INTRODUCTION

Peripheral nerve injury is a consequence of many different traumas including car accidents, combat wounds, and others. Nerve injury can severely diminish quality of life. Recovery is poor or absent in one third of patients[3], and patients who regain limb function often require months of rehabilitation. Transection injuries are particularly difficult to treat[3]. Treatment of critical injuries— Injuries where the gap between severed ends is large enough to prevent the nerve from regenerating on its own—is especially lacking in effective options.

Unlike the central nervous system, the peripheral nervous system (PNS) possesses the potential for full regeneration after injury. Regeneration occurs through a process known as Wallerian degeneration. Following injury, the damaged part of the nerve degrades and is cleared away. Next Schwann cells, specialized supportive cells in the nervous system, come in. The Schwann cells arrange themselves in lines to guide axon regrowth. Finally reinnervation, a return of nerve function, occurs when the nerve reconnects to its sensory and motor targets. However, this last step—functional recovery—is often delayed or incomplete since rapidly deposited scar tissue and an unfavorable environment impede recovery.

A common device used to treat nerve injury is the nerve growth conduit. It consists of a hollow tube that encapsulates the damaged ends of the nerve and guides regrowth. In order to effectively promote axon regeneration while preventing inflammation and scar tissue, conduits must attract Schwann cells, contain neurotrophic factors (chemical signals that allow nerves to grow), provide structural support, and protect the distal nerve stump because it secretes factors that promote and guide regrowth[3]. Nerve growth guides must also be biocompatible, so as to avoid inflammation and immune rejection, biodegradable, and flexible in order to avoid compressional damage to the healing nerve. Currently available nerve conduits have yet to meet all of these criteria[3].

My lab has developed a novel nerve-specific extracellular matrix (ECM) hydrogel that can be used as a filler for conduits to aid in the process of nerve regeneration. The ECM is the structural and chemical environment generated by the resident cells of each tissue and therefore can be expected to vary between tissue types. For this reason, we use nerve specific ECM. The ECM undergoes a decellularization process; thus it lacks cells and associated factors that cause a specific immune reaction. ECM is biodegradable, providing temporary structural support. The chemical cues contained within nerve ECM promote the growth of axons. The hydrogel is also injectable, making it an easily usable and less invasive treatment method.

Successful nerve treatment of nerve injury is judged by functional recovery. Functional recovery can be measured by assessing muscle atrophy, since earlier reinnervation results in a decrease in muscle atrophy and a corresponding improvement in functional outcomes[4]. Measurement of average minimum muscle fiber diameter can be used to monitor the degree of muscle atrophy.

OBJECTIVE

The objective of this project was to enhance the natural regenerative potential of the PNS with the use of a nerve specific ECM scaffold and improve functional recovery in treatment of critical nerve injuries.

HYPOTHESIS/SUCCESS CRITERIA

It is hypothesized that rats treated with the ECM filled conduit will experience better functional recovery than rats treated with the saline control. Success of the experimental methods will be gauged by comparing the standard deviation in average muscle diameter measurements with literature values.

METHOD

To test the effectiveness of the ECM hydrogel, a rodent model of nerve injury was implemented. A critical gap was created in the rear right leg of rats. The gap was then repaired with a filled conduit— ECM gel filled for the experimental group (n=20) and saline filled for the control group (n=20). Rats were followed to 7, 14, 28, and 90 days post injury at which point sections of the gastrocnemius muscle were taken from both the injured and non-injured leg for functional recovery analysis. The non-injured leg served as a control to measure atrophy in the injured leg.

Muscles were stained with immunofluorescence to elucidate the borders of the muscle fibers. Stained slides were imaged with a fluorescence microscope. Five images were taken per leg for each rat. The images were next analyzed in ImageJ to obtain muscle fiber diameter measurements. ImageJ was used to track the minimum diameter of each individual muscle fiber.

The collected data was analyzed in Excel. Mean and standard deviation of minimum muscle diameter was calculated for each leg of each rat. Muscle atrophy for each rat was quantified by calculating the percentage decrease in average minimum muscle fiber diameter between the non-injured leg and injured leg. Significance was determined via a two-tailed t-test comparing average atrophy in the experimental group to average atrophy in the control group at each of the four time points. A p value of less than 0.05 was considered statistically significant.

RESULTS

Results for each of the two groups were averaged at each time point. Standard deviation of the measurements were in line with literature values[4][5]. As seen in Figure 1, atrophy progressed as the time after injury increase. This is as expected and is due to the progressive muscle atrophy that occurs after nerve injury. Average muscle atrophy at 7 days was 7.08% for the experimental group and 5.81% for the control group. At 14 days the experimental group showed 29.21% atrophy while the control group showed 45.71%. Atrophy at 28 days was 48.44% for the experimental group and 57.95% for the control group. At 90 days average atrophy was 68.09% for the experimental...
group and 75.48% for the control group. At 7 and 14 days, there was no significant difference in atrophy between experimental and control groups (p=0.763 and p=0.056, respectively). There was a significant difference in atrophy at both the 28 day (p=0.039) and 90 day (p=0.003) time points with less atrophy in the experimental group at both of these points.

**DISCUSSION**

The experiment met the success criteria; measurements of standard muscle fiber diameter fell within the appropriate range for validity. Less atrophy was seen among the experimental group at the longer time points. This is indicative of faster reinnervation, and increased functional recovery. From this study, it concluded that the NS ECM gel has the potential to decrease the degree of muscle atrophy that occurs with peripheral nerve injury, though larger studies are needed to definitively prove this.

Future studies will continue evaluating functional recovery by employing more direct methods of measuring muscle function such as biomechanical gait analysis. Studies will also involve longer time points in order to see if the ECM hydrogel continues to delay muscle atrophy long term. An examination of Schwann cell response to the ECM hydrogel is also planned in order to further understanding as to how the nerve-specific ECM promotes nerve regeneration.

This study was limited due to small sample size—only 4-6 animals were included in each group. It also did not employ any direct analysis of functional recovery, so it is unclear whether the lessened atrophy among the experimental group will lead to clinically significant functional returns. These limitations will be addressed in future studies.

In conclusion, the nerve-specific ECM hydrogel shows promise in the treatment of critical nerve injuries. The benefits of this biomaterial warrant further investigation and offer potential for improved nerve regeneration strategies.

**ACKNOWLEDGMENTS**

Thank you to the Brown Lab and to the University of Pittsburgh Honors College.

**REFERENCES**