Surface Engineering of Chronic Neural Implants for Enhanced Neuro-integration

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Introduction: Implanted silicon microelectrode arrays (Si-MEAs) are neural implants with great potential to enhance both fundamental knowledge pertaining plasticity and physiology, as well as in the treatment of central nervous system (CNS) trauma by providing single-unit recordings in the adult cortex. One of the greatest obstacles to such potential is the instability of the implant-host interface due to astroglial scarring response. Studies have shown that the instability of the interface is caused by both the initial implant penetrating injury, and the subsequent mechanical mismatch between the implant and tissue that further exacerbate scar tissue and damage. Therefore, a traditional approach, such as drug delivery, might be successful in moderating host response during the initial acute phase, but be inadequate to have chronic functional consequences due to limitations in release duration. The objective of this study was to create an inherently anti-inflammatory surface by chemically immobilizing an anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH) to the implant surface, and evaluating the effect both *in vitro* and *in vivo*.

Materials and Methods: The α-MSH peptide immobilization was achieved by using an aminosilane linker group[1]. For in vitro study, 1cm x 1cm SiO_2 coated Si wafers were used to mimic the neural probes. Briefly, Si samples cleaned with piranha solution were first silanized using (3-aminopropyl)triethoxysilane (APTES). The silanized samples were then reacted with crosslinker *N*-succinimidyl-6-maleimidylhexanoate to introduce maleimide functional groups on the surface. Finally, maleimide-grafted samples were incubated in the peptide solution that allowed the peptide to be covalently immobilized to the surface via the C-terminus cysteine group. Chemical composition of the modified surface was verified by X-ray photoelectron spectroscopy (XPS).

Anti-inflammatory properties of the modified Si surfaces were evaluated in vitro by primary rat microglial culture, prepared from brains of postnatal day 1 Sprague-Dawley rats. Microglial cells (15,000/cm^2) were seeded on the peptide-grafted Si as well as unmodified control Si surfaces, and cultured for 24 hr. Lipopolysaccharide (LPS) was then added to the culture at a concentration of 1 ng/ml to activate microglia. After 24 hr, nitric oxide (NO) production was determined by measuring the accumulated levels of nitrite in the cell culture medium with Griess reagent. The relative expression of pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) from the LPS stimulated microglia culture was measured using reverse-transcription "real-time" quantitative polymerase chain reaction (RT-qPCR).

In vivo anti-inflammatory study was performed using Si microelectrodes, where α-MSH peptide was tethered following procedures described above. Bare and α-MSH immobilized Si microelectrodes were implanted into adult Sprague-Dawley rat brains. Four weeks after surgery, brain tissue response was assessed by immunohisto-chemistry staining with GFAP and ED1, markers for astrocytes and reactive microglia respectively. The tissue sections were also analyzed by in situ hybridization to detect the expression of mRNA transcript of inflammatory cytokines.

Results and Discussion: Results from the in vitro studies showed that α-MSH maintained its biological activity after immobilization, and the grafted Si surface was indeed anti-inflammatory. The level of NO production, an important physiological messenger and effector molecule in neuronal tissue, from microglia cultured on the modified surface was 50% less than the bare control Si. Real time RT-PCR analysis further indicated that, in response to LPS stimulation, a reduction in the expression level of pro-inflammatory cytokine TNF-α was observed for microglial cells growing on the anti-inflammatory surface, opposite to the increase shown by cells cultured on the bare Si surface. From the 4-week in vivo study, a comparison of horizontal tissue sections through the level of the cortex demonstrated that the presence of α-MSH resulted in a significantly reduced microglial response around the interface. The astrocytic response, indicated by the GFAP intensity, was also weakened for the modified implant. Using in situ hybridization, the spatio-temporal distribution of cytokine mRNA expression surrounding the implant was revealed.

Conclusions: Our study suggests that immobilizing anti-inflammatory molecules on the surface of neural implant could provide an effective means to attenuate long-term brain tissue response to chronic neural implants, leading to a stable implant-host interface.

References:

1. Xiao, S. et al. Langmuir 14, 5507-5516 (1998)

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