Device design factors for enhancing the functionality of chronic intracortical microelectrodes

Heui Chang Lee
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By Heui Chang Lee

Entitled
DEVICE DESIGN FACTORS FOR ENHANCING THE FUNCTIONALITY OF CHRONIC INTRACORTICAL MICROELECTRODES

For the degree of Doctor of Philosophy

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Approved by Major Professor(s): Kevin J. Otto

Approved by: George R. Wodicka 11/16/2016
Head of the Departmental Graduate Program Date
DEVICE DESIGN FACTORS FOR ENHANCING THE FUNCTIONALITY OF CHRONIC INTRACORTICAL MICROELECTRODES

A Dissertation
Submitted to the Faculty of Purdue University by Heui Chang Lee

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

December 2016
Purdue University
West Lafayette, Indiana
Dedicated to my parents, Doosung and Hyeeun Lee,
and my wife, Jayoung Maeng,
and my daughter, Jean Lee.
ACKNOWLEDGMENTS

I would like to thank, first and foremost, to the rats and mice that participated in my research. As I am aware, they did not voluntarily choose to be subjects. If anyone points out that they were in fact destined to be used as laboratory subjects anyway, I would say they could have participated in a milder project that did not require an open head surgery. I tried to be nicest as I could, frequently giving them cereal snacks within the limit of not disturbing the scientific study, although at first they were cautious like this snack doesn’t seem legit or by eating this snack I agree to receive another brain surgery. Regardless, every time I perfuse them I had to look back at the moment when they finally accepted my cereal snack offer although temporarily, but when everyone was happy. With all my heart, I greatly appreciate their sacrifice and wish them they rest in peace.

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Lastly, my parents Doosung Lee and Hyeeun Lee, my wife Jayoung Maeng, and my daughter Jean Lee, thank you for your physical/mental support and patience. I wish the days to come soon when I can return the love I received during this Ph.D. track.
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ABSTRACT


Intracortical microelectrodes are devices used in brain-computer interfaces (BCI) to help regain lost motor, sensory, and cognitive functions of individuals with neurological disorders. However, the long-term performance of microelectrode arrays is hampered by a series of inflammatory tissue responses. The consequence of the inflammatory response is the formation of a dense astroglial sheath around the vicinity of the electrode, impeding the electrical conduction between the electrode and neurons. Furthermore, due to the cascade of neuroinflammatory events, the number of neurons is significantly reduced near the electrode, manifested by decrease in signal-to-noise ratio (SNR) and the yield of electrodes. Over time, these issues eventually lead to the functional failure of the implant.

This study aims to investigate mechanical intervention strategies to mitigate the effect of the biological response and prolong the lifetime of the implanted microelectrodes. First, the longitudinal recording performance of a modified site geometry was evaluated. With planar silicon microelectrodes, sites placed on the edge outperformed the sites placed on the center, demonstrated by increased number of detectable single units with enhanced longevity. Second, the stress-strain induced biological response was studied using various flexible electrodes. Flexible electrodes indeed reduced the magnitude of the biological response than the traditional stiff silicon electrodes. Past a certain flexibility level, however, the biological response did not reduce over less soft electrodes, suggesting a flexibility threshold model. Finally, the biological response of electrodes dip-coated with polyethylene glycol (PEG) was evaluated to resolve a
potential confound of PEG-coating used for inserting flexible electrodes. Results sug-
est that dip-coating with PEG do not significantly alter the inflammatory biomarker
profiles around the device. Overall, findings from assessing the above mentioned in-
tervention strategies will help devising a complex multimodal solution for prolonging
the lifetime of neural implants.
1. INTRODUCTION

1.1 Background

Brain-computer interface (BCI) has the potential to help regain lost motor and sensory functions of individuals with neurological disorders [1, 2]. By implanting a man-made devices that can record from brain activities or artificially stimulate the brain region, we can hear what the brain says or ask it to do certain tasks. In the United States alone, the number of individuals with partial or total paralysis is estimated to be up to 400,000, and about 11,000 new cases are added every year [3]. In addition, about 3.3 million Americans suffer from visual impairment [4] and 750,000 are affected by profound hearing impairment [3]. As one might expect from the numbers in the United States, neurological disorders are prevalent all over the world. BCI leverages the fact that in most cases of neural disorders, the central nervous system (CNS) is not damaged and can still serve its administrative functions. As CNS is one of the critical organs in the body, interfacing with the CNS required a delicate approach with appropriate devices.

Implantable microelectrodes are a type of device that can selectively target certain parts of the CNS and provide an interface between the brain tissue and external equipments [1, 2]. Microelectrodes mainly serve two purposes: recording and stimulation. Recording allows to gain information from neuronal action potential, graded potential, or postsynaptic activities that are generated either spontaneously or from the movement or sensation of the body [5, 6]. Similarly, stimulating neurons to artificially generate neuronal activity can invoke a particular movement or sensation in the subject. The mechanism of the CNS generating such information is extremely
sophisticated, but a number of successful studies have decoded the neural activities to further the application of implantable microelectrodes [5–7].

One of the most successful neural prosthesis so far is the cochlear implant, a sensory prosthetic device for individuals with an impaired peripheral auditory system but intact central auditory pathways. Sounds recorded by an external microphone is transmitted to the electrodes situated on the cochlea and are converted to electrical stimuli using the well-defined tonotopic structure of the cochlea. Although not all recipients were successful, the majority of recipients reported that they at least regained the ability to understand speech [8]. Another successful application is deep brain stimulation (DBS) for relieving parkinsonian motor deficits. This treatment uses a microelectrode placed in the subthalamic nucleus (STN), globus pallidus interna (GPi), or thalamus, to quench the tremor with electrical stimuli [9]. DBS has already been established as a successful clinical treatment for Parkinsons disease, and is gradually expanding its applications to Alzheimers disease [10], obsessive-compulsive disorder [11], dystonia [12,13], and epilepsy [12]. These clinically proven implants are capable of maintaining their functionality for several years [14], presumably because their target region, electrode size, stimulation parameters, or combination of these factors make them resistant to functional degradation [15–17].

By contrast, intracortical devices, relatively tiny devices targeting superficial cortical areas of the brain for delicate neural signal acquisition/stimulation, are more susceptible to functional degradation. Chronic foreign body response (FBR), which almost always occurs upon device implantation, is thought to drive the functional degradation over time and eventually lead to electrode failure [18–20]. Non-penetrating devices such as electroencephalography (EEG) electrodes or electrocorticography (ECoG) electrodes are preferable in term of handling the FBR, however, their limitation is resolution. The trade-off between invasiveness and resolution [21] makes the highly invasive intracortical electrodes to possess a very high spatiotemporal resolution. Interfacing at the single neuron level provides a great potential for applications in
neural prosthetics, demonstrated by successes in decoding algorithms based on single unit isolation [1]. However, the chronic FBR must be controlled to fully utilize the electrodes potential in the long run.

Chronic microelectrode failure can be categorized into several major factors: biological, material, and mechanical. Biological failure mode includes the acute inflammatory response followed by the chronic FBR that eventually leads to astrogliosis formation and neuronal loss around the electrode [22–24]. Material failure mode includes chemical/electrochemical damages to the electrode such as crack or delamination of the insulation layer, site corrosion, and tip breakage that hinders an accurate electrical signal conduction [25]. Mechanical failure mode includes damages to the backbone wire or connector that is rather dependent on the external removal of the headcap [26, 27]. While material and mechanical failure modes also account for a large portion of chronic implant failure, not all implanted device experience the two failure modes. Generally material failure starts to occur beyond 6-12 months [25] and this is long after the biological failure affects the electrodes [28]. In addition, mechanical failure is generally all or nothing, meaning that once it occurs the electrode no longer functions but otherwise the electrode is intact. On the other hand, biological failure mode occurs with every single electrodes, regardless of device size, shape, and material [29]. The inevitable nature of biological failure mode requires a significant attention to be paid in resolving the biological response to microelectrodes, to lengthen the lifetime of neural implants.

1.2 Problem statement and specific aims

Maintaining the functional stability of intracortical microelectrodes over years to decades is crucial for the BCI technology to be considered a viable treatment option for individuals with neurological disorders. Despite the extensive amount of research done to prolong the lifetime of chronic neural implants, no definitive solution has been found yet. Our current knowledge on brain’s response to chronic neural
implants suggest that the problem requires a nonlinear and multimodal solution. Thus, combining mechanical, biochemical, and possibly other approaches to derive an integrative solution is imperative. The first step to derive an ultimate solution will be to identify which individual factor plays a dominant role in mediating the FBR. In particular, mechanical properties need a significant improvement to minimize the extent of injury both in acute and chronic terms. Then other intervention strategies can be followed to cope with the minimized damage. In the body of work, mechanical factors are modulated to mitigate the FBR and extend the lifetime of intracortical microelectrodes. The remainder of this chapter characterizes the typical FBR and functional degradation of neural implants. Then a problem statement is given with three specific aims that focus on mitigating/avoiding the biological response to neural implants.

**Chapter 2 reviews the biological failure mode and mechanical intervention strategies.** This review explains the mechanism of biological failure and how they have been characterized using histological and electrophysiological assessments. The failure is manifested as degradation of the number of identifiable single units and the SNR, and activated glial cells around the device with decreased neuronal density. Then we discuss why device design factors are important and how they should be modulated to lessen the injury in the insertion and indwelling period. Particularly, flexible substrates and form factors are discussed in details as proposed strategies for modulating those two factors are covered in the consecutive chapters. The review bears the message that, while many hypothetical factors exist, decomposing individual factors for independent validation is essential for deriving a multifactorial solution.

**Chapter 3 presents Specific Aim 1: modified site placement for improving the functionality of neural implants.** Previous studies have shown that inter-device factors such as size and architecture of the device heavily affect the chronic performance. This aim focuses on the within-device factor, comparing sites on differ-
ent locations along the shank cross-section. Traditional planar silicon microelectrodes have recordable site only on the center of the shank. With the modified design having sites on the center and edge, in vivo electrophysiological performance was compared between the two over a chronic period. The key finding is that recording sites placed on the edge last longer with higher yield than sites placed on the center, and this effect diminishes with narrow devices.

**Chapter 4 presents Specific Aim 2: mechanically adaptive neural implants for mitigating the tissue response.** Flexible intracortical microelectrodes are considered to lessen the biological tissue response than stiff electrodes. However, limited work has identified the impact of further increasing or decreasing the flexibility of microelectrodes on the tissue response. To this end, this aim investigated the biological impact of four types of probes having different Young’s moduli. The significance of this work is finding that the tissue response around the polyimide probes were less severe than silicon probes, but probes softer than polyimide were similar to polyimide probes. This finding suggest a possible flexibility threshold that the beneficial effect of probe flexibility diminishes with increase in flexibility.

**Chapter 5 presents Specific Aim 3: tissue responses to neural implants dip-coated with polyethylene glycol (PEG).** Flexible microelectrodes generally have difficulty with penetrating the pia mater. Thus, PEG-coating has been used as one of the simple methods for stiffening flexible devices for insertion. In Specific Aim 2, all of the probes were dip-coated with PEG and this might confound the study as its influence to the FBR has not been verified previously. In this aim, a histological comparison was performed to identify the difference between PEG-coated and non-coated devices. Since CX3CR1-GFP mouse model was used in Specific Aim 2 the comparison was performed on CX3CR1-GFP mice as well. The significance of this work is confirmation of the limited effects of dip-coating with PEG on intracortical microelectrodes, despite the reported beneficial/detrimental effects from other
applications. This finding has broad appeal to complement previous neural implant studies as well as future studies that will use the simple dip-coating of PEG.

Chapter 6 concludes with how findings from the specific aims can contribute toward identifying important mechanical aspects. These, combined with other aspects, can be valuable assets for building an ultimate multimodal solution for lengthening the lifetime of neural implants.
2. A REVIEW OF BIOLOGICAL FAILURE MODE AND MECHANICAL INTERVENTION STRATEGIES

2.1 Biological failure mode

2.1.1 Mechanism of biological failure

Degradation of the quality of recorded signals and the efficacy of electrical stimulation has been reported by a number of studies [19, 20, 30, 31]. For recording electrodes, the signal-to-noise ratio (SNR) and the number of action potentials gradually decrease over time [19, 20]. For stimulating electrodes, the required stimulation power for activating a target brain area increases [12, 15]. This functional decline is directly related to the progress of the immune response at the electrode-tissue interface which comprises acute mechanical trauma and chronic FBR.

When an electrode is inserted into the brain, it damages blood vessels, neurons, and glial cells in its path. This initiates the wound-healing response in the CNS, which involves release of erythrocytes, activated platelets, clotting factors, and inflammatory factors from ruptured blood vessels to help rebuild the damaged tissue [30, 32, 33]. It also induces morphological and metabolic changes such as intrusion of blood-borne macrophages, release of various cytokines, and edema. The induced factors change the gene expression at the injury site and recruits activated microglia/macrophages, an immediate responder to brain tissue damage, and astrocytes, a late responder that maintains the homeostasis of the brain tissue, from day one post-implantation [33, 34]. This acute mechanical trauma is likely to be transitory since a stab injury without a residing device has been reported to heal and regenerate [22, 28, 35].
The acute mechanical trauma lasts for only a few weeks and by itself is not known to be neurotoxic [36]. However, it is followed by a chronic and persistent FBR, which adversely affects nearby neurons and the electrode-tissue medium, thereby playing a significant role in the failure of electrodes. FBR can be characterized by activated microglia and reactive astrocytes occupying the surface of the electrode after the onset of the wound-healing process [33, 37]. Unless the foreign material is removed from the insertion site, the FBR persistently aggravates the nearby tissue [33]. Activated microglia attempt to phagocytose the electrode, which they will fail, they encapsulate the material forming a dense astroglial sheath (i.e., an action referred to as frustrated phagocytosis). The astroglial sheath is mainly composed of activated microglia in the inner layer followed by reactive astrocytes in the outer layer, with astrocytic sheath being the major component. The main role of astrogliosis is hypothesized as brain tissues attempting to isolate the damaged region from healthy tissues to maintain blood-brain barrier (BBB) integrity to prevent additional lymphocyte infiltration [38]. By isolating the electrode, however, it hinders the electrical conduction between neurons and the electrode contacts. In addition, it creates a neurodegenerative environment that causes nearby neurons to retract their processes (i.e., dendritic degeneration), die off, or migrate away from the electrode [19, 39, 40]. An illustration depicting the initial injury and the FBR is depicted in Fig. 2.1.

The acute and chronic tissue response is not only the result of the reactive nature of the CNS but also of material-related factors such as the toxicity of electrode derivatives [32]. However, the brain tissue mostly undergoes a very similar acute and chronic FBR regardless of the materials, size, and surgical techniques employed [29, 32]. It is critical to characterize the degree and progression of the chronic FBR which is thought to be the main cause of eventual electrode failure.
Fig. 2.1. Schematic showing acute and chronic immune response at the CNS. Acute response (A) involves vasculature damage, protein absorption, and recruitment of activated microglia and reactive astrocytes, where chronic response (B) involves formation of an astroglial sheath around the electrode and degeneration of neurons at its vicinity. Adapted from [21] with permission.

2.1.2 Histological evaluation of intracortical microelectrode

Conducting immunohistology at multiple time points after implantation can help characterize the progression of the tissue response. Typical biomarkers for the study of FBR include: neuronal nuclei (NeuN) for neuronal cell bodies, neurofilament (NF) for neurofilaments, receptor interacting protein (RIP) for oligodendrocytes, fluorojade-C for degenerating neuronal cell bodies, glial fibrillary acidic protein (GFAP) for mature astrocytes, vimentin for reactive astrocytes and fibroblasts, ionized calcium-binding adapter molecule 1 (Iba1) for microglia/macrophages, CD68(ED1) for activated microglia/macrophages, immunoglobulin G (IgG) for blood plasma, and 4’,6-diamidino-2-phenylindole (DAPI) or Hoechst for general cell nuclei. These biomarkers tell us about the composition of glial cells and their activated states, as well as the neuronal health and density.

One of the first studies to investigate the FBR to neural electrodes, Turner et al., showed the time course of the tissue response at 2, 4, 6, and 12 weeks post-
implantation [41]. They used a micromachined silicon electrode and explored the astrocytic activity near the electrode. GFAP marker showed a large quantity of astrocyte recruitment radially around the implant region as far as 500 µm at 2-4 week time points. As the post-implantation time increased to 6-12 weeks, the astrocytes became denser and more compact around the electrode forming an astroglial sheath. Other groups have verified this time course with more extensive histology and found that morphological changes were made to astrocytes turning them into reactive state [18, 22, 42]. Proliferation and hypertrophy are the two main phenotypes of reactive astrocytes and they become more prominent as astrogliosis develops. Staining for vimentin confirmed that the astroglial sheath is mostly composed of reactive astrocytes, but not mature astrocytes, and a complete sheath was formed within 4 weeks [33]. As see in Fig. 2.2, a stratified astroglial sheath with activated microglia in the inner layer and astrocytes in the outer layer is presented with ED1 and GFAP staining.

Investigators have observed the formation of a neuronal kill zone as far as 200 µm from the implant as a consequence of the neuroinflammatory events [22, 43]. Fig. 2.2 demonstrates a loss of neurons in the vicinity of the electrode with NeuN and NF staining. The exact mechanism of this neuronal loss/degeneration is not well understood, but studies have suggested that pro-inflammatory cytokines such as transforming growth factor- (TGF-), tumor necrosis factor alpha (TNF-), interleukin-1 (IL-1), prostaglandin E2 (PGE2), and interferon- (INF-) secreted by activated microglia/macrophages intoxicate neurons to turn them into a degenerative state. Studies also point out the role of reactive astrocytes releasing neurotoxic factors as a cascade reaction [44,45]. However, the inflammatory signaling pathways in the cortex are not yet conclusive and further need to be verified.

Conventional histology requires explantation of implanted devices, resulting in significant tissue loss surrounding the device. This limits the quantification of immunolabels and complicates the interpretation of brain tissue since the most important
Fig. 2.2. Overlaid images of cell type specific markers around the electrode track (orange oval drawn on the left). ED1, which stains for activated microglia, shows the inflammatory phenotype at the closest layer followed by a dense layer of GFAP representing astrocytic phenotype. Low intensity of NeuN and NF near the implant track within \( \sim 200 \ \mu m \) shows loss of neuronal cell bodies and processes. Adapted from [22] with permission.

region of interest is in the proximity of the electrodes. Recently, device capture histology (DCHist) was devised by Woolley et al. [46, 47]. DCHist leaves the device intact in situ during the histological procedure, preserving the device-tissue interface without any distortion. The key technique employed in DCHist requires a delicate and precise handling of brain tissues. First, the brain tissue needs to be extracted without pulling out the device with the acrylic headcap. Second, a relatively thick slice (150-400 \( \mu m \)) is harvested using a vibratome. Although the slice is much thicker than conventional histology slice, care must be taken to capture the device within it. Fig. 2.3 depicts a good quality DCHist processed tissue slice presented by Woolley et al. [47]. Here we only see microglial activity near the electrode but not a significant neuronal loss. Neither a dense astroglial sheath nor a neuronal kill zone is observed due to the fact that this data is from 1 week post-implantation and the tissue response is still in the acute phase.

2.1.3 Electrophysiological evaluation of intracortical microelectrode

Electrophysiology (Ephys) recordings and impedance measurements can provide real-time in vivo data from functioning electrodes in live animals. Specifically, Ephys
recording allows us to measure the combinatorial effect of the electrode-tissue interface and the healthy neuronal population around the implant. Impedance measurement supplements the information of the electrode-tissue interface.

As a robust Ephys recording over long-term is our ultimate goal, its functional stability can be used as a metric for determining the functionality of microelectrodes. Recorded raw signals from penetrating electrodes contain multi-unit action potentials from neurons, graded potentials, thermal noise, and motion artifacts. To discriminate single-unit action potentials from other signals, a number of steps are required which typically include band pass filtering of raw signals, spike detection, feature extraction, and spike clustering [48]. After the clustering, each single-unit is distinguished from the multi-unit cluster and this provides information on the number of neurons near the electrode site, the firing rate/pattern, the amplitude of the spikes, and the background noise level.

In contrast to the Ephys recording which passively records the neuronal activities, complex impedance spectra can be obtained by applying a sinusoidal voltage at
predefined frequencies and measuring the current. A typically used frequency range
starts from 10-100 Hz and goes up to approximately 10 kHz, and resistive and ca-
pacitive impedance values can be obtained with respect to this range of frequency
input [31, 49]. Most importantly, 1 kHz impedance is known to be the fundamental
frequency of action potentials. Therefore, retaining at a low 1 kHz impedance implies
having a good electrode-tissue interface. However, it cannot be directly translated
into having a good recording performance as the status of neurons cannot be derived
with impedance measurements.

With the circuit model that describes the electrode-tissue interface, a rough esti-
mation of the degree of the FBR is made possible [31, 49]. This equivalent circuit
model divides the circuit into the electrode compartment and the tissue compart-
ment [31, 50, 51]. The electrode compartment is the electrode-electrolyte interface
described by a constant phase element. The tissue compartment is subdivided into
the resistive astroglial encapsulation layer and the complex cellular compartment.
The cellular compartment comprises the resistive extracellular medium connected in
parallel with the complex cellular membrane, which is often described as a RC com-
ponent. Complex impedance spectra can be analyzed with this model to look at how
individual compartments contribute to the overall impedance. Attempts have been
made to correlate the individual circuit component values with histological quan-
tification based on this model, but ended up with minimal successes [49]. Further
investigation is needed to verify the relationship between the individual circuit com-
ponents and the quantified measurements from histological markers.

Typically, chronic neural implants experience a decrease in recordable neuronal
activities and SNR over time [20, 30, 52–54], and an increase in the impedance [20,
31, 55]. Freire et al., used a tungsten microwire array and demonstrated that there
is a clear degradation of the quality of the Ephys recording, characterized by fewer
recorded spikes and reduced peak amplitudes (Fig. 2.4 (A)) [52]. Williams et al.,
also used a tungsten microwire array and showed that there is a constant increase in
impedance, characterized by impedance spectra moving to the upper-right direction in
the Nyquist plot (i.e., greater resistive and capacitive values) (Fig. 2.4 (B)) [31]. Fig.
2.5 (A) shows quantitative analyses indicating that a similar trend is also obtained
with silicon microelectrode arrays [20]. As the FBR occurs regardless of the type
of electrodes [29], these results support the idea that the FBR is the main cause of
electrode failure and must be suppressed to enhance electrodes functional longevity.
Fig. 2.5 (B) also demonstrates that the most dramatic change in the impedance
occurs up to four weeks post-implantation, and this coincides with previous findings
that glial encapsulation is well established by four weeks.

Fig. 2.4. Qualitative result of Ephys recordings and impedance mea-
urements with tungsten microwires. (A) Spike clustering result.
Each color except gray represents individual spiking activities of neu-
rions. Adapted from [52] with permission. (B) An impedance spectra
in nyquist plot from 0-7 days post-implantation. Adapted from [31]
with permission.
2.2 Mechanical intervention strategies for mitigating the biological response

2.2.1 Form factors

Intuitively, electrodes with smaller cross-sectional area are likely to cause a smaller mechanical injury. As they disrupt less amount of tissues, a smaller injury reduces the microglia/macrophage recruitment to the electrode, which may further lessen the inflammatory cascade that is generally progressive over time [23, 24]. Smaller size not only reduces the acute injury but also reduces the chronic FBR which has to do its presence in the brain. Recent research utilizing small size electrodes has verified that smaller electrodes cause less severe astrogliosis and blood brain barrier (BBB) instability [23,56,57]. It can be due to that a large electrode block cellular signaling pathways with its volume or a larger surface area promote glial cell attachment and facilitate activation [58]. While smaller device is preferred in terms of the tissue re-
response, a major disadvantage of using small site area is the compromise in sensitivity of neural recordings or charge carrying capacity of microstimulation [59,60]. Depending on the application and the scope of use, the substrate size and site size need to be adjusted accordingly.

In addition to size, electrode architecture is another important design factor to be considered. Edell et al. have proposed several factors that may influence the FBR including the tip shape [43]. As long as the tip is sharpened, however, differently shaped tapered tips did not result in different degrees of tissue response, whether the tip was a sword- or chisel-shaped. Bjornsson et al. later tested the tip angle of a device which may affect the insertion injury [61]. Granted that this is as intuitive as smaller size is favorable, sharp tips were favorable in reducing the FBR. Blunt-tipped electrodes induced a more severe tissue response as they result in higher tissue strain and vascular damage during device insertion. The sharpness of the tip, however, may become less crucial as electrodes gets smaller.

Apart from modulating the body of the shank, site modulation can make the recordable sites undergo less severe foreign body response (FBR) than the body of the shank. Seymour et al. evaluated an electrode design that put sites on a lateral wing to separate the sites from the bulky shank [62, 63]. As the whole electrode can also be thought as two separate electrodes, the shank part and the lateral wing part, it was a reconfirmation of the concept that smaller electrodes induce less severe FBR. Their work inspired that modulating the site placement can be an effective way of avoiding the FBR.

Surface area of electrodes is a slightly different concept than the size of electrodes. Since electrode is a three dimensional structure, a solid electrode and a perforated electrode can have the same external dimension with a different tissue-contact area. Seymour et al. first brought the concept of lattice (or perforated) design [62], and this was further evaluated by Skousen et al. that the lattice design significantly
reduced the FBR [64]. Since the lattice design exhibit a smaller cross-section than the solid design, lattice structures are better suited for cells to re-establish their network and promote isotropic diffusion of cell signaling molecules [64]. Similar to small size, glial cell attachment can be reduced as well. Electrode perforation concept has also been investigated in surface electrodes to enhance the electrode-tissue interface integrity [65].

2.2.2 Flexibility

A significant difference in softness exists between the brain tissue and electrode materials. Conventionally used substrate materials such as tungsten, silicon, platinum, steel, and glass have a Youngs modulus (i.e., a measure of softness) of 6-7 orders of magnitude greater than that of the brain tissue [66, 67]. Due to this mismatch, micromotion generated by respiratory and pulsatile movement can perturb the surrounding tissue over a long period by exerting shear stress on the tissue and/or by tearing off the tissue with sharp edges [68–70].

Stiffness of an electrodes depends on material properties and architecture [71]. Studies using finite-element modeling (FEM) evaluated the extent of shear stress induced by planar type silicon probes [70, 72, 73]. These results suggest that the implanted devices indeed generate strong stress-stress in the axial and later direction, presumably causing mechanotransduction that leads to abnormal neuronal activity. In vitro cell culture studies verified that an increased level of astrogliosis and neuronal death may happen in excessive strain conditions [74]. It is not only the harmful effect of strain that makes flexible electrode more favorable. Neurons were found to extend their projections more actively to a preferred range of stiffness, mostly to materials at low Youngs modulus, which further strengthens the idea that soft materials are better for neural implant [75, 76]. These evidences suggest that using soft materials is better for the health of surrounding neurons.
Early generation electrodes specifically designed to meet the flexibility constraint were polyimide-based microelectrodes \([66,67,77,78]\). The fabrication of these devices involved metal sites sandwiched by thin films of polyimide using standard planar photolithographic techniques. Parylene-C was also considered to be a suitable flexible material that serves the same purpose as polyimide \([79–82]\). Other types of materials that are even more flexible than polyimide/parylene were also proposed such as organic nanocomposite \([83–85]\) or SU-8 \([86,87]\), or mixture of various materials \([88–91]\). These types of electrodes are effective in reducing the strain-stress between the tissue and the device caused by micromotion.

Flexible substrates are generally better than rigid substrates in every aspects, however, the major disadvantage is their need for aid in insertion. Flexible devices tend to buckle on the surface of the brain due to the difficulty in penetrating the fibrous pia mater \([70]\). Insertion vehicle has been proposed as a means to provide temporary mechanical support for inserting the device into the brain. Coating with agents that are rigid, biocompatible, and rapidly biodegradable while breaking down into biocompatible metabolites, has also been considered. Coating devices with polyethylene glycol (PEG), gelatin, carboxyl monolayer, or tyrosine-derived resorbing polymer have been demonstrated to provide sufficient rigidity for insertion \([92–95]\). However, side effects may exist as these carriers increase the insertion profile of the device, as well as degraded byproducts may alter the nearby cellular environment.

2.2.3 Iatrogenic factors

The micromotion induced stress-strain is not only tied to the flexibility of the electrode, but also tightly related to the tethering method. Functional electrodes are normally tethered to the skull directly or via tethering cables to protect the device from dislodging. This makes the device prone to inducing injuries during micromotion, as the dislocation between the skull and the brain tissue is the main source of strain-stress. By contrast, free-floating electrodes that are not tethered to the skull are
more likely to move along with the brain tissue, causing minimal strain-stress from micromotion. Histological studies have verified that tethered devices cause significantly greater immune response than un-tethered devices [42, 57, 96]. Nevertheless, current technologies do not allow functionally active electrodes to be free-floating mainly due to the wiring issues. Even putative wireless electronics will have significant momentum that will likely result in non-zero tethering forces. As strain-stress effect comes from an interplay of electrode flexibility and tethering, the two factors can be tuned jointly to overcome the confronted issue.

Another factor to be considered is the speed of insertion. A higher insertion speed is favorable as it enables the devices to neatly penetrate the tissue without rupturing the vessels or dragging the tissues [97–99]. Subbaroyan et al. have found that tissue compression upon insertion may also rupture vessels that are relatively far away from the implant site, which is more often observed with slower insertion [72]. The extent of the tissue damage was more dependent on the speed of insertion rather than the sharpness of the tip, although blunt and sharp tips both favored a faster insertion.

All in all, a number of studies have provided insights to the mechanical factors influencing the chronic functionality of electrodes. However, some studies combined their approach with several other paradigms such as surface coatings, suggesting that the results may not be solely due to the mechanical factors. Mechanical factors need to be individually validated to better understand the optimal combination of factors requiring modulation.
3. MODIFIED SITE PLACEMENT FOR IMPROVING THE FUNCTIONALITY OF NEURAL IMPLANTS

3.1 Abstract

Objective: This study aims to identify the benefit of using edge sites over center sites on a planar silicon microelectrode array. Methods: With Michigan Arrays having sites on the center and edge, we have compared the single unit activities, signal-to-noise amplitude ratio (SNR), noise level, impedance, and histology to identify the difference between the two site locations. Wide and narrow devices were used to evaluate if the difference was consistent and had a trend that meet the theoretical expectation. Results: Edge sites recorded significantly more single units than center sites on wide devices. Significant differences were observed with SNR during the first week, but not afterwards. Edge sites also generally performed better than center sites on narrow devices, but differences were smaller than wide devices. Conclusion: Edge sites are more suitable for single unit recordings. The benefit of edge sites decreases as the distance to center sites decreases. Significance: We showed that a simple alteration to the site placement can greatly enhance the functionality of silicon microelectrodes. This study promotes assessing within-device factors that can provide an alternative pathway for lengthening the lifetime of neural implants.

3.2 Introduction

Intracortical microelectrodes generally fail to record or stimulate over a chronic period [19, 20, 30, 52, 55, 100]. Several failure modes exist [100], among which the biological failure has been extensively studied for decades as all devices, without exception, eventually lose their functionality in the long-term largely due to the bi-
ological tissue response. Over time, devices typically lose the capability to record identifiable single units and the signal-to-noise amplitude ratio (SNR) of single units is degraded [20, 52, 55]. Histology revealed that microglia/macrophages, astrocytes, and infiltrated blood-derived products are activated and/or accumulated near the device and that neurons are lost [22, 24, 28, 41, 101]. A number of mechanical factors have been investigated to reduce this tissue response, including device size [56–58], architecture [43, 62, 63], flexibility [70, 72, 83, 88], tethering [42, 57], and insertion method [61, 97]. These studies provided insight to building an ideal intracortical microelectrodes, the solution to which is complex and multifactorial [102, 103].

Extensively studied device design factors have been mostly related to the substrate of the device. Substrate is indeed a critical design parameter since it is served as a mechanical support for the electrode sites and provides insulation to the underlying wires. Substrate determines the extent of injury both in acute and chronic terms, and thus comparing the biological response and/or recording functionality across different substrates has been an area of interest [29, 58, 84, 86, 104]. However, in vivo characteristics of factors within a device have not gained much attention. As Michigan Arrays usually have multiple sites along the shank, depth dependent response was an important finding that promoted researchers to control depth when conducting a comparison study [46, 105, 106]. With traditional Michigan Arrays having sites only on the center, however, the cross-sectional plane was difficult to study. Here, we present a Michigan Array design having sites mainly on the center and edge of the cross-sectional plane, to evaluate edge sites relative to the center sites.

Several studies laid the foundation of the concept of using edge sites. Seymour et al. demonstrated that a thin lateral wing attached to the main shaft induces significantly less severe tissue response than the main shaft [62], and also showed that sites with more exposed sides can perform better than one-side exposed sites [63]. What is noteworthy, however, is that the lateral wing structures used in these studies can be regarded as a separate shank, as its thickness and materials were different
from the main shank. As a number of evidences support the idea that the smaller (or thinner) the better [56–58], lateral wings superior performance over the main shank is reasonable. However, edge sites placed on the same shank as center sites, presumably going through a similar tissue response, but performing better than center sites is not a fully convincing argument. To the authors knowledge, no study has been conducted to evaluate the impact of edge sites on a single planar shank device.

The objective of this study is to identify the impact of site placement on the neural recording performance. A preliminary study of center vs. edge sites with wide devices was presented in [107], and we further extended the study by introducing narrow devices as well as quantitatively analyzing single unit activities over the course of 8 weeks. Histology was also conducted to assess the hallmarks of biological tissue response. Findings from this study are expected to help judging the importance of within-device factors, which are often overlooked.

3.3 Materials and methods

3.3.1 Device configuration

Twenty four custom designed planar silicon microelectrode arrays (sixteen GP_1x16_249 (249 µm wide) and eight GP_1x16_132 (132 µm wide) - See Fig. 3.1 (A) for dimension, NeuroNexus, Ann Arbor, MI) were used in this study. Devices were 15 µm thick and approximately 2.2 mm long. Within each 16 channel device, seven sites were on the center, eight sites were on the edge, and one site was at the tip. The device shank was connected to a ZIF connector via a flexible polyimide cable.

3.3.2 Surgical implantation

All surgeries and animal experimentation were performed under the guidance of the Institutional Purdue University Animal Care and Use Committee.
Fig. 3.1. (A) Device design layouts of wide microelectrodes (GP_1x16_249) and narrow microelectrodes (GP_1x16_132). The sword shaped device is connected to a flexible polyimide cable for easier targeting of the brain region of interest. (B) An illustrative picture showing bilateral implantation of devices into rats primary motor cortex (M1).

Surgery and electrical measurements follow the same procedure as presented in [107]. Twelve male Long-Evans rats (300-360g, Charles River, Chicago, IL) were bilaterally implanted with microelectrodes, one device per hemisphere. Animals were anesthetized with a mix of ketamine (75-95 mg/kg) and xylazine (5 mg/kg) and their consciousness was checked with a pulse oximeter and toe pinch tests throughout the surgery. Supplementary ketamine (20 mg/kg) was administered as needed to keep the animals sedated. Body temperature was maintained with a heating pad (Stryker, Kalamazoo, MI).

Animals head was shaved and fixed on a stereotactic frame. Head was wiped with three alternating washes of betadine and 70% isopropyl alcohol. An incision was made in the midline and periosteum was gently removed. Holes were made using a microdrill (OmniDrill, World Precision Instruments, Sarasota, FL) and small titanium screws (size 0-80) were placed into the holes. A 2 x 2 mm craniotomy, centered at 2 mm anterior and lateral to the bregma (M1 region), was made on both
hemispheres. Reference and ground wires of the device was wrapped multiple times around a titanium screw, and the device shank was glued to the piezoelectric actuator (Physik Instrumente, Karlsruhe, Germany) with a heated polyethylene glycol (PEG) [108]. The device was inserted into the craniotomy 1.5-1.8 mm deep at 20 mm/s (See Fig. 3.1 (B)) and saline was applied to release the actuator. The craniotomy was covered with Gelfoam (Pfizer, New York, NY) and Kwik-Sil (World Precision Instruments), followed by dental acrylic (Lang Dental, Wheeling, IL). Suture was performed to minimize the wound exposure.

3.3.3 Electrophysiology and impedance measurements

Measurements were obtained from freely moving rats inside a faraday cage. Measurements were taken daily up to one week post-implant (WPI) and three times a week thereafter until the animal was sacrificed. Neural recordings were obtained using a RZ5 Bioamp Processor (Tucker-Davis Technology (TDT), Alachua, FL) with a RA16PA Medusa preamplifier (TDT) sampled at 24415 kHz. Recordings were taken for 10 minutes per session and the raw data were collected and stored using a custom written Matlab (MathWorks, Natick, MA) script. Electrochemical impedance spectroscopy (EIS) were also performed followed by the neural recording using PG-STAT128N Potentiostat (Metrohm Autolab, Utrecht, Netherlands). Three repeated measurements of 10 Hz - 30 kHz frequency sweeps were averaged and interpolated to draw the 1 kHz impedance value.

3.3.4 Post processing of neural recording data

Neural signals were band pass filtered at 300 Hz - 3 kHz. Spikes were detected with an amplitude threshold set at 4 times the noise level \( \sigma_N = \text{median(abs(signal))}/0.6745 \) [109]) and 1.3 ms of waveform snippets were collected. To reject artifacts appearing as neural spikes, threshold crossing snippets were discarded if (1) the peak amplitude was greater than 20 times \( \sigma_N \) or, (2) a peak occurred simultaneously (within 0.2
ms) on a distant channel (greater than 150 um apart) with greater than 70% of the peak amplitude. The reasoning behind this procedure is to mainly remove impulses of movement artifacts that are generally correlated across the channels [110,111]. Spikes were sorted in the 3D principal component domain with Scanning K-means clustering method using the Offline Sorter V3 (Plexon, Dallas, TX). Sorted units with a firing rate of greater than 0.5 Hz (i.e., >300 units in the 10 minute recording session) were identified as recorded single units.

Signal-to-noise amplitude ratio (SNR) of each unit cluster was calculated as

\[ SNR = \frac{\mu_{pp}}{2\sigma_N} \]  

where \( \mu_{pp} \) is the peak-to-peak amplitude of the sorted unit template. SNR was calculated only on the channels with identified single units. Thus, average SNR only accounts for the active channels to differentiate its interpretation from percent activity.

### 3.3.5 Statistical analysis

Using Matlab, paired t-tests were run to identify the differences between site locations (i.e., center vs edge, and lower vs upper) at a given period while blocking inter-animal variabilities. In addition, an Analysis of Covariance (ANCOVA) was also conducted to identify the difference over the entire 8 weeks. No significant interaction effects were observed between any of the site locations and any times post-implant.

### 3.3.6 Histology

Animals were deeply anesthetized and transcardially perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde solution (PFA). Heads were collected and post fixed in PFA for 24 hours at 4 °C. After rinsing the heads with PBS several times, brains were carefully extracted with either explanting the device for
conventional histology (100 µm horizontal sections) or preserving the device in situ for Device-Capture Histology (DCHist) (400 µm coronal section) [47]. Brains were sectioned using a vibratome (VT1000S, Leica Microsystems, Wetzlar, Germany) and placed in Hepes-Buffered Hanks Solution (HBHS) containing sodium azide [47] at 4 °C until the immunostaining procedure.

Briefly, tissue slices were incubated in NaBH4 in HBHS (5 mg/ml) for 30 minutes. Tissues were washed 3 times with HBHS for 5 minutes each and blocked in Wash Solution (1% v/v goat serum, 0.3% v/v Triton-X in HBHS) for 2 hours. Primary antibodies (1:400 mouse anti-NeuN (MAB377, Millipore), 1:400 chicken anti-GFAP (AB5541, Millipore), 1:400 rabbit anti-Iba1 (019-19741, Wako Chemicals)) diluted in Wash Solution were applied to the tissues and allowed to incubate for 48 hours at 4 °C. Then, tissues were washed with Wash Solution 6 times for 5 minutes each followed by 6 times for 1 hour each. Secondary antibodies (1:400 goat raised matching pairs for primaries) diluted in Wash Solution were applied and allowed to incubate for 48 hours at 4 °C. After rinsing with Wash Solution 6 times for 5 minutes each and 6 times for 1 hour each, tissues were incubated in the glycerol-based U2-Scale optical clearing solution (4 M Urea, 30% v/v Glycerol, 0.1% Triton-X) [112] for 2-3 weeks. U2-Scale was also used as the mounting medium for fluorescent imaging.

Coverslipped tissue slices were imaged using a laser scanning confocal microscope (LSM710, Carl Zeiss, Jena, Germany) and Zen 2010 software (Carl Zeiss). A 10X objective was used with four laser lines to capture emitted fluorescence at different wavelengths (488/555/633 nm for immunolabels and 405 nm for reflection of the device). Laser power and gain were adjusted accordingly for multiple z-stacks as the imaging plane moved along the z-axis.
3.4 Results

Typical lifespan of animals ranged 2-6 months and they were sacrificed for endpoint histology. No mechanical failure (loss of headcap) was observed. Devices that were broken during the surgery or did not record any identifiable neural spikes were excluded from the study, resulting in 8 wide devices and 7 narrow devices for comparison. We have statistically compared the results of neural recordings and impedance measurements up to 8 WPI (7 WPI bin is an aggregate of 7-8 weeks) as the number of animals and the number of identifiable single units decreased after 8 WPI making the results difficult to interpret. Although tip sites were in the design they were excluded from statistical analyses due to the low number of tip sites.

3.4.1 Neural recording

As previously reported by a number of literatures [20, 30, 52, 53, 55], the quality of the neural recordings degraded over time regardless of the device width and site location, the two main comparisons in this study (Fig. 3.2). Unit spikes are clearly visible in the time domain at 2 DPI with higher activities on the edge sites (Channels 9-16) than the center sites (Channels 1-7). The magnified view shows synchronized unit spike waveforms recorded across the channels adjacent to each other. Two distinct single units identified on channel 11 are presented on the right showing well separated clusters in the PC domain. Unit spikes at 48 DPI are not as clearly visible as 2 DPI, also seen with the decreased signal amplitude and the decreased number of units after sorting. Recordings from this rat was one of the quality recordings in this study, and many of the recordings failed to continue recording single units well before 48 DPI.

Most of the sites gradually failed to record single units over time, but the lifetime of each site varied dramatically. Fig. 3.3 depicts the fraction of active microelectrodes, defined as the fraction of sites recorded single units in the given time interval [55], at
each DPI and WPI. For each animal, if a site was able to record single units on any day within the week the site was deemed active. This survival fraction provides an overall picture of the functionality of the sites. Single unit activities were high during the first week and then severely degraded starting from 1 WPI. However, in almost all of the time points, edge sites had higher activity of single units than center sites. This was particularly pronounced with the wide devices. Not all time points were significantly different ($p<0.05$) with paired t-tests, but the difference over the period of 0-6 DPI and 0-7 WPI were both significantly different ($p<0.05$) with ANCOVA. Although the trend of edge sites outperforming center sites still existed with narrow devices, fewer time points were significantly different ($p<0.05$) with paired t-tests, and over the period of 0-6 DPI and 0-7 WPI were both not significantly different ($p>0.05$) with ANCOVA. Also, there was no significant difference between the wide and narrow devices ($p>0.05$).
Fig. 3.3. SNR as a function of DPI (left) and WPI (right). Heatmaps show the average SNR of each animal and line graphs show the average across animals. (A) Wide devices (GP_1x16_249) and (B) narrow device (GP_1x16_132). Error bars are standard error mean. Paired t-tests were run on each DPI and WPI (significance level: * <0.1, ** <0.05). Letters in the Rat# indicate right (R) or left (L) hemisphere.

Noise levels generally increased up to 2-3 WPI and somewhat decreased in the later time points (Fig. 3.5). For both wide and narrow devices, the day of implantation (0 DPI) was not the day with lowest average noise level. Noise levels decreased for the first 1-2 DPI and then started to rise. The difference between center and edge sites were minimal and consistent over time for both wide and narrow devices. No statistically significant differences were observed (p>0.05) with center vs. edge sites on the wide devices at any given time points. Center vs. edge on the narrow devices
at 1 DPI was significantly different (p<0.05), but no other significant differences were found to support the consistency of this observation. Also, comparisons between center and edge sites over the entire DPI or WPI were also revealed to be not statistically significant for both devices. Interestingly, the difference in the average noise level of narrow devices were significantly higher than wide devices (p<0.05).

Fig. 3.4. Noise level as a function of DPI (left) and WPI (right). Heatmaps show the average noise level of each animal and line graphs show the average across animals. (A) Wide devices (GP_1x16_249) and (B) narrow device (GP_1x16_132). Error bars are standard error mean. Paired t-tests were run on each DPI and WPI (significance level: * < 0.1, ** < 0.05). Letters in the Rat# indicate right (R) or left (L) hemisphere.
As shown in the graphs, the most dramatic changes occurred during the first week. The fraction of active electrodes, SNR, and noise level mostly underwent 0-5 days of recovery phase, followed by degradation. The degradation occurred abruptly that nearly 70% of the sites actively recording single units in the first week (0 WPI) failed to record in the second week (1 WPI). At 7 WPI, almost all sites on the wide devices lost their functionality, and only a small fraction of sites on the narrow devices remained functional. Degradation of SNR did not look to be as dramatic as the degradation of fraction of active electrodes, putatively due to the lower limit of SNR being bounded by the set threshold for spike detection (SNR of 2).

3.4.2 Impedance

Impedance spectroscopy enables a rough estimation on the state of the device-tissue interface, as cellular and non-cellular compartments can be modeled as RC circuits with a constant phase element [31,49,51]. Especially the magnitude of 1 kHz impedance provides a good estimate of the signal quality of single units. Impedance generally increased during the first few WPI and then slightly decreased or plateaued afterwards (Fig. 3.6). ANCOVA on center vs. edge sites turned out to be not significantly different for wide devices (p > 0.05), but significantly different for narrow devices (p < 0.05). When we include tip sites to the statistical test, the differences among center, edge, and tip sites were statistically significant for wide devices (p < 0.05), but not significant for narrow devices (p > 0.05). The trend of impedance very closely followed the trend of noise level of neural recordings. The difference between wide and narrow devices was also statistically significant (p < 0.05), as is observed with the noise level.

3.4.3 Histology

Both DCHist and conventional histology were conducted with three labels staining for microglia/macrophages (Iba1), astrocytes (GFAP), and neuronal nuclei (NeuN). DCHist demonstrates the gross tissue response to the implanted device along the
Fig. 3.5. Average 1 kHz impedance magnitude as a function of WPI. (A) Wide devices (GP_1x16_249), (B) narrow device (GP_1x16_132), and (C) a comparison of the average of wide and narrow devices. Error bars are standard error mean. Paired t-tests were run on each WPI, but no statistically significant differences were found.

columnar layers (Fig. 3.7). Reflectance of the device shows that the device was situated approximately 1.5 mm down the cortical surface and the majority of sites were located 600-1000 \( \mu m \), which is mostly layer IV and V of rats primary motor cortex. As the focal plane of this image is on the tapered shank, and microglial and astrocytic activities are shown to be most intense around that area. The population of neuronal nuclei looked to be less dense around the tapered area, but an accurate interpretation was difficult due to the angle of the device overshadowing the tissues beneath it. Due to the limited depth of antibody penetration, GFAP and NeuN were no longer visible after 70-100 \( \mu m \) into the section, whereas Iba1 maintained its intensity up to 200-250 \( \mu m \) (data not shown), as previous reported [47].

Conventional histology is limited to a single depth, but provides a good representation of the cross-sectional view around the device (Fig. 3.8). A horizontal slice image of Rat038 implanted with a wide device shows that after 6 months a compact astroglial sheath was formed around the device. However, glial cells nearby the astroglial sheath were not much activated and shows a stabilized response. There was a neuronal void sized 30-40 \( \mu m \) from the sheath. By contrast, images from Rat049
implanted with a narrow device shows that after 2 months there was still an ongoing glial cell activation around the device, without much neuronal loss near the astroglial sheath. The size of the wide device indicate that the hole of Rat038 was stretched to the sides while the device was residing in the brain, but shrunk upon extraction. The narrow device of Rat049 did not look to cause serious deformation upon extraction.

3.5 Discussion

As reported by a number of studies, device-tissue interfaces established with current technology are highly variable in nature [20, 25, 31, 52, 55]. Often it is hard to find a meaningful conclusion as the inter-animal variability can overshadow the effect of treatments. In this study, center vs. edge sites were compared within the same device, successfully blocking the inter-animal variability. This significantly increased
Fig. 3.7. Histology images of horizontal sections from rats implanted with (A) a wide device (GP_1x16_249) for 6 months, and (B) a narrow device (GP_1x16_132) for 2 months. Sections were taken from approximately 1100 μm deep from the cortical surface. Scale bars are 100 μm. Orange rectangles shown above the Iba1 images depict size of the respective devices. (C) A finite element modeling (FEM) image from [113] showing relative concentration of MCP-1 and TNF-surrounding an implanted device. Rectangles depict center and edge sites surrounded by different concentrations of pro-inflammatory cytokines. Adapted from [113] with permission.

the statistical power, shown in the results that edge sites were almost always better than center sites at any given time point.

Single unit activities of edge sites were significantly greater than center sites on wide devices over the course of 8 weeks. The SNR was also significantly greater in the 0-6 DPI, but not in the 0-7 WPI, presumably because the number of single units decreased and the average SNR lost its statistical power. Interestingly, the difference in the SNR was not manifested in the noise level. These differences in center vs. edge sites were also observed from narrow devices, but with a smaller difference, which is intuitive. Keep decreasing the shank width would result in center and edge sites
overlapping with each other, rendering no difference between the two. Center vs. edge sites on the narrow devices did not show any statistically significant differences in the fraction of active electrodes, SNR, and noise level. As it is seen in Fig. 3.3 (B), however, the trend of edge sites outperforming center sites seen from the wide devices still existed.

The noise level and impedance were tightly correlated to each other. The difference of center vs. edge sites seen in Fig. 3.5 was also observed in Fig. 3.6. Unlike single unit-related measures, these measurements did not fluctuate much as they do not depend on the status of neurons. Although noise levels increased and single unit activities decreased over time, it was difficult to conclude if noise level was the dominant cause of signal loss. Sites with single unit activities in the chronic phase (4-7 WPI) had similar noise levels compared to sites with no single unit activities. To tease apart, it is likely that loss of identifiable single units is dictated by nearby neuronal activity (i.e., signal source) rather than the glial encapsulation itself (i.e., electrical conduction), putting aside the neuroinflammation cascade initiated by activated glial cells. We observed that narrow devices had significantly higher noise level and impedance than wide devices, which was not expected, but these did not make a large impact on the recorded single unit activities as wide and narrow devices were not significantly different in the fraction of active electrodes and the SNR.

Histology images provided a reasonable explanation on the difference between the center and edge sites. Glial cells were more likely to adhere to the surface of the planar shank rather than the blades (see Fig. 3.8 (B) and [113,114]). This phenomenon is not observed unless the device track in the tissue is well maintained until imaging. As more activated glial cells are accumulated near the center of the shank, the concentration of released pro-inflammatory cytokines may form an elliptical shape, as modeled by Skousen et al (Fig. 3.8 (C) [113]). This explains the difference in the neural recordings of center vs. edge sites observed in this study. It is likely that edge
sites were more accessible to the action potentials generated from healthy neurons near the sides of the shank.

Despite the fact that edge sites turned out to be superior to center sites, they all went into the failure mode. Fig. 3.8 (A) shows that after 6 months the tissue response was uniform and a significant area was absent with neurons around the device track. Edge sites may have better neuronal contact than center sites, but it does not prevent them from losing the functionality. As the solution to maintaining the functionality of neural implants for a lifetime is likely to be multifactorial [103], however, the findings from this study can contribute toward building a complex multimodal solution.

3.6 Conclusion

We have compared the chronic recording functionality of sites located on the center and edge of planar silicon microelectrodes. Wide and narrow devices with modified site placement were used to identify the impact of separation distance on the electrophysiological performance. Edge sites on the wide devices significantly outperformed center sites in terms of single unit recording capability. Edge sites on the narrow devices generally outperformed center sites but the difference was not statistically significant. Histology images in this study and previous studies infer that these results are attributed to edge sites superior accessibility to nearby healthy neurons. Our findings suggest that site geometry within a device is an important factor and must not be overlooked when designing novel microelectrodes.
4. MECHANICALLY ADAPTIVE NEURAL IMPLANTS FOR MITIGATING THE TISSUE RESPONSE

4.1 Abstract

One major impediment towards advancing brain-computer interface (BCI) technology is resolving the brain's biological response to neural implants. Flexible neural probes are hypothesized to reduce the chronic foreign body response (FBR) mainly by reducing the strain-stress caused by an interplay between the tethered probe and the brain's micromotion. However, a large discrepancy of Young's modulus still exists (3-6 orders of magnitude) between flexible probes and the brain tissue. This raises the question of whether we need to bridge this gap; would increasing the probe flexibility proportionally reduce the FBR? Using novel Off-Stoichiometry Thiol-Enes-Epoxy (OSTE+) polymer probes developed in our previous work, we quantitatively evaluated the FBR to four types of probes with different softness: silicon (~150 GPa), polyimide (1.5 GPa), OSTE+\text{Hard} (300 MPa), and OSTE+\text{Soft} (6 MPa). We observed a significant reduction in the fluorescence intensity of biomarkers for activated microglia/macrophages and blood-brain barrier (BBB) leakiness around the three soft polymer probes compared to the silicon probe, both at 4 weeks and 8 weeks post-implantation. However, we did not observe any consistent differences in the biomarkers among the polymer probes. This suggests that the mechanical compliance of neural probes can mediate the degree of FBR, but its impact diminishes after a hypothetical threshold level.
4.2 Introduction

One primary objective of the neuroprosthetic field has been in engineering devices that are capable of interfacing with the tissue for dozens of years in order for neural implants to be considered a viable treatment option for individuals with neurological disorders [21,102]. The functionality of implanted microelectrodes typically degrades over time and is reported to be associated with a severe foreign body response (FBR) that persists as the implant stays in the brain [22, 23]. A number of strategies have been proposed to improve the brain’s FBR to neural implants via modulating mechanical and/or biochemical aspects of the device-tissue interface [102]. In order to integrate multiple approaches to devise a complex, multimodal solution, identifying individual factors that critically impact the FBR has been of particular importance [103].

The development of micro-fabricated neural probes as an intracortical interface has come a long way from Wises approach in the 1970s [115]. While the flat sword shape design remains unchanged, the development of new materials has opened up possibilities for improved functionality. Within the device design approach [43], the mechanical mismatch of the soft brain tissue and the rigid neural implant has been thought to be one of the key components that needs mediation [70, 72, 77]. Silicon (130-180 GPa [116]), the most commonly used material for penetrating intracortical microelectrodes has a mismatch in Youngs modulus 7-8 orders of magnitude greater than the brain tissue (1 kPa [117]). It has been postulated that the strain-stress caused by the rigid backbone of neural implants and brains micromotion may persistently exacerbate the FBR, leading to electrode failure [68]. Both Subbaroyan et al. and Lee et al. utilized finite element modeling (FEM) to identify that the strain stress induced by rigid silicon probes was significantly greater than hypothetical soft probes [70,72]. The key finding was that flexible probes decrease both tangential and radial strains at any given degree of device tetheredness or device-tissue integration, which was further evaluated in vivo by Sridharan et al. [118]. In vitro cell viability
studies pertaining to mechanotransduction also suggested that excessive strain conditions decrease neuronal viability [74], whereas soft substrates allow for preferential attachment and growth of neurons over glial cells [75]. Based on these initial findings, many groups shifted gears toward developing probes made of softer materials than silicon, including polyimide [77, 78], parylene [81, 119], SU-8 [86, 87], nanocomposites [83–85, 118], and other materials [88, 90, 91, 120, 121].

Although flexible probes were found to induce significantly less strain-stress, the impact of reduced strain-stress to both the in vivo FBR and the device performance needed to be verified. Quantitative histological studies by the Capadona group and the Tyler group demonstrated that nanocomposite probes, which becomes extremely flexible (i.e., 12 MPa) when hydrated, induce significantly less neuroinflammatory response than standard silicon probes and polyvinyl acetate (PVAc)-coated silicon probes [84, 85]. In addition, several other studies showcased enhanced long-term electrophysiological characteristics in the central nervous system (CNS) with flexible surficial [122] or penetrating devices [87, 104]. It was becoming more evident that flexible materials are a favorable choice for neural implant substrates. However, questions remained regarding whether the increase in flexibility reduces the FBR proportionally, or only up to a certain level. Analogous to the critical surface area hypothesis by Seymour et al. [62], there may be a critical flexibility level, beyond which the performance benefit would plateau.

In order to evaluate probes that are softer than polyimide we used probes made of Off-Stoichiometry Thiol-Enes-Epoxy (OSTE+) polymers, which is synthesized by a radical-mediated thiol-ene click reaction. OSTE+ polymer was initially developed for microfluidic devices [123] and advanced to neural implant application [120]. The strength of the OSTE+ is its patternable nature (due to the use of UV-lithography) and tunable Youngs modulus (by simply changing the ratio of constituents). With this feature we created two types of OSTE+ probes made of the same materials that are moderately soft (OSTE+Hard) and very soft (OSTE+Soft), to solely evaluate
the effect of flexibility. Our previous in vitro study suggested that the unbound constituents of OSTE+ were effectively removed by incubating the probe in water for seven days [89]. The results of this study suggest that comparing conventional probes with OSTE+ probes would reveal the consequence of flexibility on the severity of the tissue response.

We evaluated the FBR of four microelectrodes with differing flexibility: silicon, polyimide, OSTE+$_{\text{Hard}}$, and OSTE+$_{\text{Soft}}$. Youngs moduli of the three polymer probes were obtained with dynamic mechanical thermal analysis. Then, an in vivo study was conducted to quantitatively compare the FBR of the four types of probes at 4 and 8 weeks post-implantation (WPI). The CX3CR1-GFP mouse strain [124], widely utilized in neuroscience research [125], was used to visualize microglia with endogenous green fluorescence. In order to provide the structural rigidity for insertion into mice pia mater, 4 kDa polyethylene glycol (PEG) was dip-coated onto probes.

4.3 Materials and methods

4.3.1 Fabrication of probes

As described in previous work [89, 120], OSTE+ is polymerized in two steps: 1) a fast UV initiated radical polymerization between a thiol and two allyls and 2) a slower thermal anionic polymerization between a residual thiol and an epoxy resin. The first polymerization utilizes Irgacure(R) TPO-L (BASF, Germany), an UV-initiator that crosslinks the thiol [tris(2-(3-mercaptopropionyloxy)ethyl] isocyanurate (BRUNO BOCK, Chemische Fabrik GmbH and Co. KG, Germany) with the two allyls, trimethylolpropane diallyl ether and 2,4,6-triallyloxy-1,3,5-triazine. The second polymerization utilizes DBN (1,5-diazabicyclo[4.3.0]non-5-ene) as the initiator that crosslinks the unreacted thiol and the epoxy resin (D.E.N 341 Epoxy Novolac resin, Dow Chemicals, USA).
Table 4.1
Fabrication parameters for OSTE+\textsubscript{Hard} and OSTE+\textsubscript{Soft}. Mixing ratio indicates thiol:(diallyl:triallyl):epoxy.

<table>
<thead>
<tr>
<th>Blend</th>
<th>Thiol: Allyl Mixing ratio</th>
<th>Spin speed (rpm)</th>
<th>Spin time (sec)</th>
<th>UV exposure time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSTE+\textsubscript{Hard} 65%</td>
<td>1.65:(0.05:0.95):0.65</td>
<td>2000-3000</td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>OSTE+\textsubscript{Soft} 20%</td>
<td>1.20:(0.95:0.05):0.20</td>
<td>500-1000</td>
<td>30-150</td>
<td>60</td>
</tr>
</tbody>
</table>

Thiol, allyls, and epoxy were mixed according to Table 1, with 0.2 wt% of DBN and TPO-L. Triallyl and epoxy were preheated to 67 °C before mixing. After degassing, the mixture was spin coated onto a silicon wafer having a 200 nm sacrificial layer of titanium according to Table 1. Then, a 9 µm thick PVC film was applied to wafers to prevent the mixture from sticking to the UV mask and from reacting with the oxygen. Wafers were exposed to the UV light as listed in Table 1 using a MA4 mask aligner (Carl Suss, Germany). Then, wafers were submerged in butyl acetate for 10-15 seconds to remove the PVC film and rinsed in toluene for 5 seconds. Wafers were blown dry with nitrogen gas and placed in an oven at 67 °C for 2 days. Prior to removing the sacrificial layer to release the probes, wafers were incubated in MilliQ water at room temperature (RT) for 6 days. Wafers were blown dry and slightly etched using an oxygen plasma etcher (Diener Femto, Germany) for 5 minutes in order to remove a residual layer of OSTE+ left after the development. Probes were released by incubating the wafers in 8% hydrofluoric acid solution for 24 hours. After rinsing with MilliQ water several times the probes were released using a pair of tweezers.

Polyimide (Durimide 7520, FujiFilm, Belgium) was spin coated onto a titanium coated (200 nm) silicon wafer at 1300 rpm for 60 seconds. Wafers were soft baked on a hot plate at 100 °C for 3 minutes prior to UV-light exposure for 45 seconds. Wafers
were allowed to rest overnight at RT. Then, wafers were developed using HTR-D2 (FujiFilm) for 4 minutes and rinsed with RER600 (FujiFilm). Wafers were blown dry, hard baked in an oven at 180 °C for 2.5 hours, and submerged in MilliQ at RT for 6 days. Probes were released the same way as the OSTE+ probes using hydrofluoric acid and MilliQ.

4.3.2 Fabrication of samples for dynamic mechanical thermal analysis (DMTA)

For the dynamic mechanical thermal analysis (DMTA) testing, samples of each polymer were made in blocks (1 mm thick, 10 mm wide, and 40 mm long). Samples were submerged in MilliQ at RT for 6 days after the fabrication.

For OSTE+ block samples, thiol, allyls, and epoxy were mixed and degassed the same way as the OSTE+ probe fabrication procedure. Then, the degassed mixture was poured onto a PDMS mold, covered with a polycarbonate film, and exposed to the UV-light for 40 minutes (using a Carl Suss MA4 mask aligner). After the exposure, the polycarbonate film was removed and samples were demolded and hard baked in an oven at 67 °C for 2 days.

For polyimide block samples, polyimide was poured onto a PDMS mold followed by a soft-bake at 67 °C for 2 days. The amount of polyimide was adjusted accordingly to compensate for the shrinkage. The mold was then exposed to UV-light for 40 minutes (using a Carl Suss MA4 mask aligner) and samples were demolded and hard baked in an oven at 180 °C overnight.

A dynamic mechanical analyzer (Q800, Texas Instruments, USA) was used to test the Youngs moduli of the three polymer block samples. Tension was the mode of deformation. Samples were tested from 5 °C to 45 °C with a 2 °C step size at a frequency of 1 Hz. Three samples of each polymer were tested (N=3).
4.3.3 \textit{In vivo} implantation

One day before implantation surgery, four types of probes: silicon (GP1x16,249 (NeuroNexus Technologies, USA), 249 m wide, 15 m thick, 18° tapered tip), polyimide, OSTE+\textsubscript{Hard} and OSTE+\textsubscript{Soft} were autoclaved at 121 °C for 30 minutes. All probes were then dip-coated with sterilized 4 kDa poly-ethylene-glycol in an aseptic settings and stored in a sterilized container until surgery.

All surgical procedures and animal care practices were performed in accordance with the University of Florida Institutional Animal Care and Use Committee (IACUC). Twenty-two adult mice, 20-24g, (B6.129P-Cx3cr1/J (Jackson Laboratories, USA), N=5-6 per condition) were bilaterally implanted with one probe per hemisphere. Mice were anesthetized with isoflurane (Abbott Laboratories, USA, 3% induction, 1.5% maintenance) and mounted onto a stereotactic frame. Mice were kept warm on a heating pad and their vital signs were monitored using a pulse oximeter (MouseOx, Kent Scientific, USA). After a small portion of scalp was removed, a craniotomy was made on each hemisphere centered at 1.5 mm lateral and 1.5 mm posterior to bregma. Probes were inserted 1.2 mm deep using a piezoelectric actuator (PILINE M-663, Physik Instruments, Germany) at 100 mm/s. Identifiable blood vessels were avoided to minimize iatrogenic variability. Then, the upper portion of the probe was trimmed with a pair of microscissors to detach from the actuator. Kwik-Sil (World Precision Instruments, USA) was applied to the craniotomies and tethered the probes followed by dental acrylic (Fusio Liquid Dentin, Pentron, USA) to secure the headcap.

4.3.4 Immunohistological staining

At 4 WPI or 8 WPI, mice were deeply anesthetized with 5% isoflurane and transcardially perfused with 20 ml of cold phosphate buffered saline (PBS), followed by 20 ml of cold 4% paraformaldehyde solution (PFA) [46]. After post-fixing heads for 24 hours at 4 °C brains were carefully extracted while retaining the probes in place.
Brains were cryopreserved in 30% sucrose in PBS at 4 °C for 24-48 hours. Then, brains were flash frozen in 2-methyl-butane at -40 °C for 2 minutes and transferred to a cryostat (CM1950, Leica, Germany). Brains were lightly embedded with Opti- mum Cutting Temperature (OCT) compound (Sakura Finetek, The Netherlands) and sliced into 25 μm horizontal sections with the retained probes. Tissue slices were collected on SuperFrost glass slides (Thermo Fisher, USA) and stored at 4 °C for the immunostaining process.

Tissue slices were allowed to sit at RT for 30 minutes to adhere to the slides. After washing for five minutes in PBS three times, tissue slices were blocked in blocking buffer (4% v/v goat serum, 0.3% Triton-X in PBS) for two hours at RT. Primary antibodies (refer to Table 2) were diluted in blocking buffer and applied to the slices for incubation at 4 °C for 20-24 hours. Following primary incubation, slices were washed for five minutes in PBS five times. Slices were then incubated in secondary antibodies at RT for 2 hours. Following secondary incubation, slices were washed for five minutes in PBS five times and cover-slipped using mounting medium (H-1000, Vector Lab, USA).

Table 4.2
List of primary antibodies

<table>
<thead>
<tr>
<th>Primary Antibodies</th>
<th>Target of Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CD68 (1:250, ab53444, Abcam)</td>
<td>Activated microglia/macrophages</td>
</tr>
<tr>
<td>GFP (Autofluorescent)</td>
<td>Microglia/macrophages</td>
</tr>
<tr>
<td>Anti-IgG (1:250, ab150114, Abcam)</td>
<td>Blood-brain barrier leakiness</td>
</tr>
<tr>
<td>Anti-GFAP (1:500, AB5541, Millipore)</td>
<td>Astrocytes</td>
</tr>
<tr>
<td>Anti-NeuN (1:250, MABN140, Millipore)</td>
<td>Neuronal nuclei</td>
</tr>
</tbody>
</table>
Fluorescence images were taken with a laser scanning confocal microscope (LSM 710, Carl Zeiss, Germany). A 2x2 tile was captured using a 10X objective that spans the device-tissue interface and its surroundings. For quantitative analysis, two slices per sample, roughly from 400-600 µm down the cortical column, were used to minimize the depth-dependent variability [46]. Qualitative figures presented in this paper were contrast enhanced for visual clarification. For better identification of the relationship between immunolabels, representative images are from the same animal hemisphere, horizontal slices within 100 µm to each other.

MINUTE V1.5 was used for fluorescence intensity analysis [85,126,127]. An ellipse was drawn to locate the contour of the device-tissue interface. Exclusion areas were drawn to discard areas outside the cortical hemisphere of interest. For every 5 µm concentric ring from the probe contour, average immunolabel intensities were computed. Then a 5-tap moving Gaussian window was applied to smoothen the intensity curves. All average intensities were normalized to the background, taken as the interval 600-800 µm from the probe contour. Neuronal nuclei (NeuN) were automatically counted using a custom built ImageJ [128] script.

Statistical analyses were performed using Prism 7.00 statistical analysis software (GraphPad Software, USA). Integrated intensities were binned into 50 µm intervals from 0 µm to 500 µm and averaged by each area. A two-way ANOVA was performed with post-hoc Tukeys test with probe type and distance from the probe as the two independent variables.

4.4 Results

4.4.1 Probe specification

The thickness of the polymer probes was measured using a microscope (Olympus BX40, Sweden) with a height sensor. Measured thicknesses were: 21.3 ± 1.0 m
(polyimide), 23.5 ± 2.1 m (OSTE+\textsubscript{Hard}), and 22.4 ± 2.1 m (OSTE+\textsubscript{Soft}) (mean ± standard deviation, N=15). Probes were 250 m wide and 3 mm long with a tapered tip of approximately 18 degrees in angle.

The thickness of the dip-coated PEG on the silicon probe was measured using a profilometer (Alpha Step 500, Tencor, USA). The mean thickness of the thickest portion was 46.05 ± 14.58 µm (mean ± standard deviation, N=9) on one side of the broad surface.

### 4.4.2 Dynamic Mechanical Thermal Analysis (DMTA)

Fig. 4.1 shows mechanical properties of the three polymers compared to silicon at given temperatures. Polyimide (1.5 GPa) was roughly three orders of magnitude softer than silicon (~150 GPa), approximately 5 times harder than OSTE+\textsubscript{Hard} (300 MPa) and approximately 250 times harder than OSTE+\textsubscript{Soft} (6 MPa) at 37 °C. All of the three polymers showed a decrease in Youngs modulus with an increase in temperature. OSTE+\textsubscript{Hard} showed the largest decrease in Youngs modulus in the tested temperature range. OSTE+\textsubscript{Hard} was softer than polyimide at 37 °C but harder at 20 °C. We see that the change in Youngs modulus of OSTE+ samples according to the temperature were linear-quadratic in the logarithmic scale.

### 4.4.3 Activated microglia/macrophages (CD68)

CD68 is a glycoprotein present in monocyte derived immune cells, and is most intensely expressed in their activated state [129]. Fig. 4.2 shows that, at 4 WPI, silicon probes had significantly greater CD68 fluorescence intensity than the three polymer probes in the 0-50 µm bin with the most dramatic difference within the first 20 µm. At 8 WPI, silicon probes had significantly greater CD68 than polyimide
Fig. 4.1. Mean Youngs moduli of silicon, polyimide, OSTE+\textsubscript{Hard} and OSTE+\textsubscript{Soft} (A) as a function of the temperature and (B) at 20 °C and 37 °C (with ± standard deviation). The three polymer samples were measured using a dynamic mechanical analyzer.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s Modulus at 20°C</th>
<th>Young’s Modulus at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon</td>
<td>~150 Gpa</td>
<td>~150 Gpa</td>
</tr>
<tr>
<td>Polyimide</td>
<td>1743 ± 28.2 MPa</td>
<td>1508 ± 14.9 Mpa</td>
</tr>
<tr>
<td>OSTE+\textsubscript{Hard}</td>
<td>2070 ± 69.1 MPa</td>
<td>314 ± 26.1 Mpa</td>
</tr>
<tr>
<td>OSTE+\textsubscript{Soft}</td>
<td>8 ± 0.4 MPa</td>
<td>6 ± 0.3 MPa</td>
</tr>
</tbody>
</table>

and OSTE+\textsubscript{Hard} probes in the 0-50 µm bin. Unlike 4 WPI, at 8 WPI the mean intensity of CD68 around silicon probes was more dispersed and not as concentrated
in the first 20 µm. The mean intensity generally decreased from 4 WPI to 8 WPI for all types of probes, with the greatest decrease seen with silicon. The decrease was most pronounced in the 0-50 µm bin whereas regions beyond 50 µm stayed relatively consistent.

Fig. 4.2. Activated microglial/macrophage response (CD68) to implanted silicon, polyimide, OSTE+Hard, and OSTE+Soft probes. (A) Representative CD68 fluorescent images at 4 WPI and 8 WPI. (B)-(C) Normalized CD68 fluorescence intensity as a function of distance with shaded standard error. Statistically significant differences were found in the 0-50 µm bin (p<0.05) denoted by the solid line with an asterisk. Inset in (C) is a magnification of the 0-100 µm interval. Scale bar is 100 µm.
4.4.4 All microglia/macrophages (GFP)

GFP is expressed in monocyte derived immune cells of CX3CR1-GFP transgenic mice, labeling both activated and non-activated microglia/macrophages in the brain [124]. In the 0-50 $\mu$m bin, silicon probes had significantly greater GFP intensity than polyimide probes at 4 WPI, as well as all three polymer probes at 8 WPI. GFP expression near all the probes was more amorphously aggregated than the outer region where resting microglia are detected. By 100 $\mu$m there was no noticeable difference in the distribution of microglia compared to regions beyond 500 $\mu$m. The mean GFP fluorescence intensity generally decreased from 4 WPI to 8 WPI. Unlike CD68, however, the decrease was minimal. CD68 was mostly co-localized with GFP, but not vice versa.

4.4.5 BBB Leakage (IgG)

Immunoglobulin G (IgG) is the most abundant antibody in blood serum and is often used in chronic inflammatory studies to indicate leakiness of the BBB in the brain parenchyma [39]. In the 0-50 $\mu$m bin, silicon probes had significantly greater IgG fluorescence intensity than OSTE+$_{Hard}$ probes at 4 WPI, as well as polyimide and OSTE+$_{Hard}$ probes at 8 WPI. OSTE+$_{Soft}$ probes had significantly greater IgG than polyimide and OSTE+$_{Hard}$ probes at 8 WPI in the 0-50 $\mu$m bin. Although the mean IgG intensity of silicon probes was greater than OSTE+$_{Soft}$ probes at 8 WPI, no statistical difference was found between the two types of probes. Large inter-animal variabilities were observed within the same type probes, manifesting high standard errors. This variability was especially notable around silicon probes at 8 WPI up to 100 $\mu$m from the implant. The trend of IgG generally corresponded with CD68 and GFP in terms of silicon probes exhibiting the highest FBR than the other probes. Unlike CD68, there was no notable change in the mean intensity at 8 WPI compared to 4 WPI.
Fig. 4.3. Microglial/macrophage response (GFP) to implanted silicon, polyimide, OSTE+_Hard and OSTE+_Soft probes. (A) Representative GFP fluorescent images at 4 WPI and 8 WPI. (B)-(C) Normalized GFP fluorescence intensity as a function of distance with shaded standard error. Statistically significant differences were found in the 0-50 µm bin (p<0.05) denoted by the solid line with an asterisk. Scale bar is 100 µm.

4.4.6 Astrocytes (GFAP)

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is expressed in mature astrocytes, and more intensely expressed in reactive astrocytes [36]. At 4 WPI, polyimide probes had significantly greater GFAP fluorescence
Fig. 4.4. BBB leakiness (IgG) around implanted silicon, polyimide, OSTE+Hard, and OSTE+Soft probes. (A) Representative IgG fluorescent images at 4 WPI and 8 WPI. (B)-(C) Normalized IgG fluorescence intensity as a function of distance with shaded standard error. Statistically significant differences were found in the 0-50 µm bin (p<0.05) denoted by the solid line with an asterisk. Scale bar is 100 µm.

Intensity than OSTE+Hard probes in the 0-50 µm bin. At 8 WPI, silicon probes had significantly greater GFAP intensity than OSTE+Soft probes in the 0-50 µm bin. Mean GFAP intensity profile across probes types became more similar to each other.
at 8 WPI than at 4 WPI. At both times, GFAP intensity mostly dropped down to the background level after 150 μm.

**Fig. 4.5.** Astrocytic response (GFAP) to implanted silicon, polyimide, OSTE+ Hard, and OSTE+ Soft probes. (A) Representative GFAP fluorescent images at 4 WPI and 8 WPI. (B)-(C) Normalized GFAP fluorescence intensity as a function of distance with shaded standard error. Statistically significant differences were found in the 0-50 μm bin (p<0.05) denoted by the solid line with an asterisk. Scale bar is 100 μm.
4.4.7 Neuronal nuclei (NeuN) density

Neuronal nuclei were identified with the NeuN immunolabel. Although significant differences were found with other inflammatory markers, no statistical significance was found between any two types of probes at 4 WPI and 8 WPI. Mean neuronal densities approached background levels from approximately 100 µm away from the implant. Mean neuronal densities of silicon probe and polyimide probe were higher at 8 WPI than at 4 WPI in the 0-50 µm bin, although the differences were statistically not significant. Mean background neuronal densities were 2894 ± 290 cells/mm² (4 WPI) and 2493 ± 381 cells/mm² (8 WPI) (mean ± standard deviation, N=11).

4.5 Discussion

Our work herein supports the notion that flexible probes are a favorable choice for neural implants. There was a significant reduction in the mean intensities of FBR markers surrounding the three polymer probes compared to silicon probes. However, it appears that further increasing the flexibility beyond polyimide failed to attenuate the FBR. The difference in Youngs modulus between silicon and polyimide, the stiffest of the three polymers, was roughly 100 times. Although the difference in Youngs modulus between the polyimide probe and OSTE+Soft probe was roughly 250 times, we did not find any consistent statistical differences in the inflammatory markers between the pairs of polyimide, OSTE+Hard, and OSTE+Soft probes. From this, we infer that flexibility plays a critical role when the probe is relatively hard, but its effect diminishes as the probe gets softer.

The three polymer probes generally showed a reduction in the FBR markers compared to silicon probes. CD68 intensity indicated that there was a significantly more persistent microglial and macrophage activation around silicon probes than the polymer probes. We also found that there was a large reduction in CD68 intensity at 8 WPI than at 4 WPI for all types of probes. This corresponds with previous studies
Fig. 4.6. Neuronal nuclei (NeuN) density near the implanted probes. (A) Representative fluorescent images of silicon, polyimide, OSTE+Hard and OSTE+Soft probes at 4 WPI and 8 WPI. (B)-(C) Normalized neuronal density as a function of distance with standard error. No statistically significant differences were found with Tukeys pairwise comparison (p>0.05). Scale bar is 100 µm.

that showed consistent reduction in microglial/macrophage activation after 2 WPI up to 16 WPI [28, 85]. GFP also pointed toward the same conclusion that silicon probes induced the most intense microglial/macrophage response. The overall trend at 4 WPI and 8 WPI both suggest that CD68 and GFP responses were most intense around silicon probes, despite small variations in the type of probes being significantly
different vs. silicon probes. A region with high CD68 response always accompanied GFP response. However, their relative intensities did not exactly match. It has been reported that a higher-level GFP expression was observed in monocyte-derived macrophages of CD68-GFP mice compared to CX3CR1-GFP mice [130]. It infers a preferential expression of CD68 in myeloid lineage cells and GFP in brain-resident microglia, although GFP is also weakly expressed where CD68 is expressed. With this inherent difference, consistency observed with CD68 and GFP intensity in our study strengthens the statistical significance found in immune cell distribution.

Similar to CD68 and GFP, IgG intensity indicated that silicon probes induced the most severe chronic BBB leakage. However, OSTE+Soft also had significantly higher IgG intensity compared to the other two polymer probes at 8 WPI. As OSTE+Soft only differed with OSTE+Hard in the constituent mixing ratio and the resulting softness, it is unlikely that OSTE+Soft probes chemically or physically perturb BBB more than OSTE+Hard probes. It is possible that localized IgGs seen only around the probes, within the tight astroglial junction, depends more on the insertion injury and less on the chronic BBB leakage [131]. Note that in general, IgG was particularly variable among the inflammatory markers used in this study. Even among the silicon probes only a few exhibited a very high IgG response near the probe (i.e., 2/5 at 4 WPI and 1/5 at 8 WPI, more than twice greater than the median), rendering the overall mean high. This implies that there may be an important contributor to the FBR other than the extent of the tissue damage, such as the existence or nonexistence of vasculature near the probe. It is speculated that the BBB integrity depends on the chance of mechanical/biochemical damage to nearby large blood vessels, with stiffer probes having a higher incidence of damage. Depending on the location, stiffer probes may not compromise the BBB severely, as seen from the subset of silicon probes in this study that did not show an intense IgG level. However, a joint characterization of IgG with vasculature will help to confirm that the degree of IgG response is correlated to nearby vessels.
Unlike CD68, GFP, and IgG, silicon probes did not demonstrate more severe inflammation than the polymer probes in the context of GFAP and NeuN responses. We expected to see a decrease in GFAP intensity and an increase in neuronal density around the polymer probes, as studies suggest an inverse relationship of neuronal density to astrocytic scar, microglial activation, and BBB integrity in the chronic phase [101,132,133]. Along with the statistics, however, representative images of immunolabels that are approximately from the same region also suggested that probes with high CD68, GFP, and IgG did not always exhibit a significant astrogliosis or decrease in neuronal density. It should be noted that discrepancies among studies evaluating flexible probes [84,85] indicate that differences in surgery, immunohistology, imaging, and analysis methods can impact the results more significantly than flexibility. Moreover, aforementioned studies were conducted in rats. Although not dramatically different from rats, mice are reported to show more prolonged astrocytic activation and higher neuronal density around implanted probes [126]. Taken together, previous studies are not directly comparable and discrepancies observed in this study need to be considered in their own context.

With GFAP intensity, there were significant differences between OSTE+Hard probes and polyimide probes at 4 WPI, and between silicon probes and OSTE+Soft probes at 8 WPI. However, these were inconsistent across the two time points and across the other immunolabels. With NeuN, no statistically significant differences in neuronal density were observed with any pair of probes at either time point. Although not statistically significant, neuronal densities at 8 WPI were generally higher than 4 WPI in the 0-50 µm. This is in line with previous findings that neurons around an implanted device exhibit a multi-phasic response over time [25,28,84], the mechanism of which is yet to be understood as recording/stimulation performance typically does not recover at later time points. It is worth pointing out that with OSTE+Hard and OSTE+Soft probes we did not observe such come back of neurons at 8 WPI. However, it is unlikely that this is a phenomenon pertaining to OSTE+ probes since there was
no indication of OSTE+ being neurotoxic from the results of inflammatory markers. A cytotoxicity assay (MTT assay) in our previous study confirmed that OSTE+ samples were non-toxic after a sufficient incubation in MilliQ water [89]. The size of the OSTE+ samples used in the MTT assay were 1 mm thick, roughly 45 times thicker than OSTE+ probes. Based on simple diffusion, unreacted constituents in the 22 µm thick probe would be eliminated quicker than those in the 1 mm thick sample. This suggest that OSTE+ probes are unlikely to chemically induce inflammation. Surface topology was also not a critical concern, as identified in our previous work with scanning electron microscope (SEM) showing smoothly constructed hydrophobic surfaces of OSTE+ samples [89].

We preserved the probes within the tissue during the entire procedure. This mainly prevented tissue distortion during device extraction and allowed us to image the device-tissue interface more precisely. A drawback was that silicon devices mostly shattered while cryosectioning; however, it did not severely alter the interface as seen in the figures. Reflected fluorescence from the polymer probes was detected, with polyimide being the brightest followed by OSTE+ Soft. This fluorescence further confirmed the precise location of the device. As a fluorescing device could interfere with our quantitative analysis, we took a conservative approach on the images to remove the device track prior to performing MINUTE analysis. Interestingly, we did not find any tissue slices with a hole that is far larger than the size of the device, which makes it hard to define the device contour for quantitative analysis.

A device flexibility depends on both the dimension and softness (i.e., Youngs modulus). In our study silicon probes were 15 µm thick and the three soft polymer probes were approximately 22 µm thick. As smaller probes are favorable in reducing the FBR as well as enhancing recording functionality [58], silicon devices were expected to cause a less severe FBR than the polymer probes if size was the sole variable. The difference of 7 µm in thickness means that silicon probes used in this study were softer (i.e., roughly 3 times in the planar direction \(15^3: 22^3 \cong 1:3\) and 1.5
times in the lateral direction (15: 22 ≈ 1:1.5)) than the silicon probe with the same thickness as the polymer probes. Although silicon probes were thinner they induced the most severe FBR of the four types of probes, as indicated by CD68, GFP, and IgG intensities. The impact of stiffness of silicon probes likely overshadowed the benefit of small size. A simple dip-coating in PEG was chosen as the stiffening method for insertion of the polymer probes. Our initial test on a 0.85% agarose gel block revealed that OSTE+Hard and OSTE+Soft probes always buckled, and polyimide probes buckled by chance (8 out of 10 buckled). Dip-coating with 4 kDa PEG provided sufficient structural rigidity for penetration for the polymer probes. Inserting at a very high speed (100 mm/s) also helped the probes penetrate before the PEG dissolved in the cerebrospinal fluid. Higher insertion speeds have been reported to be beneficial in minimizing the shear force [61,134] and this may have helped in minimizing the overall level of FBR in our study. No evidence of flexible probe deformation was found as probe shape and location along the depth (0-900 µm) were stable regardless of the probe type (data not shown). Note that silicon probes were also coated to prevent PEG-coating from confounding the study.

The fact that OSTE+ probes did not significantly reduce the FBR over polyimide probes suggest a threshold model for flexibility. This is further supported by OSTE+Soft probes not reducing the FBR over OSTE+Hard probes, negating the concern of comparing different materials. More evidence is required to conclude that the degree of FBR does not decrease with a material softer than OSTE+Soft. As depicted in Fig. 4.7, however, it is reasonable to speculate that the impact of flexibility in reducing the FBR would continue to diminish with increasing flexibility. A materials Youngs modulus does not have to be as low as the interacting material to prevent generating significant strain stress as long as the material is sufficiently thin. Since the probes in this study were 15-22 µm thick, the results pertain to probes in this range of thickness, as well as other design parameters. Species and organ type can also affect the outcome as tissues have different softness and this can influence their
tolerance level to the implant stiffness. It should also be noted that dummy devices used in this study were tethered to the silicon elastomer. If the device had a rigid wiring backbone, it can strengthen the degree of tetheredness to the skull and is another variable to be considered.

Resolving the biological tissue response to neural implants requires a multi-dimensional approach [103]. This includes flexibility, dimension, architecture, tethering to the skull, tissue integration, insertion profile, bioactive coatings, and drug release. One or more of these can be the limiting factor that dictates the eventual tissue response regardless of improvements in other aspects. Thus, we envision that the “threshold effect, i.e. performance limits from softer devices, can be overcome once other factors are improved. Improving the biggest pitfall step by step will be key to advancing the lifetime of neural implants.

Fig. 4.7. An illustrative graph showing hypothetical saturation of the impact of flexibility to the FBR.
4.6 Conclusion

We have investigated the biological impact of flexibility on the FBR by using four types of probes with different Youngs moduli: silicon, polyimide, OSTE+\textsubscript{Hard} and OSTE+\textsubscript{Soft}. A quantitative assessment of inflammatory biomarkers suggest that silicon probes induced the most severe FBR compared to the three soft polymer probes. No consistent statistical differences were found among the polymer probes, indicating that further increasing the probe flexibility beyond polyimide did not reduce the FBR. This suggest a possible threshold model for flexibility.

We believe OSTE+ probes are still a valuable addition to implantable electrode fabrication and designs as they performed no worse than polyimide probes. The tunable glass transition property and easy patternable nature of OSTE+ polymers can make them more useful over polydimethylsiloxane (PDMS), which is similarly soft to OSTE+\textsubscript{Soft}. 
5. TISSUE RESPONSES TO NEURAL IMPLANTS DIP-COATED WITH POLYETHYLENE GLYCOL (PEG)

5.1 Abstract

Objective: Polyethylene glycol (PEG) and the CX3CR1-GFP transgenic mouse model are frequently used tools in neural implant studies. We have evaluated the biological implications of using PEG and CX3CR1-GFP mice for chronic neural implants. Methods: Mice (CX3CR1-GFP and wild-type (WT)) received bilateral implants within the sensorimotor cortex, one hemisphere with a PEG-coated probe and the other with a non-coated probe for four weeks. Quantitative analyses were performed using biomarkers for activated microglia/macrophages, astrocytes, blood-brain barrier leakage, and neuronal nuclei to determine the degree of foreign body response (FBR) resulting from the implanted microelectrodes. Results: We observed that PEG prevented glial cell adsorption to the device, however, PEG-coated devices caused no significantly different FBR compared to non-coated controls. Likewise, the genetically modified CX3CR1-GFP mouse model did not exhibit any significant statistical difference compared to WT mice. Conclusion: We conclude that PEG-coatings and the CX3CR1-GFP mouse model do not have significant impact on the FBR. Thus, they are valid tools for chronic neural implant studies that do not bias the outcomes. Significance: Our results benefit former and ongoing neural implant research utilizing PEG and/or CX3CR1-GFP mice by resolving supposed pitfalls and confirming their limited role in ameliorating or exacerbating the FBR.
5.2 Introduction

Implantable neural probes hold the promise of providing functional recovery of various symptoms associated with traumatic injuries or neurological disorders [1, 2]. A major dilemma of using such devices is that their functionality degrades over time, which eventually leads to the inability of discriminating relevant neural signals from background noise [20, 31, 135]. In an attempt to resolve the biological aspects of this issue, researchers have modulated multiple factors including: device architecture/material type [58, 62], bioactive coatings [136–139], and drug delivery schemes [140, 141]. In parallel to these engineering mitigation strategies [103], there are ongoing attempts to discover the precise biotic and abiotic mechanisms of implant failure in order to develop strategies to improve the functional lifetime of neural implants [100].

One of the most common and indirect methods for evaluating the effectiveness of these intervention strategies is looking at markers of the FBR. Typically, implanted microelectrodes in the brain instigate a neuro-inflammatory cascade that involves infiltration of plasma proteins, monocytes, macrophages and leukocytes from breached blood-brain barrier (BBB), activation and recruitment of microglia and macrophages to the injury site, activation of astrocytes to initiate astrogliosis, and a loss of neurons near the implant [19, 22, 43, 102, 126]. A severe FBR generally results in a large neuronal loss in the long-term [127, 142]. Thus, reducing the FBR has been considered essential to achieve long-term functionality of neural implants.

A number of neural-interface strategies have used the inorganic, biocompatible polymer polyethylene glycol (PEG). Examples include using PEG as an adhesive for aid in insertion [108], a stiffening agent for inserting flexible probes [143, 144], or as a vector for molecules to deter protein adsorption [137, 138]. While the exact mechanisms are unknown, there are studies suggesting the use of PEG as a standalone treatment for spinal cord injury (SCI) [145, 146], traumatic brain injury (TBI) [147,
148], and peripheral nerve damage [149]. It was reported that PEG reduces oxidative stress and repairs damaged cell membranes which contribute to enhanced anatomical and functional recovery over a few hours [146–148] to weeks or months [145, 149]. However, the explicit consequence of using PEG for brain-implanted microelectrodes has not been investigated. This is a potential confound of neural-interface studies that utilize PEG as an insertion or stiffening aid.

There has been an ongoing effort to interrogate the cellular dynamics underlying the neuro-inflammatory cascade in response to brain injuries [150]. Transgenic mouse models expressing fluorophores in specific cell types in conjunction with fluorescence imaging techniques have proven invaluable for this purpose. CX3CR1-GFP mice express green fluorescent protein (GFP) in immune cells including microglia [124]. This strain has been actively used in studies investigating the progression of microglial response to implants, particularly during acute time points [125, 151]. Since microglia are one of the first responders to foreign objects within the brain, investigating their morphological states, from resting to activated, has provided insight into the cellular dynamics of the FBR onset. Moreover, transgenic mouse models alleviate the need for immunohistological processing and provide consistency in fluorescence intensity. However, it is important to consider the effects that genetic manipulation may have upon CX3CR1-GFP mice. Loss of the fractalkine receptor may alter microglial behavior to a neural implant, leading to an altered inflammatory cascade. Knowing if CX3CR1-GFP transgenic mice produce a similar FBR to WT mice is necessary for neural implant studies utilizing CX3CR1-GFP mice.

In this paper, we evaluated the biological response to PEG-coated silicon probes and non-coated silicon probes in two different mouse strains: CX3CR1-GFP (B6.129P-CX3CR1-/-) and WT (C57BL/6). 4 kDa PEG was chosen for its superior anti-biofouling property [152]. Statistical tests on the biomarkers of FBR were performed with both independent comparisons and paired comparisons. Furthermore, the cor-
relation between biomarkers was calculated to address possible direct and indirect relationship between the components of the FBR.

5.3 Materials and methods

5.3.1 Probe preparation and PEG-coating

Planar Michigan type silicon microelectrodes, 249 \( \mu \)m wide and 15 \( \mu \)m thick, were used in this study (GP_1x16_249, NeuroNexus, Ann Arbor, MI; also used in [107]). Probes and PEG (4 kDa MW, Sigma, St. Louis, MO) were autoclaved for 30 min at 120 °C one day prior to surgery. In an aseptic setting, PEG was melted on a hot plate at 80 °C and the probes were dip coated in PEG for 5 seconds (Fig 5.1). After allowing the probes to air dry they were sealed in a sterilized container until surgery.

A separate set of probes coated with PEG were taken for profilometry thickness measurements (Alpha Step 500, Tencor, Milpitas, CA). The thickness of the PEG-coatings was 46.05 \( \mu \)m (±4.86 \( \mu \)m, s.e.m., N=9) on one side of the broad surface. Images of non-coated and PEG-coated probes are depicted in Fig. 4.1 (A)-(B).

5.3.2 Surgical implantation

All surgeries and animal experiments were performed in accordance with the University of Florida Institutional Animal Care and Use Committee (IACUC) guidelines. Briefly, 2-3 months old (18-28 g) WT mice (C57BL/6, N=4) and CX3CR1-GFP mice (B6.129P-CX3CR1-/-, Jackson Laboratories, Bar Harbor, ME, N=4) were bilaterally implanted with a PEG-coated device in one hemisphere and a non-coated device in the other hemisphere. Mice were anesthetized with isoflurane (3% induction, 1.5% maintenance) and kept warm using a heating pad. Vital signs were monitored throughout the surgery with a pulse oximeter (MouseOx, Kent Scientific, Torrington, CT). After a small portion of scalp was removed, a craniotomy above each brain hemi-
sphere was performed using a microdrill. A probe was mounted onto a piezoelectric actuator (PiLine M-663, Physik Instruments, Karlsruhe, Germany) and inserted into one hemisphere 1.2 mm from the cortical surface at 100 mm/s. Sensorimotor cortical regions (approximately 1 mm posterior and 1.5 mm lateral to bregma) were targeted, however small deviations occurred to avoid large surface vasculature. This procedure was then repeated on the contralateral hemisphere. After the insertion in both hemispheres, craniotomies were covered with silicone elastomer (Kwik-Sil, WPI, Sarasota, FL) followed by dental acrylic (Fusio Liquid Dentin, Pentron, Orange, CA) to secure the devices. Post-operative care involved applying antibiotic ointment (Actavis, NC) around the cut areas and maintaining the body temperature. Meloxicam (Norbrook, United Kingdom) was administered pre- and up to three days post-surgery to alleviate pain.

Fig. 5.1. (A) Planar silicon probe GP_1x16_249 used in this study. (B) Probe dip-coated with PEG. (C) An image from a typical tissue slice demonstrating using Minute v1.5 for quantitative analysis of immunolabels. Yellow dotted concentric circles denote binned interval (0-50 µm, 50-100 µm, 100-200 µm, and 200-300 µm from the defined probe contour). GFP (green), GFAP (red), and NeuN (blue) are shown. Outside the red dashed circle (600 µm) was considered background. The white line denotes the border of an exclusion area in this image.
5.3.3 Tissue preparation

After four weeks of implantation, mice were deeply anesthetized with 5% isoflurane and transcardially perfused with 20 mL of ice cold phosphate buffered saline (PBS, pH 7.4) followed by 20 mL of ice cold 4% paraformaldehyde (PFA, pH 7.4) solution. Heads were then isolated and soaked in 4% PFA for 24 hrs at 4 °C for post-fixation. After rinsing with PBS, brains were carefully extracted from the head and cryopreserved in 30% sucrose in PBS for 24-48 hrs at 4 °C. Brain tissues were flash frozen in 2-methyl butane at -40 °C for 2 min and equilibrated to -20C before cryosectioning. Tissues were lightly embedded with Optimal Cutting Temperature (OCT) medium (Sakura Finetek, Alphen aan den Rijn, The Netherlands) then sectioned into 25 µm slices and transferred to electrostatic adhesive glass slides (Superfrost Plus, Thermo Fisher Scientific, Waltham, MA). Harvested slices were kept at 4 °C for no longer than one week before immunolabeling.

5.3.4 Immunohistological staining

Tissue slices were allowed to sit at room temperature (RT) for 30 min for secure adhesion onto slides. Tissue slices were rinsed three times with PBS for 5 min each to remove residual OCT and then blocked in blocking buffer (4% goat serum, 0.3% Triton-X in PBS) for 2 hrs at RT. Primary antibodies diluted in blocking buffer were applied and incubated for 20-24 hrs at 4 °C. Following primary incubation, tissue slices were washed five times with PBS for 5 min each to remove any unbound primaries. Corresponding secondary antibodies diluted in blocking buffer were then applied to the tissue slices and incubated for 2 hrs at RT. After 5 min of five subsequent washes with PBS, tissue slides were cover slipped using VectaShield mounting medium (H-1000, Vector Lab, Burlingame, CA). Antibodies used in this study are listed in Table 1.
Table 5.1
List of primary and secondary antibodies

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<td>Rat anti-CD68 (1:250, ab53444, Abcam)</td>
<td>Anti-rat IgG 647 (1:500, ab150159, Abcam)</td>
<td>Activated microglia /macrophages</td>
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</tr>
<tr>
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<td>Anti-rabbit IgG 633 (1:500, A21071, Life Tech.)</td>
<td>Neuronal nuclei</td>
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5.3.5 Imaging and quantitative analysis

Fluorescence images were taken with a Zeiss LSM 710 laser scanning confocal microscope (Carl Zeiss, Jena, Germany). A 2x2 tile was captured using a 10X objective to obtain a wider view of the device-tissue interface for quantitative analysis. To minimize the depth dependent variability [46], two slices per sample, roughly from 450-600 µm down the cortical column, were used for quantitative analysis. A 20X objective was used for representative qualitative figures. A maximum intensity projection was used for 20X stacks to span 10 µm. Qualitative figures presented in this paper were contrast enhanced for visual clarification.

We used Minute v1.5 [126, 127] for quantitative analysis. Briefly, an ellipse was drawn to define the contour of the device track. Exclusion areas were drawn to limit analysis to be conducted on only artifact-free areas in the device-containing hemisphere. For each 5 µm concentric ring, expanding from the defined ellipse, the average label intensity and the area of the ring was calculated. The strength of Minute V1.5 is that it allows us to utilize the entirety of an image, which is useful for
reducing potential bias from using only a portion of the image for quantification. An illustration is depicted in Fig. 5.1 (C).

We binned in intervals: 0-50 µm, 50-100 µm, 100-200 µm, 200-300 µm, and 300-500 µm. Average intensities of 5 µm rings were weighted by each area over the total area and summed to generate an average intensity of each binned interval. Each average binned interval was normalized to background, taken as the ring 600-800 µm away from the device contour. Neuronal nuclei (NeuN) were counted with ImageJ [128] using a custom, automated cell counting script.

5.3.6 Statistical analysis

Statistical analyses were performed using Prism 7.00 statistical analysis software (GraphPad Software, La Jolla, CA). A two-way ANOVA was made taking strain (GFP vs. WT) and coating scheme (PEG-coat vs. no-coat) as the independent variables for the test at each binned interval. Post-hoc Tukeys test was also performed to identify which two conditions made the most significant difference among the four treatments. Then, we blocked each animal to eliminate inter-animal variability and ran paired t-tests to directly compare a PEG-coated device implanted hemisphere with its contralateral non-coated device implanted hemisphere.

5.3.7 Correlation coefficient

To further assess the dependencies between immunolabels, we calculated Pearson correlation coefficient of normalized fluorescence intensities. The correlation coefficient was defined as

$$\text{CorrCoef} = \frac{E[(L_1 - m_{L_1})(L_2 - m_{L_2})]}{\sigma_{L_1}\sigma_{L_2}}$$  \hspace{1cm} (5.1)

where $L_1$ and $L_2$ are vectors representing normalized intensities of immunolabels of interest over the binned intervals, $m_{L_k}$ is the mean of $L_k$, $\sigma_{L_k}$ is the standard deviation of $L_k$, and $E[\cdot]$ is the expectation operator.
5.4 Results

5.4.1 Microglia/macrophage characteristics

To identify whether observed immune responses correspond with previously published work [22,125] we took high magnification images of microglia in the proximity of the implant to examine their morphology. Fig. 5.2 (A) shows CD68, GFP, and merged images of a device-tissue interface taken with a 20X objective. It can be seen in Fig. 5.2 (A)-(D) that activated microglia/macrophages extended their processes toward the implant track (C) and/or fused together making them harder to detect as individual cells (D). They looked largely different from ramified microglia that are in a resting state (B). This microglial activity was also identified by co-localization of GFP with CD68 immunoreactivity. Of the microglia co-localized with CD68 near the implant track, no particular morphological differences were noted between PEG-coat and no-coat groups (data not shown).

Fig. 5.2 (E) and (F) are examples of microglial adsorption to PEG-coated and non-coated devices, respectively. After four weeks less microglia adhered to PEG-coated devices than non-coated devices. It appeared that microglia did not preferentially adhere to a specific region such as electrode sites.

5.4.2 Quantitative analysis of immunolabels

Quantitative analysis of normalized CD68 fluorescence intensity vs. distance from the implant is depicted in Fig. 5.3 (A) and (C). CD68 was strongest in the 0-50 µm interval and gradually decreased to the background level in the outer regions. No statistical significance was found between CX3CR1-GFP vs. WT and PEG-coat vs. no-coat at any given interval (p>0.05). There was no significant interaction effect between the coating and animal strain (p>0.05). A paired t-test, conducted to solely compare the effect of PEG-coating, also did not indicate any statistical difference (p>0.05).
Fig. 5.2. (A) CD68 fluorescence, autofluorescent GFP, and a merged image of a CX3CR1-GFP non-coated device-tissue interface at four weeks. The solid circle highlights non-activated, ramified microglia that do not co-localize with CD68 (high-magnification image shown in (B)). The dashed circle highlights activated microglia that are extending their processes toward the implant (high-magnification image shown in (C)). The dotted circle highlights highly activated microglia/macrophages that look to be lumped together (high-magnification image shown in (D)). Autofluorescent GFP of (E) an extracted PEG-coated probe and (F) a non-coated probe. Scale bars in (A) and (E) are 100 µm and (B) is 20 µm.

In Fig. 5.3 (B), we see that the mean IgG fluorescence intensity was generally greater for WT than CX3CR1-GFP, especially in the 0-50 µm and 50-100 µm intervals. However, the difference between CX3CR1-GFP and WT was not significant (p>0.05) and inconsistent with other immunolabels which showed an opposite mean trend to what was observed with IgG (e.g., CD68 being higher for CX3CR1-GFP than WT). IgG was particularly variable in contrast to other immunolabels used in this study, as is indicated by high standard error of the means.

No statistical difference in normalized GFAP fluorescence intensity was found between PEG-coat vs. no-coat and CX3CR1-GFP vs. WT with independent two-way
ANOVA or paired t-test, as can be seen in Fig. 5.4 (A). The mean values of GFAP were stable especially at 0-50 µm regardless of the treatment type, indicated by relatively small error bars than that of other immunolabels. Fig. 5.4 (C) shows that there was no statistical difference in the neuronal density between PEG-coat and no-coat. The neuronal density at 0-50 µm was highly variable but gradually became stable at distant intervals. The background neuronal densities were 2347 (±94, s.e.m., N=8) cells/mm² for GFP mice and 2376 (±53, s.e.m., N=8) cells/mm² for WT mice, which are in the range of previously reported neuronal densities in healthy mouse cortex [153].

Fig. 5.3. Normalized fluorescence intensity of (A) CD68 and (B) IgG as a function of distance from the implant at four weeks. p-values of the two-way ANOVA and paired t-test of (C) CD68 and (D) IgG for each binned interval. Error bars denote standard error mean (N=4).
Fig. 5.4. Normalized fluorescence intensity of (A) GFAP and (B) neuronal density as a function of distance from the implant at four weeks. p-values of the two-way ANOVA and paired t-test of (C) GFAP and (D) NeuN for each binned interval. Error bars denote standard error mean (N=4).

5.4.3 Variability of FBR

Even within the same treatment groups vastly different tissue responses were observed. PEG-coated or non-coated devices, regardless of implanted in CX3CR1-GFP mice or WT mice, caused either a mild response or a severe response, as can be seen in Fig. 5.5. In mild responses, CD68, IgG, and GFAP fluorescence intensities were relatively weaker and only localized to the implant track. In severe responses, the fluorescence intensities were stronger and largely diffused to the outer intervals. Mild responses were also manifested by a relatively high neuronal density near the implant track than that of severe responses. When there was a large implant track, it always accompanied a severe response. However, a severe response did not always
indicate that its implant track was large (e.g., in Fig. 5.5 CX3CR1-GFP PEG-coat severe response).

![Image of CD68, IgG, GFAP, and NeuN immunolabels in GFP mice and WT mice that show either a mild or a severe FBR. Images in the same column are from the same animal. Scale bars are 100 µm.]

5.5 Discussion

5.5.1 Microglial morphology and adsorption to the device

As described in a number of reports, activated microglia play a key role in the neuro-inflammatory response to foreign objects by secreting various pro-inflammatory and cytotoxic factors which propagate the neuro-inflammatory cascade [125,150]. We see in Fig. 5.2 (A)-(D) that our samples reflect morphologic changes upon activation that have been well established in this field. Kozai et al. showed that microglia, presumably surveying the local environment, extend their processes toward the foreign object at the onset of the phagocytic state [125]. Microglia then migrate toward the
device surface and create a microglial sheath. We saw the extension of processes at four weeks post-implantation, indicating that there was ongoing inflammation near the implant track. The GFP in Fig. 5.2 (D), which highly co-localizes with CD68 activity, indicated maximally activated microglia or macrophages. It was unclear to differentiate between microglia and macrophages as the GFP is expressed not only in microglia but also in other monocyte derived immune cells [124].

Previous acute studies have revealed that PEG prevents protein adsorption to silicon or polymer based probes by creating a hydrophilic layer in between the device-tissue interface [137, 154, 155]. We also observed PEG-coated devices with fewer microglia on their surface after four weeks of implantation (Fig. 5.2 (E)-(F)). This infers that prevention of their processes from adhering to the probe in the initial phase may affect their attachment in the long-term as well. An in vitro study by Gutowski et al. showed that microglia are seldom found on PEG-decorated devices at 24 hours post-implantation. Unlike their study, we observed microglial adherence to PEG-coated devices at four weeks, likely established after the PEG layer dissolved away. Also suggested by previous studies [132], attachment of microglia on hydrophobic devices necessitates care while quantitatively examining the device-tissue interface after device extraction.

Investigating the GFP provides insights to the dynamics of microglia as well as the entire inflammatory cascade. In a statistical study, however, quantifying more specific microglial-activity markers, such as CD68 or CD11, refines our analyses by excluding resting microglia signatures which may obscure the inflammation topology.

5.5.2 Effect of PEG-coating and CX3CR1-GFP mouse model

Conflicting expectations may exist regarding the use of PEG coatings. It could be that 1) a larger implant track caused by the thick layer of coating exacerbate the chronic FBR, or 2) PEG have an ameliorative effect on the FBR. For CX3CR1-GFP
mice, the involvement of CX3CR1 gene in accelerating or decelerating the secretion of neurotoxic factors in microglia is controversial.

PEG has been shown to be biocompatible and provide a beneficial effect on traumatic injury sites. PEG is not readily degraded upon hydration and interacts with the surrounding tissues until it dissolves away [156]. Studies have reported that PEG reconstitutes damaged cell membranes which in turn promotes axonal regeneration in injured spinal cords [145, 146] or reduces traumatic or ischemic cell loss in the brain [147, 148]. Moreover, acute in vivo/in vitro tissue responses [137, 155] and impedance measurements [154] suggest that PEG possesses anti-biofouling properties which prevent glial cell adsorption. The hydrated PEG layer can also work as a short term diffusion barrier. Despite these reported beneficial roles of PEG no significant difference was found for the immunolabeling of the FBR vs. no-coat in this study. It is unclear whether PEG has little effect on reducing the FBR, or the beneficial effects of PEG were leveled off by a larger initial trauma caused by the thick coating. The increase in thickness of the penetrating profile was from 15 $\mu$m to $\sim$100 $\mu$m, which could be sufficiently large to induce a difference when the thickness was retained throughout the implantation period [58]. In our study, however, the thickness of the residing material was kept the same as control since the PEG layer likely dissolves away upon contact with the blood and cerebrospinal fluid. Skousen et al. found that the surface area of the residing material is a significant factor when penetrating profiles are kept similar [114]. Although we did not observe any significant difference with differing penetrating profiles of PEG-coat vs. no-coat, we cannot single out the detrimental effect of a thick penetration profile since the dissolved PEG may also influence the surroundings.

CX3CR1, a receptor present in microglia, interacts with the ligand fractalkine in neurons which is considered a mechanism of neuron-glia crosstalk. This poses the question of whether the deletion of CX3CR1 has a prominent impact on the various cells including neurons. Jung et al. found that the absence of CX3CR1 in microglia
did not alter the microglial response to various inflammation models, one being a peripheral nerve axotomy [124]. However, contradictory results were also reported that CX3CR1 deletion may have a negative or positive effect on neurons. Cardona et al. demonstrated that CX3CR1 deficient mice either stimulated with lipopolysaccharide (LPS), given neurotoxins to induce Parkinsons disease symptoms, or genetically modified to induce amyotrophic lateral sclerosis resulted in more neurodegeneration than controls [157]. By contrast, Denes et al. and Donnell et al. reported that the lack of CX3CR1 had a neuroprotective effect in brain ischemia and spinal cord injury models, respectively [158,159]. Among these, our results corresponded well with Jung et al. in which there was no significant impact with CX3CR1 deletion. It may be that there is no general rule for CX3CR1 deficient microglia to react to the induced inflammation, but rather the response largely depends on the type of stimuli and the target region of the body.

In general, the acute phase of the FBR lasts up to 1-2 weeks and the chronic phase begins around 3-4 weeks post-implantation. During the chronic phase there are less dynamic cellular changes and the FBR persists as long as a foreign device remains [19, 102]. The FBR among the studied conditions were not significantly different at four weeks. Since it is well known that the degree of the FBR of control condition (i.e., non-coated silicon device in a WT mouse) remains unchanged chronically, we expect the FBR of other conditions to remain similarly beyond four weeks.

Even if PEG-coating and/or the CX3CR1-GFP mouse model caused small differences in the FBR, the differences were not prominent compared to the innate variability of the neural implant with the current technological standard. The qualitative images in Fig. 5.5 are indicative of the highly variable nature of the FBR to implants. Even though all the surgical and care plans remained the same, there was a large difference in the tissue response within the same group. This is in line with the discrepancies observed from between studies or even within studies [102]. Rousche et al. and Williams et al. showed that a large variability existed even be-
tween different shanks within a multi-shank device [30, 31]. Vascular damage can be the most probable suspect for inducing this variability, as devices that sever large vasculature have been reported to cause more severe neuro-inflammation [114, 133]. Although surface vasculature can be avoided during implantation, vasculature that underlie brain parenchyma are hard to avoid unless identified. Emerging technologies such as utilizing 3D mapping of the brain prior to insertion may minimize the BBB damage [160]. Although the breach of the BBB is inevitable, reducing this variability will be critical in facilitating quantitative research.

5.5.3 Correlation between immunolabels

Fig. 5.6 shows Pearson correlation coefficients of normalized immunolabel intensities along the distance from the implant averaged across the animals. For NeuN, we used the inverse of neuronal density since neuronal density is inversely proportional to other inflammatory markers along the distance. Fig. 5.6 implies that dispersion profiles of GFAP, NeuN-1, IgG, and CD68 are generally correlated to each other. However, we see that NeuN-1 is relatively loosely correlated to other labels. This suggests why histology is not always a strong predictor of the quality of the neural recordings. A number of studies have failed to establish a direct correlation between neuro-inflammatory markers and recording quality. As neurons are the most important cells that are directly related to the quality of neural recordings, it is likely that the unpredictable neuronal density is the root cause of this discrepancy.

The correlation coefficient of IgG and CD68 were predominantly higher than the other pairs from the four immunolabels used in this study. This potentially indicates a direct relationship between BBB leakage and microglial activation that drives the inflammatory cascade forward. The hypothetical models suggested by the Tresco, Bellamkonda, and Capadona groups explain the mutual influence of pro-inflammatory cytokines secreted by activated microglia permeabilizing BBB and pro-inflammatory cytokines secreted by infiltrated myeloid cells stimulating microglia [58, 101, 133, 142].
Fig. 5.6. Correlation coefficients of the reactivity of immunolabels along the distance from the implant. Values shown above bars are mean. Error bars are standard error mean. (N=16)

Neurons are intoxicated by both of these activities but are not actively involved in driving the cascade, potentially explaining why NeuN-1 is only loosely correlated.
5.6 Conclusion

We have evaluated the FBR of implanted microelectrodes with regard to coating scheme (PEG-coat vs. no-coat) and animal strain (CX3CR1-GFP vs. WT) at four weeks. Although we observed less adherent-microglia on the PEG-coated devices, statistical analyses suggested that dip coating with PEG does not result in a significant decrease or increase in the FBR. Likewise, the use of a transgenic CX3CR1-GFP mouse model did not induce any significant difference in FBR compared to the WT mice. These results indicate that PEG-coating and the CX3CR1-GFP mouse model do not confound neural implant studies that use such materials for varying purposes. This study validates that 1) PEG-coating is a suitable insertion carrier or aid that can be used in applications such as insertion of flexible neural probes, and 2) CX3CR1-GFP is a suitable model that can be used for immunohistochemistry or in vivo imaging for studying the neuro-inflammatory cascade to implanted devices.
6. SUMMARY

The work in this thesis interrogates several electrode design factors by examining histological and electrophysiological hallmarks. The work presented in Chapter 3 demonstrates that, although substrate form factor dictates the overall FBR, putting sites on the edge can leverage relatively non-severe region. The work presented in Chapter 4 shows that flexible substrates do reduce the FBR but the benefit from flexibility does not increase proportionally with increasing flexibility and reaches a beneficial limit. The work presented in Chapter 5 shows that dip-coating with PEG, which is employed in many flexible electrode studies including Chapter 4, does not significantly alter the FBR and thus does not confound the histological analysis.

These findings can make a seminal contribution toward identifying the factors pertaining to an ideal intracortical implant. They raise additional design factor considerations. For example, as a bulky shank look to deteriorate the FBR, replacing the whole shank with a rigid dissolvable agent can be a viable option to minimize the tissue response. Findings also enrich scientific inquiry. For example, what might be the mechanism for the flexibility threshold hypothesis? How does mechanotransduction come into play in this scenario? By observing the increase in insertion profile from the thick PEG-coating not exacerbating the chronic FBR, is the FBR truly influenced by the chain reaction initiated from the insertion injury? Or does PEG have curative effect on the damaged cortical tissues? Answering these will likely raise more questions, but iterating this positive loop will advance the field of neural implants.

Biological response to neural implants is currently the major limiting factor for the functional longevity. With the presented findings, there is still a long way ahead to overcome the posed limitations. Very often the findings from the PNS does not pertain
to the CNS and vice versa due to the inherent difference in cellular compositions. Even within the CNS we have seen that a small modification to the design (e.g., site placement) can significantly alter the outcome. Therefore, hasty conclusions are discouraged. A careful step-by-step approach to identify the key features will be the path for developing lifetime-lasting neural implants.
LIST OF REFERENCES


K. A. Ludwig, J. D. Uram, J. Yang, D. C. Martin, and D. R. Kipke, “Chronic neural recordings using silicon microelectrode arrays electrochemically deposited with a poly (3, 4-ethylenedioxythiophene)(pedot) filmthis work was supported by the center for wireless integrated microsystems nsf eec-9986866 and the whitaker foundation,” *Journal of neural engineering*, vol. 3, no. 1, p. 59, 2006.


S. Sommakia, J. Gaire, J. L. Rickus, and K. J. Otto, “Resistive and reactive changes to the impedance of intracortical microelectrodes can be mitigated with polyethylene glycol under acute in vitro and in vivo settings,” *The chronic challenge-new vistas on long-term multisite contacts to the central nervous system*, p. 16, 2015.


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Peer-Reviewed Publications


12. D. C. Oh, **H. C. Lee**, and Y.-H. Lee, Linear hard decision combining for co-


**Patents**


**Presentations**


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