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Surface modification of neural electrodes with pyrrole-hyaluronic acid conjugate to attenuate reactive astrogliosis in vivo†

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Surfaces of neural probes were electrochemically modified with a non-cell adhesive and biocompatible conjugate, pyrrole-hyaluronic acid (PyHA), to reduce reactive astrogliosis. Poly(PyHA)-modified wire electrodes were implanted into rat motor cortices for three weeks and were found to markedly reduce the expression of glial fibrillary acidic protein compared to uncoated electrodes.

Neural prosthetic probes have been developed to electrically stimulate and/or record neural activity in the brain[1]. For patients with central nervous system (CNS) disorders, electrical stimulation via implanted neural probes has been reported to help restore normal neural functions[2, 3]. For example, some symptoms of Parkinson's disease are alleviated by deep brain electrical stimulation in patients[4]. In addition, neural prosthetic probes allow the recording of neuronal firing potentials and their patterns adjacent to the electrodes[5, 6]. However, implanted electrodes often lose their electrical connectivity within weeks to months and become unable to electrically communicate with neuronal cells due to high impedance between the implanted electrode and neural tissues[1]. The impaired electrode performance results from a thick glial scar tissue that serves as both a physical and electrical barrier between the brain tissue and the electrode via a process referred to as astrogliosis[7]. The exact mechanism for astrogliosis caused by neural probe implantation is not well known. Astrogliosis response usually includes an abnormal increase in the number of astrocytes due to the destruction of nearby neurons from CNS trauma, infection, ischemia, stroke, autoimmune responses, and neurodegenerative disease[8-10]. In addition, foreign body reaction in the brain can also induce astrogliosis[11, 12]. For example, the cells within the brain respond to a foreign neural electrode material by becoming activated and secreting cytokines and extracellular matrix, resulting in glial scar tissue[7, 13, 14]. However, this normal protective scarring response walls off the electrode connection to neurons; therefore, it is essential to reduce reactive astrogliosis to increase the connectivity and longevity of implanted electrodes.

Various factors of a neural probe can influence inflammatory astrogliosis, including probe geometry[15, 16], micro-motion between the probe and surrounding tissue[17, 18], and surface properties of implanted probes[19-21]. Among them, coating the electrode surfaces with biocompatible or bio-inert polymers can be an effective strategy to shield the foreign electrode materials and reduce astrocyte activation. Several attempts have been made to coat neural probes with poly(2-hydroxyethyl methacrylate) (pHEMA), alginate, polyethylene glycol, and silicone polymers [22-29]. However, these coatings resulted in substantial increase in electrical impedance after the surface modification and/or did not adequately reduce the glial response.

In this study, we developed a method of biomimetic surface modification of neural electrodes using naturally-derived hyaluronic acid (HA) as a component of a conductive coating. HA is a polyanionic polysaccharide that normally exists in the extracellular matrix of the brain, and is non-immunogenic, biocompatible, and generally non cell-adhesive to a variety of cell types[30, 31]. Furthermore, high molecular weight HA has anti-inflammatory activity in CNS tissues, especially to astroglial cells[31, 32]. Thus, HA was selected as the backbone for

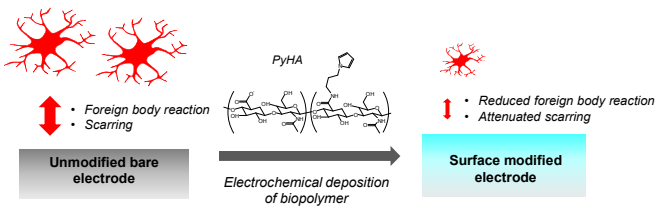

Fig. 1 Schematic illustration of the poly(PyHA)-coated neural electrode for attenuation of reactive astrogliosis. Electrochemical coating of neural electrode surfaces using the PyHA conjugate can be used to reduce the foreign body reaction and scar tissue formation.

producing electrically conductive graft copolymers used as coatings for the neural electrodes in this study. We expected that the introduction of biocompatible HA moieties on the electrode surfaces could reduce astrogliosis by attenuating the response of the astrocytes. We previously reported the synthesis of such graft copolymers, pyrrole-hyaluronic acid conjugates (PyHA), and their electro-polymerization on the surfaces of electrically conducting materials (e.g., indium tin oxide, platinum, polypyrrole)[33]. In this prior report, multiple characterization techniques including atomic force microscopy, x-ray photoelectron microscopy, water contact angle measurement, and electrochemical impedance spectroscopy were used to demonstrate the formation of a uniform 20-40 nm thick hydrophilic layer on electrodes without impairment of electrical properties. Importantly, this electrode surface modification with poly(PyHA) could prevent the adhesion of fibroblasts and astrocytes in vitro. Accordingly, in this current report, we investigate the potential of such coatings to reduce astroglial activation and scarring in vivo (**Figure 1**).

Two types of commercial neural probes (i.e., silicon microelectrodes and iridium microwire electrodes) were tested for modification and brain implantation. It is important to note that the entire surface of the iridium microwire electrode is conductive, whereas the silicon microelectrode consists of conductive and non-conductive portions. Both types of probes were electrochemically coated with poly(PyHA) by applying 20 cycles of linear sweeping potentials from 0 to 1.0 V. The electrode surface was shown to turn hydrophilic after the poly(PyHA) coating process because HA is highly negatively charged and PPy is positively charged. This electrochemical polymerization parameter resulted in a 20-40 nm thick layer of poly(PyHA)[33]. To visualize the poly(PyHA) on the modified electrodes, we immunostained the electrodes using HA binding protein (HABP). As shown in **Figure 2a and 2b**, positive staining of the poly(PyHA)-coated electrodes with HABP confirmed the successful surface immobilization of HA onto the conducting electrode surfaces. The modified iridium microwires fluoresced relatively uniformly on their outside surfaces whereas the silicon microelectrode probes showed staining on the conductive electrode pads (circular dots) and remained unstained on the surrounding insulating silicon nitride pad.

In addition, electrochemical impedance spectra (EIS) of the modified electrodes were obtained and compared with those of unmodified controls to study the electrical performance of the neural probes in phosphate buffered saline (PBS). **Figure 2c and 2d** indicate that the modified neural electrodes exhibit similar impedance spectra to those of unmodified electrodes in a range of 1 Hz - 100 kHz, indicating no severe impairment of electrical sensitivity. Therefore, the poly(PyHA) coating

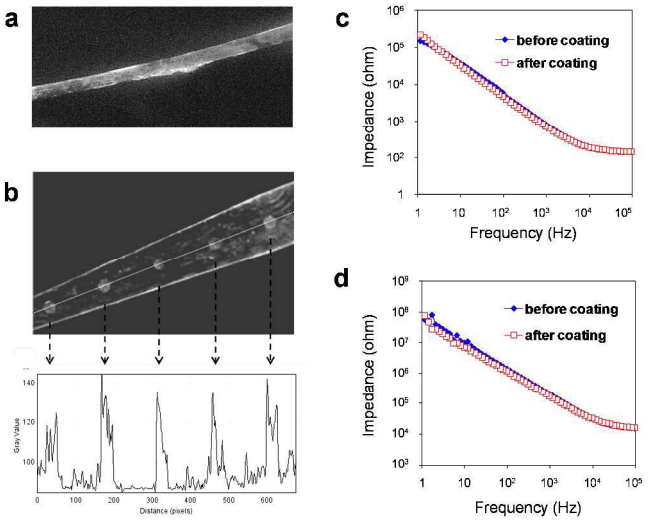

Fig. 2 Characterization of poly(PyHA)-coated neural probes. Fluorescence images of (a) an iridium microwire electrode and (b) the silicon microelectrode probe after electrochemical modification with poly(PyHA). The probes were stained with biotinylated HABP, followed by incubation with streptavidin-PE. For the silicon microelectrode probe, a fluorescence intensity profile was plotted (bottom) from the line of the top image. Impedance spectra of neural probes before and after the poly(PyHA) coating: (c) the iridium microwire electrode and (d) the silicon microelectrode.

introduces biocompatible HA moieties onto conducting surfaces while maintaining the original electrical properties of the electrodes.

Next, we examined the effects of poly(PyHA) coatings on the surfaces of both the iridium microwire electrodes and silicon microelectrodes in an in vivo study. Two groups of rats were implanted with either poly(PyHA)-coated probes (n=4) or unmodified probe controls (n=5). The probes were implanted into the motor cortices and allowed to remain there for three weeks. Astrocytes are known to become activated a few days after implantation, proliferate near the implanted site, and begin to form organized scar tissue around the implanted probes within three weeks[34]. Thus, we selected a three week time point to evaluate brain tissue responses. Staining of the retrieved modified iridium microwires revealed the presence of poly(PyHA) on the surface, whereas no distinct fluorescence was detected from the retrieved unmodified iridium microwire electrodes (controls) (Supplementary Information Figure S1). The results suggest that poly(PyHA) layers on the probes were stable after surgical probe insertion and after being placed within the brain tissue for three weeks. Previously, we found that poly(PyHA) coatings were stable after treatment with hyaluronidase up to 5 U/mL[33], a concentration which is significantly higher than the hyaluronidase activity in brain tissue (approximately 1 mU/mL)[35].

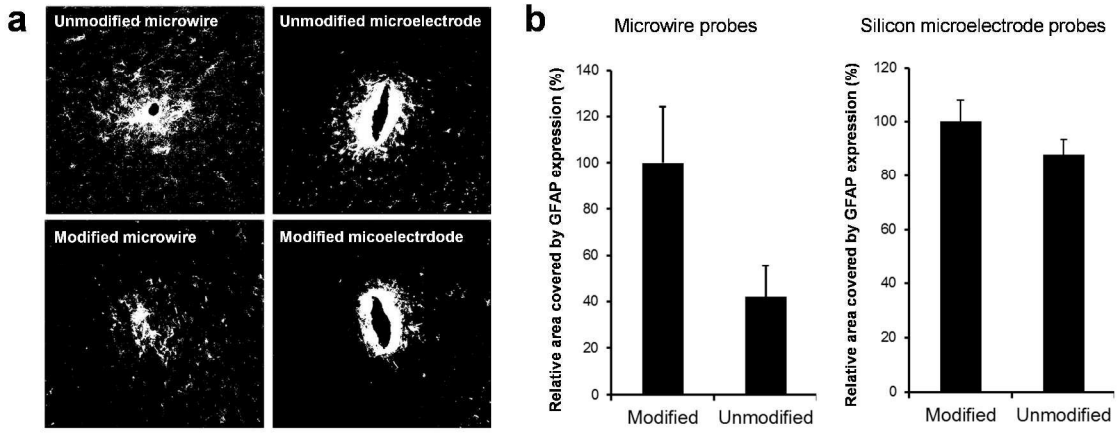

Fig. 3 Astrogliosis in brain tissues around the neural probes implanted after three weeks. (a) Representative immunofluorescence images of brain sections obtained from different animals, which were implanted with the iridium microwire probes and the silicon microelectrode probes. Brain slices were stained for GFAP, which is an astrocyte marker. (b) Analysis of GFAP expression from the brains implanted with probes. The fluorescence images were processed to binary images with the same threshold value. Area positively covered by GFAP was measured and normalized to the control (areas from unmodified probes). From each group (poly(PyHA)-coated or uncoated), averages and SEM were calculated and reported.

To assess reactive astrogliosis in the brain tissue, we examined the glia fibrillary acidic protein (GFAP) expression level as an activated astrocyte marker[36, 37]. **Figure 3a** illustrates representative immunofluorescence images of brain tissue slices stained for GFAP from animals that received modified and unmodified probes. Tissues implanted with the poly(PyHA)-coated iridium microwires displayed approximately 60% lower fluorescence intensity compared to those implanted with uncoated control iridium microwire probes (p=0.098), indicating substantial reduction in GFAP expression in the animals implanted with probes coated with poly(PyHA). However, implantation of the silicon microelectrode probes, whether modified with poly(PyHA) or left unmodified, into the brain resulted in a similar GFAP-positive response (p=0.831).

Our results indicate that the effects of the poly(PyHA) coating on reactive astrogliosis was different depending on the neural electrode type and geometry. For the iridium microwire, the entire surface is a continuous conducting substrate, which allows for uniform modification with the poly(PyHA), whereas the silicon microelectrode probe consists of an insulating silicon nitride pad with small circular conducting electrode sites. For the silicon microelectrodes, only the small conducting pads are modified with the poly(PyHA) (see Fig. 2). The presence of larger non-conducting portions (and therefore non-poly(PyHA)-coated areas) on the silicon microelectrode probes appears to play a role in inducing reactive astrogliosis as suggested by the observation of no significant differences in GFAP expression between the poly(PyHA)-coated silicon microelectrode probes and uncoated control microelectrode probes. On the other hand, the iridium microwires are essentially completely modified with the poly(PyHA), resulting in a more dramatic decrease in reactive astrogliosis and GFAP expression compared to the uncoated iridium microwires.

Regarding the possible roles of non-conducting components of probes interacting with brain tissue, to the best of our knowledge, there are no other previous studies examining this interaction specifically. We speculate that unmodified insulating parts of the silicon microelectrode probes might be recognized as foreign materials and lead to astrogliosis. It is also possible that other factors, such as probe dimension and mechanical properties, might have greater effects on GFAP expression levels than the surface properties. Hence, future work should include *i)* a more precise immunohistological analysis of neuronal and glial cell interactions with electrode coatings and insulating pads in vivo, *ii)* functional studies of the poly(PyHA)-coated neural probes with respect to electrical connectivity after long-term implants, and *iii)* systematic studies on biomimetic coatings of probes, for instance, partial coating versus complete coating of neural probes and the effects of coating thickness on electrical performance and brain tissue reactions.

Conclusions

In this study, we electrochemically coated electrode surfaces with a non-cell adhesive and biocompatible poly(PyHA) to improve brain tissue compatibility. The effectiveness of the poly(PyHA) coating to minimize astrogliosis was tested in vivo with two different neural electrodes (iridium microwires and silicon microelectrods) for at least three weeks. The modified electrodes presented HA moieties selectively on conducting electrode surfaces and maintained electrical impedances after the coating. Histological studies performed three weeks after implantation into rat cortices revealed significantly attenuated GFAP expression (astrogliosis) from the poly(PyHA)-coated

microwires compared to unmodified iridium microwires. However, no significant differences were found between the poly(PyHA)-coated silicon microelectrode probes and uncoated control microelectrode probes, necessitating further studies on functional electrical sensitivity and the roles of non-conductive pad components in tissue interactions. This novel technique for surface modification of metallic and non-metallic conducting substances can also be extended for use in other applications and medical devices (e.g., stents, biosensors) requiring the need to minimize scarring or tissue reaction.

Notes and references

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