INTRODUCTION

Often, when one experiences trauma or undergoes an invasive surgery, peripheral nerve damage results. Each year 360,000 people in the United States suffer from peripheral nerve injury [1]. Peripheral nerve injury can result in a loss of sensory and or motor function and will often lead to long-term disability in patients [2]. When a peripheral nerve is compressed or severed, the injured section of the nerve undergoes Wallerian degeneration until a gap has formed, separating the proximal and distal ends of the nerve and leaving a nerve gap [3].

When nerve gaps are too large (>15mm) for a surgeon to repair effectively by suturing the proximal end of the nerve to the distal end, there are two standard modes of repair: an autologous nerve graph and the use of a nerve conduit. In the use of autologous nerve graphs to replace the missing nerve the nerve gap, a section of a nerve from the patients own body, usually the seural nerve which runs down the back of the leg. The section of nerve removed is then sutured in place of the degenerated nerve. The autologous nerve graft method currently used is less than satisfactory, as the seural nerve is a sensory nerve, and leg sensation can be lost as a result of the surgery. The alternative, the use of a nerve conduit has been shown to have positive effects in the nerve regeneration, as it is used as a guide to help the axons of the severed nerve grow outward from the proximal end until it meets the distal end of the severed nerve. The nerve guide is effective in preventing wayward nerve growth. Its tubular shape allows nerve axons to grow straight toward the distal end rather than branching out with no specific direction. While the autologous nerve grafts and use of nerve conduits are effective in assisting axons in growth between the nerve gaps, the outcome of nerve repair with these treatments is determined by the location of the injury of the nerve and the time delay before treatment and fully functional recovery is rare [4].

Many researchers have been working to improve the functionality of regenerated nerves by not only using nerve conduits but by introducing various modifications to the conduits including addition of growth factors, supportive cells, and internal frameworks. The Brown Lab at the University of Pittsburgh has focused on the use of an internal framework and growth factors to modify a nerve conduit. We have done this by developing a nerve specific hydrogel derived from healthy porcine sciatic nerve. The nerve removed of DNA leaving behind only the extracellular matrix, which is an internal framework including growth factors that recruit Schwann cells similar to urinary bladder matrix hydrogels that have been used for regeneration of other types of tissue. Schwann cells are an integral component of nerve regeneration. The hydrogel begins as a liquid and becomes a gel at physiological temperature. Having peripheral nerve origin, the gel it maintains growth factors specific to nerves, and the hydrogel is hoped to have a positive effect on nerve regeneration [5].

To test the effectiveness of our product in vivo, we created a rat model in which the sciatic nerve was axially severed, creating a 15mm gap to simulate a severed nerve in a human. In study rats, our hydrogel was inserted into a conduit sutured to the ends of the severed nerve and taken out to 7, 14, 28, and 90-day time points. The control models were similar but had saline injected in the conduits rather than our hydrogel. To gain preliminary results, we excised and imaged a 28-day rat and imaged the nerve regrowth as shown in Figure 1.

Figure 1: Preliminary image of growth in 28 day rat model

OBJECTIVE

The preliminary image shown in Figure 1 let us know that there were definitely cells in the nerve gap during the 28 days. It was unknown if the cells in the area were blood cells, fibroblasts, or any other debris that could have washed into the area after The goal of the project then became to determine what types of cells were in the nerve gap. Specifically we were looking to determine whether or not axons were present in the gap to confirm whether or not the proximal nerve grew distally.

HYPOTHESIS/SUCCESS CRITERIA

Unpublished data collected in the Brown Lab from in vitro characterization studies of the nerve specific hydrogel proved that the hydrogel caused increased growth of neurite axons. Based on this data, it was hypothesized that the addition of hydrogel to a 15mm nerve gap rat model would not only cause beta tubulin 3 to be present in the nerve gap but also that an increase in beta tubulin 3 presence as the model was taken out to later time points. The presence of beta tubulin was to be tested via immunofluorescence staining. A resulting image with that displays color at the wavelength of the secondary antibody used would indicate that beta tubulin is present in the nerve gap, proving our hypothesis correct.

METHOD

We started our experiments with two slides with two rat model nerve sections each. Antigen retrieval was performed on the sections by washing them in a citric acid buffer for 20 minutes at 98°C followed by three five minute washes in Tris-Buffered Saline and Tween 20.
Blocking was then performed to prevent nonspecific binding of the primary and secondary antibodies. Donkey serum was used for blocking and the slides were washed in the serum for one hour.

The slides were washed in a beta tubulin primary antibody diluted in a 1:150 dilution in donkey serum overnight at 4°C. In order to confirm that the immunofluorescence staining worked correctly, primary antibody were only put on one of the sections, the positive section, of each slide so that it could be ensured that there is not too much autofluorescence in the images. Autofluorescence is caused by nonspecific binding of the secondary antibody to antigens on a tissue sample.

Following washing overnight in primary antibody the slides were washed three times in a phosphate buffer solution for five minutes each. The slides were washed in a donkey anti-mouse secondary antibody in a 1:300 dilution in donkey serum for one hour at room temperature in a closed humidity container. The slides were then washed three times in a phosphate buffer solution for five minutes each.

A DAPI coverslip was put on the slides to preserve the sections for imaging. The slides were imaged on a 20X microscope at 80% zoom and at a 594nm wavelength, the wavelength that the secondary antibody used reflected. The slides were imaged in multiple small 20X images and stitched together using Adobe Photoshop.

RESULTS

Figure 2: Beta tubulin fluorescent microscopy image of 14-day rat model. A is Positive stain on right and B is negative stain on left.

An initial image was taken to ensure that the slide staining worked and can be seen in Figure 2. The positive stain clearly displays more color that the negative stain, confirming that the stain was effective, and the fluorescence of the secondary antibody did not cause too much autofluorescence. We can safely say that the fluorescence is beta tubulin.

After the stain was confirmed, images of the other 14-day and 28-day rat model sections were taken as shown in Figure 3.

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REFERENCES

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