Bioinformatics and Bioengineering Summer Institute (2003)
Exploring the process of nerve myelination using Electronic Cell-substrate Impedance Sensing (ECIS)

I. The Scenario: Myelination of Axons

You are a neuro-biologist studying the myelination of axons in the peripheral nervous system. Myelination is the process by which axons are covered and insulated by Schwann cells. To investigate this process, you are examining Schwann cell migration onto thin gold fibers. To detect the movement and spreading of the cells onto the fibers, an Electronic Cell-Substrate Impedance Sensing (ECIS) device is used.

The device in your experiment works in the following manner. The gold fibers are placed in a fibrin gel along with cultured Schwann cells. The fibers have the ideal geometry to stimulate Schwann cell attachment and spreading. The gold fibers are connected to an ECIS machine, allowing a small current to flow through them. As Schwann cells attach and spread on the gold fibers, the ECIS device measures and records changes in the impedance of the fibers. Overall, you hope your research might help find a cure for Multiple Sclerosis, a disease causing demyelination of nerve axons.

II. The Nervous System

Since you are working with nerve cells, a little background on the nervous system should help you with your project. Overall, the nervous system is divided into two main groups: the central nervous system (CNS) and the peripheral nervous system (PNS). The central nervous system includes the brain and the spinal cord. The peripheral nervous system includes cranial and spinal nerves, ganglia, motor nerve endings, and sensory nerve endings. The PNS sends information about sensory input to the CNS and directs motor commands to peripheral tissues and sytems from the CNS. Nerve fibers or axons carry this information in the PNS. The CNS processes the information it receives from the PNS and sends out commands.

Two main types of cells comprising nervous tissue are neurons and supporting cells. The neuron or nerve cell is the functional unit of the nervous system. Its structure consists of a cell body, an axon, and dendrites (illustrated below). Collections of cell bodies outside the CNS are referred to as Ganglion. Supporting cells known as neuroglial or glial cells surround and protect neurons. In the CNS, glial cells include astrocytes, oligodendrocytes, microglia, and ependymal cells. Satellite cells and Schwann cells are the supporting cells in the PNS.

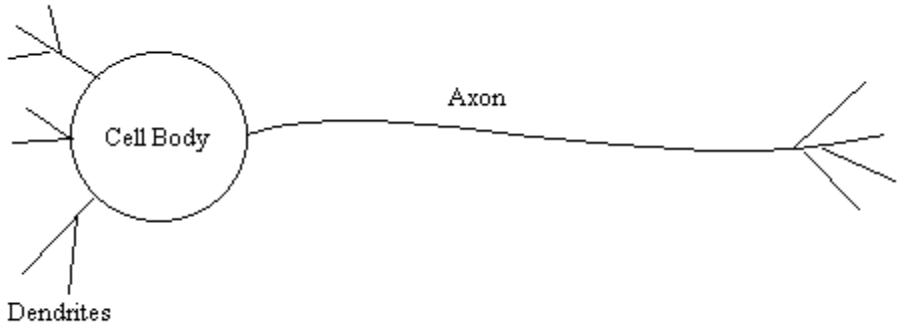

Figure 1: Neuron Structure

In the PNS and sometimes the CNS, the axons extending from cell bodies are myelinated. Myelination is the process by which axons are covered by a myelin sheath, or layers of lipid membrane. In the PNS, nerve fibers (axons) are myelinated by Schwann cells. During myelination, a Schwann cell attaches and wraps around an axon multiple times leaving behind a myelin sheath. Small gaps between adjacent wrapping are called Nodes of Ranvier. Myelin serves two functions in the PNS. It insulates the axon, and it speeds nerve conduction in a phenomenon known as saltatory conduction (see figure below). Typically, a nerve impulse is carried down an axon by skipping from node to node. At each node, an action potential arises as the local current within the axon depolarizes the node to threshold. In diseases like Multiple Sclerosis, demyelination occurs, resulting in decreased nerve insulation and conduction.

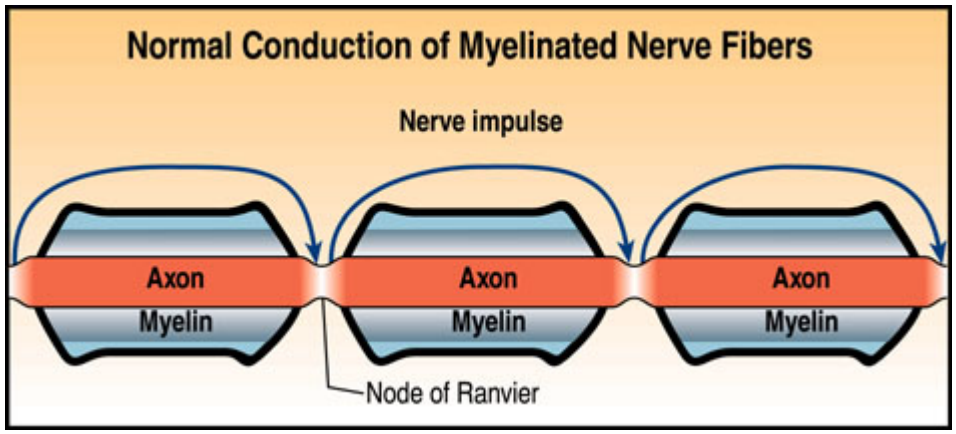

Figure 2: Myelinated Nerve Fiber
http://medlib.med.utah.edu/kw/ms/mml/ms_pathology03.html

SQ1. How is the nervous system organized?
SQ2. Why do you think unmyelinated nerves cause problems in people afflicted with Multiple Sclerosis?

III. Cellular Migration and Overview of ECIS

Since you are investigating Schwann cell attachment and spreading on gold fibers, you should know a little information on techniques used to measure cell migration and attachment.

Many different methods currently exist to examine cellular migration. The migration of cells may be studied directly or indirectly. Indirect methods include the Boyden Chamber. In this assay, cells are grown on a microporous membrane. The number of cells that migrate through the membrane are counted and compared to the cells that did not. A modified version of this method uses an agarose gel instead of a microporous membrane. Overall, this technique is very reproducible. Direct methods include image analysis and video microscopy in a Zigmond or Dunn chamber. In video microscopy, real time images of cells are taken and stored in a computer. A computer program is then used to analyze the migration of cells in response to various chemotactic agents. Overall, video analysis is very precise, but it is costly and difficult compared to the Boyden chamber method.

Electric cell-substrate impedance sensing (ECIS) is a novel process used to monitor directly cell attachment and spreading. The process is non-invasive, providing real-time data. ECIS can also be used to measure cellular migration. Overall, ECIS offers many advantages over current techniques to observe cellular migration and attachment.

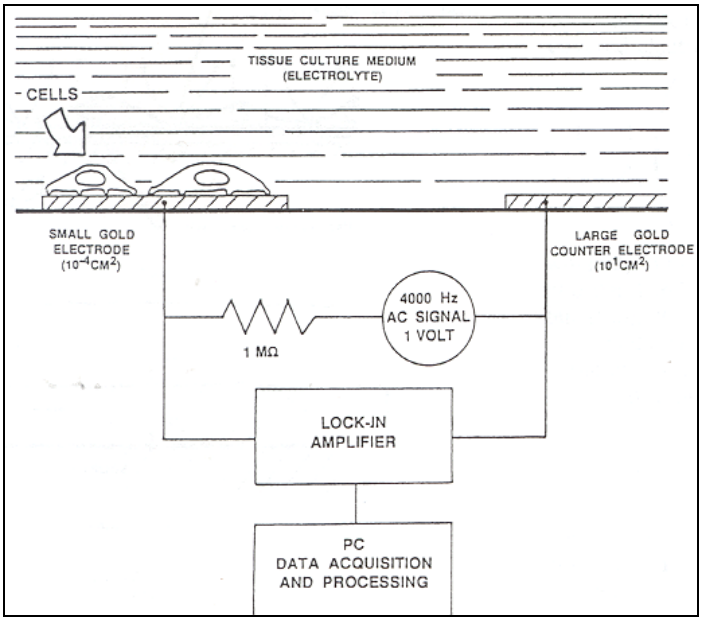

Figure 3: ECIS Diagram

Giaever, I.; Keese, C. Micromotion of mammalian cells measured electrically.
Proc Natl Acad Sci USA **88**, 7896-7900, 1991.

Electric cell-substrate impedance sensing is a complex technique that utilizes computers and electrodes to directly monitor cell migration and spreading (See Figure above). During an ECIS test, cells are placed in media over gold-film electrodes deposited on a cell culture plate. There are sensing electrodes and one reference electrode on the culture plate. The electrodes are connected to an ac power source, supplying a small current to the system. The cell media acts as an electrolyte carrying current between the sensing electrodes and the reference electrode. As cells attach and spread on the sensing electrodes, the surface area decreases inhibiting current flow and increasing resistance and impedance. The membrane of each cell also acts as a capacitor. The changes in resistance, capacitance, and impedance of each electrode is measured, recorded, and plotted over time (See Figure Below) by a computer connected to the system. The

results can then be used to analyze cellular migration and attachment in response to various environmental changes.

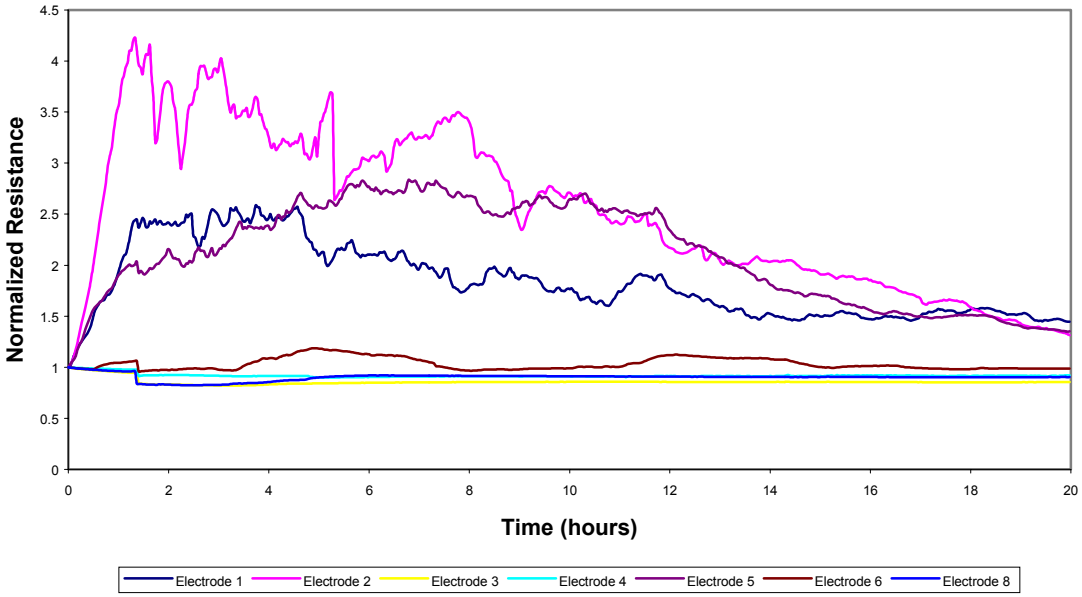

Figure 4: Example of ECIS Data

SQ3. How does an ECIS device work?

SQ4. What are some research projects that could benefit from ECIS? Can you think of any processes that involve cellular migration and attachment?

IV. The Physics of ECIS: DC vs. AC and Electrode Size

An AC signal is used to make ECIS measurements. Why is an AC signal used instead of a DC signal? Perhaps a little information on AC and DC circuits should help answer this question. AC stands for alternating current; whereas, DC stands for direct current. In a DC circuit, the current is constant. The current fluctuates and changes direction in an AC circuit. Using AC instead of DC signals to monitor the cells on electrodes has two important consequences. First, an AC source is used to prevent electrolytes in the culture media from depositing on the electrodes, which would cause the properties of the electrodes to change or polarize. In a DC circuit, electrode deposition would occur. Compared to a DC signal, the use of an AC signal also allows you measure changes in more parameters, such as impedance, capacitance, and reactance.

Impedance is the AC equivalent to resistance. When an AC current (I) is supplied to the electrode system, the resulting voltage (V) is measured. The current and voltage in the circuit change in a sinusoidal manner. They may be in phase, out of phase, or in between. Impedance (Z) is calculated by Ohm's law: $Z = V / I$. The impedance can also be broken up into two components: one part for pure resistance and the other part for the reactance of the system. The reactive part is due to the capacitance associated with the metal surfaces in the tissue culture medium. Basically, the electrode setup can be represented as a resistor and capacitor in series

(RC circuit). The impedance for each element in the circuit is calculated by the following equations: $R=V_{(in\ phase)} / I$ and $X_c=V_{(out\ of\ phase)} / I$, where R stands for resistance and X_c stands for capacitive reactance. The total impedance is calculated by the following equation:

$$Z= (R^2 + X_c^2)^{0.5}$$

X_c is calculated from the AC frequency (f): $X_c = 1/(2\pi f C)$. Since frequencey is known, the capacitance can be calculated from this term.

With all of this information, more than just changes in impedance are reported for the cells. Changes in resistance and capacitance may also be noted. All of the parameters listed above may also be used to provide further information about cell attachment and spreading on electrode surfaces. For example, the spacing beneath and between the cells and the capacitance across their membranes may be examined.

During a typical ECIS experiment, cells attach to sensing electrodes. The AC current flows from the sensing electrode through the cells and to the electrolyte (cell media). The cells impede the flow of current, increasing the impedance and resistance of the affected electrode. Next, the current flows through the electrolyte and to a reference electrode. In a typical ECIS setup, the size of each sensing electrode is much smaller compared to the reference electrode. What is important about this size difference? Maybe it has something to do with the cell media and the reference electrode. The cell media acts as an electrolyte with charged praticles moving in response to an applied voltage. The electrolyte also has its own resistance, and the reference electrode does too. To avoid measuring the resistance of the cell media and the reference electrode instead of the resistance of the sensing electrodes, the size of each sensing electrode is much smaller than the reference electrode. This causes a bottleneck effect where the sensing electrode resistance dominates the entire resistance of the system. Any small changes to the sensing electrodes, like cell attachment, will result in large changes in measured resistance.

SQ5. Why is an AC power source connected to the electrodes? What would happen if a DC power source was used?

SQ6. What would happen if the sensing electrodes were not much smaller than the reference electrode?

V. Modeling Demyelination

After you collect all of the data on Schwann cell attachment and spreading on gold fibers, you decide to model the process of demyelination. Suppose the model is an axon that has cells on it arranged as a real myelinated axon, with spaced nodes. You suppose one could calculate the speed of axonal conduction with a given spacing of nodes. Now let cells die at random but at a constant rate. With each cell death, another node is opened at a random position. How will that affect the speed of axonal conduction? How would speed degrade over time? Conceivably, such a model might give insight as to how much cell loss is required before conductance is significantly affected, which might be important in getting a feel for the progression of diseases that cause demyelination.