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# Chronic tissue response to carboxymethyl cellulose based dissolvable insertion needle for ultra-small neural probes

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ABSTRACT

Implantable neural electrodes must drastically improve chronic recording stability before they can be translated into long-term human clinical prosthetics. Previous studies suggest that sub-cellular sized and mechanically compliant probes may result in improved tissue integration and recording longevity. However, currently these design features are restricted by the opposing mechanical requirements needed for minimally damaging insertions. We designed a non-cytotoxic, carboxymethylcellulose (CMC) based dissolvable delivery vehicle (shuttle) to provide the mechanical support for insertion of ultra-small, ultra-compliant microfabricated neural probes. Stiff CMC-based shuttles rapidly soften immediately after being placed ~1 mm above an open craniotomy as they absorb vapors from the brain. To address this, we developed a sophisticated targeting, high speed insertion (~80 mm/s), and release system to implant these shuttles. After implantation, the goal is for the shuttle to dissolve away leaving only the electrodes behind. Here we show the histology of chronically implanted shuttles of large (300 μm × 125 μm) and small (100 μm × 125 μm) size at discrete time points over 12 weeks. Early time points show the CMC shuttle expanded after insertion as it absorbed moisture from the brain and slowly dissolved. At later time points neuronal cell bodies populate regions within the original shuttle tract. The large CMC shuttles show that the CMC expansion can cause extended secondary damage. On the other hand, the smaller CMC shuttles show limited secondary damage, wound closure by 4 weeks, absence of activated microglia at 12 weeks, as well as evidence suggesting neural regeneration at the implant site. This shuttle, therefore, shows great promise facilitating the implantation of nontraditional ultra-small, and ultra-compliant probes.



## 1. Introduction

Penetrating neural probes have demonstrated feasibility and great potential in human brain computer interface applications [1–3]. However, current devices face the common issues of poor reliability and longevity, which have greatly limited the widespread clinical adoption of the technology [4–7]. On a basic science level, limitations in single-unit neural recording reliability and stability have constrained longitudinal studies of memory and plasticity, particularly in awake, behaving animals [8].

The mechanisms of chronic recording failure are an active area of research with multiple identified or proposed biological and non-biological contributing factors. Non-biological factors mainly involve material failure including electrode corrosion, insulation leakage, and breaking of the lead and connectors [7]. On the other hand, the biological host tissue reactions could adversely affect the recording functionality. The host reaction begins with a penetration injury which damages the vasculature [6,9,10], tears and

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compresses the tissue and kills the neurons and glial cells along the penetration path. Activation of microglia cells occurs immediately after insertion as demonstrated by 2-photon *in vivo* imaging [11]. This acute injury may recover spontaneously to a significant degree if the implant is removed right away, so called "stab wound" injury [12]. The persistent presence of the device on the other hand triggers chronic inflammatory tissue reactions with a chronic glial scar formation and loss of neurons at the vicinity of the probe. Such consequences are likely to lead to degradation of neural recording [12–16].

Experimental and modeling evidences suggest that implant compliance (flexibility) and material softness significantly affect the degree of host tissue reaction to the implanted electrodes. Physiological motion (breathing and heartbeat), micromotion (normal movement), macromotion (falls and traumatic brain injuries) can exacerbate injuries surrounding the implants due to interfacial strain caused by the mechanical mismatch between probe and brain tissue. In addition, the friction induced stress resulting from the mismatch of mechanical properties leads to mechanosensitized inflammatory response in the surrounding tissues [17–21] as well as disruption of the blood-brain barrier (BBB) which could exacerbate tissue inflammation and neuronal degeneration [6,9–11]. Computer models have indicated that soft and flexible materials help minimize mechanical strain at the probe tissue interface [22,23].

Another major consideration is the size of the implant. Seymour et al. have shown that a neural probe with a subcellular size elicited minimal glial reaction with no neuronal loss around the implant [16]. In another study a 7 μm thick carbon fiber electrode produced much reduced tissue reaction than silicon probes (100 μm × 15 μm cross-section) and recorded stable neural signal chronically [14]. Besides the obvious benefit of reduced invasiveness, one proposed mechanism for size being an important factor is that smaller surface area leads to reduced inflammatory cell and molecule accumulation [14,24]. It is also possible that reduction in size leads to increased device flexibility, which in turn reduces the mechanical irritation.

Therefore, many research groups have devoted efforts to fabricating probes with more flexibility and/or subcellular size [7,8,14,22,25–28]. However increased flexibility and complex geometry of next generation electrodes introduces new challenges in inserting these devices into the brain. Ultra-compliant probes tend to buckle and bend prior to penetration into the brain [25]. Even when these probes penetrate the surface of the brain, they can deflect causing the electrodes to stray far from the intended brain region. Furthermore, subcellular sized device are usually not mechanically strong enough to penetrate the pia [25]. As a result, many strategies to implant flexible devices previously described elsewhere have been developed, each with varying design space limitations and tradeoffs [25]. Many of the emerging insertion strategies employ flexible polymer devices temporarily coupled to removable insertion shuttles [25,28–31]. Flexible devices are coupled to stiff shuttles through dissolvable adhesives, sleeve holes, or electrostatic interaction [25,29–31]. This approach is generally limited to tethered flexible probes with planar geometry or simple 3D geometries and becomes more challenging with high channel count 3D multielectrode arrays. As these devices become smaller and more flexible (especially in the axial direction), it will be important to consider the surface chemistries of the flexible probe and shuttle to reduce hydrophobic adhesion in the aqueous environment of the brain and cerebral spinal fluid [25,32–34].

Another approach is the use of an electromagnetic linear accelerator or coilgun [35]. While current efforts are focused on improving targeting precision, limitations also exist in compatible geometry, size, and the minimum size of ferromagnetic particles in the electrode necessary to generate velocity and overcome viscoelastic effects in tissue. Naturally, this also creates compatibility challenges with MRI/fMRI for the implanted patients. A separate approach uses thermal and water-sensitive polymers that are stiff at room temperature and dry environments, but become softer after insertion [33,34]. While promising, these materials present challenges in fabrication of functional devices due to poor dimensional stability during microfabrication. With decreasing size and elasticity, cracking of thin-film conductors become a larger concern. It may become necessary to look to more durable composite thin-film conductors [28,36–38]. An alternative approach that has been well explored employs materials to stiffen a compliant structure for cortical implantation [39]. The materials investigated include polyethylene glycol (PEG) [40] and poly(lactide-co-glycolide) (PLGA) [41] tyrosine-derived polycarbonate [42], and biodegradable silk polymer [43]. These strategies have relied on dip coating of flexible devices, which requires a certain amount of minimum stiffness and present challenges in tip sharpness and coating uniformity desired for implantation [42–44]. We have proposed an innovative platform technology to enable the fabrication and delivery of lithographically patterned ultra-small and complex neural probe via a micromolded dissolvable shuttle made by carboxymethyl cellulose (CMC) [25,28]. CMC is a natural occurring polysaccharide which is soluble in water and leaves no harmful dissolution by-products behind, thereby making it a suitable material choice for our application. The molding process allows precise design of the CMC delivery vehicle (shuttle) geometry and size for optimal outcomes. As a necessary first step, a comprehensive brain tissue reaction analysis to the CMC shuttles is reported here.

## 2. Methods

### 2.1. Fabrication

The probes are fabricated using a solvent-based spin-casting process Fig. 1a. The spin-casting fabrication method was previously described in Ref. [45] for fabrication of microneedle arrays for transdermal drug delivery. This method enables molding of solvent-based polymers. In general, the mold is placed inside one of the buckets of a centrifuge, and the dissolvable polymer mixed with the solvent (commonly in a gel form) is loaded on the mold. Centrifuging provides both the required force to completely replicate the mold geometry, and the air circulation that evaporates the solvent to create the final, solid sample.

For our application we use sodium salt of CMC (NaCMC, Mw ~ 90,000, Sigma Aldrich: 419273) as the dissolvable polymer, the solvent of which is deionized water. Hydrogel of CMC is made by mixing specific amount of dry powdered form of material with deionized (DI) water such that CMC has a 15% mass concentration by weight. The mixture is thoroughly mixed using a spatula and the obtained hydrogel is refrigerated at 4 °C for 24 h to allow for the mixture to homogenize. The hydrogel is then centrifuged at 3000 rpm for 10 min to remove any remaining bubbles.

To create the CMC shuttle (insertion needle), a Si wafer was etched using the Bosch deep reactive ion etching DRIE process with the specified needle and tab geometries (Fig. 1b). The depth of the etch pit was 170 μm, which was specified to obtain a final probe thickness of 125 μm after the shrinkage that occur due to the drying process. It is noted that there is no appreciable shrinkage in the length dimension, and thus, the length of the final needles is close to that on the Si-mold.

The steps involved in spin casting of CMC can be seen in Fig. 1a. The Si-mold is placed inside a custom made silicone (poly(dimethylsiloxane) (PDMS)) holder, which was placed in one of the swing buckets of the centrifuge (ThermoFisher Scientific – Heraeus Multifuge XR with Swinging Bucket Rotor TX 750). The buckets contain a set of PDMS inserts to ensure that the centrifugal force acts perpendicular to the surface of the Si-wafer during centrifugation at 4700 rpm. 400 mg of 15 wt% CMC hydrogel is dispensed over the Si-mold. The centrifugal forces acting on the hydrogel causes it to spread uniformly over the entire Si-surface and also fills the etch pit on the Si-mold. The mold is centrifuged for 10 h at 5 °C during which the water evaporates from the surface of hydrogel leaving behind dry (approximately 87%–90% dryness ratio) CMC on top of Si-mold. The sustained centrifugal force during spin casting ensures that the etch pit on Si-mold is always filled with CMC hydrogel and most of the shrinkage occurs from the top surface (the surface that is exposed to air). The excess CMC formed on top of Si-mold is removed by applying small amounts of DI water using a brush on CMC and squeegeeing it away with a sharp blade until the surface of Si-mold in exposed leaving behind the necessary