Pre-Implantation Electrochemical Characterization of a Parylene C Sheath Microelectrode Array Probe*

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Abstract— **We present the preliminary electrochemical characterization of 3D Parylene C sheath microelectrode array probes towards realizing reliable chronic neuroprosthetic recordings. Electrochemical techniques were used to verify electrode integrity after our novel post-fabrication thermoforming process was applied to flat surface micromachined structures to achieve a hollow sheath probe shape. Characterization of subsequent neurotrophic coatings was performed and accelerated life testing was used to simulate six months *in vivo*. Prior to probe implantation, crosstalk was measured and electrode surface properties were evaluated through the use of electrochemical impedance spectroscopy.**

I. INTRODUCTION

Chronic neuroelectrical implants in the cortex enable acquisition of neuronal activity that can be decoded and processed to realize control of prosthetic limbs [1]. However, clinical translation of cortical probes is limited by signal degradation or loss over long-term use. To date, chronic electrical recordings in humans have only been reported for durations less than five years [2-4]. One contributing factor limiting their success has been the brain's local immune response to the probe [5-6]. In addition, the mechanical mismatch between the rigid probe material and the cortical tissue results in probe breakage, repositioning, and associated tissue damage [7].

To address these concerns, a 3D biocompatible polymer sheath microelectrode array probe was developed with surface micromachined Parylene C for neuronal recording (Fig. 1). The probe has four Pt electrodes both on the interior and exterior surfaces of the Parylene C sheath; the sheath structure and interior electrodes provide a mechanism to isolate neuronal signals as neural processes grow into the sheath toward recording sites. Anti-inflammatory and neurotrophic coatings loaded on a basement matrix of Matrigel (BD Biosciences) were applied to the probes to counteract the immune response and promote the dendritic growth towards the electrodes and into the sheath. Thus, the sheath forms a path for establishing a natural anchor for the probe with the surrounding neural tissue [8].

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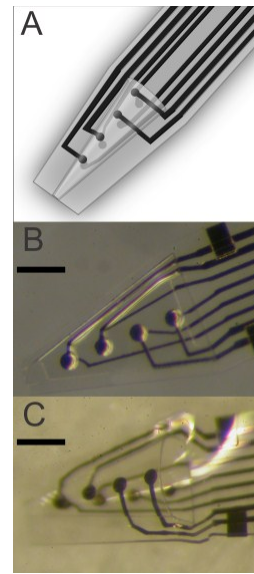
Figure 1. (A) Schematic and pictures of (B) unopened, and (C) opened Parylene C sheath microelectrode array probe. Scale bar = 180 µm.

Electrochemical methods, such as electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV), are commonly utilized to characterize electrode properties and investigate changes to the electrode surfaces [9]. In the long-term, these methods can be utilized *in vivo* to monitor electrode status and the effects of the biological response to the probe implant [10-11]. We present the preliminary electrochemical characterization using EIS of our novel probes following fabrication, thermoforming, and coating. We also present interelectrode crosstalk measurements. These pre-implantation studies are intended to establish a baseline prior to implantation and elucidate probe performance under accelerated lifetime testing. Such methods and the acquired data will enable development of reliable probes for long term use over the patient lifetime.

II. MATERIALS AND METHODS

Parylene sheath probes were fabricated using a process described in [8]. Probes were tested with the sheath either in an opened or an unopened state. The opening of the sheath was performed post-fabrication via the manual insertion of a microwire. Prior to testing, electrodes were electrochemically cleaned and probes were coated with Matrigel. For these experiments, Matrigel was diluted with $1\times$ PBS at a ratio of 790µL:210µL. Two methods were assessed to coat the probes: micropipette application and submersion with sonication agitation. Coatings were then left to gel at room temperature.

A. Electrochemical Cleaning and Characterization

Electrochemical measurements were performed in a three-electrode configuration with a Gamry Reference 600 potentiostat (Gamry Instruments, Warminster, PA). A Faraday cage was used to minimize noise.

Electrochemical cleaning was conducted in 0.05M H_2SO_4 by cycling the potential of the working electrode from -0.2 to 1.2V vs. Ag/AgCl (3M NaCl) at 250 mV/s for 50 cycles. A large area Pt plate served as a counter electrode.

EIS measurements were conducted in phosphate buffer saline (1× PBS) with an AC sinusoidal signal of 10 mV rms in the frequency range of 1–100,000 or 10–100,000 Hz. An adjacent electrode served as a counter electrode and an Ag/AgCl (3M NaCl) electrode as reference. To more closely simulate electrode performance *in vivo*, measurements were taken at 37°C.

Interelectrode crosstalk was measured to ensure proper insulation and the lack of interference between electrodes. The probes were immersed in 1× PBS along with a large area Pt plate counter electrode. For each electrode, a biphasic, balanced charge current pulse (5 µA amplitude and 200 µs phase width) was applied. Any resulting voltage across that electrode, as well as across all other electrodes on the probe, was measured. The normalized voltage signal present on each electrode was used as a measure of the crosstalk.

B. Accelerated Lifetime Testing

Accelerated aging of probes was accomplished by immersion in 1× PBS at 80°C according to the following equation,

$$t_{37} = t_T \times Q_{10}^{\frac{(T-37)}{10}} \qquad (1)$$

where t_{37} is the simulated age in days at 37°C, t_T is the number of days aged at temperature, T, and Q_{10} is the temperature coefficient. This equation is often used to simulate aging of polymers in medical devices with a temperature coefficient of 2 according to [12]. At this temperature, a simulated six months (180 days) of aging *in vivo* is completed in 9.1 days. Daily EIS measurements were taken of each electrode at 37°C.

III. RESULTS AND DISCUSSION

A. Electrochemical Cleaning

Electrochemical cleaning of the electrode surface produces a clean surface by anodizing the surface to remove any adsorbed material and subsequently reducing the oxide layer [13]. EIS was performed on electrodes prior to and following electrochemical cleaning (Fig. 2).
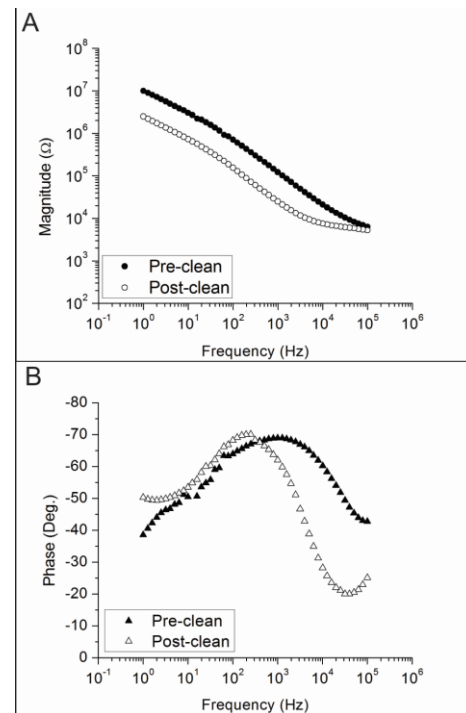

Figure 2. (A) Magnitude and (B) phase of EIS measurements before and after electrochemical cleaning of the electrode surface.

Electrochemical cleaning of the electrode surface resulted in a clear decrease in impedance and a shift in phase. The solution resistance (high frequency resistance) remained constant, indicating that the shifts were a result of the removal of contaminants and the production of a cleaner electrode surface.

B. Post-Fabrication Sheath Formation

An artifact of our post-fabrication process to form the hollow sheath is the development of tensile strain on the outer electrodes caused by the mechanical stress of opening the cone. This strain occasionally leads to cracking of the thin-film platinum electrode sites located on the sheath exterior. EIS was performed on an outer electrode in the same position on an opened (thermoformed) and an unopened (as-fabricated) probe (Fig. 3). The EIS data, specifically the phase, of the opened sheath probe indicate the existence of multiple time constants, which may be due to capacitance added to the system by the cracked electrodes. Electrodes are currently located along the central axis of the sheath and thus subject to high strain. To avoid tensile strain induced cracking of electrodes and improve process yield, the exterior electrodes will be relocated away from the central axis in future designs.
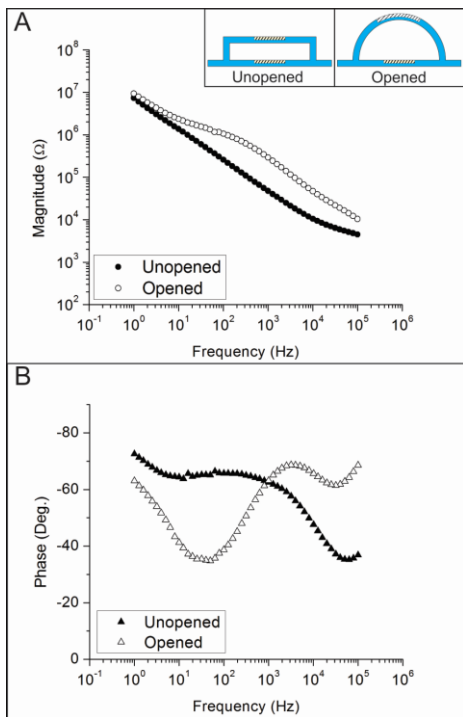
Figure 3. Magnitude (A) and phase (B) of EIS measurements for outer electrodes of unopened and opened sheath probes. Inset shows cross-sections of an unopened and an opened probe.

C. Crosstalk

No crosstalk was observed between electrodes, demonstrating proper function of and adequate insulation. A voltage signal was observed on each electrode as it was stimulated with a current pulse, as seen on the diagonal of Table I, confirming electrical conductivity of each electrode.

TABLE I. NORMALIZED CROSSTALK MEASUREMENTS ACROSS A SINGLE PROBE

Electrode	1	2	3	4	5	6	7	8
1	100	0	0	0	0	0	0	0
2	0	100	0	0	0	0	0	0
3	0	0	100	0	0	0	0	0
4	0	0	0	100	0	0	0	0
5	0	0	0	0	100	0	0	0
6	0	0	0	0	0	100	0	0
7	0	0	0	0	0	0	100	0
8	0	0	0	0	0	0	0	100

D. Neurotrophic Coatings

Due to the hydrophobic nature of Parylene C, application of coatings to the probes required the use of methods to overcome surface tension effects to allow for coverage of the inside of the sheath. Two such methods were developed and then assessed with EIS.

One method utilized a thin nylon filament to manually break the surface tension. The filament was threaded through the sheath, the chilled coating solution was applied, and then the filament was gently removed. This method allows the Matrigel to cover the inside of the sheath, but suffers from

low uniformity. EIS measurements show an increase in impedance magnitude and a shift in phase, likely due to the thick non-uniform coating (Fig. 4).
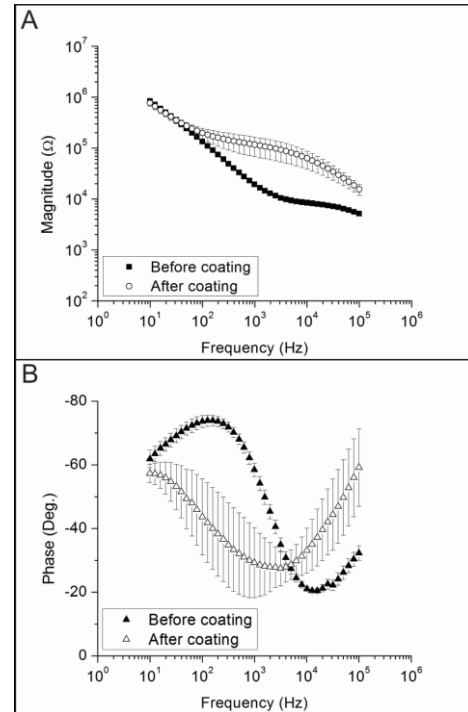

Figure 4. Magnitude (A) and phase (B) of EIS measurements before and after coating via manual breakage of surface tension (n = 4, mean ±SE).

Another method leveraged sonication: probes were soaked in chilled Matrigel solution in an ultrasonic bath. This method evenly coated the sheath with Matrigel, but did not add significant impedance to the electrode, suggesting the formation of a thinner and more uniform coating (Fig. 5).
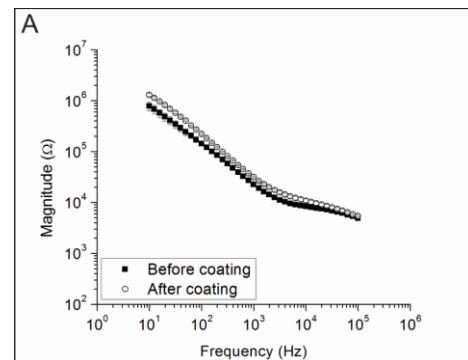

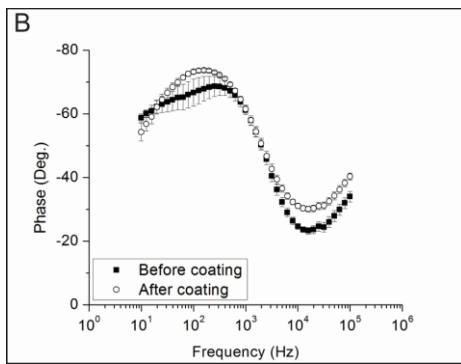Figure 5A. Magnitude of EIS measurements before and after Matrigel coating sonication (n = 4, mean ±SE).

Figure 5B. Phase of EIS measurements before and after Matrigel coating sonication (n = 4, mean ±SE).

E. Accelerated Lifetime Testing

Daily EIS measurements of a probe undergoing accelerated aging showed a decrease in impedance magnitude and shift in phase over time (Fig. 6). This trend is consistent with increasing electrode surface area. This may be due to delamination of the insulating Parylene C around the electrode site, effectively increasing the exposed electrode area. This was confirmed optically after the test. It is worth noting that the rate of change slows and begins to stabilize around Day 5, indicating that the changes to the electrodes are reaching equilibrium.

Discontinuities in measurements taken on Days 1 and 2 were observed on all electrodes measured that day and are likely due to equipment error or connection problems. However, the overall trends are still visible despite the discontinuities. Further work is underway to repeat these studies and eliminate artifacts introduced by the experimental setup.
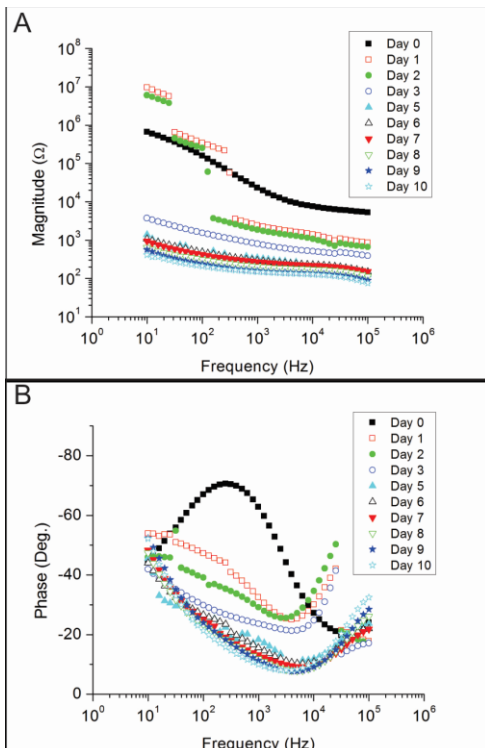

Figure 6. Magnitude (A) and phase (B) of EIS measurements over the course of 10 days.

IV. CONCLUSION

Preliminary electrochemical characterization of a novel Parylene C sheath microelectrode array probe demonstrated electrode integrity and aided in selection of a method to apply neurotrophic coatings. It was also demonstrated that electrochemical characterization is a useful tool to address the issues specific to polymer-based electrodes. Initial accelerated life testing has indicated a "break-in" period for the probe, but has demonstrated electrode functionality up to a simulated 6 months of use at 37°C. Further investigation of this process is underway and rigorous electrochemical characterization of a new probe design modified in response to this initial study will be conducted.

REFERENCES

[1] M. A. Lebedev and M. A. L. Nicolelis, "Brain-machine interfaces: past, present and future," *Trends in Neurosciences,* vol. 29, pp. 536-546, Sep 2006.

[2] P. R. Kennedy and R. A. Bakay, "Restoration of neural output from a paralyzed patient by a direct brain connection," *Neuroreport,* vol. 9, pp. 1707-11, Jun 1 1998.

[3] P. R. Kennedy*, et al.*, "Direct control of a computer from the human central nervous system," *IEEE Trans Rehabil Eng,* vol. 8, pp. 198-202, Jun 2000.

[4] L. R. Hochberg*, et al.*, "Neuronal ensemble control of prosthetic devices by a human with tetraplegia," *Nature,* vol. 442, pp. 164-171, Jul 2006.

[5] V. S. Polikov*, et al.*, "Response of brain tissue to chronically implanted neural electrodes," *Journal of Neuroscience Methods,* vol. 148, pp. 1-18, Oct 2005.

[6] C. Marin and E. Fernandez, "Biocompatibility of intracortical microelectrodes: current status and future prospects," *Front Neuroeng,* vol. 3, p. 8, 2010.

[7] A. Blau*, et al.*, "Flexible, all-polymer microelectrode arrays for the capture of cardiac and neuronal signals," *Biomaterials,* vol. 32, pp. 1778-86, 2011.

[8] J. T. W. Kuo, Kim, B. J., Hara, S.A., Lee, C., Gutierrez, C. A., Hoang, T., and E. Meng, "Fabrication of 3D Parylene Sheath Probes for Reliable Neuroprosthetic Recordings," presented at the Hilton Head 2010: A Solid State Sensors, Actuators and Microsystems Workshop, Hilton Head Island, South Carolina, USA, 2012.

[9] S. F. Cogan, "Neural stimulation and recording electrodes," *Annual Review of Biomedical Engineering,* vol. 10, pp. 275-309, 2008.

[10] J. P. Frampton*, et al.*, "Effects of Glial Cells on Electrode Impedance Recorded from Neural Prosthetic Devices In Vitro," *Annals of Biomedical Engineering,* vol. 38, pp. 1031-47, 2010.

[11] A. Mercanzini*, et al.*, "In Vivo Electrical Impedance Spectroscopy of Tissue Reaction to Microelectrode Arraysl," *IEEE Transactions on Biomedical Engineering,* vol. 56, pp. 1909-1918, Jul 2009.

[12] D. W. L. Hukins*, et al.*, "Accelerated aging for testing polymeric biomaterials and medical devices," *Medical Engineering and Physics,* vol. 30, pp. 1270-1274, 2008.

[13] D. T. Sawyer, "Voltammetric Indicator Electrodes," in *Experimental electrochemistry for chemists*, ed New York: John Wiley and Sons, 1974, pp. 60-100.