the detected spikes. The spike amplitude was calculated as the absolute value of spike's peak height, and the SNR was calculated as the ratio of average spike amplitude to noise.

RESULTS

MG on Parylene and electrode surfaces

Untreated Parylene (deposited on silicon coupons) dip coated in MG yielded an uneven distribution of thick and

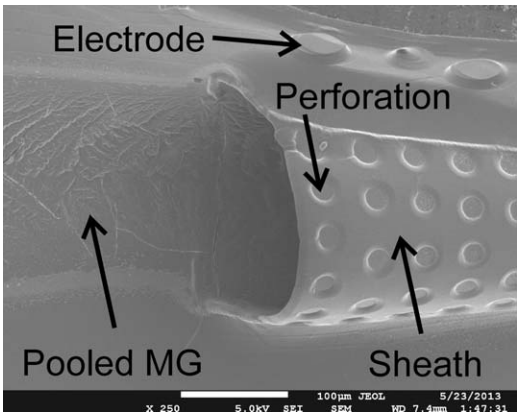

FIGURE 6. SEM image of the top portion of a perforated PSE coated using surface modification. The MG pooled toward the center of the cable near the large opening of the sheath and filled in the perforations which were added to the PSE to improve cellular communication across the Parylene substrate.

thin regions of MG similar to the coating appearance on an untreated PSE (as described in Ref. 41). The thicker regions of MG on the coupon revealed fractal patterns (Figure 5), which is a behavior predicted by diffusion-limited aggregation theory applied to collagen[46,47] and shown in many other natural and synthetic polymer systems.[48–51]

The 600 × 5000 × 10 μm Parylene coupons coated with MG by sonication or surface treatment with PDL both exhibited uneven distribution across the surface. Pools of MG formed along the center of the coupon similar to what was seen on the PSE cable just above the sheath (Figure 6). For the PDL-treated coupons, the average thickness of the coating was 12.9 ± 5.2 μm (mean ± SD, $n = 3$) in a representative sample and ranged from a minimum of 3.4 μm near the edge of the cable to 33.2 μm in the center where the foliated texture of the pooled MG is apparent. Coupons coated using sonication (no PDL) did not retain enough MG to measure the cross-sectional thickness using optical microscopy.

SEM images of electrodes on the PSE reveal Pt at the bottom of a recess formed by etching away Parylene insulation [Figure 7(a)]. When coated with PDL, crystals appeared that cross from Parylene down to the Pt surfaces [Figure 7(b)]. After coating with MG, MG was present on both the Parylene and the Pt surfaces as evidenced by the change of surface roughness around the edges of the Parylene insulation and the appearance of characteristic MG fractal patterns (Figure 5). The previous study described that electrodes on PSEs coated using sonication showed similar coverage of Pt and Parylene surfaces.[41]

EIS revealed that impedance did not significantly increase after being dip coated via sonication [Figure 8(a,d)]. For probes coated with only MG, the impedance decreased over time, indicating the gradual removal of MG on the surface of the electrode [Figure 8(c)]. In contrast, the impedance electrodes having MG loaded with DEX increased over time [Figure 8(f)]. EIS data measured *in vivo* from probes coated using the surface modification method similarly showed a change in impedance that lasted <2 weeks.

Drug release from coating

DEX/MG deposited onto the sidewall of the microwell (with no surface treatment) with a pipette was hydrated with PBS and continuous kinetic absorbance scans measured DEX release (at 242 nm) [Figure 9(a)]. These scans show the maximum absorbance occurring within 35 min after the PBS was added to the well [Figure 9(b)]. The calibration curve, of known concentrations of DEX at 242 nm, is shown in Figure 9(c).

To verify that coating on Parylene does not modify DEX release, the rate of release of DEX from MG coated onto a Parylene coupon using the PDL surface modification method was measured using spectrophotometry at 242 nm. DEX was released within 1 day of soaking in PBS (Figure 10). Sterilizing the coating using the EtO gas process did not change the release rate, but slightly decreased the amount of DEX released (9%).

The rate of NGF and BDNF release from MG deposited on a Parylene coupon using the surface modification method was measured using ELISA. These measurements indicate that the NGF eluted from the MG within 4 h [Figure 11(a)], whereas the BDNF had eluted out of the MG within 1 day [Figure 11(b)]. This is only a fraction of the total amount of NGF and BDNF initially deposited onto the Parylene (100 and 10 ng, respectively). After sterilization, the amount of NGF and BDNF was reduced further (46 and 35%, respectively) from the amount released from the nonsterilized coating.

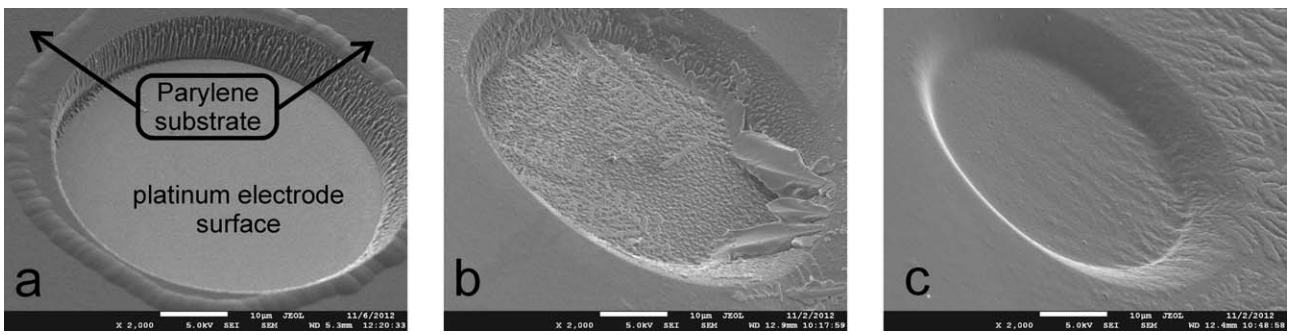

FIGURE 7. (a–c) SEM images of recessed Pt electrode on Parylene probe (a) before coating, (b) after PDL surface treatment, and (c) after coating with MG. (Scale bars are 10 μm. PSE was sputtered with Au prior to imaging.)

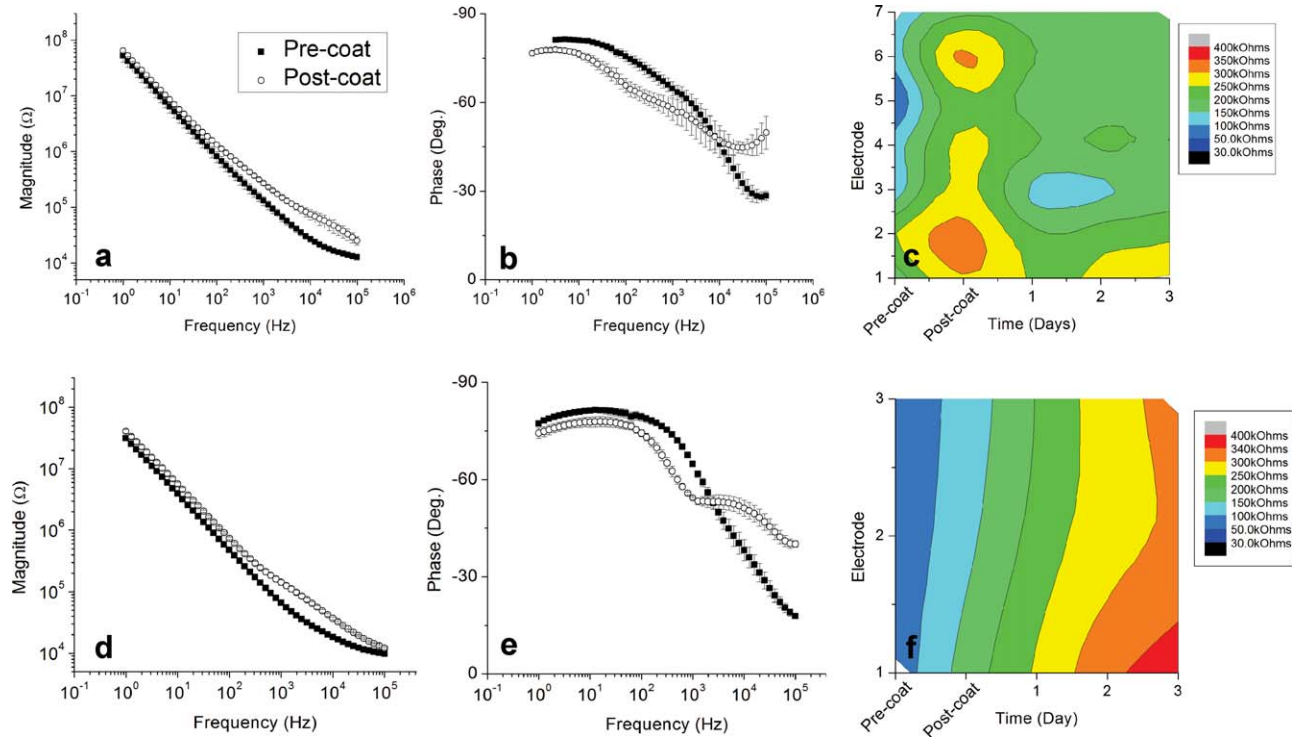

FIGURE 8. EIS data (mean ± SE) from (a–c) MG-coated ($n = 7$) and (d–f) MG/DEX-coated ($n = 3$) neural electrodes (using sonication method). (a and b; d and e) Magnitude and phase data were measured pre- and postcoating. (c,f) Impedance measured at 1 kHz before the probe was coated to 3 days after coating.

The rate of DEX release from a probe coated using PDL surface modification was also verified using spectrophotometry. For each measurement, the coated probe was dipped into a microwell containing PBS for a given time period and then removed to allow spectrophotometric scanning [Figure 12(a)]. The absorbance from the eluate of probes coated using the surface treatment method at 242 nm was 0.3 after 2 h [Figure 12(b)]. The maximum absorbance measured occurred during the first time period, which showed that the majority of the DEX eluted out before the second measurement (i.e., within 30 min).

Immunohistochemistry

The MG-coated and MG/DEX-coated probes (coated using the sonication method) were implanted in the rodent cerebral cortex for 1 month to evaluate the *in vivo* response to DEX elution from the probe. The results from this study are shown in Figure 13. The antiastrocytic GFAP antibody was used to evaluate the immune response to a chronic probe present in the cerebral cortex. As the probes with MG and DEX coatings were implanted in different animals, three-step normalization was performed. First, the GFAP immunoreactivity was calculated from the actual edge of the probe rather than from the center of the probe track to reduce the confounding effect of different track diameters (which is owing to a tapering shape of the probe). Second, a section of the image containing any artifacts (e.g., tissue folding or the presence of a large blood vessel) was removed from quantification. Third, the GFAP immunoreactivity was normalized based on the level remote to the probe (distance, 270–300 μm) to reduce animal-to-animal and section-to-section variability in the overall level of GFAP staining. The immunohistochemistry did not reveal a significant difference in astrocytic density between the probes coated with only MG compared to the probes coated with MG supplemented with DEX.

Electrophysiological measurements from a 3-month study

Probes coated with either MG, MG supplemented with DEX and neurotrophic factors (MG+) (coated using the surface modification method), or noncoated probes were implanted in the rodent cerebral cortex for 3 months to evaluate the functionality of the coated versus uncoated probes. The results from this study are shown in Figure 14. EIS data taken at 1 and 2 weeks show that the presence of the hydrogel significantly lowers the impedance of the electrode during the first week. By the second week, however, these differences are no longer significant [Figure 14(a)]. Electrophysiological data taken at 2 and 14 weeks show a significant improvement in SNR over time for two MG-coated probes, but not for a noncoated probe. Although the SNR was not significantly different among the probes at week 2, by 14 weeks the probes coated with MG and with MG supplemented with DEX, NGF, and BDNF exhibited a significantly better SNR than the uncoated probe [Figure 14(b)].

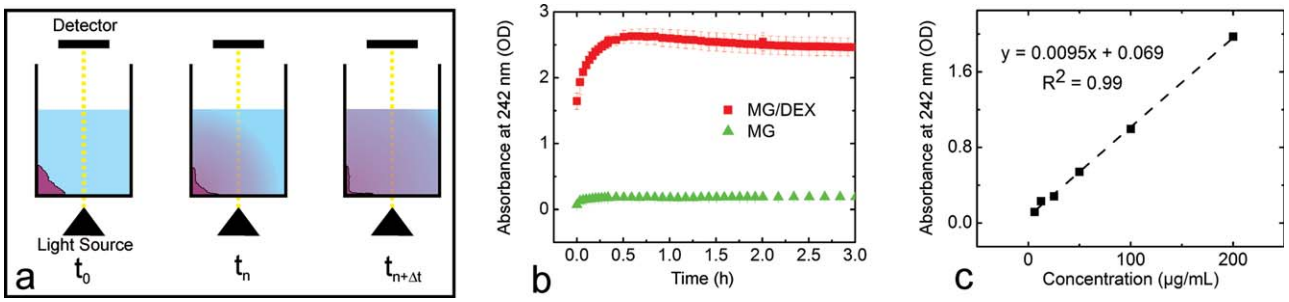

FIGURE 9. Kinetic scan of microwells with MG/DEX and MG coated on microwell sidewalls. (a) Schematic representation of test setup showing MG/DEX coating diffusing from bottom left corner of microwell. (b) Kinetic scan showing peak absorbance at 0.5 h (mean ± SD, $n = 3$–5) (c) Calibration curve of known dilutions of MG/DEX coating.

DISCUSSION

MG on Parylene and electrode surfaces

The fractal patterns formed by dried MG on Parylene-coated silicon coupons (Figure 5) demonstrate its ability to self-assemble into microstructured topography, which has been shown to be conducive to neuronal attachment.[52] The uneven distribution of MG on the 600 × 5000 × 10 μm Parylene coupons was consistent to what has been seen on the PSE cable just above the sheath (Figure 6) and demonstrates the tendency of MG to pool along concave surfaces. However, the SEM images of the electrode surfaces (Figure 7) show that MG was present on both Pt and Parylene surfaces of the probe.

As mentioned earlier, the measurements of EIS have been correlated with recording performance of electrodes *in vivo*, with impedances of <1 MΩ being preferred for recording electrodes. The lack of a significant increase in impedance associated with the coating process is expected from a hydrogel with large pores (2 μm at 50% MG[36]) and is similar to the results from other hydrogels deposited on neural electrodes, such as sodium alginate.[27] Lower impedances also portend well for *in vivo* recordings. The decrease in impedance over time associated with the MG coating suggests gradual removal of bulk coating from the probe surface. Visual inspection after soaking for 1 day also suggests a reduction in the amount of MG present on probe surfaces. The increase of impedance over time associated with DEX-loaded MG is likely owing to the water-soluble DEX used in this study that was incorporated into cyclodextrin, which has been shown to swell in aqueous solutions.[53] Even with the increase, however, the 1-kHz impedance never exceeded 1 MΩ.

Drug release from coating

Kinetic scans showed that the majority of DEX was released within the first 35 min after the PBS was added to the well [Figure 9(b)]. This time period corresponds to a diffusion coefficient of 9.4×10^{-10} m^2/s which is faster than predicted by Einstein–Stokes theory for diffusion of DEX in water (6.82×10^{-10} m^2/s [Ref. 54]) and suggests that MG does not hinder the diffusion of DEX into solution. A 24-h EtO sterilization did not affect the rate of DEX released from MG/DEX deposited onto Parylene using surface modification and reduced only the amount of DEX released by 9%.

NGF and BDNF eluted more slowly from MG than DEX (within 4 h and 1 day, respectively), which is likely owing to their larger sizes (NGF and BDNF are 27 kDa compared to DEX, which is 392 kDa), but this rate is likely too fast to have an effect on chronic recording quality. The loss of factors during the elution process could be owing to protein adhesion to the sidewalls of the wells and denaturing of the proteins. During sterilization, the extra time at room temperature and exposure to EtO could cause further denaturing of the proteins. During actual recordings *in vivo*, the proteins will not be exposed to the large hydrophobic surface area of the microwell and therefore more will be available to neurons. Sterilizing the probes prior to coating and coating under sterile conditions could eliminate the need for postcoating sterilization and preserve a larger portion of the neurotrophic factors.

The absorbance of the eluate from PSEs coated with MG/DEX using the surface modification method was 0.3 after 2 h [Figure 12(b)] and corresponds to 1.2 μg of DEX

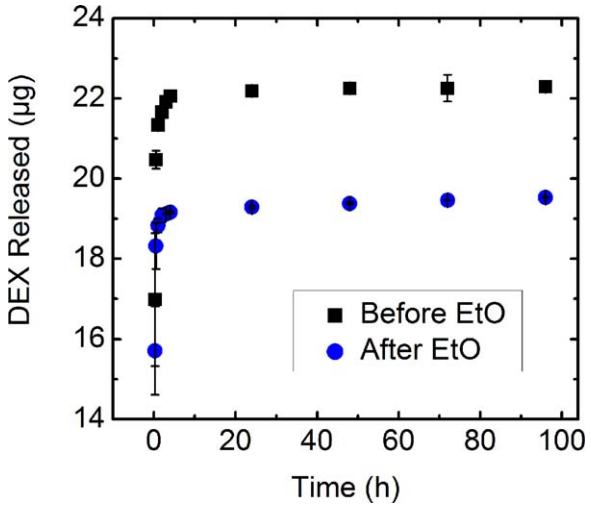

FIGURE 10. Cumulative DEX released from MG deposited on Parylene coupons using the PDL surface modification method before and after sterilization ($n = 3$, mean ± SE).

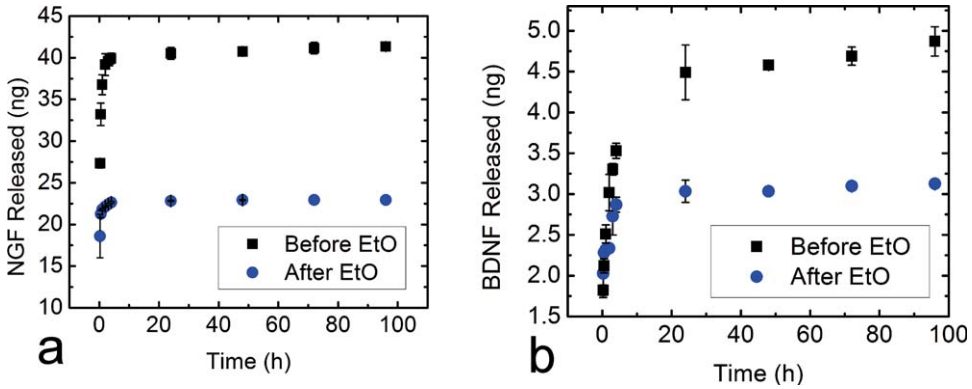

FIGURE 11. Cumulative release of (a) NGF and (b) BDNF from MG loaded with neurotrophins and deposited on Parylene using the PDL surface modification method before and after EtO sterilization ($n = 3$, mean ± SE).

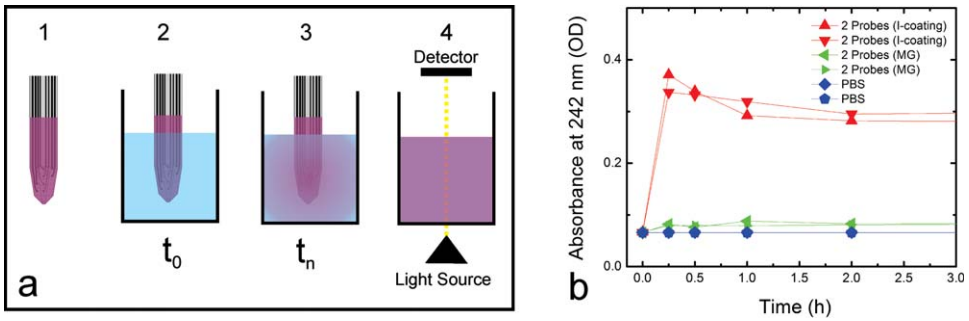

FIGURE 12. Drug loading and release from PSE coated using surface modification method. (a) Schematic representation of testing method: (1) probe coated in DEX loaded MG, (2) coated probe inserted into microwell, (3) probe soaked for time tn, (4) probe removed from microwell and absorbance of eluate measured using spectrophotometer at a wavelength of 242 nm. Probe is reinserted into well after the measurement for subsequent measurements. (b) Absorbance of eluate from DEX/MG-coated probes. (Absorbance of probe coated with MG and PBS-filled well is also provided to show the ability of spectrophotometer to selectively measure DEX concentrations using a 242-nm light source. For calibration curve, see Figure 9(c).)

being released from each probe. The maximum measured absorbance occurred during the first time period, which suggests that majority of the DEX eluted out before the second measurement (i.e., within 30 min). These results verify that the release profile measured by kinetic scans of DEX being released from MG deposited with no surface treatment is similar to the release profile from probes coated using surface modification.

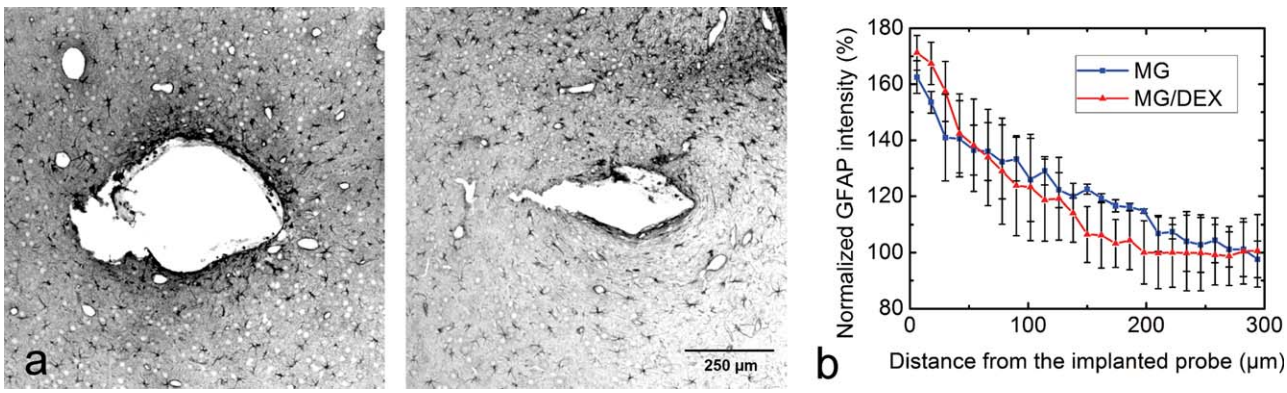

FIGURE 13. (a) Sample microphotographs of the GFAP immunostained sections of cerebral cortex through the probe tips coated with MG (left) and MG/DEX (right); (b) quantification of GFAP immunoreactivity in the sections shown in (a) at increasing distances from the probe. The immunoreactivity data are normalized to the level at the distance of 270–300 μm.

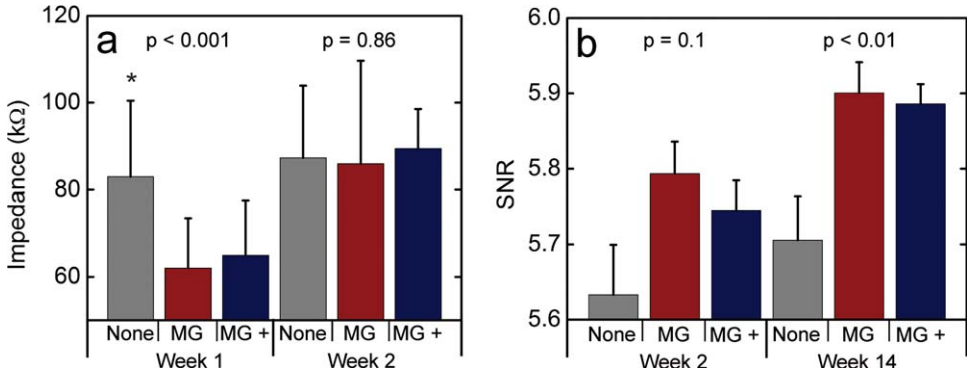

FIGURE 14. *In vivo* EIS and electrophysiological data from 3-month chronic study. (a) *In vivo* EIS data taken during the first 2 weeks comparing three probes: one noncoated, one coated with MG, and one coated with MG supplemented with DEX, NGF, and BDNF (denoted MG +) at 1 week and 2 weeks after implantation ($n = 13$–35, mean ± SD). (b) Comparison of SNR for neural activities recorded with three probes ($n = 6$–12, mean ± SD). The *p*-values are for ANOVA comparisons among three coating groups at each time point. Error bars indicate the standard deviation for sites within the probe. Asterisk indicates significant difference of noncoated group versus two other groups in the *post hoc* test.

Immunohistochemistry

The immunohistochemical results indicate no difference between the normalized GFAP intensity around the MG-coated probe as compared to the MG/DEX-coated probe, suggesting that the DEX was released too quickly to produce any significant effect on the chronic astrocytic response. These results are consistent with electrophysiological recordings, which showed little difference in SNR and noise levels between probes coated with only MG and probes coated with MG supplemented with bioactive molecules.[12] However, the histology fails to explain the reason why, in the same study, both MG supplemented with DEX and MG supplemented with neurotrophins showed a statistically higher ($p < 0.001$, and $p = 0.001$, respectively) event rate.[12]

In vivo EIS and electrophysiology

The absence of a significant difference in electrical impedance between coated and uncoated probes at 2 weeks post-implantation corroborates the conclusions from the *in vitro* data that the bulk of the hydrogel is removed from the electrodes during the first 2 weeks. The observation of lower impedance in MG-coated groups at 1 week *in vivo* instead of higher initial impedance seen in the bench top saline test is likely owing to the hydrogel being more conductive than brain tissue, but less conductive than saline.

The SNR measured i*n vivo* for 14 weeks was improved over time for probes that were coated with either MG by itself or MG supplemented by DEX, NGF, and BDNF. There was no measurable benefit from supplementing the MG with DEX, NGF, and BDNF, consistent with the drug elution data that showed that all drugs loaded into MG are eluted out within the first day. However, both MG-coated probes (supplemented and nonsupplemented) had considerably better functional performance than uncoated probes. The stability and long-term efficacy of MG were previously demonstrated in the literature where MG has been used as a scaffold to support survival and differentiation of transplanted neural precursor cells.[16,17]

CONCLUSIONS

This study showed that MG can be coated onto a Parylene-based neural probe to improve the performance. The previous studies have shown that Parylene surfaces can be modified to improve its performance for cell culturing[32,34] and used as a stencil for coculturing[55] and that MG can be used for creating realistic cell morphology[56] *in vitro*, whereas this study evaluates the behavior of MG on Parylene C for the purpose of implanting the coated Parylene surface *in vivo*. The immunosuppressant DEX and a combination of neurotrophic factors were also loaded into MG to modulate the immune response and improve neuronal integration into the probe. Although a previous 1-month *in vivo* study showed that probes coated in MG supplemented with bioactive molecules recorded an increased number of spike-like events,[12] in this study, probes coated with MG that included bioactive molecules did not perform any better than probes coated with only MG. However, probes coated in either MG or MG supplemented with bioactive molecules had a higher SNR than noncoated probes. Elution studies showed that the bioactive molecules are all released from MG within a day and therefore would have little effect during a chronic implantation. Other groups that have successfully shown decreases in astrocytic growth around probes using other coatings that released DEX over the course of days[57] or weeks.[58] To obtain the benefits of the bioactive molecules while maintaining the improved performance of MG, the bioactive molecules could be encapsulated in slow-releasing, biodegradable microparticles that could be loaded into the MG. The microparticles could be small enough to be released from the MG, but large enough remain in the insertion site, similar to other microparticles loaded into hydrogels.[19]

ACKNOWLEDGMENTS

The authors would like to thank the Center for Electron Microscopy and Microanalysis for use of their scanning electron microscopes. The authors also thank Dr Donghai Zhu

of the Keck Photonics Laboratory for help with fabrication and members of the Biomedical Microsystems Laboratory of USC for their assistance.

REFERENCES

1. Collinger JL, Wodlinger B, Downey JE, Wang W, Tyler-Kabara EC, Weber DJ, McMorland AJC, Velliste M, Boninger ML, Schwartz AB. High-performance neuroprosthetic control by an individual with tetraplegia. Lancet 2013;381:557–564.
2. Lebedev MA, Nicolelis MAL. Brain-machine interfaces: Past, present and future. Trends Neurosci 2006;29:536–546.
3. Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods 2005;148:1–18.
4. Nicolelis MAL, Dimitrov D, Carmena JM, Crist R, Lehew G, Kralik JD, Wise SP. Chronic, multisite, multielectrode recordings in macaque monkeys. Proc Natl Acad Sci 2003;100:11041–11046.
5. Najafi K., Wise KD. An implantable multielectrode array with on-chip signal processing. IEEE J Solid-State Circ 1986;21:1035–1044.
6. Campbell PK, Jones KE, Huber RJ, Horch KW, Normann RA. A silicon-based, three-dimensional neural interface: Manufacturing processes for an intracortical electrode array. IEEE Trans Biomed Eng 1991;38:758–768.
7. Kennedy PR. The cone electrode: A long-term electrode that records from neurites grown onto its recording surface. J Neurosci Methods 1989;29:81–193.
8. Kennedy PR, Bakay RA, Sharpe SM. Behavioral correlates of action potentials recorded chronically inside the cone electrode. Neuroreport, 1992;3:605–608.
9. Bartels J, Andreasen D, Ehirim P, Mao H, Seibert S, Wright EJ, Kennedy P. Neurotrophic electrode: Method of assembly and implantation into human motor speech cortex. J Neurosci Methods 2008;174:168–176.
10. Miller K, Chinzei K, Orssengo G, Bednarz P. Mechanical properties of brain tissue in-vivo: Experiment and computer simulation. J Biomech 2000;33:1369–1376.
11. Kuo JT, Kim BJ, Hara SA, Lee CD, Gutierrez CA, Hoang TQ, Meng E. Novel flexible Parylene neural probe with 3D sheath structure for enhancing tissue integration. Lab Chip 2013;13:554–561.
12. Kim BJ, Kuo JT, Hara SA, Lee CD, Yu L, Gutierrez CA, Hoang TQ, Pikov V, Meng E. 3D Parylene sheath neural probe for chronic recordings. J Neural Eng 2013;10:045002.
13. Kennedy PR, Bakay RAE. Activity of single action potentials in monkey motor cortex during long-term task learning. Brain Res 1997;760:251–254.
14. Passaniti A, Taylor RM, Pili R, Guo Y, Long PV, Haney JA, Pauly RR, Grant DS, Martin GR. A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab Investig 1992;67:519.
15. Kennedy PR, Mirra SS, Bakay RA. The cone electrode: Ultrastructural studies following long-term recording in rat and monkey cortex. Neurosci Lett 1992;142:89–94.
16. Jin K, Mao X, Xie L, Galvan V, Lai B, Wang Y, Gorostiza O, Wang X, Greenberg DA. Transplantation of human neural precursor cells in Matrigel scaffolding improves outcome from focal cerebral ischemia after delayed postischemic treatment in rats. J Cerebr Blood Flow Metab 2009;30:534–544.
17. Uemura M, Refaat MM, Shinoyama M, Hayashi H, Hashimoto N, Takahashi J. Matrigel supports survival and neuronal differentiation of grafted embryonic stem cell-derived neural precursor cells. J Neurosci Res 2010;88:542–551.
18. Lein PJ, Higgins D. Laminin and a basement membrane extract have different effects on axonal and dendritic outgrowth from embryonic rat sympathetic neurons *in vitro*. Dev Biol 1989;136: 330–345.
19. Kim DH, Martin, DC. Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery. Biomaterials 2006;27:3031–3037.
20. Abidian MR, Martin DC. Multifunctional nanobiomaterials for neural interfaces. Adv Funct Mater 2009;19:573–585.
21. Winter JO, Cogan SF, Rizzo JF 3rd. Neurotrophin-eluting hydrogel coatings for neural stimulating electrodes. J Biomed Mater Res Part B: Appl Biomater 2007;81:551–563.
22. Jhaveri SJ, Hynd MR, Dowell-Mesfin N, Turner JN, Shain W, Ober CK. Release of nerve growth factor from HEMA hydrogel-coated substrates and its effect on the differentiation of neural cells. Biomacromolecules 2008;10:174–183.
23. Winter JO, Gokhale M, Jensen RJ, Cogan SF, Rizzo JF 3rd. Tissue engineering applied to the retinal prosthesis: Neurotrophin-eluting polymeric hydrogel coatings. Mater Sci Eng C 2008;28:448–453.
24. Richardson RT, Wise AK, Thompson BC, Flynn BO, Atkinson PJ, Fretwell NJ, Fallon JB, Wallace GG, Shepherd RK, Clark GM, O'Leary SJ. Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons. Biomaterials 2009; 30:2614–2624.
25. He W, McConnell GC, Schneider TM, Bellamkonda RV. A novel anti-inflammatory surface for neural electrodes. Adv Mater 2007; 19:3529–3533.
26. He W, McConnell GC, Bellamkonda RV. Nanoscale laminin coating modulates cortical scarring response around implanted silicon microelectrode arrays. J Neural Eng 2006;3:316–326.
27. Kim DH, Abidian M, Martin DC. Conducting polymers grown in hydrogel scaffolds coated on neural prosthetic devices. J Biomed Mater Res A 2004;71:577–585.
28. Meng E, Zhang X, Benard W. Additive processes for polymeric materials. In: MEMS Materials and Processes Handbook. USA: Springer; 2011. pp 193–271.
29. Takeuchi S, Zeigler D, Yoshida Y, Mabuchi K, Suzuki T. Parylene flexible neural probes integrated with microfluidic channels. Lab Chip 2005;5:519–523.
30. Rodger DC, Wen L, Ameri H, Ray A, Weiland JD, Humayun MS, Yu-Chong T. Flexible parylene-based microelectrode technology for intraocular retinal prostheses. 1st IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS). Zhuhai, 2006; pp 743–746.
31. Yu F, Zhao Y, Gu J, Quigley KL, Chi NC, Tai YC, Hsiai TK. Flexible microelectrode arrays to interface epicardial electrical signals with intracardial calcium transients in zebrafish hearts. Biomed Microdevices 2012;14:357–366.
32. Chang TY, Yadav VG, De Leo S, Mohedas A, Rajalingam B, Chen CL, Selvarasah S, Dokmeci MR, Khademhosseini A. Cell and protein compatibility of parylene-C surfaces. Langmuir 2007;23: 11718–11725.
33. Wei L, Lakhtakia A, Roopnariane AP, Ritty TM. Human fibroblast attachment on fibrous parylene-C thin-film substrates. Mater Sci Eng C 2010;30:1252–1259.
34. Delivopoulos E, Murray AF, MacLeod NK, Curtis JC. Guided growth of neurons and glia using microfabricated patterns of parylene-C on a SiO_2 background. Biomaterials 2009;30:2048–2058.
35. Semler EJ, Ranucci CS, Moghe PV. Mechanochemical manipulation of hepatocyte aggregation can selectively induce or repress liver-specific function. Biotechnol Bioeng 2000;69:359–369.
36. Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, Kamm RD, Wells A, Lauffenburger DA, Matsudaira P. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proc Natl Acad Sci USA 2006;103:10889–10894.
37. Soofi SS, Last JA, Liliensiek SJ, Nealey PF, Murphy CJ. The elastic modulus of Matrigel^{TM} as determined by atomic force microscopy. J Struct Biol 2009;167:216–219.
38. Shih CY, Harder TA, Tai YC. Yield strength of thin-film parylene-C. Microsystem Technol 2004;10:407–411.
39. Zhong Y, Bellamkonda, RV. Dexamethasone-coated neural probes elicit attenuated inflammatory response and neuronal loss compared to uncoated neural probes. Brain Res 2007;1148:15–27.
40. Han N, Rao SS, Johnson J, Parikh KS, Bradley PA, Lannutti JJ, Winter JO. Hydrogel–electrospun fiber mat composite coatings for neural prostheses. Front Neuroeng 2011;4:2.
41. Lee C, Yu L, Kuo JTW, Kim B, Hoang T, Meng E. Drug eluting coating for 3D Parylene sheath electrode. 6th International IEEE/

EMBS Conference on Neural Engineering (NER). San Diego; 2013. pp 839–842.
42. Tonomura W, Moriguchi H, Jimbo Y, Konishi S. Parallel multipoint recording of aligned and cultured neurons on corresponding Micro Channel Array toward on-chip cell analysis. 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). Vancouver; 2008. pp 943–946.
43. Mazia D, Schatten G, Sale W. Adhesion of cells to surfaces coated with polylysine. Applications to electron microscopy. J Cell Biol 1975;66:198–200.
44. Orive G, Tam SK, Pedraz JL, Hallé JP. Biocompatibility of alginate–poly-l-lysine microcapsules for cell therapy. Biomaterials 2006;27:3691–3700.
45. Cogan SF. Neural stimulation and recording electrodes. Annu Rev Biomed Eng 2008;10:275–309.
46. Parkinson J, Kadler KE, Brass A. Self-assembly of rodlike particles in two dimensions: A simple model for collagen fibrillogenesis. Phys Rev E 1994;50:2963.
47. Witten TA Jr,Sander LM. Diffusion-limited aggregation, a kinetic critical phenomenon. Phys Rev Lett 1981;47:1400–1403.
48. Zhang D, Li J, Chen S, Li T, Zhou J, Cheng X, Zhang A. Hybrid self-assembly, crystal, and fractal behavior of a carboxy-ended hyperbranched polyester/copper complex. Macromol Chem Phys 2013; 214:370–377.
49. Gan H, Li Y, Liu H, Wang S, Li C, Yuan M, Liu X, Wang C, Jiang L, Zhu D. Self-assembly of conjugated polymers and ds-oligonucleotides directed fractal-like aggregates. Biomacromolecules 2007;8:1723–1729.
50. Wang W, Chau, Y. Self-assembled peptide nanorods as building blocks of fractal patterns. Soft Matter 2009;5:4893–4898.
51. Zhao Q, Qian J, Gui Z, An Q, Zhu M. Interfacial self-assembly of cellulose-based polyelectrolyte complexes: Pattern formation of fractal "trees." Soft Matter 2010;6:1129–1137.
52. Craighead HG, James CD, Turner AMP. Chemical and topographical patterning for directed cell attachment. Curr Opin Solid State Mater Sci 2001;5:177–184.
53. Thatiparti TR, Shoffstall AJ, von Recum HA. Cyclodextrin-based device coatings for affinity-based release of antibiotics. Biomaterials 2010;31:2335–2347.
54. Dungel P. Optical imaging of radiolabeled drugs in tissue sections using the microImager. University of South Florida; 2006.
55. Wright D, Rajalingam B, Selvarasah S, Dokmeci MR, Khademhosseini S. Generation of static and dynamic patterned co-cultures using microfabricated parylene-C stencils. Lab Chip 2007;7:1272–1279.
56. Kleinman HK, Martin GR. Matrigel: Basement membrane matrix with biological activity. Semin Cancer Biol 2005;15:378–386.
57. Zhong Y, McConell GC, Ross JD, DeWeerth SP, Bellamkonda RV. A novel dexamethasone-releasing, anti-inflammatory coating for neural implants. 2nd International IEEE/EMBS Conference on Neural Engineering (NER). Arlington; 2005. pp 522–525.
58. Shain W, Spataro L, Dilgen J, Haverstick K, Retterer S, Isaacson M, Saltzman M, Turner JN. Controlling cellular reactive responses around neural prosthetic devices using peripheral and local intervention strategies. IEEE Trans Neural Syst Rehabil Eng 2003;11: 186–188.