INTRODUCTION

Nowadays, per research study, around 5.6 million people around the United States suffer from paralysis [1]. There are many reasons that contribute to paralysis: stroke, spinal cord injuries, multiple sclerosis and so on. Patients who suffer from paralysis lose partial or fully sensory function and motor intention. Although paralysis is not life-threatening for most of the patients, impaired patients cannot be fully recovered. They also need to rely on caregiver’s assist to perform even simple daily tasks and they cannot live on their independent lives.

Fortunately, a novel clinical device named brain-control interfaces bring hope to paralyzed patients. The brain-control interfaces use neural probe to connect biological subject’s brain cortex with computer-based machine. There are two main functions of this device: deep brain stimulation and signal recording [2]. Deep brain stimulation stimulates the neurons in brain to ameliorate the symptoms for brain diseases [2], and signal recording decodes the motor intention from patients’ brain and send the neural signal to control prosthetic limbs, so that patients can restore their motor intention [2].

Although paralyzed patients need lifetime signal recording for their disease treatment, neural signal recording declines over time within brain-control interface, which has negative impact on the disease treatment. The long-term and steady signal recording requires steady neuron density around neural probe. The reason why neural signal declines is that neural implant in brain cortex causes brain tissue’s inflammatory response, which lead to loss of neurons and glial sheath formation as a result of gliosis around probe [3].

Due to the probe recording defect, researchers have conducted several experiments to improve probe signal recording. After several experiments carried out, researchers found out the cell adhesion molecule L1 coated neural probe, has shown to improve neuron outgrowth and inhibit gliosis around neural probe in brain tissue [3]. This result also shows that L1 coated neural probe helps to achieve long-term signal recording in brain tissue.

OBJECTIVE

Since the L1 coated neural probe has been proved to have the function of inhibiting gliosis in brain tissue, in order to find out whether L1 inhibits gliosis under different biological environment, further investigations of L1’s function in cell culture experiment needed to be performed. There are three main types of gliosis: macrophage activation, microglia activation and reactive astrocytes [3]. The objective of my research is to determine whether L1 inhibits macrophage activation in cell culture experiment.

HYPOTHESIS

The hypothesis in my study is L1 cell adhesion molecule will inhibit macrophage activation in cell culture experiment.

METHODS

The primary cell culture model was applied in this experiment and 96-well cell culture plate was used to incubate the cell during this experiment.

To determine whether L1 molecule inhibits macrophage activation for both inside of solution condition and in coating condition, two experimental groups and two control groups were involved in this experiment. The first experimental group was L1 soluble group, which was controlled by TCPS group. L1 soluble group contained L1 solution inside of cell media, and TCPS is the material of 96-well cell culture plate. The second experimental group was nitrocellulose fully-coated cell well with L1 half-coated cell well group, which was controlled by nitrocellulose fully-coated group. Nitrocellulose was used to immobilize L1, so that L1’s function was optimized in cell well coated environment.

To find out whether L1 molecule inhibits macrophage activation under regular condition and stimulated condition, two experimental conditions were involved in this experiment. For regular condition, the regular cell media was used among all groups. For stimulated condition, the stimulated media was used among all groups. The main difference between regular media and stimulated media was that the stimulated media contained LPS, which is the major component at the outer membrane of Gram-negative bacteria. Since macrophage intends to kill bacteria as part of immune response, LPS stimulates macrophage because LPS is mistaken for bacteria when macrophage encounters with it.

This experiment was started from isolating the primary macrophage from mouse intraperitoneal portion. The isolated macrophage solution was suspended in centrifuge. Then the cell pellet was collected from the bottom of centrifuge and was resuspended again in regular media. As a result, the total number of macrophages are calculated from hemocytometer, and the macrophage concentration was determined from total cell number and media volume.

The next step was cell well coating in second experimental group and its control group. The actual experimental set up was shown at Figure 1 below.

![Figure 1. Experimental Set up](image)

The cell wells in third row and fourth row were at first pipetted in nitrocellulose/methanol solution to fully cover the bottom, then the solution was immediately aspirated off. Only
for nitrocellulose fully-coated group with L1 half-coated group, L1 solution was then added to ensure it coated half of the bottom. Cell plating was then performed by adding regular media to all of the 28 cell wells among four groups, after that, macrophages were incubated for 6 days.

The third step was stimulating macrophages. Six days after, the regular media was aspirated off from each cell well, then regular media was added to 1-3 columns, and stimulated media was added to 4-7 columns. The macrophages were then incubated for another day.

The last step was to determine the macrophage activation rate by performing Griess Assay. Nitrite oxide is generated by macrophages as part of immune response, and nitrite oxide converts to nitrite in physiological condition. The higher nitrite concentration, the higher macrophage activation rate. Griess Assay is used to determine nitrite concentration quantitatively by measuring absorbance from cell media which contains nitrite. The nitrite standard curve was generated by using nitrite standard solution. Then the cell media in each of the cell well was extracted and released at the corresponding new cell well in a new cell plate, the griess reagent and deionized water were then added to each cell well in order. Finally, the absorbance of cell media at each cell well were measured by spectrophotometer.

RESULTS

The nitrite standard curve (Figure 2) was generated by diluting nitrite standard solution from 1µM to 15µM, and each absorbance corresponds to each concentration. The linear regression line was generated to estimate relationship between absorbance and concentration.

The nitrite concentrations for each of cell well were measured by converting each absorbance from each cell well to each concentration from nitrite standard curve. Each blue bar represents mean value from 1-3 columns in regular condition, each orange bar represents mean value from 4-7 columns in stimulated condition.

As seen in Figure 3, the L1 soluble group has significant lower nitrite concentration in both regular condition and stimulated condition compared to the regular condition and stimulated condition in TCPS group, with a statistically significant p value of less than 0.05. Additionally, in regular condition, nitrite concentration is slightly higher in nitrocellulose fully-coated with half-coated L1 than in fully-coated nitrocellulose group, with an insignificant p value of larger than 0.05. In stimulated condition, nitrite concentration is slightly lower in nitrocellulose fully-coated with half-coated L1 than in fully-coated nitrocellulose group, also with an insignificant p value of larger than 0.05.

DISCUSSION

From this experiment, the result from L1 soluble group and its control group shows L1 inhibits macrophage activation under soluble condition in cell media, however, whether L1 inhibits macrophage activation in cell well coating condition is still undetermined. Further experiments need to be conducted in the future to prove findings in this experiment are convincing as well as to obtain more consistent and more accurate results.

There are a few reasons contribute to experimental errors and limitation in this experiment. First of all, the uniformity of L1 coatings were not checked, however, L1 coatings’ uniformity affects L1’s function in inhibiting macrophages a lot, so this factor may contribute to the inaccuracy of our experiment results in two coating groups. Second, this experiment was conducted following pathogen associated molecular pathway, which did not simulate the actual neural probe implant environment very much.

Future experiments will be conducted following damage associated molecular pathway, which is closer to actual neural probe implant condition in brain tissue, because neural probe causes damage to brain tissue.

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REFERENCES

