Investigation of the Wound Healing Processes in the Region Adjacent to an Implanted Biosensor by Application of Electrical Impedance Spectroscopy

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

Materials and Methods:
The micro-electrode arrays utilized as the biosensor are intended for deep neural implantation. They were developed at the Center for Neural Communication Technology at the University of Michigan. These iridium-oxide electrodes have a surface area of approximately 170-micron^2 with a distance of 300-microns between nodes and are constructed on a silicon chip using standard electronic microfabrication techniques. Some probes have a thin coating of silicone polymer (pdms) and others are free of this contamination. We are applying the technique electrical impedance spectroscopy (EIS) to track the electrical signal changes over time.

We have preformed experiments *in vitro* using a reservoir of phosphate buffered saline (pbs) with the controlled addition of selected proteins. The probe surfaces were coated with type-1 rat-tail collagen, egg-white and fibrinogen within the reservoir. In between trials, probes were sequentially cleaned in DI-water, mildly agitated detergent solution (5-percent Tide® with enzymes), DI-water, mildly agitated SDS detergent, DI-water, ethyl alcohol, and acetone.

We have also investigated the biological encapsulation *ex ova* using the chick chorio-allantoic membrane (CAM) model. This model has the advantage that there are no challenges associated with a tethered small rodent or other animal, but the common stages of the normal mammalian foreign body response do occur.

The *ex ova* trials involved incubating the chicken eggs *in ova* for four days, cracking into sterile petri-dishes, incubating *ex ova* until day 7, implanting into the membrane, and then performing EIS measurements periodically during the following days. Gestation periods to date have been extended to more than 220-hours post-implantation with functional electrodes.

Upon completion of these *ex ova* experiments, we will track *in vivo* electrical signal changes by placing the microprobe within the subcutaneous intramuscular fascia tissue of a small rodent animal model.

Results / Discussion:
Analysis shows that we can easily differentiate *in vitro* which microelectrodes had the thin silicone surface layer by the electrode dielectric behavior being similar to a 0.8-pf capacitor with about a 10 to 20 angstrom thick silicone layer between the pbs solution and the 312-micron^2 active site. This 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 fibrinogen were each distinctly different as seen by the Bode-diagram graphs having different behavior patterns from 80 to 100-kHz, as well as differences from the de-coated samples following the cleaning process.

Data from repeated *ex ova* EIS trials confirms a predictable changing response of increasing phase shift for the frequencies from about 1-kHz to 30-kHz. We have examined histology samples of membrane tissues with implanted probe-tips which confirmed that the chick CAM wound healing processes are similar to the mammalian foreign body response. The CAM tissues were observed to have monocyte activation followed by fibrotic encapsulation adjacent to the implanted probe-tip.

The *in vivo* trials are currently being planned and we will report preliminary results.

Conclusions:
As a consequence of this project there is a potential to develop a new type of useful biosensor. This biosensor will provide a tool for accessing the biocompatibility of various coatings and surface treatments. As such, a versatile new assay becomes available. This project may provide the tools to address the problems common to *in vivo* sensors.

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