Surface Conjugation of a Superoxide Dismutase Mimetic to Mitigate Local Neuroinflammatory-Mediated Oxidative Stress Events

Jessica K. Nguyen,[1,2] Kelsey A. Potter-Baker,[1,2] Kyle M. Kovach,[2] Martin M. Gitomer,[1] Tyler W. Srail,[1] Wade G. Stewart,[1] John L. Skousen, [1,2] and Jeffrey R. Capadona[1,2]*

[1]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH
[2]Advanced Platform Technology Center, Rehabilitation R&D, LSCVA Medical Center, Cleveland, OH

Statement of Purpose: Neuroinflammatory-mediated oxidative stress events cause intracortical microelectrodes to fail chronically due to a variety of mechanisms, such as inducing corrosion of metallic contacts and degradation of insulation material, reducing blood brain barrier integrity, and directly damaging surrounding neurons.[1] We have shown that antioxidant treatment can reduce neuroinflammatory-mediated oxidative stress events and preserve surrounding neurons.[2,3] However, short-term systemic delivery was unable to sustain these benefits due to low bioavailability and fast clearance rates. Therefore, we utilized an antioxidant coated surface to provide sustained neuroprotection. Superoxide dismutase (SOD) mimetics have been previously shown to reduce formation of reactive oxygen species (ROS) and improve cell viability.[4] Here, we developed a surface functionalized with a commercially-available SOD mimetic, Mn(III)tetrakis(4-benzoic acid)porphyrin (MnTBAP), and investigated anti-oxidative capacity *in vitro*.[5]

Methods: MnTBAP molecules were immobilized onto aminated glass coverslips via NHS/EDC conjugation. Surface characterization was determined via contact angle and XPS analysis. Assessment of antioxidant activity used a nitrotetrazolium blue/riboflavin (NBT/RF) assay. Modified MnTBAP surfaces were tested *in vitro* with microglia cells. First, cytotoxicity of the surfaces was assessed via Live/Dead assay. Then, after exposing surfaces to microglia cells, intracellular and extracellular ROS were assessed via DHE and a modified NBT utilizing cell media, respectively.

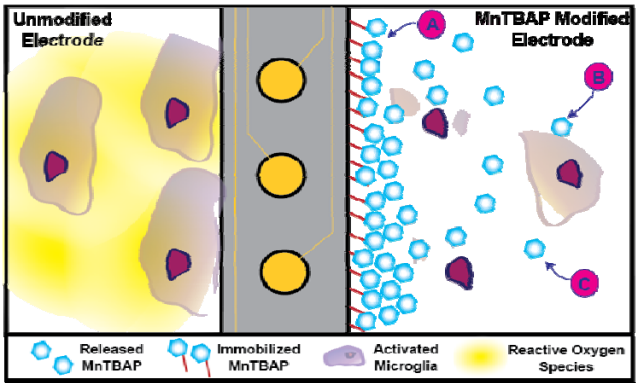

Figure 1. Mechanism of action for MnTBAP modified surfaces. Unmodified microelectrodes induce accumulation of ROS from activated microglia. MnTBAP modified surfaces neutralize ROS using three mechanisms. Immobilized MnTBAP reduces ROS at the surface of the electrode (A). An adsorbed layer of MnTBAP releases into the tissue space where MnTBAP can be internalized by microglia to reduce intracellular ROS (B) or diffuses through the tissue to reduce extracellular ROS (C).[5]

Results: Contact angle and XPS analysis of MnTBAP functionalized surfaces confirmed addition of MnTBAP onto glass coverslips. For our system, we utilized a combined surface layer of adsorbed and immobilized MnTBAP. The benefit of this system is an initial burst release neutralizes the high influx of ROS that occurs immediately after device implantation while the immobilized layer sustains activity to reduce chronic neuroinflammation. Assessment of antioxidant activity with the NBT/RF assay demonstrated that our surfaces were able to maintain antioxidant activity within our targeted therapeutic range. Further, microglia cells cultured on our surfaces confirmed cell viability and reduction of ROS both intracellularly and extracellularly compared to glass controls.

Conclusions: Microelectrodes for neural recording have limitations due, in part, to neuroinflammatory-mediated oxidative stress effects. Anti-oxidative approaches have the ability to mitigate these effects and protect neuron viability for long-term recording. Here, we developed an anti-oxidative surface coating to sustain oxidative stress effects. Our MnTBAP functionalized surface neutralizes ROS via multiple mechanisms **(Fig. 1)**. The antioxidant activity was sustained over time and reduction of ROS was seen intra- and extra-cellularly in *in vitro* studies. These results show the promise of this surface coating to improve the lifespan of neural electrodes and other implanted biomedical devices susceptible to ROS damage, such as hip implants and cardiac leads. Future studies will investigate the utility of MnTBAP modified surfaces to reduce neuroinflammatory events *in vivo*.

References:

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