Improving outcomes in immediate and delayed nerve grafting of peripheral nerve gaps using light-activated sealing of neurorrhaphy sites with human amnion wraps

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Background

- Unsatisfactory outcomes following microsurgical peripheral nerve repair are linked with needle trauma, suture foreign body reaction, inflammation and scarring, axonal escape and neuroma formation
- Limitations more pronounced following nerve gap and grafting when regenerating axons must traverse 2 coaptation sites.
- Light-activated sealing of amnion nerve wraps around coaptation sites is superior to standard suture in rodent models of end-to-end repair (figure 1)

Figure 1. Sequence of amnion wrap application and light-activated sealing. Nerve end apposed. Nerve ends and amnion wrap stained with RB for 60 seconds. Amnion wrapped circumferentially around nerve ends. Wrap/nerve interface illuminated for 60 Seconds. Nerve rotated 180 degrees and illumination repeated for posterior wall

- Limited success when applied to nerve grafts due to proteolytic degradation of amnion during extended recovery
- Crosslinking amnion improves wrap durability resulting in superior outcomes in comparison to suture when applied to 15mm rodent isografts (PHASE 1 – unpublished data)
- Previous studies assessed light-activated repair when performed immediately after injury, a situation rarely encountered clinically
- Extended periods of delay are detrimental to recovery due to chronic axotomy and denervation

Hypothesis

- Light-activated sealing of nerve grafts remains efficacious following a clinically relevant delay and may reduce the detrimental impact of delay when compared to gold standard, immediate suturing

Experimental Approach

- 40 male inbred, Lewis rats, randomized 4 groups (n=10)
- 15mm left sciatic nerve gaps created and bridged with isografts
- Repairs performed either immediately or after a 30-day delay
- Isografts secured with either light activated sealing or conventional epineurial suture (figure 2)

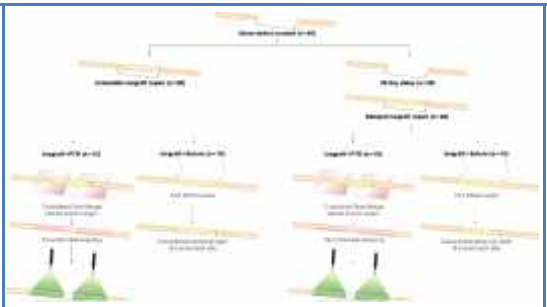

Figure 2. Experimental methods for immediate and delayed nerve graft repair

- 5-month follow-up
- Outcomes assessed using monthly walking track analysis and sciatic function index (SFI), gastrocnemius muscle mass retention and nerve histomorphometry

Results

- Crosslinked amnion wraps still present after 5-months (figure 3A)
- On gross observation, photochemical repairs had less scar tissue formation (figure 3B+C)

Figure 3. Gross observations following sacrifice. (A) Amnion nerve wraps still present after 5-months follow-up. (B) Relative absence of extraneural adhesion formation around PTB sites in comparison to (C) standard epineurial suture

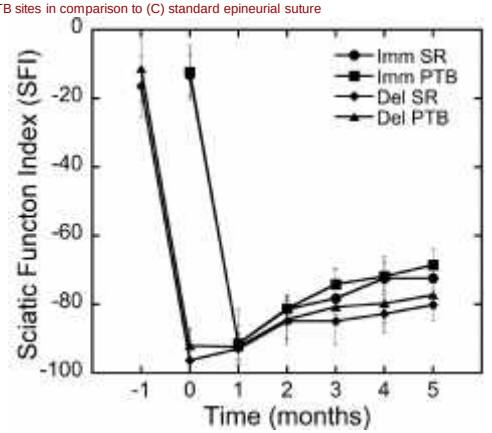

Figure 4. Sciatic Function Index of immediate and delayed groups. Immediate PTB repairs recovered greatest SFI after 5-months although this was not statistically significant in comparison to immediate suture. Similarly, no significant difference existed between delayed PTB and delayed suture. Immediate suture was significantly better than delayed suture (p=0.003). Immediate PTB was significantly better than delayed PTB (0.002). Immediate PTB was significantly better than delayed suture, which performed poorest out of all groups (p<0.0001). No significant difference existed between immediate suture and delayed PTB.

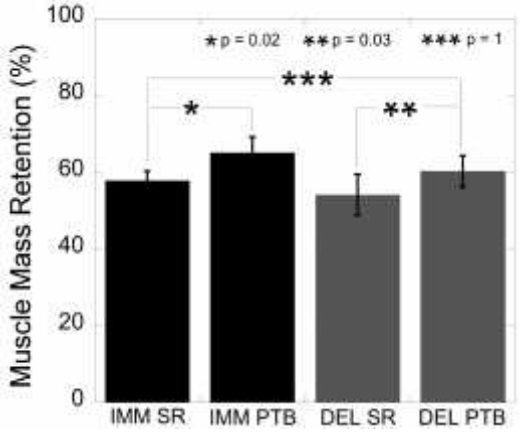

Figure 4. Gastrocnemius Muscle Mass Retention for immediate and delayed repair groups. Immediate PTB group recovered significantly greater muscle mass than immediate suture. Muscle mass was not significantly different between delayed PTB group and delayed suture.

Histomorphometry 5mm distal from distal isograft coaptation site (Mean+/-SD)

Experimental Group	Total axon count (x0.001)	Axon Density (mm^2 x 0.001)	Nerve fiber diameter (µm)	Axon diameter (µm)	Myelin thickness (µm)	G-ratio
Immediate Suture	7.34+/-4.38	24.83+/-4.23	5.75+/-1.67	4.15+/-1.38	1.60+/-0.48	0.72+/-0.06
Immediate PTB	7.29+/-4.63	23.25+/-2.25	6.30+/-1.69*	4.45+/-1.35*	1.85+/-0.61*	0.70+/-0.07*
Delayed suture	4.04+/-1.42	24.17+/-2.70	5.40+/-1.32	3.88+/-1.06	1.53+/-0.50	0.72+/-0.07
Delayed PTB	5.77+/-1.31	23.00+/-2.37	5.81+/-1.44	4.22+/-1.25	1.59+/-0.44	0.72+/-0.07

Table 1. Histomorphometric analysis. Due to large standard deviation, axon counts were not significantly different. Measurements were significantly greater in the immediate PTB group in comparison to immediate suture. There was no significant differences between immediate suture and delayed PTB. (*statistically significant in comparison to immediate suture).

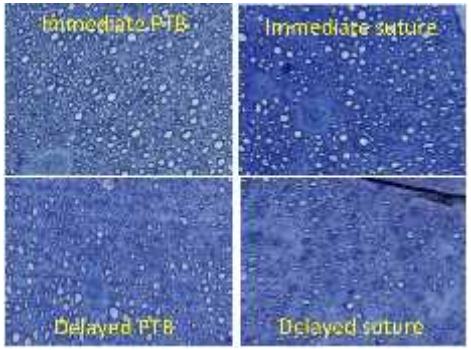

Figure 5. Nerve histology sections 5mm distal to distal nerve graft coaptation site

Conclusions

- Immediate light activated sealing of nerve ends is superior to standard suturing
- Following delayed repair, light activated sealing may help reduce the detrimental effects of chronic axotomy and denervation

Acknowledgement:
Funding for this study was provided by USAMRAA W81XWH-09-2-0001