3D PARYLENE SHEATH PROBES FOR RELIABLE, LONG-TERM NEUROPROSTHETIC RECORDINGS

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ABSTRACT

Parylene C neural probes with a 3D sheath structure are introduced as a novel interface for long-term intracortical neural recording. 3D sheath structures were assembled from surface micromachined Parylene microchannels by thermoforming the thermoplastic around a solid microwire mold. Multiple Pt electrodes lined the interior and exterior of the sheath. Electrochemical characterization of the electrodes confirmed impedance values (50-250 kΩ at 1 kHz) suitable for neural recordings. A novel insertion approach was developed that temporarily stiffens the neural probes for surgical implantation and optimized in agarose brain tissue model. Sheath probes implanted into rat cortex recorded neural signals for four weeks. To achieve long-term, reliable recordings, the sheath structures will be coated with eluting neurotrophic factors to promote and attract neural ingrowth towards electrode sites.

INTRODUCTION

A critical challenge in brain-machine interfaces (BMI) is the lack of reliable chronic recordings from the cortex using current neural interface technologies. Recorded neural signals inevitably degrade over time; this is attributed to a number of factors including the mechanical mismatch between rigid neural probes and cortical tissue and associated chronic irritation. Together these lead to neurotoxic factor release from microglia as a result of inflammatory response causing gradual retraction of neural processes from recording sites and encapsulation of the probe by astrocytes and microglia [1]. There are only a few recordings in both humans and animals reported that have lasted beyond a year, however these modest successes are inadequate to realize practical clinical devices.

Only two microelectrode technologies have progressed to clinical studies. The first consists of an array of tapered p+ silicon pins with a single Pt recording site located at each tip [2-3]. Clinical trials with these probes are ongoing. Interestingly, the most reliable long-term recordings reported in human to date were achieved using manually fabricated neurotrophic cone electrodes [4-5]. Glass cones were formed from pulled micropipette tips and recordings were achieved using microwires inserted at different depths along the interior of the cone (2-4 microwires per cone). In the neurotrophic cone electrodes, neurotrophic factors were released from the cones into the surrounding neural tissue following implantation. Long-term recordings without the typical signal degradation or loss associated with other microelectrode approaches was hypothesized to arise from dendritic ingrowth post-implantation (~3 months) into the cones toward recording sites attributed to the presence of neurotrophic factor gradients. Although long-term reliable neural recordings were demonstrated, the manual fabrication process and limited recording sites impeded manufacturability and widespread adoption of this technology.

The realization of practical neural prostheses requires improvement in long-term intracortical recordings. Probes constructed from flexible polymer materials can partially alleviate the mechanical mismatch. Microfabrication technologies overcome manufacturing challenges and achieve batch fabricated devices with consistent repeatability. Recording sites constructed with thin film metal electrodes increases the number and density of recording sites per probe for greater spatiotemporal signal information without increasing footprint compared to microwire approaches. Finally, management of the inflammatory response and promotion of tissue integration post-implantation can be achieved with slow release of appropriate bioactive coatings to the surrounding tissue.

To achieve a stable long-term intracortical interface, we previously introduced novel microfabricated structures in the form of sheaths or cylinders with Pt electrodes (Figure 1) [6-8]. Here, improved sheath probe design and the first intracortical recordings using thermoformed neural probes are described.

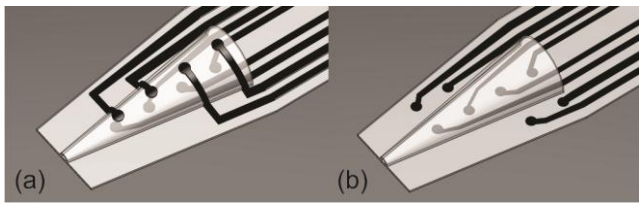

Figure 1: Conceptual drawing of Parylene sheath probes for long-term intracortical recordings. The 3D sheath structure allows for ingrowth of neural processes toward recording electrodes. Probes were fabricated having electrodes either on (a) the outer sheath surface or (b) the sheath periphery.

METHODS

Material Selection

Parylene C, a USP Class VI biocompatible polymer that can be processed with standard micromachining technologies [9], was selected as the structural material. Parylene C possesses a low Young's modulus [10] in comparison to traditional probe materials such as Si or glass. Recordings sites were constructed from thin film Pt, a non-corrosive and inert metal widely used in neural interfaces [11]. This material combination simplifies fabrication as no adhesion layer between Pt and Parylene is

required [12].

Device Design

Probes consist of sheath structures with four Pt electrodes (45 μm diameter) on each of the inner and outer surfaces (eight total electrodes). Two probe types were fabricated differing only in the location of the outer electrodes – either directly on the sheath or the flaps ("wings") peripheral to the sheath. Sheath dimensions and electrode placement were selected to match the anatomy of rat barrel motor cortex to target recordings of vibrissal (whisker)-related neuronal activity.

Fabrication

A bare Si wafer with native oxide was used as a carrier substrate during the microfabrication process and aided in the subsequent release of Parylene probes from the wafer.

Sheath probes having sheath-top electrodes were fabricated by first depositing 5 μm Parylene (Figure 2). A liftoff process using negative photoresist (AZ 5214 E-IR) was utilized to pattern inner sheath electrodes created with e-beam deposited Pt (2000 Å). A 1 μm Parylene insulation layer was then deposited and selectively plasma etched to expose electrodes and contact pads. Sheath outlines were constructed by patterning sacrificial photoresist (AZ 4620, 9.6 μm) and overcoating with 5 μm Parylene. A dual layer liftoff scheme (AZ 1518/AZ 4620) with negative sidewall profile was utilized to pattern outer electrodes on top of the sheath structure and was necessary to ensure that resulting wire traces were continuous from the top of the microchannel structure to the base [13]. Pt was then e-beam deposited (2000 Å) to form the outer electrodes. A final 1 μm Parylene insulation layer was deposited and plasma etched to create openings for outer electrodes and contact pads. A final plasma etch was performed to create sheath openings and cut out the individual probes. Probes were released from the substrate and sacrificial photoresist removed with an acetone soak.

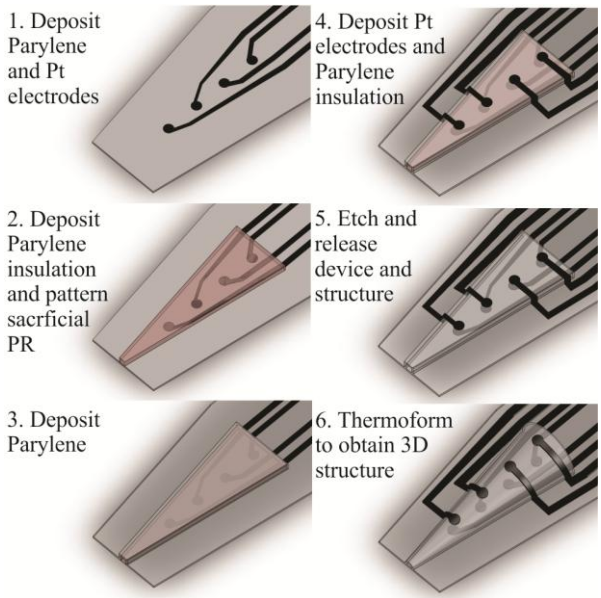

Figure 2: Fabrication process for sheath probe having "top" electrodes on sheath surface. Only the final outline of the device is shown.

The "wing" sheath probes simplified the fabrication process by moving the outer electrodes to the periphery; this reduced the number of steps required and also prevented occasional cracking of the top electrodes encountered during the sheath forming process (Figure 3). As before, a bare Si wafer with a native oxide layer was coated with 5 μm Parylene. Electrodes, traces, and contact pads were liftoff patterned using AZ 5214 E-IR and e-beam deposited Pt (2000 Å). A 1 μm Parylene insulation layer was then deposited, patterned, and plasma etched, exposing recording sites and contact pads. Next, AZ 4620 (9.6 μm) was patterned and covered with 5 μm Parylene to create sheath structures. Selective plasma etching of Parylene exposed peripheral electrodes, contact pads, and sheath openings while simultaneously defining each individual probe. Finally, the probe was lifted from the wafer surface and an acetone soak released the sheath structure.

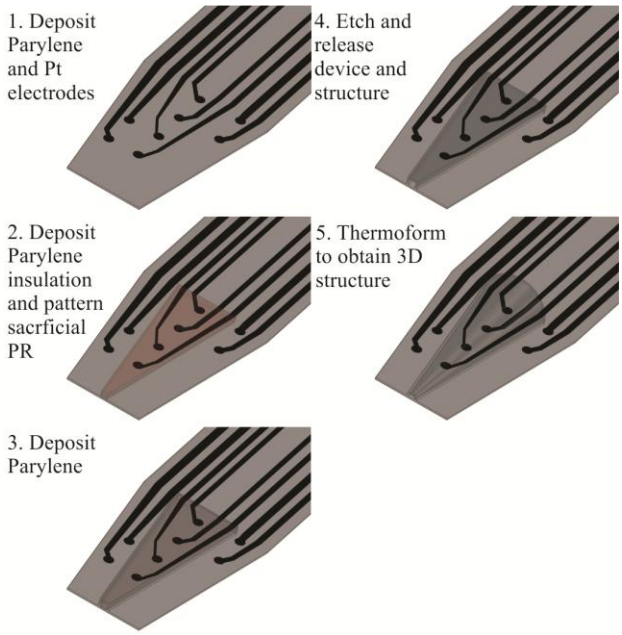

Figure 3: Fabrication process for sheath probe having peripheral "wing" electrodes. Only the final outline of the device is shown.

Parylene Thermoforming

Conical and cylindrical 3D sheath structures were created by thermoforming Parylene around a custom tapered stainless steel or tungsten microwire mold (Figure 4). Etched microwires with tapers to match the desired probe shape and to facilitate insertion into the microchannels were purchased from MicroProbes for Life Science. A microwire tip was aligned and inserted into the sheath underneath a microscope to open the structure. The assembly was held in a custom aluminum jig and placed into a vacuum oven. Thermoforming was performed with a controlled temperature ramp to 200 °C and held for 48 hours followed by a controlled cool down. Nitrogen purging prevented Parylene oxidative degradation by minimizing oven oxygen content. The microwire was then removed and the sheath retained its 3D structure. The thermal annealing process also served to improve adhesion

between Parylene layers [13].

A linear contact pad array located at the end of the Parylene ribbon cable was attached using adhesive to a thin polyetheretherketone (PEEK) sheet and inserted directly into a zero insertion force (ZIF) connector. This method allowed simple, reversible electrical connections with contact pads (Figure 5a).

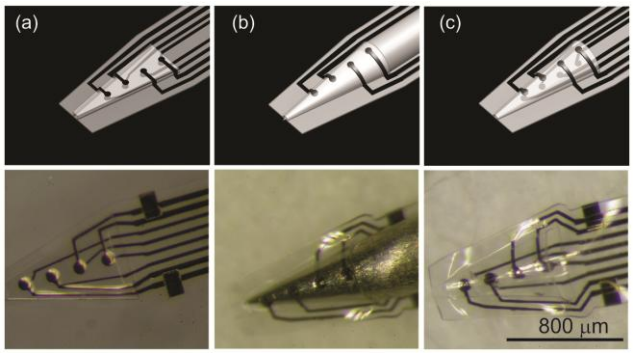

Figure 4: Thermoforming process steps. (a) Released probes were (b) shaped around a microwire mold and thermally treated. (c) Subsequently, wires were removed to reveal the final structure.

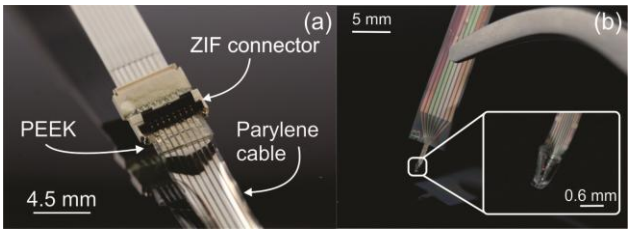

Figure 5: (a) Sheath probe with integrated Parylene cable attached to a ZIF connector for external electrical connections. (b) Released sheath probe. The 3D Parylene sheath structure holds its shape post-thermoforming.

RESULTS

3D Sheath Probe Fabrication

Both sheath probe types with different sheath shapes (A, B, and C) were successfully fabricated and thermoformed (total of six different designs, Figure 5b, Table 1). Thermoformed Parylene sheaths were mechanically robust and could withstand repeated indentation by a probe.

Table 1: Dimensions of the different fabricated sheath structures for both top and wing electrode designs.

Sheath Shape	Base Opening Diameter	Tip Opening Diameter	Sheath Length
A, tapered	300 µm	50 µm	800 µm
B, tapered	450 µm	50 µm	800 µm
C, cylindrical	300 µm	300 µm	800 µm

Outer Sheath Surface Electrodes

The dual photoresist layer liftoff method successfully patterned the outer surface electrodes and traces despite the step from the periphery to the top of the sheath. Lead resistances were measured by directly probing the top electrodes and corresponding contact pads (typically 325-389 Ω). No lead discontinuities were observed. However, some sheath-top electrodes exhibited tensile fracture at locations with high strain. Interestingly, inner sheath electrodes did not crack.

To mitigate yield loss attributed to electrode cracking, the second sheath probe type moved outer electrodes to the periphery and away from potential strain induced by reshaping. These devices did not exhibit any electrode fracture post-thermoforming and may experience improved recordings due to their edge location which provides greater access to surrounding neurons [14].

Electrochemical Characterization

Electrodes housed within a Faraday cage were assessed using a Gamry Reference 600 potentiostat for cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) characterization. Probes were immersed in 0.05 M sulfuric acid (H_2SO_4) and a separate Ag/AgCl reference electrode was used for CV measurements. One probe electrode was set as the working electrode while an adjacent Pt electrode was used as the counter. Voltage was cycled between -0.2 to 1.2 V with a scan rate of 250 mV/sec. 50 scans were taken to ensure a stable voltammogram was recorded.

The classic voltammogram of Pt immersed in sulfuric acid was obtained for all thin film Pt electrodes. Peaks corresponding to hydrogen adsorption and desorption as well as Pt oxide formation and reduction were observed (Figure 7).

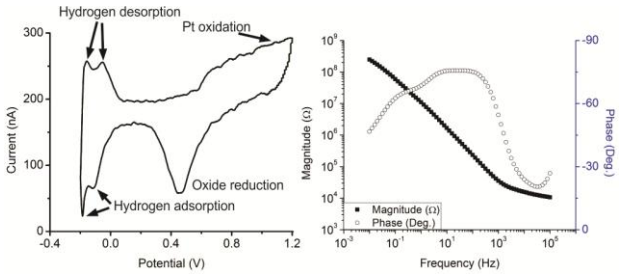

Figure 7: Representative (left) CV in H_2SO_4 and (right) EIS in 1XPBS of a single recording site.

The sheath probe was immersed in 1X phosphate buffer solution (PBS) for EIS with a separate Ag/AgCl reference electrode. Impedances for all electrodes were recorded over 1-10^5 Hz (Figure 7). Impedances ranging from 50-250 kΩ at 1 kHz were obtained indicating acceptable electrode properties for neural recording. Impedances of less than 1 MΩ portend neural recording success with high SNR upon probe implantation [15].

Implantation Procedure

An implantation procedure was developed in which sheath probes were temporarily affixed to a microwire with water soluble polyethylene glycol (PEG 8000) on a custom introducer tool [8]. A stereotaxic frame was used to implant the assembly into a 0.5% agarose gel having similar shear and elastic moduli as the brain tissue in order to optimize the insertion procedure. The probe was released from the introducer tool with a saline flush which dissolved the temporary coating PEG. Finally, the introducer tool was retracted while leaving probes in place.

In Vivo Intracortical Recordings

Probes with wing electrodes were implanted into the rat barrel motor cortex following a protocol approved by the Huntington Medical Research Institutes Institutional Animal Care and Use Committee (IACUC). Neural recordings were successfully obtained at weekly intervals post-implantation and neural signal was found to improve over time with increasing SNR and spike rate (Figure 8).

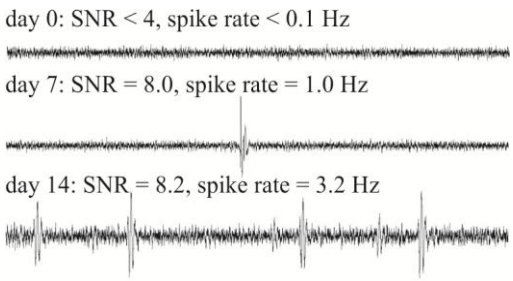

Figure 8: Representative rat neuronal activity from a sheath probe recording site at different days post-implantation.

CONCLUSION

Novel 3D Parylene sheath probes constructed by micromachining followed by thermoforming to improve long-term intracortical recordings were achieved. Probes featured multiple Pt recording sites on both the interior and exterior sheath surfaces. Electrochemical characterization of Pt electrodes was performed and confirmed acceptable properties for neural recordings. A novel implantation method was developed for this polymer probe architecture and demonstrated both in the agarose brain model and *in vivo*. Intracortical recordings from rat were obtained over weeks with improving neural signal seen over time.

Future work entails evaluation of the efficacy of long-term cortical recordings on different sheath designs, development of deposition processes for decorating bioactive coatings onto probe surfaces, and development of sheath probe arrays for recording from large areas and multiple sites of interest. This probe architecture will also be investigated for peripheral and central nervous system recording applications.

ACKNOWLEDGEMENTS

The authors thank Dr. Donghai Zhu and members of the USC Biomedical Microsystems Lab for their assistance. The authors would also like to thank the following individuals at the Huntington Medical Research Institutes: Ms. Kate Nelson and Dr. Saiyun Hou for performing the chronic animal implantations, Ms. Nijole Kuleviciute for collection of *in vivo* impedance spectroscopy data, and Dr. Martin Han for providing access to the neurophysiological recording system.

This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) MTO under the auspices of Dr. Jack Judy through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-11-1-4207.

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