Light Activated Sealing of Nerve Graft Coaptation Sites Improves Outcome Following Large Gap Nerve Injury

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Introduction: Outcomes following large deficit nerve injury and nerve graft reconstruction are poor. In addition to large regenerative distances, excess suture material and subsequent scar tissue formation at the repair site is a contributing factor. Alternatives to suture repair such as fibrin glue and laser welding are limited by poor bond strength, high rates of dehiscence and thermal damage. Photochemical tissue bonding (PTB) uses visible light to create sutureless, non-thermal, watertight bonds between two closely apposed tissue surfaces stained with a photoactive dye. When used in conjunction with a human amniotic membrane nerve wrap, our group has used this technology in rodent models of simple end-to-end repair, showing superior functional and histological outcomes in comparison to conventional epineurial suture. When applied to large deficit injury and nerve graft reconstruction, this technique has been limited by proteolytic degradation of amniotic membrane wraps and photochemical bonds during long recovery periods. Chemical crosslinking of nerve wraps prior to PTB may improve wrap durability and efficacy of technique. By investigating several candidate nerve wraps and fixation techniques, we have attempted to ascertain the optimal approach for these challenging injuries.

Methods: Three candidate nerve wraps (human amnion, crosslinked human amnion, crosslinked swine intestinal sub-mucosa (SIS)) and 3 fixation methods (epineurial suture, fibrin glue, PTB) were investigated. Wrap crosslinking was performed using (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS). Tensile properties of each nerve wrap and bond strengths of each repair technique were assessed following crosslinking with 1mM, 2mM, 4mM and 8mM EDC using a micro-tensiometer. Following digestion in type-2 collagenase, wrap durability was assessed by fluorescamine degradation assay. 110 inbred male Lewis rats were randomized into 11 groups (n=10). All rats had 15mm left sciatic nerve defects created and repaired with exchanged isografts. 9 groups had isografts secured by one of the aforementioned wrap/fixation combinations. 2 groups were used as positive (epineurial suture only) and negative (no repair) controls. Following surgery, walking track analysis was performed at monthly intervals and sciatic function index (SFI) calculated. Following sacrifice after 150-days, left (experimental) and right (control) gastrocnemius muscles were excised for calculation of muscle mass retention and repaired nerves were excised for histomorphometric analysis. Statistical analysis between treatment groups was performed using ANOVA and the post hoc Bonferroni test.

Results: Mean maximum load to failure and Young’s modulus for amnion and SIS wraps all increased significantly with increasing EDC concentration. Bond strength was maintained up to a maximum EDC
concentration of 4mM EDC. Crosslinking with 8mM EDC had a detrimental impact on bonding. No significant difference in protein liberation was observed between amnion wraps crosslinked with 4mM EDC and wraps crosslinked with 8mM EDC. Significant reductions in protein liberation were observed at all concentrations of crosslinked SIS (Fig 1). At sacrifice, crosslinked nerve wraps were still identifiable. No evidence of un-crosslinked nerve wraps could be found. Those nerves repaired photochemically had considerably less perineural adhesion formation compared with standard repair. Nerves repaired using xHAM + PTB experienced greater recovery of SFI although this did not reach statistical significance compared with standard repair (-67.93 +/- 5.11 vs -71.69 +/- 4.80) (Figure 2). Those nerves repaired with xHAM + PTB also recovered greatest gastrocnemius muscle mass retention and this result was statistically significant in comparison to standard repair (67.3% +/- 4.44 vs 60.0% +/- 5.16; p=0.02). No significant difference in axon diameter existed between treatment groups. Fiber and axon diameter and myelin thickness were all significantly greater in the xHAM + PTB group in comparison to standard repair (6.87μm +/- 2.23 vs. 5.47μm +/- 1.70; 4.51μm +/- 1.83 vs. 3.50μm +/- 1.44; 2.35μm +/- 0.64 vs. 1.96μm +/- 0.47).

Discussion: Whilst preserving the bonding ability of PTB, chemical crosslinking of nerve wraps improves their tensile strength and resistance to biodegradation. The use of crosslinked amnion and PTB to create a water-tight, sutureless bond at nerve graft coaptation sites has produced superior functional outcomes following large deficit injury when compared to standard repair.

Significance: Sealing the neurorrhaphy sites with PTB and biocompatible can improve functional outcomes following injuries leaving large nerve gaps.

![Human amnion degradation](image1)

![SIS degradation](image2)

Fig 1. (A) Type-2 collagenase degradation for amnion. (B) Type-2 collagenase degradation for SIS.
Figure 2. SFI for select groups. Those nerve repaired using xHAM+PTB recovered greatest SFI although this was not statistically significant compared with standard graft and epineurial suture (67.9+/-5.1 vs -71.7+/-4.8). HAM+PTB performed less well than xHAM+PTB and standard graft+suture although these differences were not significant (see table 2). xSIS+PTB performed significantly worse than xHAM+PTB and standard graft+suture (see table 2).