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Protease-degradable PEG-maleimide coating with on-demand release of IL-1Ra to improve tissue response to neural electrodes

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ABSTRACT

Neural electrodes are an important part of brain-machine interface devices that can restore functionality to patients with sensory and movement disorders. Chronically implanted neural electrodes induce an unfavorable tissue response which includes inflammation, scar formation, and neuronal cell death, eventually causing loss of electrode function. We developed a poly(ethylene glycol) hydrogel coating for neural electrodes with non-fouling characteristics, incorporated an anti-inflammatory agent, and engineered a stimulus-responsive degradable portion for on-demand release of the anti-inflammatory agent in response to inflammatory stimuli. This coating reduces *in vitro* glial cell adhesion, cell spreading, and cytokine release compared to uncoated controls. We also analyzed the *in vivo* tissue response using immunohistochemistry and microarray qRT-PCR. Although no differences were observed among coated and uncoated electrodes for inflammatory cell markers, lower IgG penetration into the tissue around PEG+IL-1Ra coated electrodes indicates an improvement in blood-brain barrier integrity. Gene expression analysis showed higher expression of IL-6 and MMP-2 around PEG+IL-1Ra samples, as well as an increase in CNTF expression, an important marker for neuronal survival. Importantly, increased neuronal survival around coated electrodes compared to uncoated controls was observed. Collectively, these results indicate promising findings for an engineered coating to increase neuronal survival and improve tissue response around implanted neural electrodes.

1. Introduction

Neural electrodes are an important part of brain-machine interface devices that may one day restore functionality to patients with spinal cord injury, prosthetic limbs, and sensory impairments [1–4]. However, the recording ability of the majority of electrodes fails within days to weeks after implantation [5], rendering the current technology inconsistent and unstable. While many modifications have been made to improve long-term neural electrode functionality, many issues still persist including acute and chronic inflammation, microglia and astrocyte recruitment, scar formation, and death of neurons surrounding the implanted electrode [6–10]. In addition, microvasculature is compromised upon electrode implantation causing blood-brain barrier (BBB) breach. The severity of BBB breach is an important determinant in the long-term tissue response to implanted devices, with BBB breach causing increased inflammation and neuronal death as well as correlating with decreased electrode recording functionality [11,12]. This combination of responses will eventually cause the electrode to cease functioning.

Numerous electrode coatings have been developed to improve electrode performance as well as the *in vitro* and *in vivo* response to electrodes. Conductive coatings are a widely-tested option as they can improve the electrical performance of the electrode [13,14]. Combinations of poly(3,4-ethylenedioxythiophene)/poly(styrene sulfonate) (PEDOT/PSS) or polypyrrole (PPy) with a peptide-derivative from laminin have shown promising results to decrease impedance on the active sites of electrodes, making it easier for neuronal signals to reach the electrode surface [15–17].

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Additional research with PEDOT/PPy nanotubes showed improved electrical properties as well as improved neurite outgrowth on the electrode surface [18]. Others have tried passive polymer coatings to reduce protein adsorption and cell adhesion on the electrode surface. Polyaniline-coated platinum electrodes [19] and low-protein binding polymer films on silicon electrodes [20] showed reduced protein adsorption, while PEG-NIPAm microgel coatings also showed reduced cell adhesion and cell spreading *in vitro* compared to unmodified controls [21]. Poly(vinyl alcohol)/poly(-acrylic acid) coatings reduce protein adsorption and astrocyte recruitment around the electrode site [22], while combination PEG/polyurethane coatings have reduced glial scarring and neuronal death around PEG/PU coated electrodes [23]. Several groups have also investigated the effectiveness of incorporating bioactive factors into a coating. Bezuidenhout et al. demonstrated that loading dexamethasone into degradable and non-degradable PEG hydrogels improves tissue responses [24]. Further studies showed reduced inflammatory response and increased neuronal survival with dexamethasone-releasing coatings [25–28]. Dexamethsone treatments have also yielded reduced astrocytic response in an *in vivo* model [29,30]. Incorporating α-melanocyte stimulating hormone into a nitrocellulose coating on the electrode surface reduced LPS-stimulated nitrite production *in vitro* [31] and incorporation of TGF-β on a laminin coating yielded reduced astrocytic recruitment on the electrode surface compared to laminin alone, indicating a potential target for reducing astrocytic scar formation [32]. Additionally, multi-functional coating approaches have attempted to address several problems simultaneously. Abidian and Martin incorporated slow-release dexamethasone into an alginate hydrogel with PEDOT functionalization to improve electrical impedance with promising release characteristics *in vitro* [33], while Wadhwa et al. showed similar results with a polypyrrole coating and dexamethasone release *in vitro* [34]. Potter et al. utilized a poly(vinyl alcohol) material to improve the mechanical characteristics of the electrode to reduce mechanical mismatch while also incorporating curcumin to mediate the inflammatory response [35] with promising results at 4 weeks post-implantation, but all improvements were lost by 12 weeks. Although there have been many attempts to improve electrode performance and tissue response, a solution has not been found that can address all problems associated with implanted neural electrodes.

Previous work from our group has shown that modifying the surface of an electrode with a non-fouling coating alone is not sufficient to reduce inflammatory cell recruitment or neuronal loss for electrodes implanted in the rat brain [21]. These findings prompted us to re-engineer the coating to incorporate an anti-inflammatory agent into the non-fouling coating with the goal of reducing inflammation and neuronal loss in the surrounding tissue. Interleukin 1 (IL-1) is an important cytokine in the inflammatory cascade both in the brain and throughout the body, and the presence of IL-1 can promote production of additional cytokines in the inflammatory cascade. Interleukin-1 receptor antagonist (IL-1Ra) is a 17 kDa protein that has been implicated as an important mediator of inflammation in diseases and conditions that contain IL-1 as part of the inflammatory cascade [36,37]. In the central nervous system, IL-1Ra reduces inflammation in the spinal cord, and it is also implicated in the recovery process after brain ischemia as well as stroke [36,37]. IL-1Ra also has neuroprotective effects when released by microglia [38]. Taub et al. examined the effects of IL-1Ra integrated into a laminin coating on neural electrodes and noted moderate improvement of the astrocyte response to the IL-1Ra-coated electrodes compared to uncoated controls, however no other cell types were analyzed [39]. Additionally, IL-1Ra is already approved for use in humans as a therapeutic for other inflammatory conditions such as arthritis [36]. Based on these data, we hypothesized that IL-1Ra is a suitable candidate as an immunomodulator to improve the tissue response to implanted neural electrodes.

Rather than simply adding IL-1Ra to the coating for passive release, we engineered an on-demand release characteristic to the coating. MMPs are up-regulated in many disease states and conditions that cause increased inflammatory response [40,41], including neurodegenerative diseases [42], central nervous system injury [43–45] brain injury [46,47], and around implanted neural electrodes [11,48]. Since MMPs are up-regulated in the inflammatory cascade in the brain, it is possible to utilize MMPs that are already present in the inflamed tissue to serve as the stimuli to break down a material, such as a hydrogel, that contains MMP-degradable motifs to release therapeutic agents. MMP-degradable hydrogels have been used for diverse applications including encapsulation of mesenchymal stem cells [49], fibroblasts [50,51], vascular smooth muscle cells [52], drugs [53], and biomolecules such as RGD and VEGF [54–56]. The MMP-degradable nature of these hydrogels allows for host cells to remodel the gels, allowing for cell ingrowth as well as release of any incorporated bioactive factors.

In the present study, we engineered a neural electrode coating with three essential components:

1) a protein adsorption- and cell adhesion-resistant PEG hydrogel;
2) incorporated anti-inflammatory IL-1Ra;
3) protease-sensitive crosslinkers for on-demand release of IL-1Ra in response to proteases which are up-regulated during inflammation.

We hypothesized that a coating comprising a protein adsorption/cell adhesion-resistant layer with controlled on-demand release of the anti-inflammatory agent IL-1Ra would improve the tissue response and neuronal survival near the implant–tissue interface.

2. Materials and methods

2.1. PEG-maleimide hydrogel coating of electrodes

Electrodes were purchased from NeuroNexus Technologies (CM16 A4x4-4 mm-200-200-1250) and consist of a silicon substrate with iridium wires and active sites. Each electrode is 4-mm long with four active sites on each of four prongs, and each active site has an area of 1250 μm^2. In some instances, silicon wafers were used as a surrogate for the Si substrate of the electrodes. Silicon substrates were cleaned to remove contaminants using serial 5-min incubations in trichloroethylene (JT Baker), acetone (Sigma–Aldrich), and methanol (Sigma–Aldrich). Substrates were then rinsed with absolute ethanol (Decon Labs). The surface was functionalized using a silane-based adhesion layer grafted onto the silicon oxide layer of the electrode/wafer. Briefly, the substrates were incubated for 2 h in 2.5% silane-PEG-maleimide (Nanocs) in DMSO, then rinsed with absolute ethanol and PBS. Multi-treatment PEG-mal coatings were deposited using a dip-coating technique developed for this research. Silane-PEG-maleimide-modified samples were incubated in solution with crosslinking peptide, either GCRDGDQGIAGFDRCG (GDQ) or GCRDVPMSMRGGDRCG (VPM) (peptides were custom synthesized by AAPPTec), for 2 min, rinsed with PBS, incubated for 2 min in 20 kDa 4-arm PEG-maleimide (Laysan Bio), and rinsed with PBS (Fig. 1a). Alternating incubations in crosslinking peptide followed by PEG-maleimide were repeated to achieve the desired number of treatments per coating, with each set of peptide and PEG-maleimide considered as one complete treatment. For samples presenting only the PEG hydrogel (designated as PEG), the samples were coated with six treatments of PEG-maleimide and GDQ, whereas the coatings containing IL-1Ra (PEG+IL-1Ra) were coated with two treatments of PEG-maleimide and GDQ followed by four treatments of PEG-maleimide/IL-1Ra and VPM. Although the coatings do incorporate different crosslinkers, this hydrogel system with both crosslinkers has been extensively characterized by our lab and there are minimal differences in hydrogel structure. Coating deposition was verified by X-ray photoelectron spectroscopy (XPS). Coating thickness was analyzed by wet-cell ellipsometry of coatings on Si wafers. Ellipsometry measurements were performed using a GES5 variable angle spectroscopic ellipsometer (Sopra) and the accompanying GESPack software package. Briefly, a total of six spectra for at least two test points on each sample in deionized water were scanned from 350 nm to 800 nm at 10 nm intervals using an incident angle of 70°. The thickness of the