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ABSTRACT

Neural prostheses aim to restore lost motor functions in tetraplegic humans by steering motion related signals from neurons to external devices like robotic arms. While they perform effectively during acute recordings, they often fail to function in chronic applications due to the lack of biocompatibility and due to the inflammatory response of the immune system in the brain. Hence, there is a need for neuronal adhesion, proliferation and neurite extension so that the proximity of the neurons near the electrodes is increased. To circumvent this problem, *in vitro* studies were carried out by covalently tethering laminin on glass surfaces with nerve growth factor in the culture medium at various concentrations using oxygen plasma. PC12 cell line was used as a model system to study the extent of neurite extension by culturing them on the laminin coated glass surfaces which were exposed to NGF. PC12 cells sprouted neurites depending on the concentration of NGF in the medium and best results were found at laminin concentration at 0.01mg/mL and an NGF concentration of 10000ng/mL. In an attempt to translate the model system for device implantation, laminin was immobilized with NGF and studied for neurite extension. Similar results compared to 0.01mg/mL laminin and 10000ng/mL NGF in solution were obtained at 0.05mg/mL laminin and 5000/10000 ng/mL NGF indicating that the synergistic presence of laminin and NGF at optimal concentrations may ultimately enhance axonal guidance and regeneration *in vivo*.

INTRODUCTION

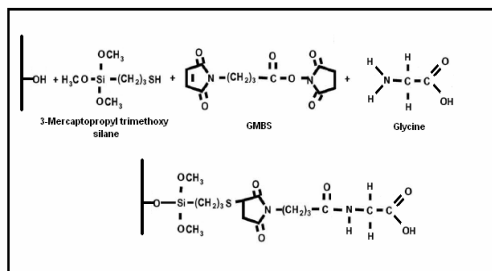
Traumatic injuries of the central nervous system and neurological disorders such as stroke, paralysis, etc continue to wreak distressing motor deficits for patients across the world. Every year, spinal cord injuries alone are responsible for the occurrence of about 11,000 new cases of permanent paralysis in the United States[1]. Neuroprosthetic devices have rekindled hope in patients suffering from partial or full paralysis[2]. They are implants made out of dozens of wire electrodes, which aid in sampling extra cellular potentials from portions of the cortex in the brain. The potentials recorded from neurons adjacent to the electrodes will be correlated to observed physical motion, which helps in translating the neuronal activity directly into robotic arm movements. While neuroprosthetic devices perform well during acute recordings, they often fail to perform during chronic applications[3]. Biocompatibility is a significant problem to solve for these of devices [4]. Glial scar formation and inflammation of the brain tissue due to immune response are the major modes of failure of Neuroprosthetic devices. This scar formation leads to increase in impedance of the prosthetic device and inhibits the device to send and receive electrical signals from the neurons. Hence, there is a need for neuronal adhesion, proliferation and neurite extension so that the proximity of the neurons to the electrodes is increased and thereby pushing the glial cells and astrocytes away from the electrodes.

METHODS

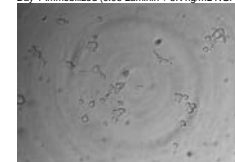
Tissue Culture:

PC12 cells frozen in a 1mL vial maintained at -137°C by using liquid nitrogen were thawed to room temperature and mixed with culture medium in 1:12 ratio (v/v). The culture medium contained 500mL of F-12K medium+ 75 mL of 15%horse serum+ 15mL of 2.5% premium fetal bovine serum+ 1.5mL of (25 units/mL) penicillin streptomycin. The PC12 cells solution was then suspended into a 75-cm² polystyrene culture flasks and incubated (37°C, 5%CO₂ and 95% air). The cell growth was monitored by using a hemacytometer and the cell concentration was measured at regular intervals of time: 1, 3, 5, 7, 9, 12, 14 days. Propagation of the PC-12 cell culture occurred every 7 days.

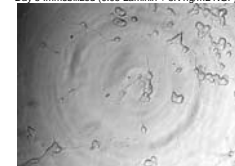
Surface Modification:



Day 1 Immobilized (0.05 Laminin + 5K ng/mL NGF)



Day 5 Immobilized (0.05 Laminin + 5K ng/mL NGF)



RESULTS

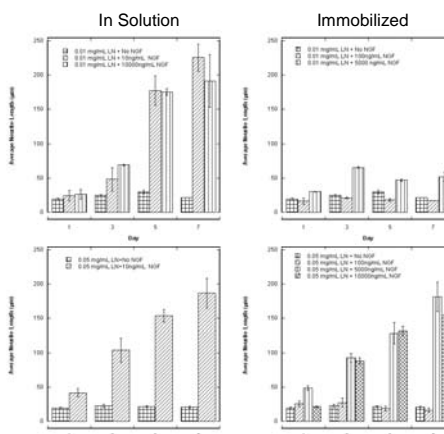
Laminin with NGF in solution and Laminin with NGF immobilized neurite length assay:

The average neurite length of PC12 was quantified using image analysis. The samples sprouted neurites depending on the NGF concentration and they appeared to sprout their neurites to a maximum length on day 7. However, maximum average neurite length observed in Laminin without NGF was around 22.8 microns, which is of small magnitude. There was a significant difference between the three samples since the amount of NGF was drastically different in them. We can note that the neurite length for Laminin 0.05mg/mL with 10ng/mL NGF in solution was lower than the sample which had Laminin 0.01mg/mL with 10ng/mL NGF in solution, although it showed better extension than the control.

Laminin with NGF in solution and Laminin with NGF immobilized neurite number assay:

Neurite number was obtained by manually counting the number of neurites extended per cell soma for the longest 10-15 neurites in the image domain. The neurite number followed a similar pattern like length where the number was directly dependent on the NGF concentration (in solution). The maximum was observed on day 7 but the magnitudes were similar. For Laminin at 0.05mg/mL and 10ng/mL NGF in solution, the number of neurites showed limited growth after day 3. As described in the previous section, a similar pattern was observed with the immobilized NGF in different concentrations (as in neurite length) and the neurite number started falling after day 3 for Laminin 0.05mg/mL+no NGF and 100ng/mL NGF but on the other hand the neurite number was found to increase when NGF concentration was as high as 5000ng/mL and 10000ng/mL.

AVERAGE NEURITE LENGTH



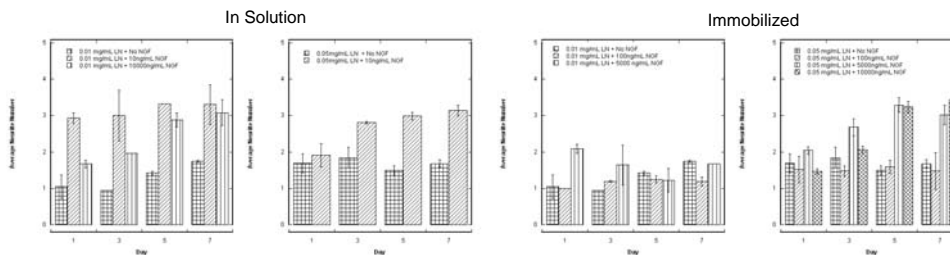
CLASSROOM CONNECTION

An Inquiry into Physiology of Termites and Blackworms

Students will be given the opportunity to investigate the behavior and physiology of termites and blackworms. Worker termites have an ability to follow an ink tracing on a piece of paper. Students will be presented with this phenomenon, then design an experiment to test other ink tracings. And, data will be collected and analyzed using easily available tools. Students may develop deeper hypotheses about the basis of the worker termite behavior, and they will be able to design further experiments to test these ideas. Similarly, students will determine the effect of varying concentration levels of common toxins on the circulatory system of blackworms.

Though the RET laboratory experience was not upon animal behavior, the intent of this lesson is to demonstrate how a living organism can sense and respond to its environment. Similarly, we are attempting to encourage the growth of nerve cells and attachment to an implant device. In addition, chemical sensation is based in the nervous system. As we learn about the development and structure and function of these systems in living organisms, we can use termites and blackworms as interesting, hands-on exploration of the content.

AVERAGE NEURITE NUMBER



References
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Images
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