Strain-induced scarring and its effects on microelectrodes

During the award period of this grant we made significant progress toward developing coatings for electrodes for improved neural recordings.

The Specific Aims as listed in the original grant are:

Specific Aim 1: Determine how strain modulates the production of inflammatory cytokines and scar-associated chondroitin sulfate proteoglycans.

Specific Aim 2: Determine whether strain-induced scarring within organotypic hippocampal slice cultures affects the recording stability of Si-microelectrodes.

Specific Aim 3: Develop coatings for Si-microelectrodes that locally release methylprednisolone (anti-inflammatory agent), and investigate their influence on scar formation and recording stability in organotypic slice cultures.

The specific aims have been slightly modified from the original proposal in that instead of using organotypic slice cultures to test our coatings and recording stability, we decided it was best to do this *in vivo* in the rat cortex. We generally believe that the *in vivo* cortical 'test-bed' is more rigorous than the organotypic slice cultures originally proposed. Additionally, it is more clinically relevant to test the hypothesis directly *in vivo*. None of the goals of the project were changed as a result, rather only the test bed, which in our opinion, was enhanced.

Some of the research plans have been modified, as obtaining a quantitative measure of recording stability from organotypic hippocampal slice cultures was problematic in many ways. Besides the initial startup effort of maintaining viable slices for many weeks, we were also faced with simulating the injury and presence of an inserted silicon electrode. In view of major efforts we had made towards a solution, a reevaluation of this approach was undertaken. Since our ultimate goal is *in vivo* testing, the decision was made to discontinue work toward developing an *in vitro* "test bed," and to focus our efforts on *in vivo* experiments. For *in vivo* recordings, we have purchased and are waiting for the arrival of a Plexon 16-channel recording system. This system will provide the capability to record neuronal activity from Michigan chronic electrodes. So our hypothesis will be directly tested in an *in vivo* system as opposed to the hippocampal slice culture based organotypic model as we originally described in our proposal.

The research conducted over the grant period has resulted in the preparation, submission, and publication of several manuscripts. The list follows:

1. Zhong Y, **Bellamkonda RV**. 2005. Controlled Release of anti-inflammatory agent alpha-MSH from Neural Implants. Journal of Controlled Release 106(3):309-18.
2. Lee HJ, **Bellamkonda RV**, Sun W, Levenston ME. 2005. Biomechanical analysis of silicon microelectrode induced strain in the brain. Journal of Neural Engineering 2(4):81-9.
3. He W, McConnell G, **Bellamkonda RV**. 2006. Nanoscale laminin coating modulates cortical scarring response around implanted silicon microelectrode arrays. Journal of Neural Engineering, 3: 316–326.

4. McConnell G, Schneider TM, Owens DJ, **Bellamkonda RV**. 2007. Extraction Force and Cortical Tissue Reaction of Silicon Microelectrode Arrays Implanted in the Rat Brain. IEEE Transactions on Biomedical Engineering 54(6 Pt 1):1097-107.

5. Zhong Y and **Bellamkonda RV**. 2007. Dexamethasone Coated Neural Probes Elicit Attenuated Inflammatory Response and Neuronal Loss Compared to Uncoated Neural Probes. Brain Research 1148:15-27.

6. Zhong Y and **Bellamkonda RV**. 2008. Biomaterials for the central nervous system. Journal of the Royal Society Interface, 5:957.

7. McConnell G, **Bellamkonda RV**. 2009. Implanted neural electrodes cause chronic, local inflammation that is correlated with local neurodegeneration. *Accepted, Journal of Neural Engineering*.

8. McConnell GC, Butera RJ, **Bellamkonda RV**. 2009. Bioimpedance modeling to monitor astrocytic response to chronically implanted electrodes. Journal of Neural Engineering 6:055005.

Our achievements on this project were primarily on two fronts. 1) We designed and developed novel coatings for implantable electrodes to promote integration and decrease scarring, without interfering with the electrical properties of the electrodes (see 1-6 below); and 2) we developed novel means of non-invasively monitoring tissue response to implanted materials (see 7-11 below).

Descriptions of the findings are below:

1. Presence of chronically implanted electrodes causes local inflammation and neurodegeneration (publication #11)

We have recently demonstrated that the chronic presence of implanted electrodes, but not acute injury, causes a persistent inflammation around the electrodes which in turn leads to a neurodegenerative state around the electrode. This finding is novel and has profound implications for feasibility of chronic recording from implanted electrodes. It is important to note that reliable chronic recordings are a necessary and integral part of successful development of neuroprosthetics.

2. Anti-inflammatory dexamethasone releasing coatings (publications #2 and 7)

We developed a novel nitrocellulose-based coating for the sustained local delivery of the anti-inflammatory drug dexamethasone, a synthetic glucocorticoid that effectively reduces inflammation in the CNS. The coating released bioactive dexamethasone over a period of 16 days and caused a reduction in inflammation as shown by reduction in staining for reactive astrocytes, reactive microglia, and chondroitin sulfate proteoglycans. The coating was found to effectively reduce scar tissue formation without adversely affecting the electrical performance of the electrodes.

3. PEI and laminin adsorption for neuro-adhesive coatings (publications #1 and 5)

We developed a coating to support neuron adhesion and growth through the physical adsorption of laminin. Using the layer-by-layer (LbL) technique, alternating layers of laminin and polyethyleneimine (PEI) were absorbed onto the electrodes. *In vitro*, neurons attached to the surface and had long neurites creating a dense neuronal network. Electrostatic LbL assembly enables nanoscale bioactive coatings, and PEI–LN multilayers significantly enhance

cortical neuronal attachment and differentiation *in vitro* with no deleterious effects on impedance of the electrodes. Further, this LbL coating could be applied on top of the nitrocellulose-based dexamethasone coatings for a combinatorial approach. *In vivo*, the coating reduced inflammation after 4 weeks.

4. Anti-inflammatory α-MSH releasing coatings (publication #3)

We developed a novel nitrocellulose-based coating for the sustained delivery of the anti-inflammatory neuropeptide, α-melanocyte stimulating hormone (α-MSH). Sustained release was attained over 21 days *in vitro*, and the α-MSH released on day 21 was still bioactive and successfully inhibited nitric oxide (NO) production by LPS-stimulated microglia. The release rate can be tailored by varying the amount of drug initially loaded, with higher initial loading increasing the mass released but not the percent of drug released. These coatings have the potential to reduce inflammation at the electrode–brain interface *in vivo*, and facilitate long-term recordings from Si-multi-electrode arrays without adversely affecting the electrical performance of the electrodes.

5. Immobilized α-MSH coatings (publication #9)

To address the chronic phase of the inflammatory response due to the continuing presence of the electrode, we developed a method to intrinsically modify the electrode surface to render it anti-inflammatory. α-MSH was selected as the agent because of its known anti-inflammatory effect on monocytes, macrophages, as well as microglial cells. Electrical performance of the electrodes was preserved with the coatings, and the coatings led to a significant reduction of reactive microglia and macrophages around the peptide-tethered probes compared to uncoated probes at both 1 week and 4 weeks *in vivo*.

6. Evaluating recording stability in primates

During the grant period, we established a base-line for primate chronic electrode studies. Chronic electrodes were implanted in the cerebral cortex, and in some of the implants, the exposed tissue was treated with dexamethasone administered in the fluid surrounding the arrays, in agar above the cortex or in one case from a gel coating applied to the microwires. There seems to be no clear detriment to the use of dexamethasone chronically applied to the peri-electrode tissue. The dexamethasone-gel microwires recorded unit activity for several months and seemed to perform as well or better than non-treated microwires. However, at this point, there is too much variability in the non-coated controls and not enough treated microwire experiments to make a claim about the benefits of the treatment.

Unfortunately, we did not test the coated electrodes in the primate model for several reasons. First, we were unable to test recording properties in rats due to difficulty in coating functional electrodes (which are more fragile than the non-functional electrodes because of the ribbon cable). There was also a large variability of tissue response with the chronic recording functional electrodes (presumably due to the exposed connector), which would require larger sample sizes, which would not be desirable with the primate model. Another complication encountered was the need to insert the functional electrodes by hand (due to their more fragile nature compared to the non-functional electrodes), whereas we were able to standardize the implantation techniques for the non-functional electrodes by using a stereotactic frame.

7. Non-invasive evaluation of coating performance using impedance spectroscopy (publication #10)

Several techniques were evaluated as candidates for the non-invasive evaluation of the coatings' performance, including optical coherence tomography (OCT) and impedance spectroscopy. OCT was not compatible with our application because of its inability to image through the blood (red blood cells scattered the light). However, impedance spectroscopy was able to non-invasively quantify cellular concentration and GFAP expression in the immediate vicinity of the electrode. We therefore pursued the measurement of *Py* value as a fast measurable, and easily interpretable, parameter to predict changes in inflammation surrounding microelectrodes. The electrical properties of the scar tissue are a function of the scar's molecular/cellular makeup. Our results corroborate previously reported correlations between impedance spectroscopy and histology. Particularly, the more reactive the response to microelectrode, the more pronounced the semi-circular arc in the spectrum at high frequencies.

8. Force extraction measurement of neuro-integration (publication #6)

A technique was developed to determine the extent of neuro-integration through correlating relative integration to the force required to extract the electrode. Over four weeks, the extraction force increased with respect to time after electrode implantation. This technique is useful in determining the extent of mechanical integration of the electrode when evaluating the effectiveness of electrode coatings and also to inform modeling studies that estimate strain maps around implanted electrodes.

9. Finite Element Method (FEM) modeling of electrode-brain interaction (publication #4)

FEM was used to model the extent of strain in the cortex due to micromotion of the electrode relative to the brain. Our analysis demonstrated that when physical coupling between the electrode and the brain increased, the magnitude of micromotion-induced strain of tissue around the electrode decreased as did the relative slip between the electrode and the brain, thus validating the approach of developing neuro-integrative coatings.

10. Image analysis technique (publications #2, 5-11)

An image analysis technique was developed for analysis of the tissue probe interface using custom software in MATLAB. For each image, a point was manually selected within the probe site and an edge detection algorithm was used to locate the probe-tissue interface. This edge functioned as the zero point for all further distance calculations. Equidistant, equiangular radial lines (n = 120) were drawn around the edge-detected interface and the mean vector was calculated for each image. The average integrals of the 'mean intensity versus distance from probe site' vectors for all images were also used in comparing coated and control conditions. This technique proved valuable in determining staining intensity around the probe site and the extent of inflammation as a function of distance from the site. This technique has been adopted for other applications in the laboratory as well.

11. Neuronal density dependent upon depth in the cortical column (publication #8)

Neuronal density and proximity to recording electrodes are good indicators of recording longevity from chronically implanted microelectrodes. We therefore studied the effect of depth to neuronal density and found that neuronal density in the cortex responds differently to injury based on the location within the cortical column. This data highlights the need to account for depth information in evaluating tissue reaction to microelectrode implantation.

In conclusion, we have made significant progress toward the aims, developing several potentially viable coatings for electrodes capable of preventing inflammation and encouraging

integration, bringing us closer to having reliable, long-term recordings. Furthermore, we developed the means to non-invasively monitor the effectiveness of the coatings *in vivo*, which is a valuable tool for evaluating compatibility and integration of implanted electrodes.