Investigation of the Foreign Body Response with an Implanted Biosensor

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Background:
One of the principal challenges for the long-term implantation of biosensors is that the normal physiological responses of the immune system create a fibrotic capsule of scar tissue surrounding the implanted sensor. This tissue acts to isolate the device from the local environment it is intended to sense, causing a degradation of the signal. We hypothesize that this degradation or change in biosensor signal character is itself an indicator of the physiological responses to the implantation of the foreign body and can be interpreted to track the progressive stages of this response.

Materials and Methods:
We have selected a micro-electrode array built upon a silica needle substrate as the biosensor for this investigation. This particular electrode device is designed and intended for deep neural implantation. The iridium-oxide electrode surfaces have a surface area of approximately 320-μm^2 (~18-μm diameter) with an inter-electrode distance of 300-μm between nodes and are constructed on a silicon chip using standard electronic microfabrication techniques.

We are applying the technique of electrical impedance spectroscopy (EIS) to track the changes of the electrical signal over time. We have also designed and tested a circuit for a four-electrode tissue impedance measurement method. This four-electrode impedance method has the ability to determine the thickness of adjacent material layers using mathematical algorithms developed by Schlumberger.

We have performed experiments *in vitro* using a reservoir of phosphate buffered saline (PBS) with the controlled addition of selected proteins. The probe surfaces were coated by adsorption with Type-1 collagen, egg-white and fibrinonectin within the reservoir. Between trials, probes were thoroughly cleaned with detergents, solvents and electronic methods.

We have also investigated the foreign body response using the chick chorio-allantoic membrane (CAM)
ex ova model. Following implantation of the electrode array probe, the chick CAM develops the common stages of the normal mammalian foreign body response.

The CAM trials involved incubating the chicken eggs *in ova* for four days, cracking them into sterile Petri-dishes, incubating *ex ova* until day 7, implanting the electrode probe into the membrane, and then performing EIS measurements periodically during the following days. Gestation periods have been extended to more than 220-hours post-implantation with functional electrodes. Future *ex ova* experiments will include utilizing the four-electrode tissue impedance measuring method. In addition, experiments are planned where the electrode array will be coated with substances believed to alter the foreign body response. We will compare the electrical sensitivity behavior of the coated electrodes to uncoated electrodes.

We have begun investigation *in vivo* tracking over time the electrical signal changes by placing the electrode array subcutaneously into the musculature tissue of a small rodent animal model.

Results to date:
Analysis shows that we can easily differentiate *in vitro* which micro-electrodes had the thin silicone surface layer by the electrode dielectric behavior. This layer is within the expected range of thickness measurement compared to our time-of-flight secondary ion mass spectroscopy characterization of the probe surface.

The *in vitro* EIS trials with probes coated with collagen, egg-white and fibronectin were each distinctly different as seen by the Bode-diagram plots having different behavior patterns from 750-Hz to 80-kHz, as well as similar differences from the de-coated samples following the cleaning process.

Data from repeated implants in the chorio-allantoic membrane (CAM) of fertilized chicken eggs
ex ova confirm that EIS behavior shows a predictable changing response of increasing phase shift for the frequencies from about 750-Hz to 30-kHz. Histological examination of samples of membrane tissues with implanted probe-tips confirmed that the chick CAM wound healing processes adjacent to the implanted probe are similar to the mammalian foreign body response. The CAM tissues were observed to show ectoderm hyperplasia and to have monocyte activation and giant cell formation adjacent to the implanted probe-tip.

The *in vivo* trials of the biosensor using a Sprague-Dawley rat for subcutaneous implantation of the electrode array are currently underway and will be reported in this paper.

Conclusions:
This project presents the potential to develop a novel type of biosensor. This biosensor will provide a tool for assessing the biocompatibility of various coatings and surface treatments. The problems common to many *in vivo* sensors could be addressed with this versatile new tool.