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Neuroadhesive L1 coating attenuates acute microglial attachment to neural electrodes as revealed by live two-photon microscopy

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ABSTRACT

Implantable neural electrode technologies for chronic neural recordings can restore functional control to paralysis and limb loss victims through brain-machine interfaces. These probes, however, have high failure rates partly due to the biological responses to the probe which generate an inflammatory scar and subsequent neuronal cell death. L1 is a neuronal specific cell adhesion molecule and has been shown to minimize glial scar formation and promote electrode-neuron integration when covalently attached to the surface of neural probes. In this work, the acute microglial response to L1-coated neural probes was evaluated *in vivo* by implanting coated devices into the cortex of mice with fluorescently labeled microglia, and tracking microglial dynamics with multi-photon microscopy for the ensuing 6 h in order to understand L1's cellular mechanisms of action. Microglia became activated immediately after implantation, extending processes towards both L1-coated and uncoated control probes at similar velocities. After the processes made contact with the probes, microglial processes expanded to cover 47.7% of the control probes' surfaces. For L1-coated probes, however, there was a statistically significant 83% reduction in microglial surface coverage. This effect was sustained through the experiment. At 6 h post-implant, the radius of microglia activation was reduced for the L1 probes by 20%, shifting from 130.0 to 103.5 μm with the coating. Microglia as far as 270 μm from the implant site displayed significantly lower morphological characteristics of activation for the L1 group. These results suggest that the L1 surface treatment works in an acute setting by microglial mediated mechanisms.

1. Introduction

Chronically implanted neural electrodes have emerged as basic neuroscience research tools and effective therapeutics [1–6]. Specifically, microscale recording and stimulation electrodes have played a fundamental role in understanding and modulating the basic neural circuitry underlying complex neural networks [7–11]. In human clinical applications, implantable brain computer interface devices have demonstrated great promise in the ability to restore functional motor control [12,13]. However, the recording potential of implanted neural electrodes is limited by instability since the signal quality degrades over months to years post implant [7,14–17]. The degradation of the signal quality over time is understood to be a combination of material failure and biological factors [18].

Material failures largely result from corrosion and delamination of the electrode sites [19–25], cracks in the electrical traces [26–28], and delamination of insulation materials [18,25,28–30], all of which are exacerbated by perpetual strain caused by tissue micromotion during movement [28,31,32]. Biological failure modes of neural interfaces result from multiple sources that ultimately

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lead to meningeal cell invasion and fibrous encapsulation [14,33], insulting glial scar encapsulation, and neural degeneration [34,35]. Electrode insertion is a traumatic process, and even insertions that avoid large pial arteries and veins will break capillaries in the cortex, causing blood-brain barrier disruption [18,36,37]. This leads to an influx of plasma proteins that adsorb onto the surface of microelectrodes [18,36] and infiltration of inflammatory cells [38–40]. The combination of plasma proteins and cells, necrotic cell debris, and mechanical strain imposed by probe insertion causes an upregulation of proinflammatory cytokines that initiate the cascade of reactive tissue response [18,41–44]. Using two-photon microscopy, we have observed that nearby resident microglia immediately activate by retracting most of their processes while extending a few processes towards the probe in order to cover the surface of the implant with a lamellipodia sheath [41]. Over the following days, microglia and astrocytes aggregate at the surface of the implant to form an electrically insulating astroglial sheath [35,38,39,45,46]. Additionally, chronic inflammation leads to neural degeneration which presumably diminishes signal [18,24,42]. Lastly, failure is also fueled by the decrease in metabolic supply and neurotoxic waste product removal from loss of blood flow perfusion and increased metabolic consumption from inflammation [18,41,42].

In order to improve neural interface performance longevity, neural engineers have explored numerous intervention strategies. This includes changing the footprint of the probe or the probe's electrode sites [18,36,44,47–51,160], altering recording site materials [48,52–57], applying flexible geometries or soft materials [26,27,36,58–62,161,162], creating dissolvable insertion shuttles for softer probe materials [63], locally delivering anti-inflammatory or neuroprotective drugs [64–75], and modifying the probe's surface chemistry [36,76–78].

One promising method involves covalently attaching L1 cell adhesion molecule (L1) to the surface of the probe. L1 is a transmembrane cell surface glycoprotein that functions through homophilic interactions with L1 molecules on other cells to mediate cell recognition and cell interactions [79,80]. It has been shown to play a critical role in neuronal adhesion, axonal growth, neural migration, neural differentiation, and neuronal survival [81–89]. L1 is also implicated in improving regeneration following lesions in both the central and peripheral nervous systems [90–97]. Several studies have shown that L1 promotes neuronal cell attachment and growth while inhibiting glial and fibroblast cell attachment *in vitro* [98–101]. In the context of neural implants, our group has shown that covalent attachment of brain tissue derived L1 to neural probes can reduce glial scarring, while simultaneously encouraging neuronal attachment to the probe's surface for at least 2 months post-implant [61,76,77]. While these studies suggest L1 can modify the behavior of glial scars, the mechanism behind this is unclear. In the present work, we use two-photon microscopy (TPM) to study the dynamic microglial response to L1 coated microelectrodes for the first 6 h post-implant in living mice, as the first step to uncover the mechanisms. Compared to uncoated microelectrodes, there was significantly less microglial coverage of the L1 probes from 8 min–6 h post-implant, despite similar degrees of microglial process extension toward both coated and control probes. This suggests that L1's mechanism for preventing glial attachment and scarring occurs rapidly after initial contact.

2. Methods

2.1. Neural probes and L1 protein immobilization

All studies were performed using four-shank NeuroNexus 16-channel, 15 μm thick, 3 mm long SOI silicon probes (NeuroNexus Technologies, Ann Arbor, MI) mounted on dummy boards.

For quantitative analysis, L1 immobilization was conducted along the entire shank of the probes (n = 7), and all control probes (n = 7) were pristine, uncoated arrays that were washed with ethanol and phosphate buffer solution. L1 immobilization on the silicon dioxide surface and iridium oxide electrode pads were carried out as previously described with minor modifications [77,99]. Briefly, probes were cleaned and functionalized with either HNO3 (Sigma Aldrich) or by serial washes in acetone, 50% (v/v) MeOH/H2O, and chloroform before oxygen plasma cleaning (30W) for 1 min (Harrick Plasma, PDC-001) [102]. Probes were silanized by immersion in 2% (3-mercaptopropyl) trimethoxysilane (Sigma Aldrich) solution with 4-maleimidobutyric acid N-hydroxysuccinimide ester (2 mM, Sigma Aldrich) for 1 h. Finally, probes were fully immersed in a 100 μg/ml solution of purified L1 protein (purified at our lab) for 1 h at 4 °C, and stored in sterile 1× phosphate buffer solution (Sigma Aldrich) until implantation. In an additional validation experiment (n = 1), following silanization of the probe's full surface, the probe was dipped only ~150 μm in the L1 solution. This half-coating design allowed for comparison between L1 and no L1 conditions on the same probe (Supplemental Fig. 1). The L1 modified probes were stored in saline for up to 1 h prior to implantation. Previous studies have shown that the L1 coating procedure yields a uniform 6.37 nm thick coating with 0.53 g cm^{-3} density and increased hydrophobicity (water contact angle: 69.8 ± 1.7° for L1 coated v. 27.3 ± 1.4° for unmodified control) [99].

2.2. Surgery and probe insertion

Surgical procedures were conducted as previously described with 14 adult CX3CR1-GFP transgenic mice with GFP expression in macrophages and microglia controlled by the CX3CR1 promotor (Jackson Laboratories, Bar Harbor, ME) [41]. A cocktail of intraperitoneally (IP) administered ketamine/xylazine (90/8 mg kg^{-1}) was used to induce anesthesia, with depth of anesthesia assessed by monitoring the toe-pinch response, breathing, and heart rate. After animals were secured in a stereotaxic frame, scalps were shaved, cleaned with 70% ethanol, and resected. Calvarial periosteum was scraped off with cotton swabs, and a thin layer of Vetbond (3 M) was applied to dry the skull. A 1–1.5 mm tall well of light-curable cement (Composite Flowable; Henry Schein, NY, USA) was set around the margin of the exposed skull, following which a ~4–6 mm craniotomy was performed with a high-speed dental drill over the visual cortex (V1 and V2; centered approximately 2–3.5 mm caudal to Bregma and 1–3 mm lateral from midline). The craniotomy site was frequently washed with saline to remove bone fragments and prevent thermal damage of the underlying brain. A dental cement well was cured around the margin of the craniotomy to hold a saline immersion with the microscope objective. After the skull was thinned, it was carefully removed with fine-tip forceps. Following craniotomy, animals were placed under a two-photon microscope using a 16x, 0.8 numerical aperture water immersion objective (Nikon Inc., Milville, NY). Probes were stereotaxically targeted within the V1/V2 portion of the craniotomy and inserted in a rostral direction into the cortex at a 30-35° angle and parallel to midline at 50–100 μm s^{-1} (oil hydraulic microdrive; MO-81, Narishige, Japan) to a final resting depth of 250–300 μm (layer II-III) beneath the surface of the brain (Fig. 1). Major blood vessels were identified prior to insertion and avoided. All animals had a similar density of capillaries within the imaging window. Little or no bleeding was observed during insertion, though some spontaneous bleeding was observed throughout the 6 h experiment. Immediately prior to imaging, sulforhodamine 101 (SR101) was injected IP as a vascular contrast agent (red; 0.02–0.04 cc; 1 mg ml^{-1}). Updates