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Improving the Brain Machine Interface via Multiple Tetramethyl Orthosilicate Sol-Gel Coatings on Microelectrode Arrays*

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***Abstract*—The failure of neurally implanted microelectrode arrays has been highly correlated with the foreign body response (FBR). In order to maintain high fidelity signaling across the brain machine interface, the FBR must be minimized. Multiple thin coatings of Tetramethyl Orthosilicate have the potential to reduce the acute FBR through their ability to release a model therapeutic drug for 4 to 10 days, their positive effects on electrode performance (e.g. a minimal change in impedance at 1kHz, less than 10%, and an increase in charge carrying capacity, up to 49%), and their low impact on device thickness.**

I. Introduction

Intracortically implanted neuroprosthetic devices show great promise as a research avenue to improve the quality of life of people with amputation or spinal cord injury. Loss of limb function currently affects 238,000- 332,000 people in the United States [1]. The cost of treatment for an individual with paralysis in the United States is between 1.5-4.6 million dollars for lifetime medical care depending upon the severity and age of injury onset [1]. Chronic stimulation and recording with microelectrode arrays (MEAs) from a neuroprosthetic device offers an approach towards restoring function to the amputated or paralyzed [2], [3]. However, the long-term effectiveness of current devices *in vivo* is limited by the foreign body response (FBR) of the nervous system. During implantation, the blood brain barrier (BBB) is ruptured and blood plasma containing macrophage antibodies enter the implantation site. Minutes after implantation, microglia become activated and within 6-24 hours migrate towards the implant site [4]–[6] . Astrocyte activation also begins within the first 24 hours, of the implantation, with full activation seen around day 6 [4]. Within weeks, activated astrocytes form a compact glial sheath around the implanted device, a hallmark of the sustained FBR, which mechanically and electrically isolates the device from the surrounding tissues [7]–[10]. Sustained sheath presence has been associated with an increase in electrode impedance [11] and a decrease in the signal to noise ratio [12], rendering the device ineffective by hindering its ability to transmit and receive signals. A solution to this problem is to mitigate inflammation prior to full onset.

Histological techniques have allowed researchers to compare device architecture in the hopes of decreasing the FBR associated with the initial trauma of implanting devices. The results of numerous studies have shown that insertion speed [13] and device geometry [14], [15] are paramount when reducing the resulting disturbance of the implantation. Advances in these techniques, *e.g.* allowing for device capture histology [16]–[19], have also provided more intimate knowledge of the brain device interface. The results of these studies suggest that thinner devices displace less tissue and allow for easier communication between cells, while sharper devices create less damage than blunt devices when penetrating the tissue [14]. Research in the field has delved into the coating of MEAs with peptides, polymers, or hydrogels [20]–[26], some which release therapeutic factors, to alleviate the FBR by decreasing inflammation and glial reactivity [7], [20], [23], [25], [26]. The problem with many of these treatments, however, is that they often increase the thickness of the device drastically or can have undesirable effects on impedance.

Orthosilicates sol-gels have been shown to create thin film coatings capable of sustained controlled release for several days [27]. A single layer of Tetramethyl Orthosilicate sol-gel has also shown negligible effects on electrode impedance while at the same time increasing the Charge Carrying Capacity (CCC) [28]. Here we report research investigating multiple thin film TMOS sol-gel coatings as a drug delivery mechanism for the mitigation of the acute FBR, without negatively impacting impedance and CCC. Via two separate experiments we evaluate the ability of TMOS sol-gel coatings to 1) elute a protein therapeutic analog, Bovine Serum Albumin (BSA), from a macro scale silicon wafer chip model, while 2) insignificantly affecting *in vitro* MEA electrochemical properties.

II. Materials and Methods

A. Wafer Dicing

4 inch Silicon Wafers (Silicon Quest International, San Jose, CA 95134)) were cut to size (10mmX5mm) using a K&S 4526 (Kulicke and Soffa, Fort Washington, PA).

B. MEA and Wafer Cleaning

In this study, single shank, Michigan Electrode Arrays (NeuroNexus, Ann Arbor, MI) and silicon wafer chips were

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cleaned to ensure the surface of the materials were not contaminated (ensuring an even coating of TMOS without cracking). Cleaning was performed with piranha solution, prepared by mixing equal parts 13M Sulfuric Acid (Sigma Aldrich, St. Louis MO) and 30%w/v Hydrogen Peroxide Sigma Aldrich, St. Louis MO. Samples were placed into a 20 mL glass vial and 10 mL of piranha solution was slowly added. The samples were allowed to react with the solution for 5 minutes, before the solution was removed and the process repeated. Afterwards, the devices and chips were triple rinsed with Double Deionized (DDi) water and isopropanol (Sigma Aldrich, St. Louis MO), to rinse any contaminants from the surface of the substrate, and allowed to dry overnight.

C. TMOS Sol-Gel Synthesis

Sol-gel synthesis was performed in a manner similar to that previously described [28]. In short, within a 20 mL glass vial containing a stir bar, 3.8 mL of TMOS (98% Purum, Sigma Aldrich, St. Louis MO) was mixed with 1.8 mL of DDi water and 55 µL of 1.0 M Hydrochloric Acid (Amresco, Solon, Ohio), added as a catalyst. This mixture was allowed to stir at room temperature at atmospheric pressure for three hours prior to being sealed and stored at -20°C overnight.

D. Protein Encapsulation within Sol-Gel

Sol-gel was loaded with BSA (Sigma-Aldrich, St. Louis, MO) immediately prior to coating of wafer chips. This was essential as the addition of BSA initiated rapid polymerization of the sol-gel within minutes. In an ice bath, a 1.5 mL micro centrifuge tube was filled with 700 µL of 4.6% BSA W/V in Phosphate Buffer (Sigma-Aldrich, St. Louis, MO), 300 µL of TMOS sol-gel, and 100 µL of Methanol (98% Purum, Sigma Aldrich, St. Louis, MO). Mixtures were vortexed for 5 seconds at 2000 RPM and then used to coat silicon chips immediately.

E. Thin Film Deposition

Deposition was performed in a manner previously described [28]. In short, clean dry samples were affixed to an actuator which was used to dip-coat the chip into the TMOS sol-gel solution at 70mm/min, until fully submerged, then removed at the same rate. The wafer was then allowed to dry for 10 minutes. Once dry, the chip was placed into a saturated Sodium Chloride solution to stabilize the surface properties of the coating for future dip-coating [27]. Chips were submerged for 10 seconds and then allowed to dry for a further 10 minutes. Subsequent dip-coating depended upon coating paradigm. After finalizing the coating paradigm, wafers were allowed to dry overnight at room temperature.

Drug release coating paradigms on the silicon chips consisted of 1 Protein Layer + 2 Neat Layers (1PL+2NL), 4 Protein Layers + 1 Neat Layer (4PL+1NL), or 4 Protein Layers + 2 Neat Layers (4PL+2NL), wherein each protein layer is a TMOS sol-gel coating encapsulated with BSA and each neat layer was native TMOS sol-gel. Each wafer chip paradigm had an n=3 for a total of 12 chips. Coatings on the four MEAs were comprised of 6 Neat Layers.

F. *In vitro* Protein Release and Analysis

Wafer chips were incubated in PBS at 37°C for 30 days. PBS was replaced at hours 2, 4, 8, 24, and then subsequently every 24 hours thereafter. Protein release was analyzed via the Thermo Scientific Pierce Coomassie (Bradford) Protein Assay (Thermo Fisher Scientific, Waltham, MA), a colorimetric analysis to determine protein concentration via a Microquant spectrophotometer (BioTek Instruments Inc, Winooski, VT).

G. Electrical Set-up

Each silicon-substrate shank of the MEAs consisted of 16 iridium sites (64 sites in total) with nonfunctional sites (15 in total for the experiment) ignored for the purpose of this study. The sites were spaced between 100 – 150 µm apart and were 15 µm in diameter. All of the electrochemical measurements were performed using an Autolab PGSTAT128N (Metrohm Autolab B.V., Utrecht, The Netherlands) in a three-electrode configuration. Each iridium site acted as a working electrode, and a platinum wire with a large surface area functioned as the counter electrode. The working and counter electrodes were submerged in 1X PBS (154 mM NaCl, 5.8 mM NaH2PO4 and 1.1 mM KH2PO4) along with a saturated calomel electrode (Thermo Fischer Scientific, Fair Lawn, NJ) that acted as the reference electrode. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed before and after each layer of TMOS coating.

H. Electrochemical Measurements

The EIS was performed with two multi-sine waveforms at 25 mVrms. Each multi-sine waveform consisted of 15 logarithmically spaced frequencies with one ranging from 10 Hz – 1 kHz, and the other ranging from 100 Hz – 10 kHz. The overall trend of the impedance across the frequencies was observed, and the complex impedance was more closely examined at 100 Hz, 1 kHz, and 10 kHz.

CV was performed by scanning a voltage range that does not induce the electrolysis of PBS (-0.6 V to +0.8 V at a rate of 1 V/s) while measuring the resulting current. The voltage range was scanned three times and the last two scans were averaged. The resulting hysteresis curve in the cyclic voltammograms was used to derive the Charge Carrying Capacity (CCC) of the electrode which is a measure of electrode performance.

I. EIS and CCC Normalization and Analysis

Because of MEA variability from device to device and site to site, changes in EIS and CCC were normalized with 1 being the value for each electrode site of the untreated devices. Normalized values for each site were then averaged and a standard deviation found. These normalized averages and standard deviations were then analyzed via ANOVA at each level of coating (Untreated, 1 NL, 2NL, 3NL …6NL).

III. Results and Discussion

A. In vitro Protein Release Analysis

By altering the number of PL's and NL's it was demonstrated that release duration can be tuned from 2 to 10 days. As seen in Figure 1, the 1PL+2NL chips released BSA for 2 days, the 4PL+1NL chips released BSA for 6 days, and the 4PL+2NL for 10 days. This data is promising as previous research has shown that microglia become activated and begin moving towards the implanted device between 6 and 24 hours after implantation [4], [5], [29], whereas astrocytes are not found around the device for 6 days [4]. These separate timelines would allow for time specific therapies to be released directly at the implantation site, avoiding issues commonly associated with systemic injections such as liver/renal clearance and passage across the BBB. As these coatings are performed at 0°C, and the sol-gel forms at room temperature, there is also less concern with therapeutic degradation when compared to other methods of orthosilicate formation (often requiring high temperatures).

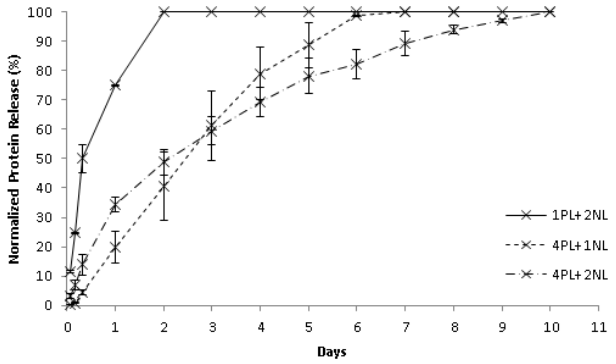

Figure 1. *Normalized Protein Release from Wafers*. Effect of altering the number of (PL) and (NL).

B. EIS Analysis

Previous research has also shown that changes in impedance were minimal after coating with a single TMOS sol-gel layer. The results in multiple coated TMOS sol-gel MEA's were similar. A statistically significant (p<.05) decrease in impedance occurred at 100 Hz, while no statistically significant change (p<.05) occurred at 1 kHz. A statistically significant increase (p<.05) in impedance occurred at 10 kHz. These findings, shown in Figure 2 and listed in Table 1, corroborate with the previously published 1 coat EIS analysis [28]. These small changes are mostly unimportant, as the changes in impedance are small, and therefore, unlikely to have relevant effects upon stimulation at these frequencies. Furthermore, the effects at 1 kHz, the fundamental frequency for recording action potentials, is not significant.

C. Charge Carrying Capacity Analysis

Previous research has shown an increase in charge carrying capacity after coating with a single layer of TMOS [28]. This trend was also observed when multiple layers of TMOS sol-gel were coated upon devices as seen in Figure 3.

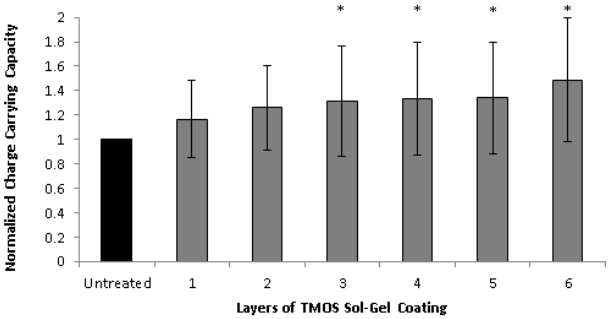

Figure 3. *Effect of Multiple TMOS Coatings on Charge Carrying Capacity* * Denotes a significant difference from the untreated MEA measurements (p<.05).

As can be seen, a trend is clear, showing a 17-49% increase in CCC with the additional layers of TMOS compared to the uncoated devices. Statistical analysis in the form on an ANOVA was performed and the results indicated that increases in CCC are significant for coatings 2-6 (p<0.05). These results, shown in Table 1, suggest an improvement in CCC for the MEAs.

TABLE I. CHARGE CARRYING CAPACITY AND EIS ANALYSIS

Analysis	Number of TMOS Sol-Gel Coatings					
	1NL	*2NL*	*3NL*	*4NL*	*5NL*	*6NL*
CCC	1.17 ±0.32	1.26 ±0.34	1.31 ±0.45	1.33 ±0.46	1.34 ±0.46	1.49 ±0.51
EIS (100 Hz)	0.85 ±0.13	0.79 ±0.12	0.76 ±0.12	0.73 ±0.13	0.73 ±0.15	0.67 ±0.14
EIS (1 kHz)	0.95 ±0.10	0.95 ±0.15	0.96 ±0.20	0.94 ±0.22	0.93 ±0.20	0.96 ±0.23
EIS (10 kHz)	1.12 ±0.13	1.23 ±0.32	1.33 ±0.47	1.35 ±0.49	1.30 ±0.48	1.46 ±0.49

(All data is normalized to uncoated control)

IV. CONCLUSION

TMOS sol-gels offer an innovative opportunity for acute treatment of the FBR across the brain machine interface

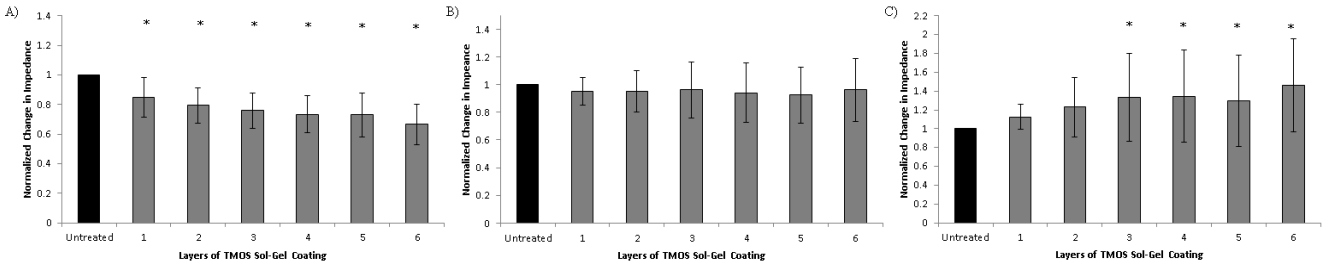

Figure 2. *Effect of Multiple TMOS Coatings on Impedance* A) Impedance at 100 Hz B) Impedance at 1 kHz C) Impedance at 10 kHz.

* Denotes a significant difference from the untreated MEA measurements (p<.05).

because of its ability to control therapeutic release for a period ranging from 3-10 days. Before being studied *in vivo*, release analysis must be performed with a desired therapeutic such as an anti-inflammatory peptide or growth factor, as size and zeta potential will greatly effect elution from the thin film coatings. The initial protein release discussed in the experiments above occurred on a macro scale for ease of drug elution analysis; future research will also delve into releasing therapeutics directly from the MEA surface and nanoquantification of therapeutic release (a more costly set of experiments), after which *in vivo* implantation, histological response, and electrochemical property changes can be assessed.

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