Improving Electrophysiological and Histological Outcomes by Photochemically Sealing the Peripheral Nerve Repair Site

INTRODUCTION:
Microsurgical suture repair remains the current gold standard for peripheral nerve repair, but often results in incomplete functional recovery. Photochemical tissue bonding (PTB) is a sutureless tissue repair process that can seal the repair site without trauma or foreign body reaction, thus allowing prompt restoration of the normal endoneural environment for healing. In PTB, a photoactive dye is applied to tissue surfaces that are then placed in contact and irradiated with green light, which, excess, is absorbed at a power that does not thermally damage the tissue, unlike thermal laser welding techniques, and therefore preserves the nerve structure and surrounding tissues. The light activates the dye leading to immediate formation of covalent crosslinks between proteins on both surfaces, thereby forming a water tight seal.

There also has been ongoing interest in nerve wrapping as a means to improve outcomes following peripheral neurotomy. Autologous monkey vein is most commonly used, the use of human amniotic membrane (HAM) has been reported as a method for reducing scar formation. This study determines whether isolation of the nerve repair site using photochemical tissue bonding (PTB) in combination with human amniotic membrane can improve both functional and histological recovery.

METHODS:
Twenty-four female New Zealand White Rabbits (2-2.5 kg) were used and sterile technique followed. All animal procedures were approved by the IACUC. The right common peroneal nerve was exposed through a dorso-lateral muscle splitting incision and sharply transected 5 cm distal to the sciatic notch. The nerves were repaired immediately with three 10/0 nylon epineurial sutures. Animals were randomized to one of three experimental groups:

1. Standard Neurorrhaphy (SN)/ Control Group (n=8)
2. Standard Neurorrhaphy (SN) & HAM wrap (n=8)
3. Standard Neurorrhaphy (SN), HAM / PTB (n=8)

Human placenta was obtained with the approval of the Institutional Review Board (IRB). The placenta was washed several times with Earle’s Balanced Salt Solution (EBBS) and the amniotic membrane was peeled away from the chorion and placed on nitrocellulose paper (epithelial side down). The amnion was cut into segments and stored at -100°C in 1% osmium tetroxide solution at 4°C for 48 hours, post fixed in 1% osmium tetroxide, followed by dehydration and embedding in epoxy. Cross sections (1 µm) were made 5 mm proximal and distal to the neurorrhaphy site and stained with 0.5% toluidine blue. Five images were taken from each proximal and distal segment in the experimental nerves at sites evenly distributed within the cross section. Fifty fibers were randomly selected in each image (total of 250 fibers per nerve sample). Total fiber diameter and axon diameter were measured using Image J™ Software. Myelin thickness was derived from the difference between the fiber and axon diameter. The g-ratio of each fiber was calculated as a ratio of the axon diameter to the fiber diameter. Statistical significance was set at p<0.05. Statistical significance was assessed by a one-way ANOVA for each time point using an unpaired t-test. A linear regression model of overall percentage change in time (using the slope of the percent change in amplitude per 30 days as a variable) comparing each group was also analyzed for the electrical data, thus allowing for an overall recovery rate to be expressed as a percentage and analyzed for significance.

RESULTS:
Nerves treated with amnion wraps, sealed with PTB demonstrated a statistically significant improvement across both functional and histological parameters. Functional recovery, as measured by repeated electrophysiological studies over time showed an overall increased rate of recovery (as measured by percentage change over time with linear regression), which was 26.2% greater than that of the control (Standard Neurorrhaphy) group. This difference was statistically significant (p<0.05). The SN & HAM group showed an overall rate of recovery that was 13.7% higher than the animals in the SN group, however this finding did not achieve statistical significance. These results are shown below in Figure 1. Nerves treated with PTB sealed amnion wraps had significantly (p<0.001) greater axon (5.08 ± 1.06 µm) and fiber diameters (7.46 ± 1.37 µm) as well as myelin thickness (2.39 ± 0.71 µm) and G-ratio (0.68 ± 0.07) distal to the repair site compared to standard neurorrhaphy alone (4.98 ± 1.81 µm, 6.77 ± 1.94 µm, 2.39 ± 0.71 µm and 0.71 ± 0.09 respectively). Nerves treated with PTB sealed amnion wraps also revealed a decrease in epineurial / extraneural axonal regeneration suggesting improved preservation of the neural microarchitecture.

DISCUSSION
In conclusion, we have shown that our photochemical technique along with a HAM wrap results in a strong reliable nerve repair that shows an improvement in both neurophysiological and histological outcomes over standard neurorrhaphy. This technique does not add significantly to operating time, does not require additional microsurgical training and would be implemented easily into clinical practice by microsurgeons.

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