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GFAP Inflammatory Response to Dextran-Coated Silicon Electrodes

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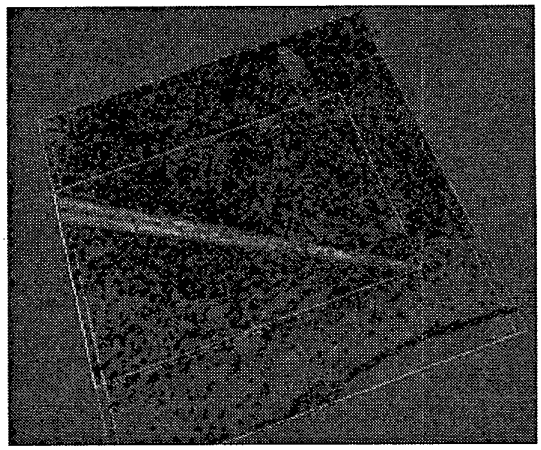
Abstract-**Recent advances in three-dimensional, confocal imaging techniques and image analysis software allow for previously unattainable measurements in the neural environment. Specifically, relative quantitative measurements of the amount of glial fibrillary acidic protein (GFAP) surrounding a neural implant can be determined. In this study, dextran coatings have been applied to silicon neural implants. Dextran coatings have been shown to limit non-specific cell adhesion *in vitro*. Thus, it is believed that these coatings will limit the amount of gliosis surrounding a neural implant and ultimately improve signal-to-noise ratio. To test this hypothesis, Sprague-Dawley rats were implanted with both dextran-coated and non-dextran-coated silicon electrodes. Using immunohistochemical staining in conjunction with confocal imaging and image analysis techniques, the differences in GFAP upregulation between dextran-coated and non-treated silicon neural implants were investigated.**

Keywords-**GFAP, astrocyte, confocal, neural, imaging, gliosis**



I. INTRODUCTION

It has been demonstrated that astrocytes are the primary component of the glial sheath that forms during gliosis in response to a penetrating neural injury [1-4]. Furthermore, it is this sheath that is the ultimate cause for the failure of electrodes for the recording of single unit neural activity [1]. The ability to measure this sheath would be a valuable tool that could help researchers better understand the formation of this sheath and develop methods to stop to stop it from forming

As confocal imaging systems become a more prevalent method for analyzing cellular and tissue features in both *in vivo* and *in vitro* systems, including those for assessing biocompatibility, it would be beneficial to quantify some of the data acquired from the images. Until recently, qualitative observations on the fluorophors imaged via confocal systems were all that was possible. However, advances in image processing found in software packages like ImarisSurpass from Bitplane AG allows for the segmentation of surface rendered volumes. Figure 1 shows an example of a volume model of glial fibrillary acidic protein (GFAP) surrounding a neural implant. As a result of this segmentation, the measurement of their subsequent internal volumes is possible. With careful methodologies and analysis techniques one can add some measure of quantification to confocal volume data by standardizing the data so that relative quantitative measurements can be made. Using these techniques, the volumetric change in the up-regulation of GFAP in rat CNS tissue was investigated. This study focused the implantation of silicon electrodes to determine the difference in the GFAP inflammatory response to dextran-coated versus control neural implants.

II. METHODOLOGY

Six Sprague-Dawley nts were surgically implanted with non-functional silicon electrodes as per Rousche *et al.* [5]. Prior to implantation electrodes were coated with dextran using methods modified from Massia *et al* [6]. At a 2-week time point, the rats were euthanized via overdose injection of sodium pentobarbital and perfusion fixed with 4% paraformaldehyde in physiological saline as per the protocol by Turner and Shain [1].

A vibrating microtome was used to cut 125 ?m thick sections of brain tissue. Tissue sections were stained for GFAP according to a modified protocol described by Turner and Shain [1]. Sections were imaged on a Leica TCS SP2 spectral confocal microscope. The images were cropped in the z-dimension to ensure uniformly stained sections were being analyzed. Next, using Bitplane's Imaris? software, a series of individual images 25 ?m in width were created adjacent to the implant site. Then, Bitplane's ImarisSurpass volume rendering software was used to measure the volume of the GFAP in the individual images using precisely the same criteria for each image. The volume of GFAP from an image a given distance from the implant site was used as a control. Each image was normalized according to this control. The volume per distance from the implant site was plotted in Excel and an equation was fit to the curve to determine the slope of the

line. Comparisons were made between the responses.

III. RESULTS AND DISCUSSION

Analysis of the tissue sections shows that the reaction curves are similar regardless of the specimen thickness (x-z plane). Thus, the controls placed on data acquisition appear to make quantitative comparisons justifiable. (Figure 2 shows an example of the response of two implants.)

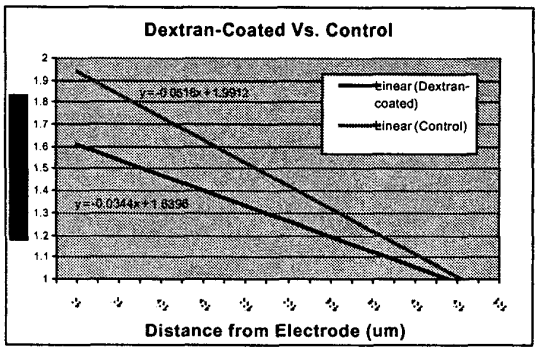


Figure 2. Inflammatory response of a dextran-coated versus non-dextran coated silicon electrode.

IV. CONCLUSION

This study points out the limitations of quantitative measurements from confocal microscope data on biological tissue. However, it is further demonstrated that with careful controls, it is possible to make quantitative comparisons of data relating to physical volumes if and only if acquisition and analysis parameters are kept constant. These preliminary studies suggest that dextran-coated electrodes can limit the extent of the inflammatory response, in particular, the amount of GFAP expression. Hence, it is reasonable to conclude that functional electrodes coated with dextran may yield signals with higher signal to noise ratios.

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