31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Effects of adsorbed proteins, an antifouling agent and long-duration DC voltage pulses on the impedance of silicon-based neural microelectrodes

Salah Sommakia, Jenna L. Rickus, and Kevin J. Otto, *Member, IEEE*

Abstract—**The successful use of implantable neural microelectrodes as neuroprosthetic devices depends on the mitigation of the reactive tissue response of the brain. One of the factors affecting the ultimate severity of the reactive tissue response and the *in vivo* electrical properties of the microelectrodes is the initial adsorption of proteins onto the surface of the implanted microelectrodes. In this study we quantify the increase in microelectrode impedance magnitude at physiological frequencies following electrode immersion in a 10% bovine serum albumin (BSA) solution. We also demonstrate the efficacy of a common antifouling molecule, poly(ethylene glycol) (PEG), in preventing a significant increase in microelectrode impedance. In addition, we show the feasibility of using long-duration DC voltage pulses to remove adsorbed proteins from the microelectrode surface.**

I. Introduction

Implantable neural electrodes have the potential to treat a multitude of neurological disorders such as deafness, blindness and paralysis. Their successful chronic implementation, however, is hampered by the reactive response of the brain tissue culminating in the formation of a glial scar. The glial scar surrounds the implanted electrode, physically isolating it from the rest of the brain tissue, and electrically insulating it, resulting in increased impedance and lower signal to noise ratio (SNR) values[1]. This deterioration in electrode performance often leads to the failure of the implanted electrode as a viable neuroprosthesis.

The reactive tissue response begins from the moment the implanted electrode pierces the brain tissue, rupturing microcapillaries, disrupting the blood-brain barrier (BBB) and lysing cells. This results in the extravasation of blood components into the brain and the accumulation of cellular and myelin debris at the implantation site. Most electrode materials are prone to protein adsorption, also referred to as biofouling, which can ultimately affect the biocompatibility of the implanted electrode[2]. This debris, in addition to macrophages from the ruptured vasculature and extravasated proteins like thrombin and fibrin, adsorb onto the surface of the electrode resulting in cytokine and chemokine gradients around the electrode. These gradients trigger various signaling cascades, leading to the influx and activation of microglia and astrocytes. Activated microglia form the central core of the chronic glial scar[3, 4], while tightly bound activated astrocytes form the thick outer layer of the glial scar[5-7]. This is usually accompanied by a gradual loss of neural density and signal strength over time[8, 9].

While the exact mechanisms guiding the progression of the reactive tissue response are not well known, it has been suggested that the intensity of the chronic glial scar is related to the amount of initial injury inflicted upon the brain tissue during electrode insertion[10]. This is an important point because most neurointegrative approaches currently under investigation attempt to modulate later stages of the glial scar formation, either by attempting to reduce astrocyte activation or attract neuronal growth in close proximity to the implanted electrode. There are few reports in the current literature of attempts to modulate the severity of the glial scar by intervening at the earliest stages of the injury, and none attempting to suppress biofouling in the brain. In addition to their role in driving the reactive tissue response of the brain to implanted electrodes, adsorbed proteins can contribute to the decline of device performance[11].

One approach that has demonstrated its efficacy at restoring SNR and lowering impedance for a brief time following the formation of the glial scar is the application of DC voltage pulses with relatively large amplitudes for a few seconds[12, 13]. The exact cellular mechanism of this process, termed rejuvenation, is not completely understood, but it may involve a temporary loosening of the tight junctions between the activated astrocytes in the glial scar. One likely effect of electrode rejuvenation could be the induction of protein desorption off the electrode surface with the application of these voltage pulses.

In this paper, we investigate the effects of immersion in a protein solution on the impedance magnitude of neural microelectrodes. We adapt the rejuvenation procedure as part of the electrode cleaning process, in a step we term anodic cleaning, showing that the induction of

This work was supported by the Purdue Research Foundation.

S. Sommakia is with the Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47906 USA (e-mail: ssommaki@purdue.edu).

J. L. Rickus is with the Weldon School of Biomedical Engineering and the Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47906 USA (email: rickus@purdue.edu).

K. J. Otto is with the Weldon School of Biomedical Engineering and the Department of Biological Sciences, Purdue University, West Lafayette, IN 47906 USA (email: kotto@purdue.edu).

protein desorption is a valid explanation for the efficacy of electrode rejuvenation. In addition, we investigate the effects of an antifouling material, poly(ethylene glycol) (PEG), on the impedance magnitude of the microelectrodes in the protein solution. PEG is a hydrophilic molecule known for its antifouling properties[14] as well as its surfactant properties which have been employed to induce membrane sealing and promote neuron healing following traumatic brain injury[15]. Our results indicate the potential for the use of PEG to reduce protein adsorption on the surface of implanted neural microelectrodes with the goal of reducing the intensity of the chronic glial scar.

II. Materials and Methods

A. Microelectrodes

The microelectrodes employed in this study were silicon-substrate microelectrode arrays provided by the Center for Neural Communication Technologies (CNCT) at the University of Michigan. The arrays consisted of a single silicon shank with 16 iridium electrodes with a diameter of 40 µm spaced 100 µm apart. The electrode shanks had a length of 1 mm, a thickness of 15 µm, and a width ranging from 100 µm at the base to 25 µm at the tip. A total of 3 electrodes was used for this study.

B. Electrochemical measurements

Electrochemical measurements were made using an Autolab potentiostat PG-STAT12 with a built-in frequency response analyzer [13]. A three-electrode cell configuration was used with the microelectrode site functioning as the working electrode (WE), a large-area Pt wire functioning as the counter electrode (CE), and an Accumet, gel-filled, KCl saturated calomel electrode [16] (Thermo Fischer Scientific, Fair Lawn, NJ) functioning as the reference electrode (RE).

Cyclic voltammetry (CV) was performed by sweeping the applied voltage from -0.6 to +0.8 V at a scanning rate of 1 V/s to determine sites with broken or poor connections, designated as sites exhibiting a maximum current below 1 nA. These sites were discarded from the analysis thus yielding a total of 30 functional electrode sites on 3 different electrodes.

Electrochemical impedance spectroscopy (EIS) was used to measure the impedance of the electrode sites with the application of 16 sequentially applied sinusoidal waves at logarithmically spaced frequencies ranging from 46 Hz to 10kHz, with an amplitude of 25 mV_{RMS}. For each electrode, EIS was performed first in a 1X phosphate buffered saline (PBS) with a concentration of 154 mM NaCl, 5.8 mM NaH_2PO_4 and 1.1 mM KH_2PO_4PBS. Following that, the electrodes were immersed in a 10% solution of bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) in PBS, the concentration of which was chosen to mimic protein concentration in rat cerebral cortex[17], and EIS measurements were run after a five minute interval. The electrodes were then cleaned with ddH2O as recommended by CNCT. This was followed by a round of anodic cleaning where a DC potential of 1.5 V was applied for a duration of 10-15 seconds. The electrodes were then treated with the antifouling agent before being immersed in BSA again for 5 minutes and submitted to further EIS measurements. Changes in the impedance were then evaluated using student's t-tests with $p < 0.05$ indicating statistically significant differences between groups.

C. Antifouling agent

To block the adsorption of proteins, the electrodes were dipped in a 50% solution of poly(ethylene glycol) (PEG, Sigma-Aldrich, St. Louis, MO) with a molecular weight of 3392 for one minute and then left to dry for an additional minute before immersion into the BSA solution. To mimic a topical application of PEG, an additional 0.1 mL of PEG was applied with a Pasteur pipette to the electrode shank as it entered the BSA solution.

III. Results

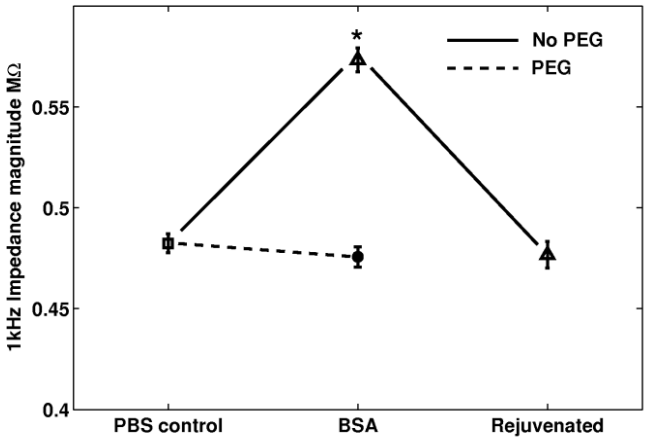

Fig. 1: The immersion of the electrode shank in a 10% BSA solution resulted in a statistically significant (p<0.05) 19% increase in the mean impedance magnitude at the physiologically relevant frequency of 1 kHz (BSA open triangle). The application of PEG to the electrode prior to immersion in BSA prevented the increase of the impedance magnitude (filled circle). The impedance magnitude of untreated electrodes was returned to baseline levels using anodic cleaning (Rejuvenated open triangle), the application of long duration DC voltage pulses. Error bars represent standard error measurements.

A. Effects of electrode immersion in BSA solution

EIS measurements demonstrate a statistically significant increase (p<0.05) in the mean impedance magnitude at the physiologically relevant frequency of 1 kHz following immersion in the BSA solution. The increase is roughly 19%, from 0.48 MΩ in PBS controls to 0.57 MΩ for electrodes immersed in the 10% BSA solution, as shown in Fig. 1.

Analysis of the EIS spectrograms obtained from the PBS controls and electrodes immersed in the BSA solution reveals an increase in the impedance magnitude across the frequency spectrum in BSA solution compared to PBS with a larger increase at the lower frequencies, as shown in Fig. 2. These results indicate a change in the electrochemical properties of the electrodes due to protein adsorption onto the surface of the microelectrode sites.

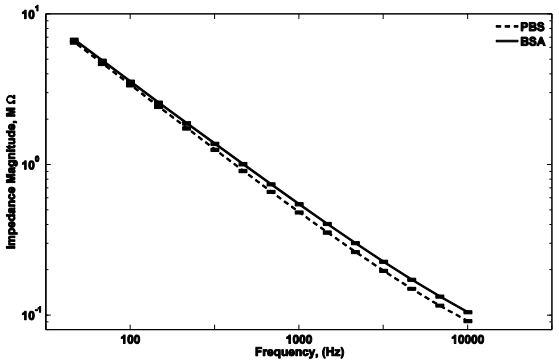

Fig. 2: Impedance magnitude spectrograms obtained from electrodes in PBS (dashed line) and BSA solution (solid line) in a log-log scale. The spectrograms demonstrate increased impedance magnitude in the BSA solution across the frequency spectrum.

B. Effects of PEG on electrode impedance

When PEG was applied to the microelectrodes prior to immersion in the BSA solution, the resultant change in impedance at the physiologically relevant frequency of 1kHz was not statistically significant, as shown in Fig. 1. Comparison of the EIS spectrograms of the PBS controls and PEG-coated electrodes in the BSA solutions reveals a less pronounced change in the impedance magnitude compared to the uncoated electrodes characterized in the PEG solution. Examination of the spectrograms shows a slight decrease in impedance magnitude at the lower end of the frequency spectrum that is not observable towards the higher end of the frequency spectrum, as shown in Fig. 3.

These results indicate the effectiveness of the applied PEG coating in blocking the adsorption of BSA proteins onto the microelectrode sites, preventing a significant increase in the measured impedance magnitude at 1kHz.

C. Effects of anodic cleaning on electrode impedance

After removing the electrode from the BSA solution, it was cleaned with ddH_2O, as recommended by the manufacturer, and then subjected to anodic cleaning. As observed in Fig. 1, the impedance magnitude of microelectrodes immersed in BSA solution increases compared to the PBS controls but is returned to baseline impedance magnitudes levels following the application of the DC voltage pulses during the anodic cleaning procedure. This indicates the removal of the adsorbed proteins from the microelectrode surface under the influence of the DC voltage pulses applied to the microelectrodes.

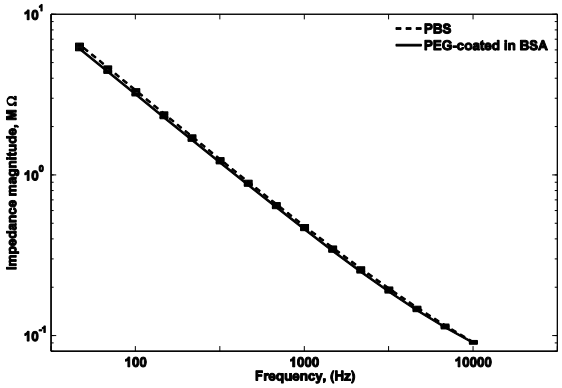

Fig. 3: Impedance magnitude spectrograms for electrodes in PBS (dashed line) and BSA solution after coating with PEG (solid line) in a log-log scale. The spectrograms show a slight decrease in the impedance magnitude in the BSA solution with the PEG coating at the lower frequencies. This decrease in impedance is not obvious at higher frequencies. The spectrograms indicate that the PEG coating has modulated the degree of protein adsorption onto the surface of the electrode sites.

IV. Discussion and Conclusions

Our results show a quantifiable, statistically significant increase in impedance magnitude following the immersion of the microelectrodes in a BSA solution with a concentration mimicking natural protein concentration in the cerebral cortex. Our current setup investigates the effects of BSA proteins on the impedance magnitude of the microelectrodes after immersion for a short period of time. Since the brain contains different proteins, the precise changes in impedance magnitude as well the timeline will differ from our *in vitro* results. The various chemical structures and binding affinities will likely have a great effect on the changes in impedance magnitude and the adsorption profile.

Several issues need to be considered when designing a neurointegrative approach incorporating an antifouling agent like PEG to counter protein adsorption. In this study, we used two concurrent delivery methods: the simple adsorption of PEG onto the electrode surface and the application of liquid PEG directly on the surface of the BSA solution in proximity to the immersed electrode shank, to simulate topically applied PEG in an *in vivo* setting. Given the high water solubility of PEG, it is expected that it will dissolve in the BSA solution fairly rapidly and diffuse away from the electrode, although the speed at which the dissolution and diffusion might occur could be tempered by the proteins present in the BSA. Depending on the interactions between the diffusing PEG and the proteins, the effects of PEG in blocking protein adsorption might last for a few hours.

Future studies will examine PEG stability to determine how long the effects of PEG applied as described

in this paper will last. If the goal is to solely block the adsorption of proteins during the initial injury phase, then a time window of a few hours may suffice since after that time various cells involved in the reactive tissue response will have arrived at the electrode surface. If, however, the goal is block the adsorption of other inflammatory components, such as activated microglia, then an effective long-term approach would likely involve the permanent immobilization of PEG directly onto the surface of the electrode sites. In this case, a binding platform, such as a thin-film silica sol-gel, may prove useful [18].

The *in vivo* effects of rejuvenation protocols on adsorbed proteins are still not well understood. The induction of protein desorption *in vivo* is likely only a partial explanation for the *in vivo* success of electrode rejuvenation. A deeper understanding of the role of the binding affinities of proteins to the microelectrode surface and adsorption timelines is necessary to elucidate the mechanisms underlying the temporary efficacy of *in vivo* electrode rejuvenation in restoring microelectrode functionality. In our experimental setup, the anodic cleaning procedure was only efficient when performed out of the BSA solution and in PBS. We were unable to observe a rejuvenating effect when the DC voltage pulses were applied to electrodes immersed in the BSA solution. A possible explanation is that the BSA proteins have high binding affinities to our electrode site material resulting in rapid adsorption onto the already rejuvenated/anodically cleaned sites before the procedure is complete for all electrode sites. It is worth noting that the ddH_2O rinse by itself did not reduce the impedance of the electrodes significantly.

In conclusion, we have presented a quantification of the effects of surface adsorbed proteins on the impedance magnitude of neural microelectrodes *in vitro*. We have shown that the protein adsorption is preventable through the use of an antifouling molecule, PEG. We have demonstrated the possibility of using long-duration DC voltage pulses to remove the adsorbed proteins and restore baseline impedance magnitudes.

V. Acknowledgment

The authors would like to thank the Center for Neural Communication Technology for providing the silicon microelectrode arrays (NIH/NIBIB P41-EB002030),

References

[1] R. Vetter, J. Williams, J. Hetke, E. Nunamaker, and D. Kipke, "Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex," *IEEE Transactions on Biomedical Engineering*, vol. 51, (no. 6), pp. 896-904, 2004.

[2] D. Williams, I. Askill, and R. Smith, "Protein adsorption and desorption phenomena on clean metal surfaces," *Journal of biomedical materials research*, vol. 19, (no. 3), 1985.

[3] V. Polikov, M. Block, J. Fellous, J. Hong, and W. Reichert, "In vitro model of glial scarring around neuroelectrodes chronically implanted in the CNS," *Biomaterials*, vol. 27, (no. 31), pp. 5368-5376, 2006.

[4] R. Biran, D. Martin, and P. Tresco, "Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays," *Experimental Neurology*, vol. 195, (no. 1), pp. 115-126, 2005.

[5] D. Edell, V. Toi, V. McNeil, and L. Clark, "Factors influencing the biocompatibility of insertable silicon microshafts in cerebral cortex," *IEEE Transactions on Biomedical Engineering*, vol. 39, (no. 6), pp. 635-643, 1992.

[6] J. Turner, W. Shain, D. Szarowski, M. Andersen, S. Martins, M. Isaacson, and H. Craighead, "Cerebral astrocyte response to micromachined silicon implants," *Experimental Neurology*, vol. 156, (no. 1), pp. 33-49, 1999.

[7] J. Fawcett and R. Asher, "The glial scar and central nervous system repair," *Brain Research Bulletin*, vol. 49, (no. 6), pp. 377-391, 1999.

[8] M. Nicolelis, D. Dimitrov, J. Carmena, R. Crist, G. Lehew, J. Kralik, and S. Wise, "Chronic, multisite, multielectrode recordings in macaque monkeys," *Proceedings of the National Academy of Sciences*, vol. 100, (no. 19), pp. 11041-11046, 2003.

[9] X. Liu, D. McCreery, R. Carter, L. Bullara, T. Yuen, and W. Agnew, "Stability of the interface between neural tissue and chronicallyimplanted intracortical microelectrodes," *IEEE Transactions on Rehabilitation Engineering*, vol. 7, (no. 3), pp. 315-326, 1999.

[10] D. Szarowski, M. Andersen, S. Retterer, A. Spence, M. Isaacson, H. Craighead, J. Turner, and W. Shain, "Brain responses to micro-machined silicon devices," *Brain Research*, vol. 983, (no. 1-2), pp. 23-35, 2003.

[11] N. Wisniewski, F. Moussy, and W. Reichert, "Characterization of implantable biosensor membrane biofouling," *Fresenius'Journal of Analytical Chemistry*, vol. 366, (no. 6), pp. 611-621, 2000.

[12] K. Otto, M. Johnson, and D. Kipke, "Voltage pulses change neural interface properties and improve unit recordings with chronically implanted microelectrodes," *IEEE Transactions on Biomedical Engineering*, vol. 53, (no. 2), pp. 333-340, 2006.

[13] M. Johnson, K. Otto, and D. Kipke, "Repeated voltage biasing improves unit recordings by reducing resistive tissue impedances," *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, vol. 13, (no. 2), pp. 160-165, 2005.

[14] N. Alcantar, E. Aydil, and J. Israelachvili, "Polyethylene glycol-coated biocompatible surfaces," *Journal of biomedical materials research*, vol. 51, (no. 3), pp. 343-351, 2000.

[15] A. Koob, B. Duerstock, C. Babbs, Y. Sun, and R. Borgens, "Intravenous polyethylene glycol inhibits the loss of cerebral cells after brain injury," *Journal of neurotrauma*, vol. 22, (no. 10), pp. 1092-1111, 2005.

[16] M. San, Demirta, scedil, R. Burgut, A. Birand, Ba, lami, and F. li, "Left Ventricular Systolic and Diastolic Functions in Patients With Sickle Cell Anemia," *Int J Angiol*, vol. 7, (no. 3), pp. 185-7, May 1998.

[17] M. Banay-Schwartz, A. Kenessey, T. DeGuzman, A. Lajtha, and M. Palkovits, "Protein content of various regions of rat brain and adult and aging human brain," *Age*, vol. 15, (no. 2), pp. 51-54, 1992.

[18] A.L. Pierce, S. Sommakia, J.L. Rickus, and K.J. Otto, "Thin-film silica sol-gel coatings for neural microelectrodes," *Journal of Neuroscience Methods*, vol. 180, (no. 1), pp. 106-110, 2009.