The Effect of Multiple Thin-Film Coatings of Protein Loaded Sol-Gel on Total Multi-Electrode Array Thickness*

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Abstract— **Tetramethyl orthosilicate shows promise as a thin-film delivery vehicle for multi-electrode arrays for drug release and electrical performance; however, its effect upon device footprint has yet to be assessed. Using a previously established silicon wafer chip model, the thickness of one, two, and four protein doped coatings of sol-gel were analyzed via profilometry. Coating thickness was found to be 0.4μm, 1.1μm and 2.2μm on each side of the device. This addition to a native MEA is minimal when compared to other drug delivery paradigms currently associated with neural implants.**

I. Introduction

As of 2014, the United States had 276,000 people living with spinal cord injury (SCI), with lifetime treatment costs projected between 1.1 and 4.7 million dollars per person, depending on age of onset and injury location[1]. Intracortical multi-electrode arrays (MEAs) may provide patients suffering from spinal cord injury induced paralysis improved quality of life and a decrease in caretaker dependence. The integration of intracortical MEAs with computer assisted robotic devices would provide recovery of impaired motor control and computer-based assistance.

This potential is related to the ability of MEAs to stimulate or record from small neuronal populations; however, this also requires the devices to be highly invasive. The implantation of MEAs is a traumatic procedure which activates the foreign body response (FBR)[2], [3]. The FBR consists of two phases: acute and chronic; the acute phase lasts for approximately 2 weeks, associated with the activation and migration of microglia around the site of implantation. This activation has also been associated with a decrease in signal to noise ratio of electrophysiological recordings and an increase in impedance[4], [5] of the device tissue interface. The chronic phase of the FBR is associated with the presence of activated astrocytes[6] and the formation of the glial encapsulation. The glial encapsulation is temporally correlated to loss of device recording and stimulation functionality[2], [3].

In attempts to mitigate the FBR, researchers have altered the physical attributes of the device and delivered anti-inflammatory drugs. In altering the device, it was found that thin devices elicit less of a FBR response than thicker devices[7]. It has also been shown that systematic injections of anti-inflammatory chemicals can have an ameliorative effect upon the FBR, reducing glial encapsulation[6], [8]. However, systemic delivery can have negative side effects on the patient[9]. This study evaluates local delivery of chemicals, potentially from the device itself, which would provide targeted therapy and lower the necessary therapeutic dose.

Many drug delivery technologies require thick coatings to release a therapeutic dose of drugs for extended periods of time[10], while other adhesion-based technologies often have drawbacks including low loading concentrations and short term delivery[3], [8]. This can result in one of two outcomes: poorly functioning devices with large coatings that do not elicit an exaggerated immune response, or thin devices that perform well for short periods of time before losing functionality because of the activation of the FBR.

Tetramethyl Orthosilicate (TMOS) sol-gels are capable of releasing therapeutic drugs[11], [12]; cell seeding[13]; and peptide tethering[14]. In the past, we have demonstrated TMOS' ability to coat NeuroNexus devices[15], [16]; its ability to release protein in a controlled release profile[16]; and shown that multiple coatings of sol-gel had no significant effect upon impedance at 1kHz and actually increased charge carrying capacity in an *in vitro* model[16].

One aspect that has yet to be analyzed, however, was the effect of multiple coatings of protein loaded sol-gel on the thickness of a device. This study utilized the previously established silicon chip model to correlate coating thickness to the number of coatings, in an attempt to determine the viability of TMOS as a functional delivery vehicle.

II. Materials and Methods

A. Wafer Dicing and Cleaning

Four inch, silicon wafers (Silicon Quest International, San Jose, CA 95134) were cut to size (10mmX7mm) using a *7100 Dicing Saw* (Advanced Dicing Technologies, Horsham, PA 19044). Wafer chips were then cleaned using a piranha solution to remove contaminants and ensure an even sol-gel deposition.

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In short, a 1:1 mixture of 13M Sulfuric Acid (Sigma-Aldrich, St. Louis, MS 63178) and 30% hydrogen peroxide (Sigma-Aldrich) was added to the diced wafer chips and allowed to react. Once cooled, the wafer chips were then removed and triple rinsed with double deionized (DDI) water (Barnstead, Fisher Scientific, Pittsburgh, PA 15275) before being rinsed once with isopropanol (Sigma-Aldrich) and dried at 37°C overnight.

B. *Sol-gel Synthesis*

The formulation of protein doped sol-gel was accomplished as previously described [13]. A 5:1 Water:TMOS (Sigma-Aldrich) sol-gel was synthesized with an acid catalyst, then chilled overnight at -20°C. Bovine serum albumen (Sigma-Aldrich), dissolved in phosphate buffer was then added to the sol-gel, and mixed immediately prior to deposition upon the chip.

C. *Sol-gel Deposition*

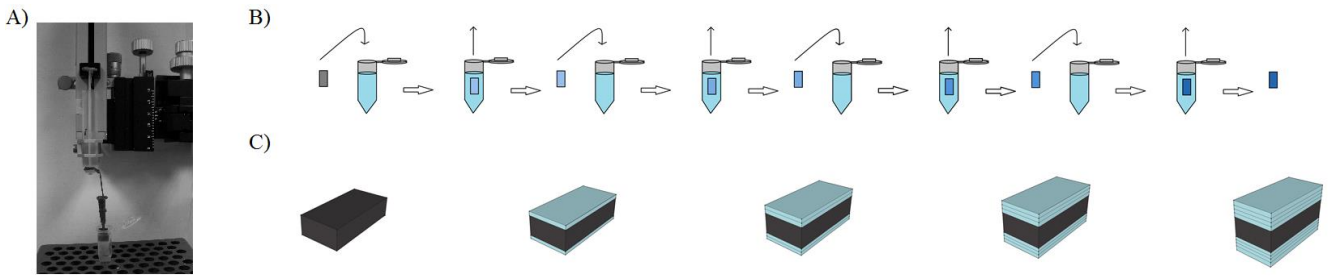
Wafers were dip-coated using a retrofitted stereotaxic injector (Stoeltling, Wood Dale, IL 60191) at 70mm/min and allowed to dry for 30 seconds; placed in a [0.04] phosphate buffer, saturated with NaCl, for 15 seconds; and then rinsed 3 times in DDI water for 15 seconds. This process was then repeated to achieve one, two, or four protein-doped layers, each with a sample size of n=3. Wafer chips were then stored overnight in water until just prior to profilometry analysis.

Deposition is demonstrated in Figure 1: *A* shows the dip-coating set-up, while *B* demonstrates the dip-coating procedure and *C* visualizes the deposition upon an uncoated wafer chip with protein-doped layers of TMOS.

D. *Profilometry*

An *Alpha Step 500* profilometer (KLA-Tencor, Milpitas, California 95035) was used to measure thickness of TMOS sol-gel coatings. Wafers were coated as described above, then one edge of the wafer was cleaned, using a razor blade to scrape the coating from the chip. The profilometer was then used to measure the change in height between the cleaned wafer surface and the intact coating; it should be noted that the Alpha Step 500 allowed for visual inspection of the wafer surface to ensure that the coating was fully removed. Each chip was analyzed at three locations: Left Edge, Right Edge, and Center. All data for each coating paradigm were averaged and the standard deviation calculated.

Figure 1. Sol-Gel Deposition



III. Results and Discussion

We have previously demonstrated that this coating technology is capable of tunable controlled release of BSA, as a model protein therapeutic, for time periods between 1 and 10 days. This was accomplished by the deposition of up to 6 sol-gel coatings[16]. Functional NeuroNexus devices were also coated with up to 6 layers of sol-gel and it was found that the charge carrying capacity was significantly increased by about 50% and electrical impedance was not significantly increased at 100Hz or 1 kHz[15], [16]. (Summarized in the appendix.) While this initial research was promising, without evidence of a small impact on device thickness, this technology cannot be implemented.

A. *Profilometry Analysis*

The data from the thickness measurements are shown in Table I. Coatings ranged from 0.4 ± 0.1μm to 2.2 ± 0.5μm. It should be noted, however, that these measurements were conducted on dehydrated sol-gel samples, as the water in the hydrated samples interfered with the accuracy of the measurements.

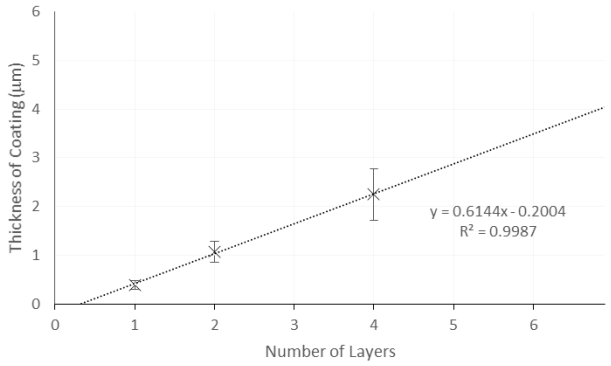
TABLE I. Thickness of Sol-Gel Coatings

Number of Protein Doped Layers	***Thickness (μm)***	***StDev (μm)***
One Coating	0.4	0.1
Two Coatings	1.1	0.2
Four Coatings	2.2	0.5

B. *Linear Regression Analysis*

The coating thickness data were plotted along with the linear regression model to correlate the number of coating layers with thickness (Figure 2). It also extrapolates the data up to 6 coatings. The data are highly correlative (R^2 = 0.9987); however, as shown in Table 1, the coatings vary slightly in thickness. This change in thickness could be attributed to fluctuations in temperature and pressure during sol-gel deposition [17] or perhaps because the sol-gel adheres to the native silica differently than to other sol-gel layers.

Figure 2. Linear Regression of TMOS Layers vs Thickness



Although we have only analyzed up to four coatings in this study, the extrapolation of the linear regression shows that 6 coatings (as conducted in our previous drug delivery and electrical analysis) would have an estimated thickness of 3.5µm. Maximizing the number of coatings without dramatic increase in thickness is important as protein loading and duration of release are directly correlated with the number of deposited layers[16].

C. Extrapolation to MEA Devices

As previous research has shown that both device shape and thickness are important to reduce the FBR[7], it was necessary to investigate TMOS feasibility as a drug delivery vehicle by considering the total thickness added to the device (as both sides of the device are coated with this method). Equation 1 details the calculation for finding the increase to device footprint

$$\frac{2*Thickness\ of\ Coating+Device\ Thickness}{Device\ Thickness} * 100\ . \quad (1)$$

Table II shows the relative increase in thickness per side, total increase to thickness, and percent increase to total thickness for a device with a footprint of 25µm, a standard size of a NeuroNexus device.

TABLE II. EXTRAPOLATED INCREASE TO DEVICE THICKNESS

Number of Protein Doped Layers	*Thickness (µm)*	*Total Impact (µm)*	*Increase to Device Footprint (%)*
One Protein Layer	0.4	0.8	3.0
Two Protein Layers	1.1	2.1	8.4
Four Protein Layers	2.2	4.5	18
Six Protein Layers*	3.5	7.0	28

*Extrapolated from Linear Regression

As previously mentioned, minimizing the increases in the total size of the device is critical to both minimizing the body's initial response to the implant, but also in allowing the neurons in the area to remain as close to the electrode sites as possible.

D. Comparison in Delivery Technologies

Our previous drug release and electrical data focused on 6 coatings of protein loaded sol-gel. A 7µm (28% increase) in device footprint is not prohibitive to device functionality, especially when compared to other coating strategies whose thicknesses also range from a few microns[18]–[20] to a few hundred microns[21].

Sol-gel dip coating coats the entire device, which allows for a larger volume in which to load a therapeutic when compared to technologies that rely upon electroplating on electrode sites[19] or adhesion directly to the device surface[18]. Unlike layer-by-layer technologies, the incorporation of new therapeutics does not require the implementation of new synthesis pathways. However, sol-gels do possess some negative attributes: as a glass-like substrate sol-gels do not reduce the hardness mismatch with the tissue interface, sol-gel solutions contain alcohols which can be harsh on peptides and proteins or can have a negative impact on the solubility of some therapeutics, sol-gel delivery has thus far been limited to timescales only associated with the acute phase of the FBR.

E. Future Uses of the Technology

To this point TMOS has been modeled for the release of a therapeutic peptide using BSA. However, the incorporation of drug loaded nanoparticles would greatly increase its potential. These nanoparticles when incorporated with the current methodologies would present a therapy capable of multiple cell types with specific therapeutics and at relevant time-points, while extending the duration of therapeutic release. Because the release would occur locally around the implant and not be distributed systematically, normal issues with micro and nanoparticles concerning bypassing the liver, kidneys, and blood brain barrier would not be a concern; likewise, side-effects could also be minimized.

IV. CONCLUSIONS

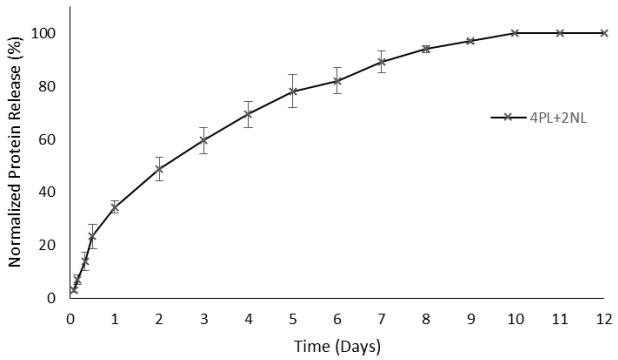
TMOS sol-gel is a viable option for protein and drug delivery, without interfering with the electrical capability of MEA devices. This research further champions TMOS as a viable drug delivery vehicle, elucidating the minimal effect of multiple coatings on total MEA footprint. Current release strategies have only involved the release of a model protein, BSA, but future studies will include the analysis of the release of biologically relevant drugs, such as dexamethasone or minocycline and peptide sequences such as $MK2_i$, an inhibitor to a pro-inflammatory pathway. *In vivo* analysis to ascertain the increased duration of device functionality that this strategy is capable could then be performed.

APPENDIX

Below, previously published data concerning a 6 coating loaded sol-gel paradigm are summarized (Figure I), followed by electrical impedance spectroscopy (EIS) and charge carry

capacity (CCC) data collected from type A1-16 NeuroNexus devices (Table I).

Figure I. Normalized Protein Release vs Time



Protein delivery in the 4PL +2 NL coating paradigm (4 protein doped sol-gel layers, 2 non-doped layers) lasted for ten days. This period of time correlates well with the acute phase of the FBR.

Table I. Electrical Characterization of 6 Sol-gel Coatings

CCC	*EIS (100Hz)*	*EIS (1kHz)*	*EIS (10kHz)*
1.49 ± 0.51	0.67 ± 0.14	0.96 ± 0.23	1.46 ± 0.49

Data Normalized to Uncoated Devices

Electrical analysis of 6 sol-gel coatings showed a significant increase in charge carrying capacity, no significant increase in EIS at 100Hz or 1kHz, and a significant increase in EIS at 10kHz (α= 0.05). These data suggest that these coatings will not hinder device function.

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