The Application of Photochemical Tissue Bonding (PTB) for Large Gap Peripheral Nerve Injury

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Background

- Unsatisfactory outcomes following microsurgical peripheral nerve repair are linked with needle trauma, foreign body reaction, inflammation and scarring, axonal escape and neuroma formation
- These limitations are more pronounced following large deficit injury requiring nerve grafting when regenerating axons must traverse 2 coaptation sites.

Hypothesis

- Photochemical sealing of nerve graft coaptation sites using a durable, biocompatible nerve wrap will create a sutureless water tight seal, leading to superior outcomes compared to conventional methods.

Aims

1. Determine mechanical properties, seal strength and resistance to biodegradation of candidate biological nerve wraps.
2. Determine efficacy of regeneration in a rat model of large deficit injury as a function of nerve wrap/fixation method.

Experimental Approach

Aim 1

- Candidate nerve wrap biomaterials:
  - Processed human amnion (HAM)
  - Swine intestinal submucosa (SIS)
- Wraps crosslinked with EDC(1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/NHS (N-hydroxysuccinimide)) to improve durability.
- Mechanical properties of nerve wrap materials determined by tensiometry.
- Resistance to biodegradation measured using collagenase digestion and fluorescamine assay.
- Nerve wraps stained with 0.1% Rose bengal (RB, Fig 1) and bonded to ex-vivo rat sciatic nerves using 532 nm light (0.5 W/cm^2 for 2 mins per coaptation site).
- Bond strength between nerve wrap and epineurium tested using tensiometer (Fig. 2).

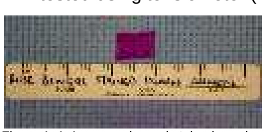

Figure 1. 1x1cm rose bengal stained amnion

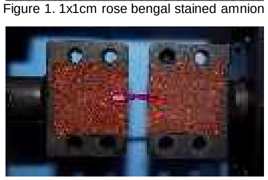

Fig 2. Sciatic nerve + RB stained HAM wrap sealed with PTB in tensiometer

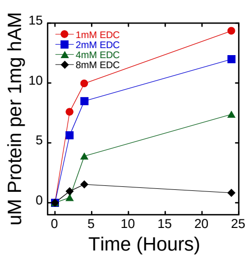

Fig 3. HAM degradation reduced >10 fold when increasing EDC/NHS concentration from 1/0.25mM to 8/2mM

- Tensile strength and Young's modulus of HAM and SIS increases (stronger) significantly with EDC/NHS concentration.
- Photochemical bond strength of epineurium/wrap interface is maintained until 8mM EDC/2mM NHS.
- Crosslinking HAM and SIS wraps reduces rate of proteolytic degradation (Fig 3).

➢ 4mM/1mM EDC/NHS selected for in vivo study based on optimal bond strength and resistance to collagenase degradation of xHAM and xSIS in vitro.

Aim 2

- 110 male Lewis rats
- 1.5cm left sciatic nerve defect created and repaired with isograft (Figure 4).
- 11 treatment combinations (n=10)

HAM **xHAM** **xSIS**	**X**	**Suture** **PTB** **Fibrin Glue**	**+**	**No repair** **Standard graft**

- Sacrifice after 150-days. Outcomes assessed by monthly walking track analysis (sciatic function index (SFI)), gastrocnemius muscle mass retention and, nerve histomorphometry.

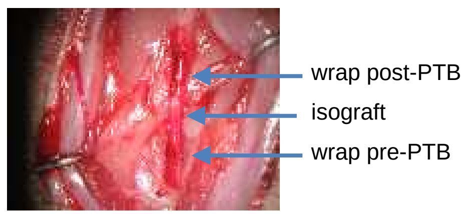

Fig 4. In-vivo rat sciatic nerve graft with coaptation sites wrapped with amnion and sealed with PTB

Outcome assessment

- Greatest recovery of SFI and muscle mass retention occurred in xHAM+PTB group (Table 1 and 2)

Experimental group	Mean 5-month SFI	SD	P value *
Negative control	-96.23	3.66	<0.0001*
Positive control	-71.69	4.8	1
HAM+suture	-77.85	6.27	0.39
HAM+fibrin	-75.15	4.62	1
HAM+PTB	-74.50	4.53	1
xHAM+suture	-76.82	2.68	1
xHAM+fibrin	-74.95	3.99	1
xHAM+PTB	-67.93	5.11	1
SIS+suture	-80.31	3.23	<0.01*
SIS+fibrin	-78.77	3.89	0.11
SIS+PTB	-84.97	6.02	<0.01*

*Statistical significance between treatment group and positive control group

Table 1. Mean 5-month SFI data for all treatment groups. Greatest functional recovery occurred in xHAM+PTB group although this was not significant compared with positive control group (-67.93+/-5.11 vs -71.69+/-4.80). Recovery was significantly worse in those nerves repaired with commercially available SIS material.

- No significant difference in axon counts in distal nerve segments between treatment groups
- Nerve fiber diameter, axon diameter, myelin thickness and G-ratio (corrected indicator of myelination) significantly greater in xHAM+PTB group in comparison to standard repair group (Table 4; Figure 5)

Experimental group	Mean left gastrocnemius muscle mass retention (%)	SD	P value*
Negative control	9.2	0.92	<0.0001*
Positive control	60.0	5.16	1
HAM+suture	56.0	5.60	1
HAM+fibrin	59.8	5.43	1
HAM+PTB	62.5	4.01	1
xHAM+suture	57.7	5.12	1
xHAM+fibrin	62.7	4.30	1
xHAM+PTB	67.3	4.44	0.02*
SIS+suture	54.9	4.46	0.68
SIS+fibrin	58.5	5.44	1
SIS+PTB	54.1	3.18	0.37

*statistical significance between treatment group and positive control group

Table 2. Mean left gastrocnemius muscle mass retention for all treatment groups. Greatest recovery of muscle mass occurred in xHAM+PTB group and this was significantly different in comparison to positive controls (67.3%+/-4.44 vs 60.0%+/-5.16; p=0.02). These results correlated well with SFI score.

Distal nerve histomorphometric measurements (mean +/- SD)

Experimental Group	Total axon count (x0.001)	Nerve fiber diameter (µm)	Axon diameter (µm)	Myelin thickness (µm)	G-ratio
No repair	0.04+/-0.05	4.14+/-1.15	3.13+/-1.17	1.01+/-0.48	0.74+/-0.12
Standard Graft Suture	7.61+/-3.42	5.47+/-1.70	3.90+/-1.44	1.96+/-0.47	0.62+/-0.06
HAM+suture	10.41+/-3.99	5.07+/-1.58	3.44+/-1.39	1.63+/-0.59	0.67+/-0.17
HAM+fibrin	10.42+/-1.54	5.22+/-1.67	3.44+/-1.45	1.78+/-0.45	0.64+/-0.09
HAM+PTB	9.31+/-4.19	5.19+/-1.76	3.47+/-1.51	1.72+/-0.43	0.68+/-0.09
xHAM+suture	9.79+/-3.35	5.14+/-1.66	3.54+/-1.47	1.59+/-0.36	0.67+/-0.06
xHAM+fibrin	10.87+/-4.32	5.24+/-1.69	3.52+/-1.46	1.72+/-0.42	0.65+/-0.09
xHAM+PTB	9.66+/-5.08	[illegible]*	[illegible]*	[illegible]*	0.64+/-0.08
SIS+suture	9.36+/-2.41	4.85+/-1.47	3.31+/-1.29	1.53+/-0.38	0.67+/-0.08
SIS+fibrin	6.91+/-2.62	5.18+/-1.30	3.58+/-1.56	1.59+/-0.52	0.68+/-0.11
SIS+PTB	7.84+/-2.04	4.81+/-1.49	3.33+/-1.33	1.45+/-0.34	0.68+/-0.08

Table 3. Histomorphometric measurements for all treatment groups. Nerve fiber diameter, axon diameter and myelin thickness significantly greater in xHAM+PTB group in comparison to positive control. *statistical significance in comparison to +ve control

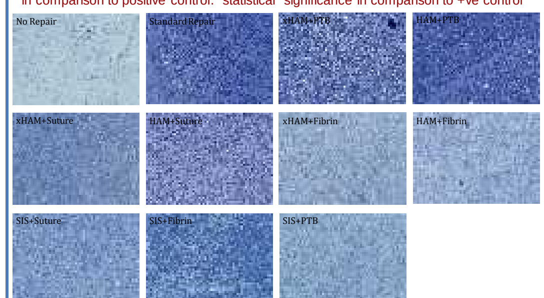

Figure 5. All treatment groups regenerated axons through isograft and into distal nerve segment. Gross examination shows more successful neuroregeneration in those nerves repaired using photochemically sealed crosslinked amnion. This finding is supported by histomorphometric analysis

Conclusions

➢EDC/NHS crosslinking of biological nerve wraps increases strength and resistance to proteolytic degradation.

➢Crosslinked amnion wraps photochemically sealed to neurorrhaphy sites of nerve grafts results in superior functional and histological outcomes compared with standard epineurial suture

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