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Acute *in vivo* Recording with a Generic Parylene Microelectrode Array Implanted with Dip-coating Method into the Rat Brain*

Huijing Xu, *IEEE, Member*, Kee Scholten, *IEEE, Member*, Wenxuan Jiang, *IEEE, Student Member*, Jessica-Lizbeth Ortigoza-Diaz, *IEEE, Student Member,* Zhouxiao Lu, *IEEE, Student Member*, Xin Liu, *IEEE, Student Member*, Ellis Meng, *IEEE, Fellow* and Dong Song, *IEEE, Senior Member*

***Abstract*—Flexible polymer-based microelectrode arrays (MEAs) can reduce tissue inflammation and foreign body response and greatly prolong the lifetime of neural implants. However, standard and customized polymer devices are only accessible to limited groups. To better promote the development and application of polymer MEAs, we have launched the Polymer Implantable Electrode (PIE) Foundry and developed a 64-channel Parylene C-based MEA with generic electrodes layout that can be used to record from both cortical and sub-cortical regions in rodents. In addition, a practical dip-coating protocol for the insertion of the flexible standard Parylene MEA is developed.**

I. Introduction

Penetrating microelectrode arrays (MEAs) are one of the most commonly used tools for electrophysiological studies of brain functions. Microelectrodes placed adjacent to neurons can directly monitor the activities of individual neurons. In particular, invasive penetrating MEAs provide the best spatial and temporal resolution for neuroscience studies. This advantage makes them one of the most powerful tools for real-time investigations of neuronal properties of different cell types and a necessary instrument to study the correlation between neural activities and behaviors [1]. The advancement of manufacturing metal-based microwire arrays and the fabrication of high-density silicon-based multi-shank arrays made it possible to monitor unitary activities of large populations of neurons simultaneously [2][3]. However, the performance of rigid MEAs typically degrades over time and longevity of recording remains one of the major challenges faced by rigid MEAs [4].

Flexible polymer-based probes can significantly reduce inflammation and tissue response and obtain stable long-term recording from the brain [5][6]. However, limited accessibility of polymer MEAs has largely restricted the development and application of this new technology to a few research groups that is able to build their own devices. Supported by the NIH BRAIN Initiative, we established the PIE Foundry** as a shared-resource center to build and test polymer MEAs for neuroscience researchers. The aim of the PIE Foundry is to develop a platform for rapid manufacture of standard and customized polymer MEAs for chronic neural recording and stimulation (Fig. 1). For researchers who are interested in ready-to-use polymer MEAs, we have developed a Parylene C-based MEA with generic electrode layout. This "standard" penetrating Parylene MEA is suitable for both cortical and sub-cortical recordings in small animal models such as rodents.

In addition, implantation of flexible MEAs often requires special procedures such as the usage of specifically fabricated shuttle devices [7] or precisely aligned stiffener [8]. These methods are usually not easy to adopt for practitioners in the broader community. In this study, we experimented different insertion techniques and developed an easy-to-operate dip-coating protocol using polyethylene glycol (PEG) for the implantation of the generic Parylene MEA.

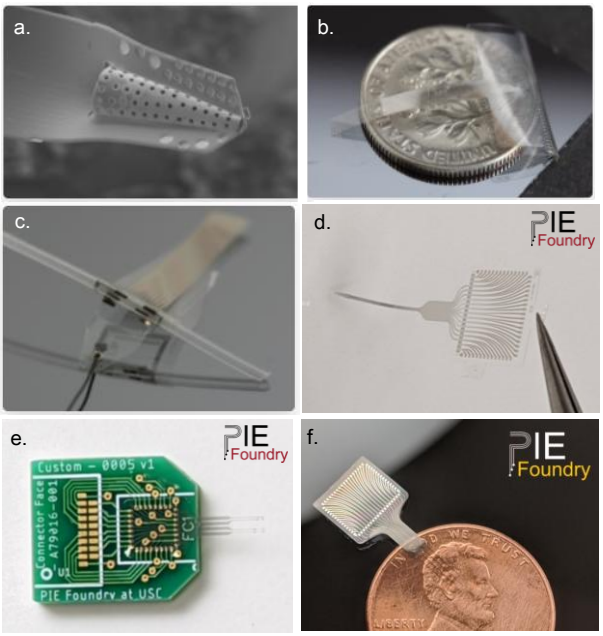

Figure 1. Examples of polymer devices developed and fabricated by co-founders of the PIE Foundry. a: A flexible Parylene neural probe with 3D sheath structure for enhancing tissue integration [5]. b: A multi-shank Parylene MEA for simultaneously targeting for multiple sub-regions of the rat hippocampus[9]. c: A cuff electrode with microfluidics to interfacing with peripheral nerve [10]. d: A Parylene probe with eight tetrodes for chronic recordings from mouse striatum. e: A 32 channel, multi-shank MEA for multi-regions recordings from the mice hippocampus and, f: the standard Parylene MEA for cortical and sub-cortical recordings from rodent's brain.

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H. J. Xu is with the Center of Neural Engineering, University of Southern California, Los Angeles, CA 90089 USA (phone: 213-740-8061; email: huijingx@usc.edu).

K. Scholten, J Ortigoza-Diaz, X. Liu and E. Meng are with the Biomedical Microsystem Laboratory, University of Southern California, Los Angeles, CA90089 USA (email: kscholte@usc.edu, ellis.meng@usc.edu).

W. X. Jiang, Z. X. Lu and D. Song are with the Center of Neural Engineering, University of Southern California, Los Angeles, CA 90089 USA (email: jian393@usc.edu, luzhouxi@usc.edu, dsong@usc.edu)

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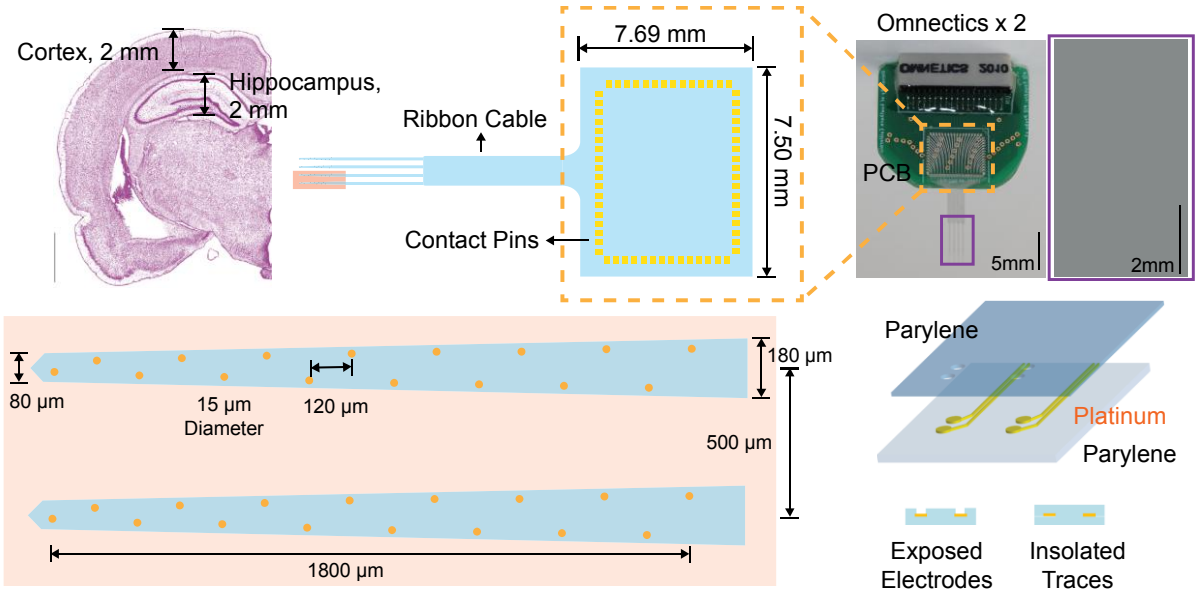

Figure 2. Layout, structure and packaging of the PIE Foundry standard Parylene MEA. Picture on the left of the top row shows the size of rat cortex and hippocampus. The middle figure illustrates the overall geometry and dimension of the Parylene MEA. Pictures on the right shows a fully packaged MEA and the zoomed-in view of four Parylene shanks. The bottom left figure shows detailed dimensions of the width of individual shanks, spacing between shanks, spacing between electrodes and diameter of recording electrodes. The bottom right picture illustrates the structure of the Parylene MEA in cross-section.

II. Material and Method

A. The Standard Parylene MEA

The 64-channel standard penetrating probe array consists of four 5 mm long Parylene shanks separated by a 500 µm distance (center to center). To reduce tissue damage and ease the insertion of the flexible MEA, shanks are tapered in width from 180 µm to 80 µm. On each shank, 16 platinum (Pt) electrodes with 15 µm diameter are evenly distributed along both edges of the Parylene shank. The center-to-center vertical distances between electrodes are 120 µm. The recording region spans 1800 µm vertical distance which is enough to cover multiple layers of the rat cortex and large sub-cortical structures such as the hippocampus.

Each individual electrode is connected to a small Pt contact pad with 3µm thin Pt traces. Metal traces are sandwiched between two layers of 10 µm thin ParyleneC films. The 8×8 mm^2 Parylene pad that contains all 64 contact pads is directly bonded to an 18×17 mm^2 customized PCB using a novel polymer-ultrasonic-weld method [11]. This method significantly reduced the size of the PCB and eliminated the usage of extra connectors such as the zero-insertion-force (ZIF) connectors. Two stainless steel wires are soldered onto the PCB as ground wires. To minimize the size of the PCB, two 36-pin Omnetics connectors are soldered on both sides of the PCB, to connect the Parylene MEA with data acquisition systems. All soldering pins and the entire PCB are coated with epoxy (Epotek 302M) for insulation and protection.

The standard MEA was fabricated with conventional microlithography techniques. As illustrated in Figure 2, a base layer of 10 µm Parylene C was deposited on a silicon wafer followed by the deposition of a 200 nm thick Ptlayer. The recording site, connecting traces and contact pads were contained in the metal layer and defined by lift-off. The metal layer was then encapsulated by another layer of 10 µm thick Parylene C. Electrodes and contact pad were subsequently exposed by deep reactive ion etching (DRIE) with an oxygen plasma, and a second DRIE step defined the array outline and shape. Finally, the devices were released from the silicon wafer in a water bath. Connectivity of electrodes and traces were evaluated with visual inspection under microscope and electrochemical properties of each individual electrode were evaluated using electrical impedance spectroscopy.

B. Dip-coating Protocol

Polyethylene glycol (PEG) with 8kDa molecular weight was melted and heated to just above 100°C for sterilization. The melted PEG was then kept between 75 to 80°Cona hotplate. The Parylene MEA, together with its packaging, was pre-sterilized by soaking in 70% ethanol for 5 minutes. After the device was fully dried, it was attached onto a manipulator which can smoothly rise or lower the assembly. First, the entire device was lowered into the PEG solution until the bottom edge of the PCB was in contact with the solution. Then the device was slowly retrieved from the PEG at a speed about 20 µm/sec. A thin PEG film is often formed between Parylene shanks. If the shanks in the array are aligned, no further operation is needed. Otherwise, the shanks are submerged into pre-sterilized distilled water for 2 minutes. The thin PEG film dissolves quickly, and the shank tips are lowered into the PEG solution to re-coat (Fig. 3).

C. Animal Preparation

Sprague-Dawley (SD) rats older than twelve weeks and weighing between 250 to 450g were used for the implantation of the standard Parylene MEA. All procedures were approved by the University of Southern California Institutional Animal Care and Use Committee. During the implantation surgery, rats were anesthetized intra-operatively with a mixture of isoflurane and air. Toe-pinch withdrawal reflexes were tested

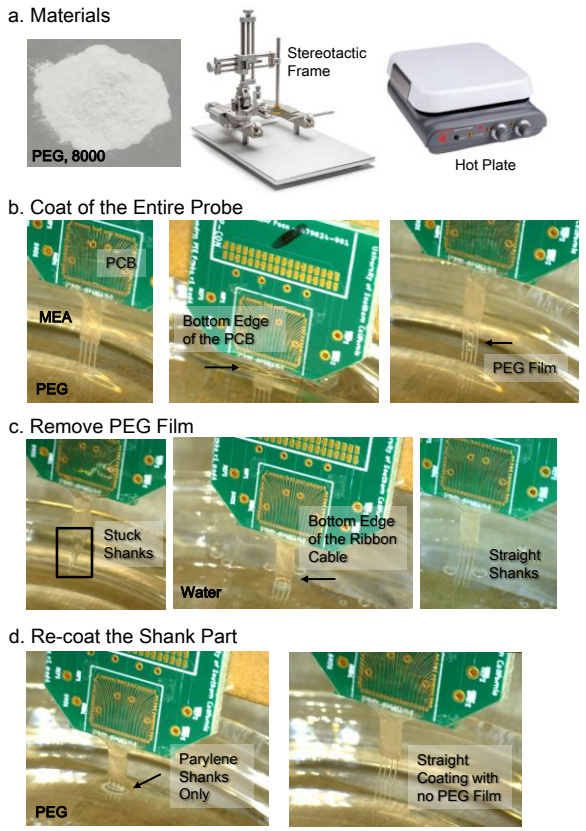

Figure 3. Procedure to dip-coat the Parylene MEA with PEG. a: Material and equipment used for dip-coating. b: Coating of the entire device with PEG. The right photo shows a coated MEA with thin PEG films between shanks. c: Steps to remove PEG film by water immersion. d: Procedure to coat only the shanks with PEG. The right photo shows a properly coated MEA with no excess PEG.

frequently to ensure proper depth of anesthesia. Each animal was fixed on a stereotactic frame with ear bars, and a 2×4 mm^2 piece of skull above the dorsal hippocampus on the right hemisphere was removed. Both the dura and pia layers were carefully removed before the insertion of the MEA. Small drops of saline were applied to the exposed brain surface to prevent drying of the tissue. The PEG coated Parylene MEA was slowly inserted to a depth of 3.8 mm measured from the surface of the skull. Signals from all 64 channels were monitored during the insertion and the final depth of the MEA was adjusted according to the quality of neural signals. After the microelectrode array reached desired depth, dental cement was applied to seal the bone window and fix the location of the MEA by attaching it to five anchor screws pre-secured on the skull. Two ground wires were twisted onto the screw that was in contact with cerebrospinal fluid and tips of ground wires were inserted into a small hole drilled above the cerebellum.

After all neural signals were collected, the animal was deeply anesthetized and perfused with 4% paraformaldehyde (PFA). The brain was carefully dissected and preserved in PFA for histological studies.

D. Histology

Horizontal brain slices (10 µm thick) from one animal implanted with the Parylene MEA for one month were prepared using cryostat and stained with crystal violet to verify the depth of the insertion and to evaluate damages caused to surrounding tissues by the implanted MEA.

III. Results

A. Insertion of the Standard Parylene MEA

The insertion of the flexible standard MEA with dip-coated PEG was first tested using a brain phantom made with 0.6% agarose gel. Two dip-coated devices with thin PEG films between the shanks were inserted into the agarose to a depth of 4 mm (Fig. 4). The PEG film dissolved immediately upon contact with the agarose gel and did not affect the insertion of the Parylene MEA. Feasibility of the dip-coating protocol was further tested on rat brains. Six Parylene MEAs were dip-coated following steps described above and inserted into the brain of anesthetized rats. Two devices had thin PEG films between shanks and the remaining four devices were coated twice to remove the PEG film. All six MEAs penetrated brain tissue smoothly and were successfully inserted without buckling. Brain slices (10 µm) collected from the brain of an animal following implantation of the Parylene MEA for one month verified that the Parylene MEA was successfully inserted to the depth of 3.00 mm. In addition, the preliminary histology result shows that no severe immune response was observed at the implantation site and the Parylene MEA caused very limited damage to surrounding neurons.

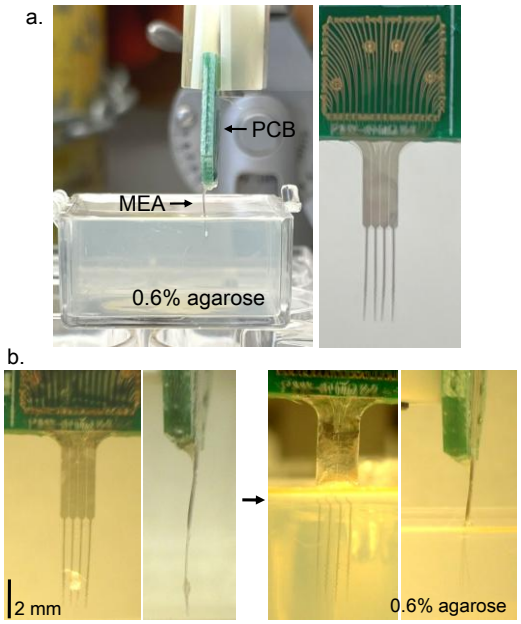

Figure 4. Insertion of dip-coated Parylene MEAs into brain phantom. a: Bench top testing setup. 0.6% agarose gel is used to mimic the texture of the brain tissue. The photo on the right shows an un-coated Parylene MEA. b: Photos on the left show the front and side view of a PEG-coated Parylene MEA with a small block of PEG on the shank. Photos on the right show front and side view of the coated MEA after insertion into the agarose gel ~4 mm deep.

B. In vivo Recording with the Standard Parylene MEA

Neural activities were recorded during the implantation of two Parylene MEAs. One prototype MEA with 48 functional electrodes was implanted to a depth of 3.0 mm. Unitary activities and local field potentials from both the cortex and

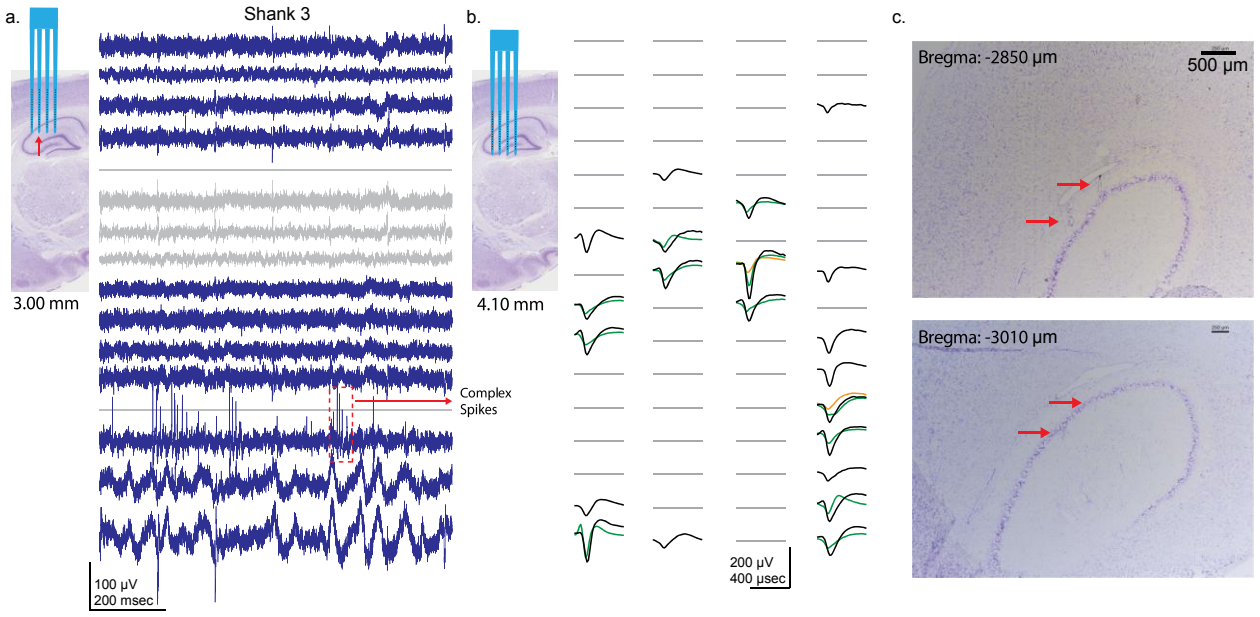

Figure 5. Acute *in vivo* recording with dip coated, standard Parylene MEAs from ratcortex and hippocampus. a: Data collected from a PIE Foundry standard MEA implanted to a depth of 3.00 mm. 800 msec of high-pass filtered (>300 Hz) neural activity recorded by 16 electrodes on a single Parylene shank is shown. Single spikes from the cortex recorded with superficial electrodes and complex spikes from the hippocampus recorded with bottom electrodes were recorded simultaneously. Not functioning channels are shown as gray. b: Data collected from standard MEA implanted to a depth of 4.10 mm. 34 units from multiple hippocampal sub-regions were recorded. Multiple units recorded with the same electrodes are color-coded. c: Nissl stain of 10 µm horizontal brain slices collected from an animal implanted with the Parylene MEA for one month. The MEA was dip-coated and inserted to a depth of 4.05 mm during the surgery. Red arrows indicate locations of two Parylene shanks. No severe tissue response is observed and damage to surround tissue is limited to the width of the Parylene shank.

the hippocampus were recorded along an individual shank. Another fully functional Parylene MEA was implanted to a depth of 4.10 mm. All four shanks reached the hippocampal region and recorded complex spikes from hippocampal pyramidal cells. A total of 34 units were recorded with the standard Parylene MEA (Fig. 5).

IV. Discussion and Future Works

In this work, we designed and manufactured a polymer-based MEA with a standard electrode layout. A practical dip-coating protocol for the insertion of flexible polymer MEAs was also developed. To further evaluate long-term performances of the Parylene MEA, we will implant the MEA chronically and collect neural signals frequently over a long period of time. Immuno-histochemistry studies will be conducted to evaluate the tissue responses to the MEA in more details.

One of the major goals of the PIE Foundry is to produce and distribute polymer devices with arbitrary geometries, sizes and channel counts for the broader neuroscience community. To achieve this goal, we will design, fabricate, and test different MEAs for different animal models and different experimental paradigms and include them into the PIE Foundry's library of polymer devices. In addition, we will collaborate with researchers from the neuroscience and neural engineering community to develop highly customized polymer devices for each group's specific research need.

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