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Ultra-compliant neural probes are subject to fluid forces during dissolution of polymer delivery vehicles

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***Abstract*— Ultra-compliant neural probes implanted into tissue using a molded, biodissolvable sodium carboxymethyl cellulose (Na-CMC)-saccharide composite needle delivery vehicle are subjected to fluid-structure interactions that can displace the recording site of the probe with respect to its designed implant location. We applied particle velocimetry to analyze the behavior of ultra-compliant structures under different implantation conditions for a range of CMC-based materials and identified a fluid management protocol that resulted in the successful targeted depth placement of the recording sites.**

I. Introduction

Brain-machine interfaces (BMI) are a promising therapeutic technology for the treatment of disease- and injury-related loss of bodily function [1]. BMI enable the recording of neural activity from targeted areas of the brain of an implanted individual, decoding of the person's volitional intent and use of the decoded signal to actuate a prosthetic device that performs the desired bodily function.

While there are several types of interfaces being researched today, intracortical neural implants offer the spatial and temporal resolution and signal to noise ratio necessary to achieve metabolically and cognitively light, high degree-of-freedom (DOF), real-time prosthetic control. A number of intra-cortical devices are in use in BMI research, but a critical issue hindering their chronic clinical deployment is the loss of signal strength over the period of several years due to reactive tissue response.

Blood brain barrier (BBB) breach during implantation, relative probe-tissue micromotion and the strains it induces, chronic leaky BBB and the surface conditions of the implant have been identified as factors driving the reactive tissue response [2-5]. To test the significance of probe size and compliance on reactive tissue response, we developed ultra-compliant meandering platinum-parylene-C (Pt-Px) probes with sub-cellular dimensions [6]. The size and compliance of the ultra-compliant probes require they be embedded in a rigid, needle-shaped delivery vehicle for implantation into neural tissue. The Pt-Px probes are embedded in the delivery vehicle using a molding process described in reference [6]. Sodium carboxymethyl cellulose (Na-CMC) was selected as a base material for the delivery vehicle due to its biocompatibility, its ease of molding using spin-casting and its strength in penetrating the epidermis of the skin for micron-scale transverse cross-sections [7]. The histological effect of CMC was determined in a previous study [8] in which cortical tissue was shown to approach baseline reactivity 12 weeks after implantation in rats. Several saccharides (glucose, sucrose and maltose) were selected as additives to explore the range of control they could provide over the dissolution properties of the delivery vehicle.

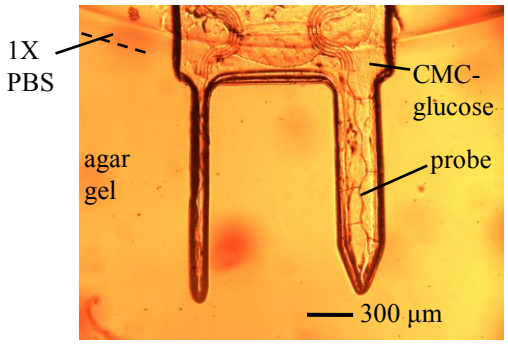

Figure 1 Optical image of a molded 85%CMC-15%glucose delivery vehicle with embedded ultra-compliant 8 µm wide, 2.5 µm thick platinum-parylene neural probe.

During in vitro implantation tests of the ultra-compliant probes in an agarose gel brain phantom we observed fluid structure interactions between the probe and the dissolving delivery vehicle that caused displacement of the electrode site from its designed target location. An image of a molded 85%CMC-15%glucose delivery vehicle with embedded probe is shown in Figure 1 at the moment of implantation in 0.6% w/v agarose gel. This paper presents our findings on the impact of fluid forces on ultra-compliant structures during the dissolution of polymer composite delivery vehicles. Particle velocimetry quantified the axial velocity of the probe after implantation. The probe behavior was compared to the absorption of liquid into the delivery vehicle from the brain phantom, which was quantified as the area of the needles. A protocol for managing the dissolution process is presented and the successful targeted delivery of ultra-compliant neural probes in ex vivo bovine neural tissue is demonstrated.

II. In Vitro Neural Probe Testing

A. Ultra-Compliant Probe Preparation

Ultra-compliant probes suspended over a needle-shaped bottom mold were made using the integrated nanofabrication process described in reference [6]. The moldable polymer

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delivery vehicles comprise a section for manual handling and two needles of different widths: 100 µm and 300 µm that were defined lithographically. An open resin top mold was mounted on the bottom mold using a Laurier M9A bonder.

Four different delivery vehicle polymer compositions were used in this study to determine the material effect on fluid-structure interaction. The base material was 90 kDa Na-CMC (Sigma-Aldrich, USA) with a degree of polymerization of 400. Solutions were made with pure CMC and with 180 kDa glucose (Sigma Aldrich, USA) at 15% and 30% w/w concentrations and with 342 kDa sucrose (Sigma Aldrich, USA) at 15% w/w concentration using DI water. Delivery vehicles were spun cast as described in reference [6] to thicknesses in the range from 125 µm to 150 µm.

To mimic in vivo conditions as closely as possible, the delivery vehicles were heated to a temperature of 110 °C for 30 minutes, following our pre-implantation sterilization protocol. The delivery vehicles were sealed in a stainless steel container to constrain them during heating and prevent curling under thermal stress gradients.

0.6% w/v agar gel brain phantoms were prepared with 1X phosphate buffered saline (PBS) and dispensed to transparent containers with a 10 mm by 5 mm opening and a depth of 20 mm. This size was chosen to provide sufficient image clarity but a high enough volume of agar to consider it an infinite medium with respect to the implanted delivery vehicles over the timescale of the implantation test.

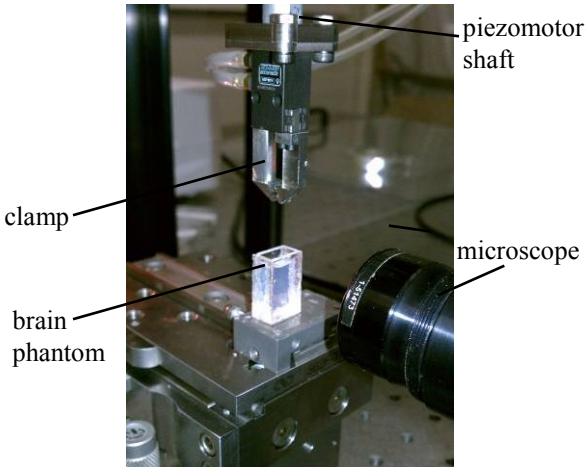

Figure 2 In vitro test bed for the study of fluid-structure interaction between an ultra-compliant neural probe and the dissolution of a polymer composite delivery vehicle.

B. In Vitro Testing

The in vitro test bed is shown in Figure 2. It comprises manually controlled stages with sub-millimeter position control for a camera, an implantation device and the sample stage. Implantation tests were monitored using a side-mounted CMOS microscope camera capturing at 24 fps with transmission illumination to accentuate the opaque structures and feature edges. A lithographically patterned calibration plate with 50 µm pitch measurement features was used to determine the size of the field of view (FOV) for the study. The same microscope magnification setting was used for each test. The agar brain phantoms were mounted on a 5-DOF stage to enable orientation of the surface of the agar to be normal to the delivery vehicle implantation direction. 5µL of PBS were dispensed to the surface of the agar to mimic the surgical conditions during in vivo implantations.

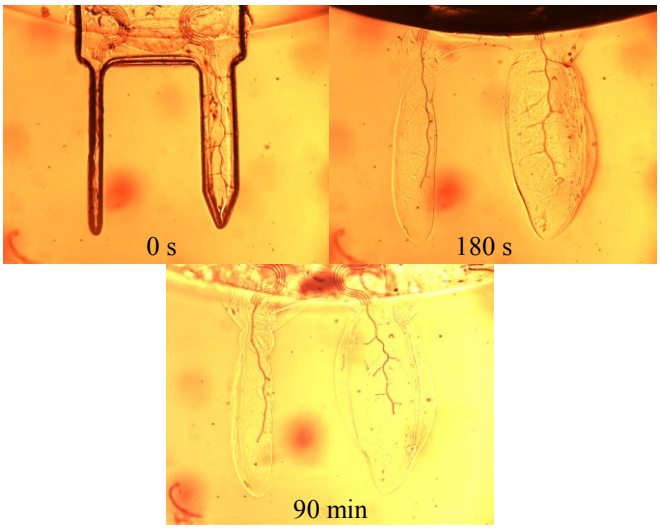

Figure 3 Representative frames of an 85%CMC-15%glucose delivery vehicle with embedded ultra-compliant probe at various times after implantation in agar gel.

The delivery vehicle was placed in a pneumatic clamp that had an 8 N closing force, which was mounted on a PI M272 piezomotor. The delivery vehicles were implanted through the center of the agar surface at a speed of 80 mms^{-1}. 8 minutes after implantation, the surface of the agar was flushed with a large volume of PBS to mimic the cleaning of the surface of the skull after implantation, prior to sealing the craniotomy with dental acrylic. Three repeatability tests were done for the 85%CMC-15%glucose polymer composite.

C. Ex Vivo Testing

Fresh, unfrozen bovine neural tissue was soaked in 1X PBS at room temperature for 1 hour prior to implantation. 1000 mm^3 sections were cut from the brain and mounted in plastic sample containers. The insertion apparatus used for the in vitro tests was used for the ex vivo tests. The tissue surface was oriented so the implantation would be normal to the tissue surface. Two implantation conditions were tested: 1. with a layer of 1X PBS on the tissue surface, and 2. through a tissue surface that was dried immediately prior to implantation. Pure CMC delivery vehicles with embedded ultra-compliant neural probes were implanted into the tissue at 80 mms^{-1} in both cases. The samples were allowed to sit at room temperature for a period ranging from 5 min to 20 min and were then flash frozen in liquid N_2.

The implanted samples were cryo-sectioned in 30 µm slices up to a depth of 1650 µm and mounted on microscope slides. Samples were optically imaged sequentially to follow the track of the embedded CMC delivery vehicle needles and Pt-Px fragments. Sequential tracking was necessary because the blade edge displaced some pieces of Pt-Px completely from the CMC needle track and could possibly have carried some pieces from one section to the next. Only Pt-Px pieces that tracked across multiple adjacent sections were accepted in this analysis and imaged using fluorescence microscopy at 370 nm after illumination with UV light.

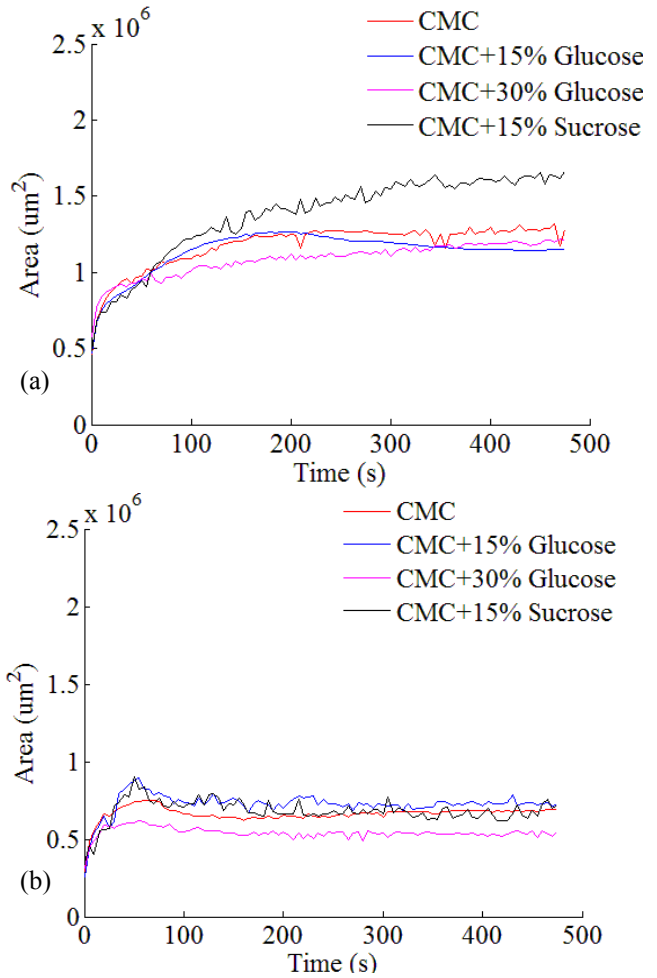

Figure 4 Needle area variation with time following implantation in agar gel for a variety of polymer composites for (a) 300 µm wide needles and (b) 100 µm wide needles.

D. *Video Post Processing*

Video data was post-processed using Matlab. Custom scripts were written to use the image processing capability of Matlab to parse the needles within the captured images in a subset of the frames over the 8 minute capture period. A representative set of images taken at various time points in a video is shown in Figure 3.

Needle area was determined by identifying the continuous edge of each needle within a pre-selected region of interest (ROI), counting the number of pixels within the boundary and scaling it according to the calibrated size of the FOV. The position of the probe was tracked in a frame every 5 s and the displacement of the probe in two subsequent frames over that time period was computed. Axial velocity was computed as the displacement in the vertical direction toward the agar surface divided by the interval between frames.

III. Experimental Results

A. *Needle Area*

The needle area variation following implantation for each polymer composite for the 300 µm and 100 µm needles is shown in Figure 4. All polymer composites for both needle sizes expanded rapidly after implantation in the brain phantom. The needles expanded to about 2.3 times their original size, and in the case of the sucrose composite, the large needle expanded to almost 3 times it original size. With the exception of the large 85%CMC-15%sucrose needle, the expansion stopped within the recording time.

Images at long times following implantation, like the one at 90 min shown in Figure 3, verified that expansion definitely stopped and in some cases it was observed that the needles had shrunk. After the expansion period, bulk flow of material out of the needle was observed in all cases, but unfortunately this effect can only be conveyed by inspection of the video, because of its dynamic nature.

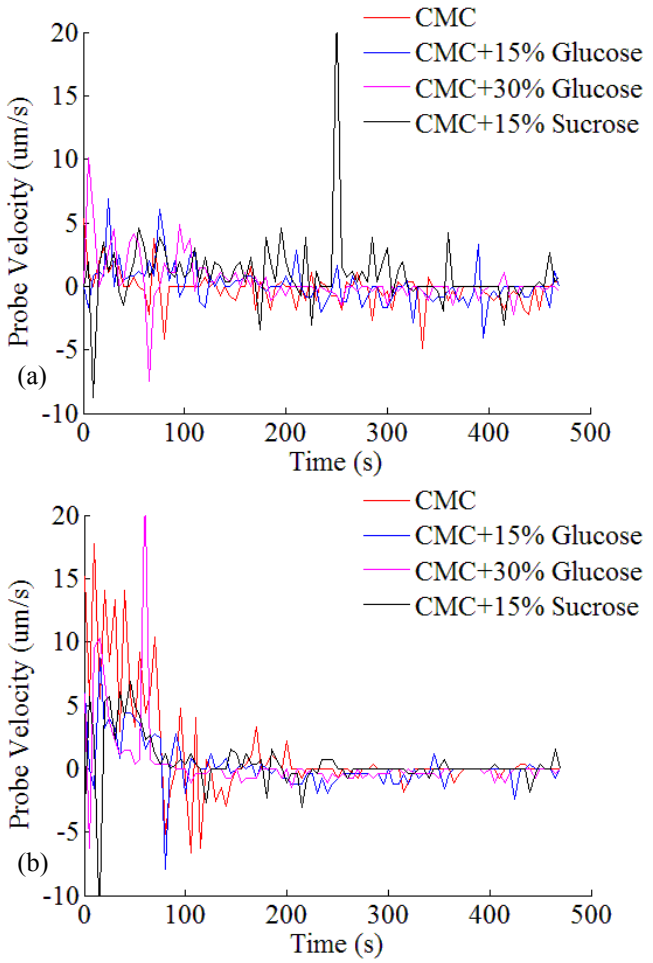

Figure 5 Axial probe velocity variation with time following implantation in agar gel for a variety of polymer composites for (a) 300 µm wide needles and (b) 100 µm wide needles.

B. *Axial Probe Velocity*

Axial probe velocity variation following implantation is shown in Figure 5 for each polymer composite for 300 µm and 100 µm delivery vehicle needles. Probe motion under the action of fluid forces during the expansion of the needles and bulk expulsion of the gelled delivery vehicle was extremely non-uniform. Probe velocity was generally greater shortly after implantation and slows at times coincident with the end of the period of needle expansion. However, spikes in axial velocity were observed minutes after expansion had stopped, indicating instability in the gelled media.

C. Ex Vivo Sections

Pt-Px was observed in the ex vivo bovine neural tissue sections up to a depth of 1650 μm only for the implantations done through 1X PBS. No Pt-Px fragments were found in any of the sections taken from tissue that was dried immediately prior to delivery vehicle implantation. Figure 6 shows a representative fluorescence microscope image of the large needle track for a section taken at a depth of 1500 μm. The diameter of the CMC track is about 1.5 times its pre-implantation size 20 minutes after implantation, which is smaller than the expansion observed in the in vitro tests.

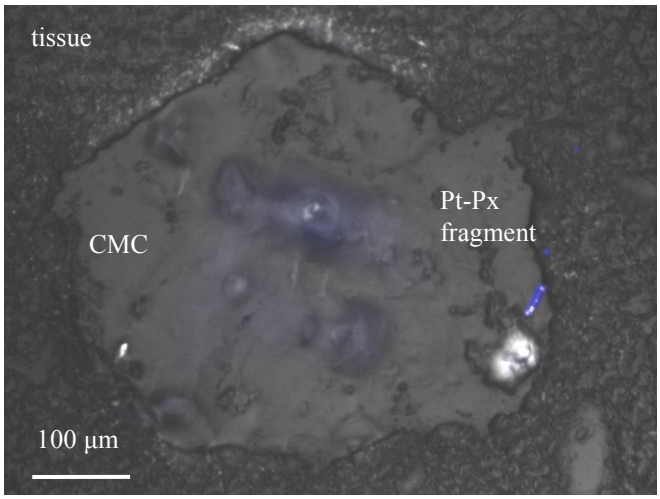

Figure 6 Fluorescence microscope image of the track of a 300 μm wide CMC delivery vehicle needle in an ex vivo bovine neural tissue cryo-section taken at a depth of 1500 μm. A fluorescing Pt-Px section is shown.

IV. Discussion

Significant sporadic probe displacements were observed in the in vitro tests during liquid absorption for all of the polymer composite delivery vehicles. The axial velocity of the probes was dependent on the size of the needle in which they were embedded with higher axial velocities of up to 15 μms^{-1} being observed for the 100 μm wide needles. The axial probe velocity is greater during the initial expansion of the delivery vehicle needles as the needles change from solid to gel and the diffusive flux of liquid inward to the delivery vehicle from the implanted media is expected to be greatest. This study was not able to resolve consistent differences between the probe motion during the implantation for different polymer composites.

The expansion of the delivery vehicles is rapid immediately after implantation and reaches a maximum within 80 s for the 100 μm wide needles and within 200 s for the 300 μm wide needles. Prior to reaching maximum size, material inside the delivery vehicle was observed to flow out of the needles. This suggests an interplay between the viscosity of the gelled delivery vehicle and the stress field of the media into which it's implanted. As the delivery vehicle absorbs liquid from the implanted media its viscosity appears to decrease to a point at which the pressure exerted by the media causes it to be ejected. The interesting observation is that delivery vehicle bulk flow doesn't correspond to an increase in axial probe velocity.

Ex vivo bovine neural tissue sections confirmed the successful targeted delivery of ultra-compliant neural probes to tissue structures up to 1650 μm deep. The absence of Pt-Px structures from delivery vehicles that were not implanted through a PBS layer on the surface of the tissue indicates a significant role for diffusive flux management in the successful placement of the probes.

The swelling and extended in vivo dissolution time of the CMC-based delivery vehicle raises significant questions about material selection and its interplay with delivery vehicle design. CMC was chosen as a base material because of prior work in skin [7], but neural probe delivery, particularly the quasi-static character of the implanted media, has uncovered phenomena that bear further scrutiny. The degree of delivery vehicle swelling in vivo, its absorption, locking up and withholding of liquid from the tissue, its biochemical effects during clearance, and the ultimate time for complete dissolution must be understood and engineered for this method to become an acceptable means for delivering ultra-compliant functional implants.

V. Conclusion

Ultra-compliance is a logically desirable trait in implantable devices as the tissues of the body are typically very soft in comparison to the materials used to fabricate implants. However, the implications of ultra-compliance must be carefully considered when planning the delivery method. In the case of ultra-compliant neural probes, the use of a dissolvable polymer composite delivery vehicle required the understanding and management of the fluid-structure interactions that arose after implantation to achieve successful targeted placement in neural tissue.

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References

[1] J.L. Collinger et al., "High-performance neuroprosthetic control by an individual with tetraplegia," *The Lancet*, vol. 381, pp.557 - 564, 2013.

[2] V.S. Polikov et al., "Response of brain tissue to chronically implanted neural electrodes", *J. Neurosci. Meth.*, vol. 148, pp. 1 – 18, 2005.

[3] R. Biran et al., "Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays", *Exp. Neurol.*, vol. 195, pp. 115 – 126, 2005.

[4] J. P. Seymour and D. R. Kipke, "Neural probe design for reduced tissue encapsulation in CNS", *Biomat.*, 28, pp. 3594 – 3607, 2007.

[5] B.D. Winslow, P.A. Tresco, "Quantitative analysis of the tissue response to chronically implanted microwire electrodes in rat cortex," *Biomat.*, 31, pp. 1558-1567, 2010.

[6] P.J.Gilgunn et al., "An ultra-compliant scalable neural probe with molded biodissolvable delivery vehicle," in *Proc. IEEE MEMS 2012*, Paris, France, Jan. 29 - Feb. 2, 2012.

[7] G. Erdos et al., "Topical patch vaccines target antigen to cutaneous dendritic cells efficiently inducing potent cell mediated immune responses," *Immunology 2009,* May 8-12, 2009, Seattle, WA.

[8] T.D.Y. Kozai et al., " Insertion and chronic implantation of biodissolvable delivery vehicle for ultra-small, ultra-compliant neural probes ," in *Neural Interfaces Conference 2012*, Salt Lake City, Utah, June 18 - 2-, 2012.