Controlled Neuron Growth By Patterned Protein Gradients

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Abstract:

Neuron guidance is mainly controlled by the growth cone, located at the end of an axon. Extra-cellular components, including proteins, control the directional growth of these neurons; this allows for the possibility to manipulate extra-cellular factors to aide in the accurate rewiring of damaged neurons.

The objective of this project is to create a standard platform on which to study neuron guidance by protein gradients using an electrospray system. The electrospray system is first studied using polystyrene latex (PSL) particles. This method deposits a uniform distribution of particles on a substrate and can be controlled to spray at constant velocities or accelerations. Neuron cells from extracted dorsal root ganglia (DRG) of fetal chickens are used to observe growth patterns under varying conditions, including various protein gradients and the presence of different proteins.

Introduction:

In the United States, there are approximately 200,000 people with spinal cord injuries, with another 11,000 new injuries each year. The majority of injuries occur between the ages of 16 and 30; therefore the preponderance of patients live many years paralyzed. Currently, there are no treatments for such injuries, resulting in a push to find a regeneration technique.

Control of immature neuron growth is instigated at the end of the axon in a region known as the growth cone. Within the growth cone are two sensory structures: filopodia, finger-like projections, and lamellipodia, web-like structures (Figure 1). These structures sense the presence of various extracellular components, such as proteins. These proteins either repel or attract axon growth, in processes known as chemorepulsion and chemoattraction, respectively. By controlling the location, concentration, and gradient of the guidance proteins, the directional growth of the neurons can be controlled also.

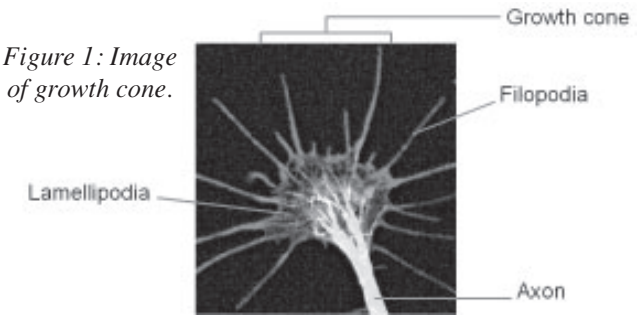

Figure 1: Image of growth cone.

An electrospray system is one method by which particle location can be controlled. A minute amount of solute is sprayed and deposited onto a surface with the aid of a strong electric field present under the surface. The repelling charges of the particles prevent agglomeration on the substrate surface. Guidance proteins are electrosprayed onto a pattern of extra cellular matrix molecules that are micro-contact printed onto the substrate. Combining these two techniques creates a guided pathway on which the neurons grow. The objective of this project is to use the attraction properties of certain proteins deposited by the electrospray system to guide neuron growth.

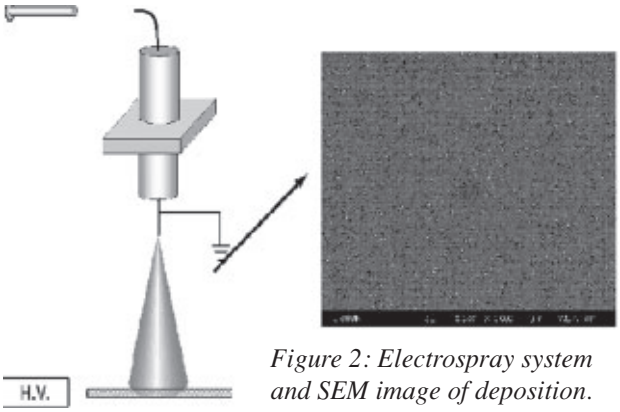

Figure 2: Electrospray system and SEM image of deposition.

Methods:

The electrospray system was first tested using a 57 nm PSL particle solution at a concentration of 10 drops PSL/10 mL deionized water. The solution was pumped from a syringe into a capillary with a constant flow rate. Under the capillary, a stencil taped

to a silicon wafer was placed on a plate with a charge of 5-7 kV, producing a uniform distribution of particles. This plate was then placed on a computer-controlled stage, with the capability of moving the substrate at either a constant velocity or constant acceleration (Figure 2). The stage was moved at constant velocities of 25 μm/s, 50 μm/s, and 75 μm/s, and constant accelerations of 1.20 μm/s^2, 1.98 μm/s^2, and 2.75 μm/s^2.

Dorsal root ganglia (DRG) were harvested from embryonic stage nine chicken fetuses. Half of the DRGs were chemically dissociated into individual neurons. Both the DRGs and dissociated neurons were placed on a silicon wafer stamped with lines of laminin 1 μm wide. The neurons grew for 2 days in an incubator.

Results:

Electron microscope analysis of the electrosprayed silicon wafers showed that the distribution of particles was uniform with little agglomeration (Figure 2). The deposited concentration of the PSL particles was determined by converting the images to black and white and analyzing the fraction of each color with an intensity histogram software program. The samples were also analyzed using a fluorescent microscope. The florescent intensity was measured for each sample to determine the concentration gradient qualitatively. The samples generated by moving the stage at a constant acceleration created a non-linear gradient described by Equation 1, which determines the surface concentration at any point x on the line (Figure 3).

Analysis of the directed neuron growth showed no neuron growth for either the DRGs or dissociated neurons.

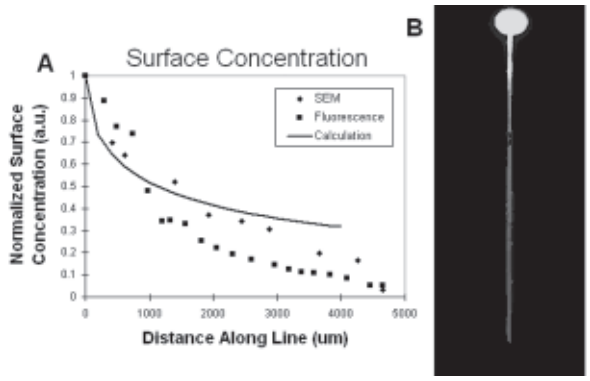

Figure 3: (A) Comparison of SEM and Fluorescent samples with calculation; (B) Fluorescent image of sprayed line.

$$\#/\mu m^2 = \frac{C \cdot Q}{A}\left(\frac{\sqrt{V_0^2 + 2ax + 2ad} - \sqrt{V_0^2 + 2ax}}{a}\right)$$

Equation 1: C=concentration of solution, a=acceleration rate, Q= flow rate of solution, d=diameter of sprayer, A= spray area, x=distance along line, V_0=initial velocity of stage.

Conclusions:

The electrospray system has the capability to create uniform concentration patterns or nonlinear concentration gradients; however, a method to create a linear gradient is still needed. These gradients all contain a uniform deposition with little agglomeration of particles.

Directed neuron growth was not successful; however, we believe this was due to contamination of the sample, which allowed for the growth of a fungus. Previous experiments show that neurons are able to grow on stamped lines of the extracellular matrix molecule laminin, as seen in Figure 4.

Future work on this project includes the development of a method to spray a linear concentration gradient as well as the ability to spray proteins. As of now, the spraying of proteins has been unsuccessful, possibly due to the proteins being denatured by the strong electric field. Once these technical difficulties are solved, this approach will be useful for studying the guided growth of neurons.

References:

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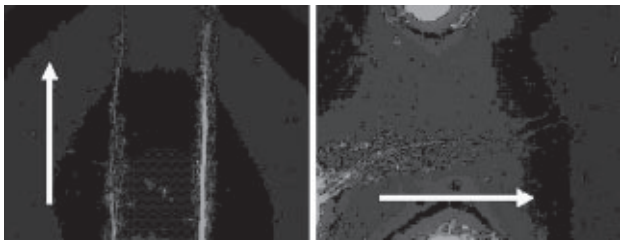

Figure 4: Neuron growth along laminin lines.