Analyzing the Impact of Bis-Acrylamide Stiffness on Neuronal Development

Margaret Chou
Margaret.y.chou@gmail.com

Priya Dave
pjdave4@gmail.com

Cayanne Liew
cayanneliew@hotmail.com

Jason Qin
jasonzqin@gmail.com

Matthew Reda
sciencemr@aol.com

NJ Governor’s School of Engineering & Technology 2011

1 Abstract
The rigidity of bis-acrylamide gels affects neuron development, especially in dendrite and axon growth. The bis-acrylamide to water ratio determines the texture of the culture; the higher the amount of liquid bis-acrylamide, the stiffer the culture. In this experiment, we sought to determine which type of gel, the stiffer or the softer, the neuronal cells favored and on which they developed more on. This required the team to develop many unique designs as the two different gels must be perfectly aligned in height without any inconsistencies so that the neurons can cross both variations without any external influences. After designing the experiments, we selected two unique ones that were expected to overcome both time and material constraints. The ultimate model required a minute circular piece of gel as the center and a differing gel as the perimeter. Unfortunately, the designs were compromised due to toxicity and the neurons on the gels did not survive. The thickness of the gel caused the toxic acrylamide monomer to reside within the gel; however, composing a thinner gel or rinsing the gel more would have resolved this issue. In another experiment, conducted earlier by our mentor, we were able to collect various Green Fluorescent Protein [GFP] values of other cells to determine that they proliferated more in the stiffer gels. The proliferation marker used to detect GFP, KI-67, had larger overall values in the stiffer gels, which proved that the cells divided. Altogether, our general research proved that neuronal cells were more successfully developed in the stiffer gels.

2 Introduction
Spinal cord injuries often leave people permanently paralyzed and may eventually cause death. Two and a half million people worldwide live with spinal cord injuries and 12,000 to 15,000 new spinal cord injuries occur each year in the United States [1]. Unfortunately, most of these injuries occur in younger individuals from 15 to 35 years of age,
leading to lives of debilitation. Spinal cord injuries are categorized into various vertebral segments, which are determined by the spinal roots that exist throughout the divisions. The segment levels are cervical, thoracic, lumbar, sacral and vertebrae. These injuries are detected by the first segment that shows an abnormality through the loss of motor function or sensation in a specific region on the body. Overall, the injuries are classified in the American Spinal Injury Association [ASIA] impairment scale with each subsequent letter acting as a decrease in the degree of severity; A is the most severe with complete loss of motor and sensory function and E is normal function [2].

The major factor reason that spinal injuries are permanent is that sensory axons must grow back into the spinal cord in order to restore function. However, they generally will not do so because of axonal growth inhibitors in the spinal cord, which are particularly located at the Peripheral Nervous System-Central Nervous System [PNS-CNS] junction at the dorsal root entry zone. Secondly, the cauda equina, the structure in the lower region of the spine that consists of nerve roots, contains the ventral roots of the spinal cord, through which the motor axons of the spinal cord pass to move one’s muscles. If the injury to the ventral root is close to the motor neurons that sent the axons, the injury may damage the motor neuron itself. Both of these factors significantly reduce the likelihood of neurological recovery in a cauda equina injury [3].

The primary response to a spinal cord injury is cell death of neurons and oligodendrocytes along with damaged axons. Then, inflammation and glial scar formation occurs at the site of injury; this is where axonal regeneration is inhibited. Currently, there exist three major spinal cord injury treatments: the administration of Methylprednisolone, which is a steroid, the usage of hypothermia, and the process of surgery. However, each of these operations has major disadvantages. For the first treatment, a time constraint reduces the effectiveness of the steroids. For the other treatments, high risks of fatality impede the success of the processes. At present, therapies that can completely restore spinal cord functions to patients do not exist.

An idealistic treatment would be able to repair a spinal cord void and supply extraneous neurons and oligodendrocytes to the damaged area. In an ideal and successful situation, the therapy would stimulate and restore connections among neurons, encourage complete motor recovery, and re-establish sensations [4].

For this experiment, stiff and soft bis-acrylamide gels were used to determine which stiffness augmented neurons growth and allowed a stronger adhesion to the gel during development. Overall, the goal was to develop a procedure that would ensure full neuron maturity, and allow these neurons to be used in future spinal cord treatments.

3 Background

3.1 Neurons

Neurons, or nerve cells, transmit chemical and electrical signals throughout the human body and the transmissions occur at the connections of the neural networks, also known as the synapses. The easily excited neuron consists of various parts ranging from a cell body, a soma, dendrites, to an axon.
The dendrites act as a receiver and relay information to the cell body, while the axon operates as a courier and transports impulses away [5]. Nerve cells, the major components of the brain, the spinal cord, and the peripheral ganglia, exist as a major component to human survival.

Neurons can specialize into sensory neurons or motor neurons, both taking major roles in their respective systems. Sensory neurons respond to changes in an organism’s environment and react to diverse stimuli such as light, touch, sound, and pain by sending signals to the brain and the spinal cord, from where the motor neurons take over. These neurons receive the signals from the brain and the spinal cord and stimulate a motor reaction in the body by producing muscle contractions, signaling glands, and creating other physical responses. Aside from the two major forms of neurons, interneurons also exist as the messengers between neurons that exist in the same areas of the spinal cord and the brain.

Neurons are also influenced by the behavior of other cells, such as glial cells, oligodendrocytes, and astrocytes. Glial cells provide protection, support the neurons of the brain, and also play a key role in homeostasis and the formation of myelin, which aids in the rapid transfer of signals within a neuron. Oligodendrocytes insulate the axons of the nervous system, allowing for effective and efficient transfer of information between neurons and signal receivers. Lastly, astrocytes ensure a proper environment by contributing nutrients to neurons and nervous tissue, protecting the blood-brain barrier cells, repairing brain injuries, and maintaining extracellular matrix ion balance.

When there is damage to neurons, the severity of the injury and its consequences depend primarily upon where in the body the neurons reside. For example, the neural axons of the peripheral nervous system can regenerate, while the neural axons of the spinal cord are incapable of the action. The difference between the two regions resides in the fact that the PNS provides a suitable environment for the growth and proliferation of neurons with its constant creation of new growth cones, the elongation of axons, and the formation of synapses. Other researchers such as Albert Aguayo et.al. have created this proper environment of the peripheral nervous system in the spinal cord region in their experiments. [6]

3.2 Gels

Polyacrylamide is formed by the polymerization of the monomer molecule-acrylamide cross-linked by N,N'-methylene-bis-acrylamide [BIS]. BIS and acrylamide are nonreactive when alone or mixed. To start the reaction, an activator and a catalyst are needed. In this case, the activator was ammonium persulfate [APS], which generates free radicals, and the catalyst was -N,N,N',N'-tetramethylethylenediamine [TEMED] which acted as an oxygen scavenger.

This experiment takes advantage of the acrylamide gel system’s unique quality, which is that the initial concentrations of acrylamide and BIS control the hardness of the gel. [7] The hardness of the gels affects the way the cells grow, allowing for the testing of mechanical factors that promote cell proliferation. By understanding the mechanical factors, in this case stiffness of the gels, that cause cells to grow, new
treatments can be developed that rely on mechanical factors instead of chemical factors.

3.3 Effect of Gel Stiffness on Neuronal Development

Previous studies have determined that the stiffness of the gel on which a neuron is grown determines the growth patterns of the neuron. As gel stiffness increases, dendrite branching on neurons typically increases as well [8]. From 300 Pa to 3000 Pa, the number of primary and secondary dendrites created by the neurons increased [8]. Furthermore, the number of neurons that adhere to and grow on the gels increases in relation to gel stiffness. As stiffness increases, more neurons grow on the gels [9].

3.4 ImageJ

ImageJ is a software program designed to display, analyze, process, and save images. It can calculate area and pixel value statistics such as maximum, minimum and mean intensities of pixels contained within a certain selection. It also supports basic image processing functions necessary to analyze the photos of the cells [10]. The cells analyzed were stained with KI-67 and DAPI. KI-67 is a GFP whose gene is a marker for expression. The glowing green stain displays that the cells are actively growing and dividing. DAPI, on the other hand, is a reporter of the presence of DNA, also showing cell growth [11].

4 Methods

4.1 Materials

This experiment used many materials typical of designs involving the growth of neurons on bis-acrylamide gels. A 40% bis-acrylamide solution was used in various quantities to create the 3%, 5%, and 8% bis-acrylamide solutions. Solutions of APS and TEMED, once added, caused the liquids to polymerize. These solutions were created as needed in the experiment and stored in 1.5 mL Safe-Lock Tubes. To create the APS solution, a minute and subjective amount of the APS solid was measured in mg, and one-hundred times that value of water was added in terms of µL (e.g. for 2.7 mg of APS, 270 µL of water were added). The TEMED solution was not diluted; pure TEMED liquid was used. The bis-acrylamide solutions were stored in 15 mL centrifuge tubes. In the final design, two Polydimethylsiloxane [PDMS] rings and multiple Petri dishes of 60 mm in diameter were used. The gel was transferred using 10 µL, 50 µL, and 1000 µL pipettes. To color the gels in the outer layer of the design, a modest yet unweighted amount of Sulforhodamine B sodium salt was used to create a pink colored solution. In an uncompleted design, culture plates containing 48 wells, 24 wells, and 12 wells created by Corning ® Costar® were used (see Fig. 2). Later, various media and a solution of Sulfo-SANPAH [SS] were also used to prepare the gels for the application of neurons.
4.2 Creation of Bis-Acrylamide Solutions and Mixtures: Primary Design

Part 1: Different compositions of bis-acrylamide solutions:

This experiment required the use of three various gel textures, with the bis-acrylamide to water ratio in each sample determining the stiffness or softness of the gels. 3% bis-acrylamide solutions were created by adding 450 µL of 40% acrylamide solution to 5.43 mL of distilled water. Similarly, 5% solutions were created by adding 750 µL of 40% bis-acrylamide to 5.13 mL of distilled water. Finally, 8% bis-acrylamide solutions were created by adding 1.2 mL of the 40% bis-acrylamide to 4.68 mL of distilled water.

The 3% bis-acrylamide gel corresponds to a stiffness value of .3 kPa ± .1 kPa; the 5% bis-acrylamide gel corresponds to 5.8 kPa ± .4 kPa; the 8% bis-acrylamide corresponds to 25 kPa stiffness. These measurements correspond to amplified values of those given in the Figure 2 below.

Part 2: Formation of hardened gels:

The design of the experiment involved first creating discs of 30.5 mm in diameter in the center of PDMS rings. Before the addition of the bis-acrylamide the PDMS rings must be placed into a Petri dish of 60 mm in diameter. To begin the creation of the gels, 60 µL of the APS 6 µL of the TEMED were added immediately prior to the use of any liquid bis-acrylamide since the solutions quickly harden into gels once both are added. 2.7 mL of 5% bis-acrylamide solutions were added to two wells in two Petri dishes. The liquids were allowed to solidify into gels, which are shown in the figure below. If the gels did not sufficiently solidify, the gels were contained in a 5% CO₂-regulated incubator at 37° C overnight. After the gels had hardened, the ring was removed. This process is shown in Figure 3 below:

<table>
<thead>
<tr>
<th>Components</th>
<th>3%</th>
<th>5%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% acrylamide/bis (29:1) stock</td>
<td>37.5</td>
<td>75</td>
<td>62.5</td>
</tr>
<tr>
<td>dH₂O</td>
<td>452.5</td>
<td>905</td>
<td>427.5</td>
</tr>
<tr>
<td>APS (10%, 10mg APS w/ 90 µL)</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>TEMED (1:10 dilution)</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Stiffness (kPa)</td>
<td>.3 ± .1</td>
<td>5.8 ± .4</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 2: The bis-acrylamide creation table used for the productions of different gels. This table describes the amount of each ingredient needed for 500 µL and 1000 µL solutions (seen on the left and right side of each group of cells related to each concentration of bis-acrylamide in the solution, respectively).
Figure 3: The inside gel in a petri dish. The PDMS ring has been removed. The inner ring was molded using the PDMS ring as the container.

To one of the dishes with 5% gel, 3% bis-acrylamide liquid including APS and TEMED, as well as a solution of Sulforhodamine B sodium salt, were added. The second solution was added to form an outside ring for the first gel—the inner circle. 8% bis-acrylamide liquid, containing the Sulforhodamine B sodium salt, was added to the other dish containing the 5% gel. The solutions were added until the dishes were covered and the liquids appeared to be at the same level as the 5% gels. Finally, the top of a cover to a petri dish was covered with a hydrophobic solution and was used to press down upon the solution, in order to assure that both gels would be of same height after the outside gel hardened. In one dish, only the cover was used to press upon the gels; in the other dish, another petri dish and a PDMS ring were both added on top of a petri dish cover for additional weight. The additional weight was added after it was discovered that the petri dish itself was not sufficient to create an even surface between both gels.

4.3 Addition of Neurons

After the gels formed, they were prepared for the addition of neurons. It should be noted that neurons were only plated onto the gels in the design to be described in 4.5 First Design due to time constraints. We did not have time to prepare the gels and plate them with cells. For the first design, the gels used in section 4.5 were first transferred to solutions of 12 well culture plates with well diameters of 22.1 mm. Then, SS and Poly-D-lysine hydrobromide [PDL] were created. The SS solution was created by adding 5 mL of Hepes to 1.6 µg of SS, then leaving the solution in a 37° water bath for several minutes. The PDL solution was created by adding 1.1 µg of PDL to 5 mL of Hepes. All equipment was sterilized, and the petri dishes containing the gels were sterilized under ultraviolet rays for 2000 seconds. Using a pipette, SS was added to the wells to cover the gels. The gels were put in UV chamber for 400 seconds. Subsequently, the excess SS was removed using a weak vacuum and a new solution of SS was applied to the gels, which were once again left under UV light for 400 seconds. After these 400 seconds, a buffer was added to the wells containing the gels, and the plate was gently shaken by hand. The solution in each well was removed using a weak vacuum. Figures 4 and 5 below show the sterilization of the gels. Finally, about 1 mL of PDL was added to each well, and the plate was left overnight in a 4° C refrigerator to absorb the PDL.
The following day, the PDL was drained from the wells in a sterile environment. A medium was then added to cover the gels. After all gels were coated, the medium was removed, and another layer was applied. The gels were left in a 4°C refrigerator overnight.

Next, the media on which the cells were to be grown on were created. In a sterile environment, 24 mL of Neurobasal Medium was added to a centrifuge tube. To this, 250 µL of a penicillin/streptomycin solution, followed by 250 µL of glutamax, were added. Then, 500 µL of B27 were added to the vial. The medium was evenly distributed to the five wells containing
gels, and the gels were left overnight in a refrigerator at 4°C.

After the gels were left overnight, cells were added using the procedure described by Dr. Michelle Previtera [8]. KI-67 was used as a proliferation marker to record cell growth.

4.4 Design Ideology

There are a few factors that influenced the decision to choose this design. We wanted to create gels combinations of different stiffnesses that did not have gaps between them. Also, it is important that the gels be of even height, so that neurons may easily grow from one gel stiffness to the other. In this design, after the second gel is added, the bottom of the gels becomes the medium in which the neurons are cultured. Because the bottom of the well creates an even surface for the gels to form, the flat surface created provides the even surface needed for efficient testing of the impact of gel stiffness on neuron development.

4.5 First Design

We tested this design before using the design explained above. The design of the experiment involved first creating discs of 11.0 millimeters in diameter in wells on a 48 well culture plate. 300 µL of 5% bis-acrylamide solutions containing APS and TEMED were added to each of 6 wells of the 48 well plate, and 300 µL of 8% bis-acrylamide solutions were added to 3 wells of the same plate.

For this design, three combinations of bis-acrylamide mixtures of different stiffness levels were created. The hardened gels were removed from their wells using microspatulas, and added to culture plates with 24 wells. The gels were placed in the center of each well. To three of the wells with 5% gels, 300 µL of 3% bis-acrylamide liquid added with APS and TEMED, as well as a solution of Sulforhodamine B sodium salt, were added. 300 µL of 8% bis-acrylamide liquid, containing the Sulforhodamine B sodium salt, were added to each of the other three wells containing the 5% gel. Finally, 300 µL of the 3% gel also containing the Sulforhodamine B sodium salt were added to the three wells containing the 8% gel. Unfortunately, four of the gel combinations fell apart, leaving only one gel of the 3% and 5% combination, two of the 5% and 8% gel combinations, and two of the 8% and 3% gel combinations.

Cells were then added using the design referenced above. [8]

During the process of testing the design, we encountered various issues, to be discussed in depth in the results section. As a result of these issues, we ultimately did not use this design.

4.6 Other Designs

Due to the short timeframe of the New Jersey Governor’s School of Engineering and Technology (NJGSET) Program, the research attempted was limited by the number of hours we could dedicate toward testing experimental designs and culturing cells. We created various other designs that hold potential to also function as efficient ways of creating the combined bis-acrylamide mixtures.

One design created is somewhat similar to the first design attempted. First, three gels of 3%, 5%, and 8% each should be created in the 48-well plate.
300 µL of each gel should be added into all of their respective wells. Then, once these gels harden after 15 or 20 minutes, a second layer of gels is created (refer to Figure 5). The second layer of gels contains the Sulforhodamine B sodium salt. For the wells containing 3% gels, 300 µL of a 5% gel is added; for 5% gels, 300 µL of an 8% gel is added, and for the 8% gels, 300 µL of a 3% gel is added. After these gels form, the gels are removed using a microspatula. An exacto-knife should then be used to cut the sample vertically, such that slices that contain both gels from a given combination are made. The long, flat surfaces of these gels can then be used to culture the cells. A problem with this design, however, is the difficulty of accurately cutting a small slice of the gel layers in a simple and efficient way, such that the gels do not become separated and the heights of the gels the same upon final inspection. During our time at GSET, we attempted to create this design (Fig. 6). Unfortunately, the gels did not adhere together, causing us to discard this design before attempting to cut the vertical sample.

Another possible design also uses a well to hold the bis-acrylamide liquids. The design involves the use of three rectangular plastic dividers 11.0 mm in width, and three circular pieces of plastic 11.0 mm in diameter with a 1 mm wide hole placed in center of one quadrant of the circle. The dividers are placed into the middle of three wells of a 48-well cell culture plate. One half of the wells are then filled with 150 µL of 3% bis-acrylamide solution containing both APS and TEMED. The liquid forms a gel, and then the dividers are removed, washed, and placed into three new wells.

Figure 6: The inability of the two different gel types to adhere to one another

The process of adding liquids is repeated, except with 5% bis-acrylamide solutions containing APS and TEMED. Then, the process is once again repeated with the 8% bis-acrylamide solution containing APS and TEMED in three new wells. Now, the 11.0 mm circles are placed on top of the wells with the 3% gel. The holes should be lined up with the empty half of the wells. Push the circles down until they push firmly against the gel. Add 5% bis-acrylamide solution containing APS and TEMED and colored with the Sulforhodamine B sodium salt through the holes until the liquid begins to overflow through the top of the circles. Let the gel harden and/or use an exacto-knife to cut the excess gel off from the rest of the gel. Repeat this process, adding 8% solution to the wells with the 5% gel, and 3% solution to the wells with the 5% well. Hypothetically, this design should allow for the creation
of gels of uniform height without any holes in between the gels. The gels may be moved using a microspatula.

### 4.7 Additional Research

Our research group also helped analyze data completed by Dr. Michelle Previtera. We used ImageJ to find the intensities of the images of the cells dyed with KI-67 and the size of the cells. The data that was gathered via ImageJ was then given to Dr. Michelle Previtera for normalization and statistical analyses. Figures 7 and 8 below are examples of images analyzed using ImageJ.

In her research, Dr. Previtera grew neuronal precursor cells as neurospheres in media (DMEM:F12 (Gibco), B27, and Pen/Strep, bFGF, EGF) for 6 days. Cells were passaged and grown for another 4-6 days. Cells were passaged again on day 4-6 and plated on 0.3, 6, and 25 kPa gels (3, 5, and 8% gels respectively). Cells were fixed with 4% paraformaldehyde for 10 mins and stained for Nestin (Millipore), KI-67 (Millipore) and DAPI (Sigma) on DIV (Days in vitro). Cells were imaged with a 20x objective.

![Figure 7: These cells were stained by KI-67](image-url)
4.8 DataNormalization

Data normalization is the structuring of data in a way that eliminates repetition and provides a fast and efficient way to finding necessary information by using keys, columns, and tables. [11] Overall, the goal of normalization is to remove the variability within an experiment. Dr. Previtera normalized the means of the three, five, and eight percent gels’ by dividing their values by the average mean value of the three percent gel. In order to normalize the gels’ maximum value, she divided the data of all three gels by the average maximum value of the three percent gel. Lastly, for the gels’ minimum value, she divided the figures by the average minimum value of the three percent gel.

5 Results

5.1 Experimental Results

Due to time constraints and unforeseen difficulties, we were unable to culture cells on the primary design listed in methods section 4.2. Ultimately, the gels were uneven, and we did not have time to prepare the gel for the addition of more cells. The gels were uneven because the outside ring of gel was of higher height than the inside ring, despite our attempts to use the cover of a petri dish to press down upon the two bis-acrylamide gels. We attempted to alleviate this in a second design by adding a weight, in the form of a PDMS ring and an empty petri dish held on top of the petri dish cover used to press down on the solutions. Unfortunately, this weight caused the outside liquid solution to be pushed out of the edges of the petri dish. As a result, the second petri dish was also uneven; the inside ring was of greater height than the outside ring. Additionally, the dye diffused into the inner gel (Fig. 9). It is possible this diffusion was caused because the gels are porous when solidified, and the liquid dye was able to diffuse through the pores into the inner gel.
5.2 Additional Research Results

As stiffness increased, the normalized mean, minimum and maximum intensity values increased. This indicates proliferation is occurring more as stiffness increased. This matches previous results showing the percentage of KI-67 stained cells increased as stiffness increased (data not shown). Figure 10 (below) details the results of this experiment.

5.3 First Design

We encountered various issues when testing the first design. This design used the concept of surrounding a smaller section of gel with an outside layer of a different gel. An issue regarding this concept was ensuring that the gels remaining aligned in height even after flipping the gels over, i.e. after making the previous bottom of the gel the new top. When this was attempted, in many cases, the inside gel was of greater height than the outside gel, and the outside gel would slide down, hitting the bottom of the well. As such, the gels could not remain level.

Because of the time constraint previously mentioned, the whole design testing process needed to be conducted on an abbreviated schedule. Normally, the bis-acrylamide needs at least a week to polymerize sufficiently. For the designs tested, however, the bis-acrylamide was permitted only 15-20 minutes to harden before the additional layer was added. Also, after both layers were combined, they were only given at most one day to solidify and combine. The gels were fragile and difficult to handle due to this fact, and as a result, the softer bis-acrylamide gels did not completely solidify, particularly in the first design, leading to many issues involving creating a gel combination without gaps in between the gels. Also, this made it difficult to transport the gels between the wells of different sizes and to remove the gels from their wells throughout the experimentation process, since the gels would not adhere to one another during the transfer process. Furthermore, some toxic liquid bis-acrylamide monomers remained in the gel (to be explained in more detail in the analysis section). Subsequently, the toxicity of the solution killed many of the neurons used in the first design (see Fig. 11, 12, and 13) and we were forced to discard this design.
Figure 10: Normalized KI-67 intensity values of DIV 5 NPCs grown on 0.3-25 kPa gels. Neurospheres were grown, dissociate, plated, and fix onto various gels. A. Mean value of pixel intensity. B. Minimum (white) and Maximum (grey) mean values of pixel intensity. C. Representative images of A and B. Scale bar= 200 μm. Asterisks indicate statistical significance.

Figure 11: A picture of the 5% gel surrounded by the 8% gel. The picture shows cells in the 8% gel. The dots represent dead neurons.

Figure 12: A picture of the 5% gel surrounded by the 8% gel. This picture shows cells in the 5% gel. The dots once again represent dead neurons.
Various improvements may be made to the design of the experiment. If we had more time, we would have been able to assure that the gels would fully solidify before adding cells and transferring them before containers, such as in the first design. For both the first design (4.5) and the primary design (4.2), letting the gels solidify fully before adding the other gel, cells, or transferring the gels could have allowed the experiments to be more successful. For the first design, the cells would not have died, and for the primary experimental design, the inside gel might not have been contaminated by the outside gel (as shown by the pink color of the inside gel).

Another possible improvement to the first design is adding more of the outside gel. If the outside gel is added in such an amount that it covers the first gel, then when the gels are removed from the wells, they are less likely to fall apart. The excess outside gel may act as an even bottom for both gels, with the face of the gels previously touching the bottom of the wells once again acting as the top of the gels. The gels should not face the problem of the gel of lower height not attaching successfully to the other gel and sliding down to the bottom of the 12-well plate.

When it was discovered that the gels of the primary design were not of even height, it was suggested that we flip them, such that once again the bottom becomes the top of the gel. However, when we attempted this, the gels fell apart because they were attached to the hydrophilic surface of the petri dish. In our attempts to remove the gels from the petri dishes, we were able to remove both from the dish. One suffered fairly minimal damage, but for the other, the two gels fell apart. A possible improvement is applying a hydrophobic solution to the inside of the petri dishes used, such that it would be easier to remove the gels from them without the gels falling apart.

6 Analysis/Discussion

6.1 Design of Gels

We developed several experimental conceptions. One major problem lied in the assumption that the two gel types would form chemical bonds with one another, therefore sticking together. Unfortunately, this was not the case; after polymerization, the two gels did not co-polymerize to one another and simply disintegrated. Especially in the first attempted design, the inability of the gels to adhere to each other was an important issue. Because the gels did not adhere successfully, we did not use this design as our primary design. Also, the gels in each combination were not of even height,
causing a new variable to be introduced and influencing cell development. Furthermore due to time constraints and the thickness of the gels, a much larger amount of the liquid acrylamide monomer remained, compared to previous experiments conducted involving bis-acrylamide. Therefore, after the neuronal cells were plated, they ultimately died because of the toxicity from the liquid bis-acrylamide. As a result, we decided to experiment on a different prototype- the primary design described in 4.2. Unfortunately, because we did not have sufficient time, we were unable to plate cells onto these gels to examine if the neurons would have survived.

Overall, our results did not correspond to our hypothesis, determined after our side project conducted with Dr. Previtera, that the gels would function effectively in promoting neuron growth. We had expected our neurons to grow from the softer gels towards the stiffer gels. With additional time, it is possible that our group would have been able to design a model that functions in testing this hypothesis. However, due to the time constraints, our results are inconclusive. They have, though, provided us with valuable insight about the design and experimentation process for bis-acrylamide related research, which maybe be instituted in the future for an improved design.

6.2 Future Research

Future research could involve designing more experiments that could use bis-acrylamide gels to determine if neurons preferred growing on gels of greater or less stiffness. We could also examine the impact of biodegradable scaffolds of different rigidities on neuronal development within the spinal cord itself. This would be a continuation of our research on the effect of mechanical factors on the proliferation of neurons. In combination with hormones and proteins that restrict the growth of scar tissue, different scaffolds, could promote the growth of neurons within the spinal cord.

Current research has only tested the effect of a two dimensional surface’s stiffness on cell growth. By creating three dimensional architecture, it would be possible to test the effects of a surface that surrounds the cell. Future research that involves surrounding cells with a three dimensional architecture would be able to better simulate environments the cells encounter within the body.

7 Conclusion

In our experiment, our objective was to examine how neurons would journey from pliable to rigid gel. In our design attempts, we learned a great deal of information about neurology and experimental design. As high school students, we entered this project with only a basic understanding of the human nervous system. Through our research, though, we have learned a great deal about not only human physiology, but also current and novel research being conducted in the field of human neurology. Also, through this experience, we learned how to design experiments that are practical and may be useful for additional research in the future. In fact, work on our primary design is continuing under Dr. Previtera even after the NJGSET program ends. Furthermore, our time spent under Dr. Previtera’s guidance has also taught us
lab techniques that we may apply in the future.

Although the experiment did not produce optimal results lack of time, we did, however, realize through the additional analysis of the work done by Dr. Previtera that cells preferred to proliferate in cultures that have stiffer gels. With this ideology, we could potentially invent not only an effective but also a safe way to aid patients with spinal cord injuries. For instance, if a part of the spinal is damaged, doctors can inject lab-grown cells into patients, helping them restore their spinal cord. Consequently, this experiment provided the fundamentals of the recovery process. Although this may be a minuscule part in the process, it is by far one of the most vital ones because it helps us comprehend neurons by nurturing them in the type of habitat they prefer best. By gaining an understanding of this, we can have hopes of discovering a unique and resourceful way of saving the wounded.

8 Acknowledgements

We would like to thank our project mentors, Dr. Noshir Langrana and Dr. Michelle Previtera, who aided us in this research project. We would also like to express appreciation toward our RTA mentor, Namrata Kulkarni, who has greatly assisted us in our research. Also, special thanks go out to the New Jersey Governor’s School of Engineering and Technology Coordinator, Jean Patrick Antoine, the Head Counselor Daniel Cobar, Dean Ilene Rosen, and the Governor School’s Board of Overseers Ronald D. Wilson, Chair, and Anthony Grillo, Brian D. Perkins, Vice Chairs. Additionally, we would like to show gratitude to the sponsors of Governor School of Technology and Engineering 2011, Rutgers University, the Rutgers University School of Engineering, Morgan Stanley, the State of New Jersey, Lockheed Martin, Automated Control Concepts Inc., Silver Line Building Products, NJSCR #08-3080-SCR-E-0, Sharon Ma and Nan Yao (parents of Johnathan Yao, GSET 2010), the Tomasetta family and Laura Overdeck without whom this fantastic opportunity would not have existed.

9 References


Web. 16 July 2011.
<http://webspace.ship.edu/cgboe
theneuron.html>.


<http://www.udel.edu/biology/f
schmieg/411acrylamide.htm>.


tro.html>.


m/en-us/library/aa291817(v=vs.71).aspx>