Micro-Electrode Signal Degradation as an Indicator of the Biological Processes Involved in the Foreign Body Response

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Introduction:

One of the principal challenges of the long-term implantation of biosensors is that there are normal physiological responses of the immune system which create a fibrotic capsule of scar tissue surrounding the implanted sensor. The fibrotic tissue acts as barrier to separate the device from the local environment, thereby impeding proper sensor signaling. We hypothesize that this degradation in signal 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.

Concept for this Research Project:

Utilize the degradation over time of an electrode signal as an indicator of progressive capsule formation due to the Foreign Body Response (FBR).

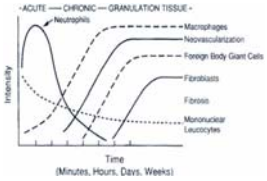
Question:

Is there sufficient sensitivity to discriminate the stages of the FBR by applying the technique of Electrical Impedance Spectroscopy (**EIS**) across implanted micro-electrodes?

Background:

Steps in the natural FBR process:

- Protein adhesion
- Inflammation (with macrophage attraction)
- Neovascularization
- Fibrous collagen capsule formation
- Vascular retraction with continued collagen thickening
- Capsule contraction with collagenous capsule density increasing



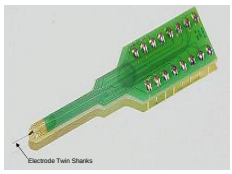
Behavior of conventional micro-electrodes:

- Micro-electrodes are implanted to sense or stimulate electrical potentials at or within biological structures.
- The foreign body response causes fibrotic encapsulation which separates the electrode from the adjacent biological structures.
- This separation causes electrical signal degradation, due to increased electrical impedance, eventually rendering the electrode non-functional.

•If sensitivity is sufficient, a new tool becomes available:

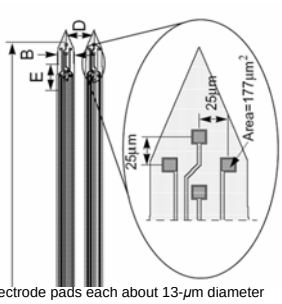
Utilize the variations in the behavior of signal degradation to compare the effects of coatings and/or surface treatments on the electrodes to alter the foreign body response.

Selection of Micro-electrode:



Electrode Assembly on Connector Board

Actual size compared to penny



Electrode pads each about 13-*µ*m diameter

Center for Neural Communication Technology – U of Michigan.

This resource center was formerly supported by the National Institutes of Health, but now has been "spun-off" into a commercial venture called **NeuroNexus Inc.**, of Ann Arbor, Michigan, USA

Appropriateness of Electrical Impedance Spectroscopy (EIS):

Complex Impedance is measured directly in the frequency domain by applying a single-frequency voltage to the interface and measuring the phase shift and amplitude (real & imaginary parts) of the resulting current at that frequency. The frequency applied is then incrementally changed while the behavior at each new frequency is recorded. The data is then analyzed over the range of frequencies applied. Instrumentation currently used is from 20-Hz to 100-KHz but it is anticipated that it may be necessary to use frequencies as high as 4-MHz. Higher frequencies may be appropriate for sensing adhesion of molecules such as proteins, while lower frequencies may be more useful for sensing thicker collagenous deposits.

Research Plan:

•Aim 1: *In vitro* trials- Apply the technique of EIS upon a micro-electrode assembly held in a reservoir cell. Sequentially expose the micro-electrode to various biological coatings that simulate the processes of protein adhesion, cell attachment and capsule formation.

•Aim 2: *Ex ova* trials: Verify the hypothesis that the stages of the FBR can be detected with EIS by implanting microelectrodes into the chorio-allantoic membrane of fertilized chick embryos. Track the changes in impedance versus time and correlate to histological samples.

•Aim 3: *In vivo* trials: Utilize in a small mammal animal model the micro-electrodes, EIS techniques, histology correlations and analysis developed in Aims 1 & 2 to demonstrate the successful development of this tool for evaluating coatings to alter or improve the biocompatibility of electrode surfaces.

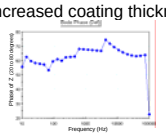
Experimental Details:

Aim 1 *In vitro:* Reservoir and Coatings:

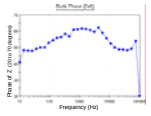
Phosphate buffered saline (PBS at pH 7.2) filled pyrex reservoir at 22°C. Protein coatings: collagen-1, fibronectin, egg white (completed), planned: thrombin, then whole blood, then cell suspensions.

Preliminary outcome of *in vitro* trials:

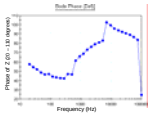
Predictable changes in impedance varying with frequency were observed as various substances came into proximity with the electrode surfaces. Our predictions were based upon how increasing the molecular weight would increase the dielectric constant for each coating material. The phase shift of a capacitor varies with the dielectric constant. Increasing phase changes with a drift towards lower frequencies was observed as the density of adjacent molecules increased. Increased coating thickness had a similar trend.



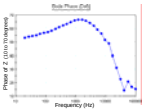
Clean



Fibronectin



Egg white (mixed proteins)



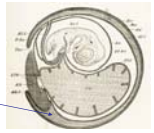
Collagen-1

Ex ova Experiments:

In ova development of the chorio-allantoic membrane

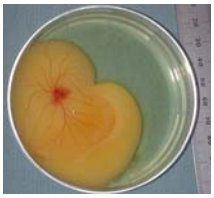
Day 5

Preferred site for implantation at Day 7



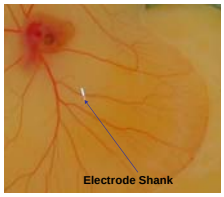
Day 7

Lillie, F.R. "The Development of the Chick" Holt & Co., NY (1908)



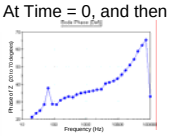
Day 7 – Ready for implantation

The chick chorio-allantoic membrane beginning from Day 7 is reactionary to foreign bodies

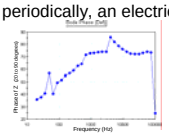


Detail of shank implanted

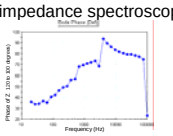
At Day 7 a micro-electrode assembly is implanted into the membrane. This causes a wound. At Time = 0, and then periodically, an electrical impedance spectroscopy data set is generated.



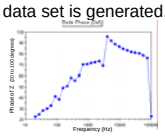
5-minutes post implant



12-hours post implant



2-days post implant

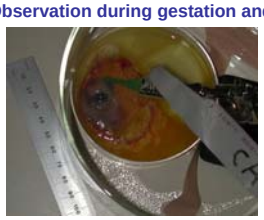


7-days post implant

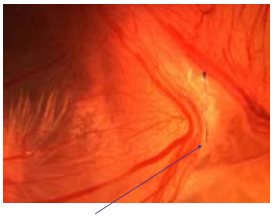
Interpretation and Analysis of EIS Data:

Gestation periods to date have been extended to more than 200-hours post-implantation with functional electrodes. Data from repeated EIS trials confirms a predictable response of increasing time delay (phase shift) for the frequencies from about 30-KHz to 1-KHz. We have preliminary histology samples which are in-process. They were observed to have visible encapsulation as shown in the photograph below.

Observation during gestation and the FBR



120-hours post implantation with 12-days gestation



Detail of probe tip implanted inside a growing chick chorio-allantoic membrane at Day-15

Future Aim 3 *in vivo* trials:

Correlate data from *in vitro* reservoir and *ex ova* models to *in vivo* animal trials. Electrodes may be implanted in the lumbar or trapezeus muscles of a rodent model for the FBR. It is essential to perform histology to verify the EIS data interpretations. A future experimental advantage would be telemetry with a micro-transmitter to allow natural animal subject behavior. Application of selected coatings to alter the FBR *in vivo* and then to track the altered response is the ultimate goal of this project.

Acknowledgements:

This work is supported by UWEB (NSF EE9529161).

Advice and assistance from Professor Emeritus Sandy Spelman.

Cooperation and support from the entire laboratory group of Professor Karl Böhringer, especially Yanbing Wang, Xiaorong Xiong, and Ashutosh Shastry

Chicken eggs were supplied by Summit Farms through the Department of Comparative Medicine.

Micro-electrodes were supplied by the University of Michigan's Center for Neural Communication Technology (CNCT), NIH P41 Research Center

Discussions and assistance from: Colleen Irvin, Elizabeth Leber and the entire UWEB team!