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: Photochemical tissue bonding uses visible light to create sutureless, watertight bonds between two apposed tissue surfaces stained with photoactive dye. When applied to nerve grafting, photochemical tissue bonding can result in superior outcomes compared with suture fixation. Our previous success has focused on immediate repair. It was the aim of this study to assess the efficacy of photochemical tissue bonding when performed following a clinically relevant delay.

Methods: Forty male Lewis rats had 15-mm left sciatic nerve gaps repaired with reversed isografts immediately (n = 20) or after a 30-day delay (n = 20). Repairs were secured using either suture or photochemical tissue bonding. Rats were killed after 150 days. Outcomes were assessed using monthly Sciatic Function Index evaluation, muscle mass retention, and nerve histomorphometry. Statistical analysis was performed using analysis of variance and the post hoc Bonferroni test.

Results: In both immediate and delayed groups, photochemical tissue bonding showed a trend toward greater recovery of Sciatic Function Index, but these results were not significant. The Sciatic Function Index was significantly greater when performed immediately. Significantly greater muscle mass retention occurred following photochemical tissue bonding in both immediate and delayed repairs. Values did not differ significantly between immediate and delayed groups. Histomorphometric recovery was greatest in the immediate photochemical tissue bonding group and poorest in the delayed suture group. Fiber diameter, axon diameter, myelin thickness, and G-ratio were not significantly different between immediate suture and delayed photochemical tissue bonding.

Conclusions: Light-activated sealing of nerve grafts results in significantly better outcomes in comparison with conventional suture. The technique not only remains efficacious but may also help ameliorate the detrimental impacts of surgical delay. (*Plast. Reconstr. Surg.* 137: 887, 2016.)

Early investigators postulated that delaying nerve repair until cell bodies had been metabolically primed and distal nerves had been sufficiently cleared of debris would translate into optimized regeneration and recovery. Similarly, others theorized that predegeneration of nerve grafts would also result in superior outcomes. Despite early promise, these predictions were not substantiated in animal models.[1] Contemporary studies have highlighted the importance of expeditious nerve repair. Fu and Gordon observed that poor recovery was a result of the combined effects of chronic axotomy and chronic denervation of the distal nerve and muscle and delineated the relative contributions played by each.[2,3] These and other studies have confirmed

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that chronic denervation of the distal nerve and target muscle is the most important contributing factor.[2–5] Denervated Schwann cells are less able to support regenerating axons, and chronically denervated muscle undergoes atrophy, is less able to recover from this atrophy, and has also been shown to impart a retrograde inhibitory influence on regenerating axons, the effects of which worsen with time.[5]

Following trauma, priority is given to patient stabilization and wound decontamination before the onset of definitive reconstruction. As a result, long delays before nerve repair can be unavoidable. Denervation times are further extended following proximal limb injury and when substantial nerve gaps exist. Autologous nerve grafts represent the current gold standard method of nerve gap repair although, following major tissue loss and limb amputation, when demand for autograft often exceeds supply, the use of acellular nerve allografts is an alternative option. Outcomes in these circumstances are notoriously poor. Improving regenerative support within grafts and expediting rates of axonal regeneration aims to reduce the deleterious effects of denervation and is the focus of much research. A limitation of many studies is that the intervention under investigation often occurs immediately following nerve repair, a situation that simply does not exist clinically. As a result, some studies may not readily translate into clinical practice.

Researchers have demonstrated superior rates of regeneration and recovery following electrical stimulation.[6–10] At the cellular level, others have reported success following the supplementation of stem cells and neurotrophic factors.[11–14] We propose a novel method that may enhance outcomes following delayed nerve gap repair by sealing the neurorrhaphy site, capturing endogenous regenerative factors within, and excluding unwanted mediators of scarring and inflammation. Light-activated sealing of human amnion nerve wraps around coaptation sites is an alternative fixation method to conventional suture. A photochemical reaction between amnion and epineurium, both of which have been stained with a photoactive dye, results in the formation of watertight bonds. In recent studies, this technique, known as photochemical tissue bonding, has been found to result in superior outcomes in comparison with conventional suture when tested in rodent models of end-to-end[15–17] and immediate isograft repair.[18] The technique has also remained efficacious when used with acellular nerve allografts.[19] Crosslinking of amnion before sealing offers protection from proteolytic degradation over prolonged periods of recovery associated with nerve grafting. The technique has also been applied successfully to peripheral nerve repair using photochemically sealed chitosan adhesive films.[20] This study assesses whether photochemical sealing of nerve grafts remains efficacious following a clinically relevant surgical delay and, indeed, whether outcomes can be enhanced in comparison with conventional suture repair.

METHODS

Amnion Nerve Wrap Preparation

Human amniotic membrane was obtained from elective cesarean section patients who had been screened for human immunodeficiency virus types 1 and 2, hepatitis B, hepatitis C, human T-cell lymphotrophic virus, syphilis, cytomegalovirus, and tuberculosis. Once removed from the placenta, amnion was irrigated with phosphate-buffered saline (Sigma-Aldrich, Co., St. Louis, Mo.). Membranes were mechanically deepithelialized using a cell scraper, cut into strips, wrapped around nitrocellulose paper, and placed in a storage solution containing a 1:1 mix of 100% sterile glycerol and Dulbecco's Modified Eagle Medium (Gibco, Grand Island, N.Y.), penicillin-streptomycin-neomycin (Gibco), and amphotericin B. Human amniotic membrane was stored at −80°C and, when required, thawed, dried onto nitrocellulose paper, and cut into 1 × 1-cm sections. Nerve wraps were cross-linked, as described previously in a recent publication, using water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-hydroxysuccinimide (Sigma-Aldrich) reconstituted with 2-(*N*-morpholino) ethanesulfonic acid buffer (Sigma-Aldrich).[18]

Sciatic Nerve Injury and Reconstruction

The Institutional Animal Care and Use Committee at the Massachusetts General Hospital approved all procedures. Forty inbred Lewis rats weighing 250 to 300 g were randomized to one of four experimental groups described below. A further 10 Lewis rats were used as isograft donors. This breed was selected to permit immunotolerant isograft exchange between rodents for nerve gap reconstruction. Induction and maintenance anesthesia was achieved using isoflurane (Baxter Healthcare Corp., Deerfield, Ill.), 5% induction and 2% to 3% maintenance. Two surgeons performed all procedures together. The lead surgeon

was a senior plastic surgery trainee with microsurgical experience. The assistant surgeon was a general surgical trainee who received microsurgical training before rodent operations. A dorsolateral, muscle-splitting incision was made on the left hindquarter of each animal and, under the operating microscope, the sciatic nerve was mobilized along its length. To standardize nerve graft location, a site 5 mm proximal to the trifurcation was marked. Using digital calipers, grafts measuring 15 mm proximal to this mark were excised. Groups 1 and 2 had nerve gaps repaired immediately, whereas groups 3 and 4 had nerve gaps repaired following a 30-day delay (Fig. 1). Wounds were closed in three layers with 4-0 Vicryl (Ethicon, Inc., Somerville, N.J.) (muscle and deep dermal) and 4-0 Monocryl (Ethicon) (subcuticular). Topical antibacterial ointment was applied liberally to wounds, and bitter apple sprayed onto the left foot to discourage automutilation. Rodents were housed in the Massachusetts General Hospital small-animal facility and had access to food and water as needed.

Immediate Repair: Groups 1 and 2

Two rodents were anesthetized simultaneously. Following the excision of 15-mm segments of sciatic nerve, nerves were reversed and immediately exchanged as isografts between animals. In group 1, isografts were secured using six 10-0 Ethilon sutures (Ethicon), representing the current standard of care. Following repair, any protruding axons were trimmed and allowed to retract within the coaptation site. In group 2, isografts were secured using photochemical sealing. Amnion nerve wraps were prepared as described

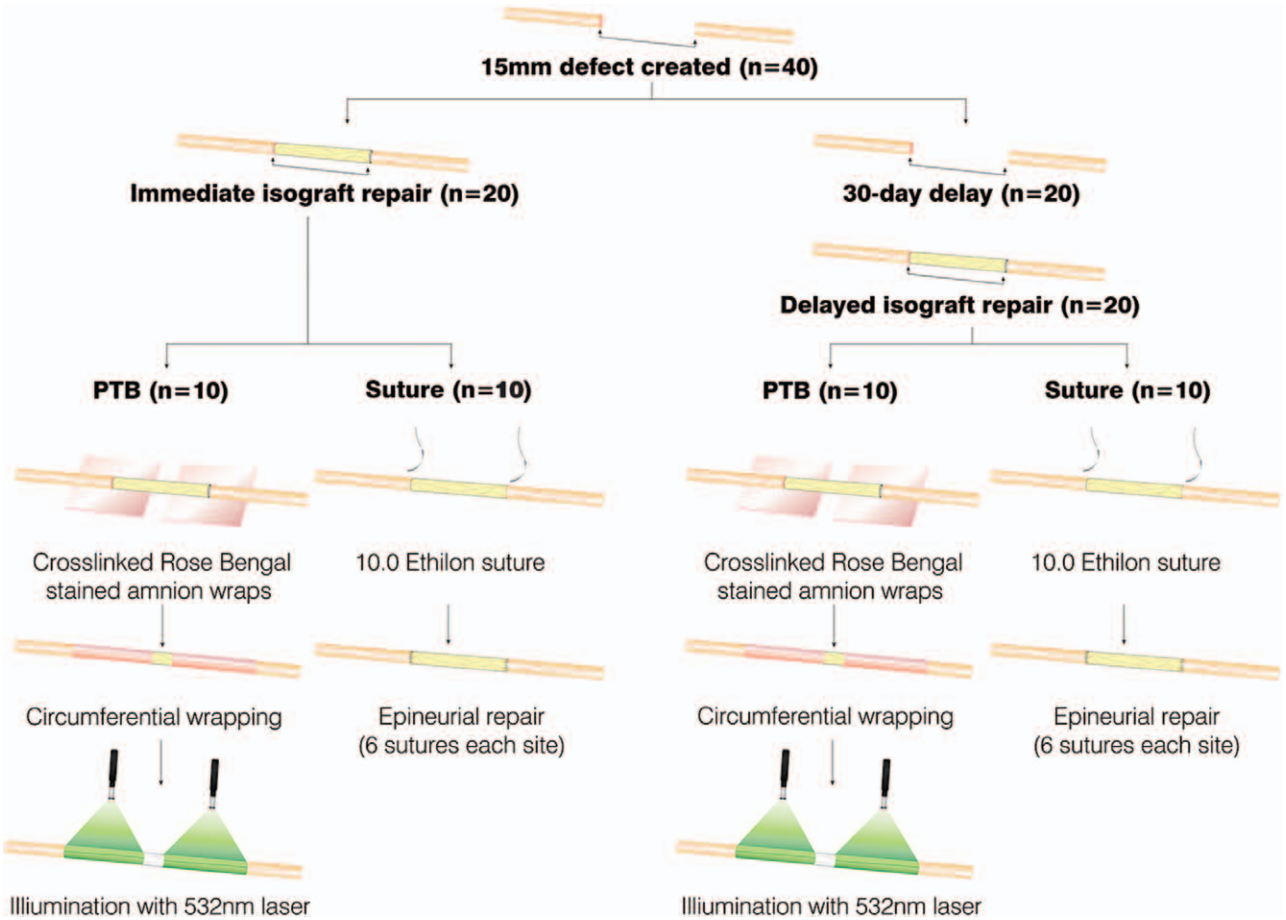

Fig. 1. Methods of immediate and delayed nerve repair. Forty rodents had 15-mm left sciatic nerve gaps created. These were repaired with reversed isografts. In two groups, repair occurred immediately after injury and involved the exchange of isografts between two simultaneously anesthetized rodents. Isografts were secured with either photochemical sealing (n = 10) or conventional epineurial suture (n = 10) using 10-0 Ethilon. In the remaining 20 rodents, repair occurred following a 30-day delay, during which proximal nerve ends were buried into adjacent muscle to prevent regeneration and reinnervation of the distal stump. After this delay, all wounds were reopened and gaps repaired using isografts harvested from 10 donor Lewis rats. As above, these isografts were secured with either photochemical sealing or conventional suture. *PTB*, photochemical tissue bonding.

above. To overcome tension between nerve ends, isografts were tacked into place using two 10-0 Ethilon sutures. Before transfer into the surgical field, wraps and coaptation sites were stained with 0.1% (weight/volume) Rose Bengal (Sigma-Aldrich) for 60 seconds. After 60 seconds, excess dye was removed. Rose Bengal–stained wraps were wrapped circumferentially around sciatic nerves, ensuring that a minimum of 5-mm overlap existed. The area of overlap was irradiated for 60 seconds using a 532-nm potassium titanyl phosphate laser (Laserscope, San Jose, Calif.) at an irradiance of 0.5 W/cm^2. The nerve/wrap was then rotated 180 degrees to irradiate the back wall in the same manner for an additional 60 seconds (Fig. 1).

Delayed Repair: Groups 3 and 4

After the creation of 150-mm sciatic nerve defects, a small incision was made in adjacent muscle, and proximal nerve ends were buried and secured using two 10-0 Ethilon sutures. Distal nerve ends were left free. Wounds were closed as described and the animals were returned to the animal facility. After 30 days, wounds were reopened and nerve ends dissected and mobilized. Simultaneously, fresh isografts were harvested from donor Lewis rats, immediately reversed, and transferred into the nerve gap. In group 3, isografts were secured with 10-0 Ethilon suture, and in group 4, isografts were sealed photochemically as described previously (Fig. 1).

Outcome Assessment

Walking Track Analysis

Walking track analysis was performed immediately before surgery for baseline Sciatic Function Index. In the delayed groups, Sciatic Function Index was also performed following the 30-day delay, immediately before isograft reconstruction. Following isograft repair, walking track analysis was performed at 30-day intervals. After dipping both hind paws in water-soluble ink, rats were encouraged to walk up a 10 × 60-cm, partially enclosed ramp lined with white paper and set at an incline of 30 degrees to horizontal. Print length, toe spread, and intermediary toe spread were measured from footprints using digital calipers. Mean values from three normal and experimental prints were entered into the Sciatic Function Index formula described by Bain and colleagues.[21]

Muscle Weight Retention and Nerve Histomorphometry

After 150 days, animals were killed using carbon dioxide inhalation. Immediately after this, both gastrocnemius muscles were harvested and had wet weights measured for calculation of left-side percentage muscle mass retention. Nerves were harvested 5 mm proximal and distal to isograft coaptation sites. Following fixation, dehydration, and embedding in epoxy resin, nerves had 1-μm sections cut from proximal and distal ends. Once prepared, nerve sections were scanned digitally using a Hamamatsu NanoZoomer 2.0-HT slide scanner (Meyer Instruments, Houston, Texas) and read using NDP.com software (Hamamatsu Corp. Bridgewater, N.J.). The specific details of these methods are available in a recently published article. The identity of digital images was concealed and all histomorphometric analysis (axon count, fiber diameter, axon diameter, myelin thickness, and G-ratio) was conducted by two blinded researchers.

Statistical Analysis

Statistical analysis was performed using KaleidaGraph for Windows v4.1 (Synergy Software, Reading, Pa.). Sciatic Function Index data were analyzed using repeated measures analysis of variance to detect the existence of significant differences over time. All remaining analysis between treatment groups was performed using analysis of variance and the post hoc Bonferroni test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Gross Observations

Two rodents in the delayed photochemical tissue bonding group had to be killed prematurely because of intractable foot ulcers. Unfortunately, one of these animals was disposed of before necropsy could be performed. In the remaining rodent, the isograft was found to be in continuity but had experienced considerable atrophy at the midportion of the graft (Fig. 2, *above*, *left*). No other episodes of dehiscence occurred in the remaining groups, with all nerves showing evidence of regeneration. Cross-linked amnion nerve wraps were identifiable following sacrifice in all groups, showing that cross-linking protects enzymatic degradation for a minimum of 5 months (Fig. 2, *above*, *right*). Extraneural scarring appeared qualitatively reduced in those nerves repaired photochemically, an observation consistent with previous studies (Fig. 2, *below*).

Sciatic Function Index

After 5 months' follow-up, greatest recovery of Sciatic Function Index occurred in the

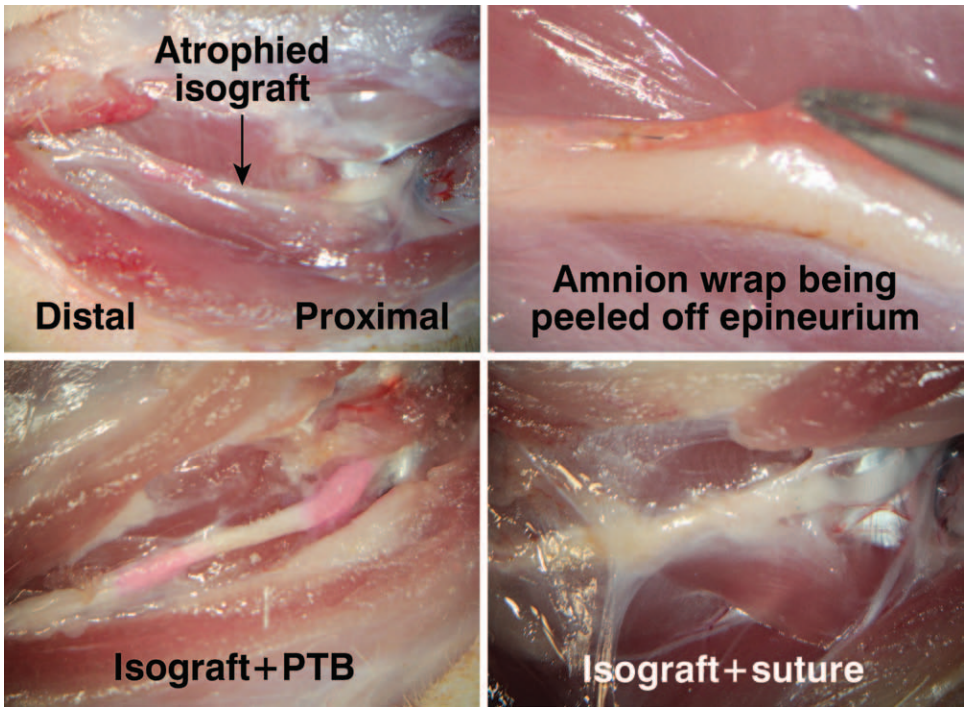

Fig. 2. Gross observations after the animals were killed. Two animals in the isograft plus photochemical tissue bonding (*PTB*) group were killed before the completion of 150 days' follow-up because of intractable foot ulcers. Necropsy of one animal showed intact photochemical bonds but a severely atrophied graft. The remaining animal was disposed of before examination could take place (*above*, *left*). Amnion wraps were found to be present, evidence of successful retardation of proteolytic degradation (*above*, *right*). Qualitatively, isograft plus photochemical tissue bonding nerves had less extraneural scar tissue formation than isograft plus suture groups (*below*).

Table 1. Mean Sciatic Function Index for All Treatment Groups over the 5-Month Follow-Up Period*

	Mean SFI				
Experimental Group	**1 Mo**	**2 Mo**	**3 Mo**	**4 Mo**	**5 Mo**
Immediate suture	−91.4 ± 10.2	−81.4 ± 4.2	−78.2 ± 4.5	−72.4 ± 6.3	−72.3 ± 4.7
Immediate PTB	−91.4 ± 5.2	−81.3 ± 3.6	−74.1 ± 4.7	−71.8 ± 4.2	−68.5 ± 4.7
Delayed suture	−92.9 ± 4.5	−84.9 ± 6.7	−84.9 ± 6.8	−82.8 ± 5.4	−80.1 ± 4.4
Delayed PTB	−92.4 ± 3.3	−84.4 ± 5.6	−80.7 ± 5.2	−79.7 ± 5.4	−77.3 ± 4.1

SFI, Sciatic Function Index; PTB, photochemical tissue bonding.
*Immediate photochemical tissue bonding of isografts recovered greatest Sciatic Function Index after 5 months, although this was not statistically significant in comparison with gold standard immediate suture. Similarly, no significant difference existed between delayed photochemical tissue bonding and delayed suture. Sciatic Function Index in the immediate suture group was significantly better than in the delayed suture group ($p = 0.003$). Likewise, immediate photochemical tissue bonding recovered a significantly greater Sciatic Function Index in comparison with delayed photochemical tissue bonding ($p = 0.002$). Immediate photochemical tissue bonding was significantly better than delayed suture, which performed poorest of all groups ($p < 0.0001$). No significant difference existed between immediate suture and delayed photochemical tissue bonding.

immediate photochemical tissue bonding group. This result was not statistically significant in comparison with gold standard immediate suture, although it was statistically better than that of the delayed photochemical tissue bonding group (−68.5 ± 4.7 versus −72.3 ± 4.7, $p = 0.41$; and −68.5 ± 0.47 versus −77.3 ± 4.1, $p = 0.002$, respectively). Recovery in the immediate suture group was statistically better than delayed suture, which performed poorest of all groups (−72.3 ± 4.7 versus −80.1 ± 4.4). There was no significant difference between immediate suture and delayed photochemical tissue bonding (−72.3 ± 4.7 versus −77.3 ± 4.1) (Tables 1 and 2).

Gastrocnemius Muscle Mass Retention

Muscle mass retention was greatest in the immediate photochemical tissue bonding group,

Table 2. Bonferroni All-Pairs Comparison for Treatment Groups*

Group Comparison	SFI (5-mo)	Muscle Mass	Axon Count	Fiber Diameter	Axon Diameter	Myelin Thickness	G-Ratio
Immediate suture vs. immediate PTB	0.41	0.02	1	<0.0001	<0.0001	<0.0001	<0.0001
Immediate suture vs. delayed suture	0.003	0.10	0.23	<0.0001	<0.0001	<0.0001	1
Immediate suture vs. delayed PTB	0.17	1	1	1	0.35	1	0.06
Immediate PTB vs. delayed suture	<0.0001	<0.0001	0.24	<0.0001	<0.0001	<0.0001	<0.0001
Immediate PTB vs. delayed PTB	0.002	0.16	1	<0.0001	<0.0001	<0.0001	<0.0001
Delayed suture vs. delayed PTB	1	0.03	1	<0.0001	<0.0001	0.001	0.12

SFI, Sciatic Function Index; PTB, photochemical tissue bonding.
*No significant differences in Sciatic Function Index were detected between immediate suture and immediate photochemical tissue bonding or delayed suture and delayed photochemical tissue bonding. Significant differences were detected between immediate and delayed suture and immediate and delayed photochemical tissue bonding, highlighting the detrimental impact of delay. No significant difference existed between immediate suture and delayed photochemical tissue bonding. Muscle mass retention was significantly improved following photochemical tissue bonding repair in both immediate and delayed groups. The effects of delay were not significantly different for suture or photochemical tissue bonding fixation. As with Sciatic Function Index, immediate suture was not significantly different compared with delayed photochemical tissue bonding. No significant differences in axon count or density existed between groups. Fiber diameter, axon diameter, and myelin thickness and G-ratio were not significantly different between immediate suture and delayed photochemical tissue bonding. With the exception of G-ratio, all other histomorphometric parameter comparisons were significantly different between treatment groups, with immediate photochemical tissue bonding achieving greatest recovery and delayed suture being poorest.

Table 3. Gastrocnemius Muscle Mass Retention for All Groups*

Experimental Group	Mean Left Gastrocnemius Muscle Mass Retention (%)	SD (%)	*p*
Immediate suture	59.0	3.6	—
Immediate PTB	64.9†	3.9	0.02
Delayed suture	54.1	5.3	0.10
Delayed PTB	60.2	4.1	1

PTB, photochemical tissue bonding.
*Groups compared statistically to immediate suture. Delayed suture and delayed photochemical tissue bonding did not differ significantly in comparison with the immediate suture group. See Table 2 for Bonferroni all-pairs comparison.
†The immediate photochemical tissue bonding group recovered significantly greater muscle mass than immediate suture.

and this was statistically significant in comparison with the immediate suture group (64.9 ± 3.9 percent versus 59.0 ± 3.6 percent, $p = 0.02$). A similar significant improvement was also observed in the delayed photochemical tissue bonding group in comparison with delayed suture (60.2 ± 4.1 percent versus 54.1 ± 5.3 percent, $p = 0.03$). With regard to the surgical delay, no significant difference existed between immediate suture and delayed suture, or immediate photochemical tissue bonding and delayed photochemical tissue bonding groups. Muscle mass retention was not significantly different between immediate suture and delayed photochemical tissue bonding (59.0 ± 3.6 percent versus 60.2 ± 4.1 percent, $p = 1$) (Tables 2 and 3).

Nerve Counts and Histomorphometry

No significant differences in axon counts existed between any of the treatment groups. The immediate photochemical tissue bonding group recovered greatest fiber diameter, axon diameter, myelin thickness, and G-ratio, and this was statistically significant in comparison with immediate suture and all remaining groups (Fig. 3). With the exception of G-ratio, all measurements were significantly greater in the delayed photochemical tissue bonding group in comparison with the delayed suture group. With regard to surgical delay, fiber diameter, axon diameter and myelin thickness were significantly greater in the immediate suture group compared with the delayed suture group. No significant difference in G-ratio was detected. All measurements in the immediate photochemical tissue bonding group were significantly better compared with the delayed photochemical tissue bonding group. No significant difference in any histomorphometric measurement existed between immediate suture and delayed photochemical tissue bonding (Tables 2 and 4 and Fig. 3).

DISCUSSION

This study verifies that light-activated sealing remains superior to conventional suture when performed both immediately and after a clinically relevant delay. Although this relationship was not significant for Sciatic Function Index, mean values were greater for light-activated sealing. Both muscle mass retention and nerve histomorphometry were significantly improved in immediate and delayed photochemical tissue bonding groups, suggesting that this may be a more sensitive measure than Sciatic Function Index in detecting a difference in regeneration. Sciatic Function Index and histomorphometric outcomes between immediately sutured nerves and delayed, photochemical repairs were not significantly different. This suggests that

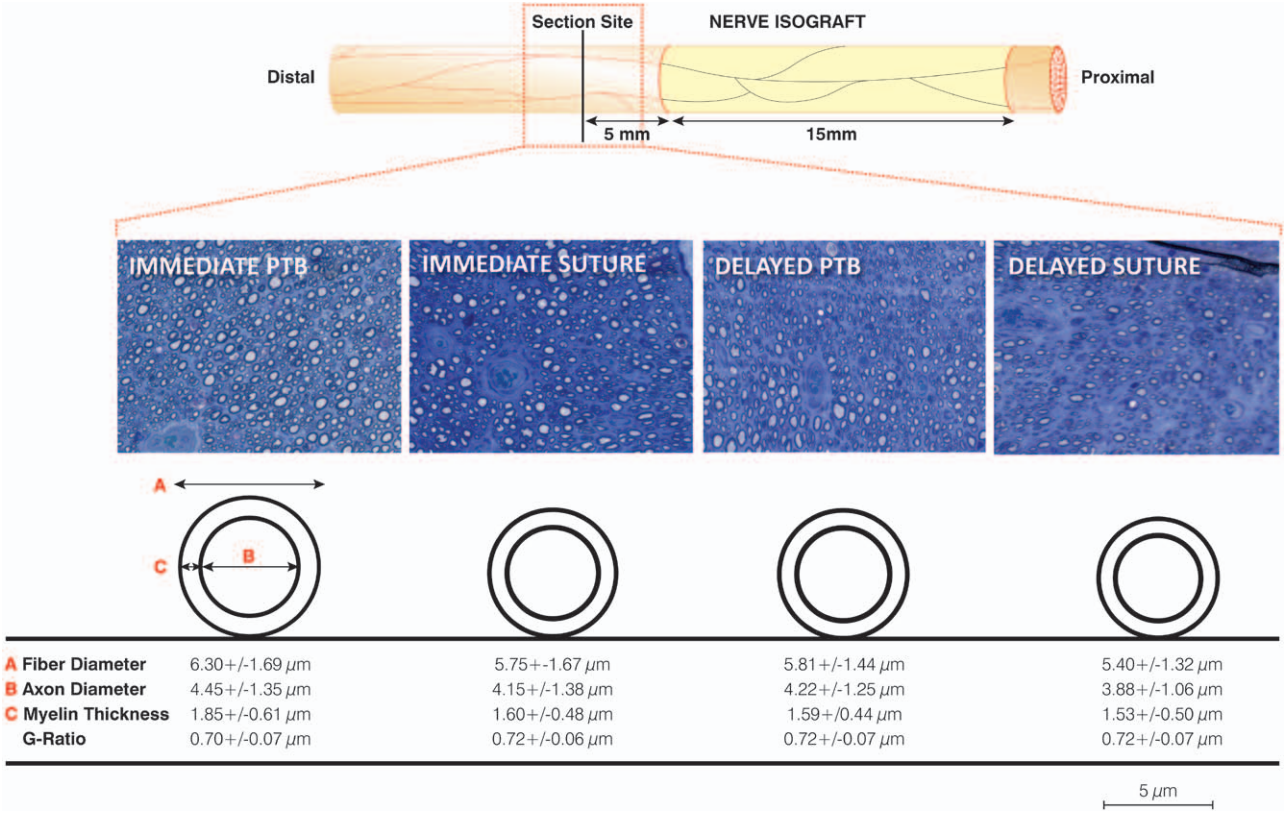

Fig. 3. Histology of distal nerve sections from each group and schematic depiction of nerve fiber and axon diameters. Sections were taken 5 mm distal to the distal isograft coaptation site. With the exception of the two rodents that were killed prematurely, all rodents successfully regenerated axons through isografts into the distal nerve stump after 5 months. Axons in the immediate groups were more abundant, although this was not significant. Histomorphometric measurements were generally greater in immediate repairs versus delayed repairs and in those repairs performed photochemically versus suture. No significant differences existed between the immediate suture and the delayed photochemical tissue bonding (*PTB*) groups.

Table 4. Histomorphometric Analysis 5 mm Distal to the Distal Isograft Coaptation Site for All Treatment Groups*

Experimental Group	Total Axon Count (× 0.001)	Axon Density (mm^2 × 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

PTB, photochemical tissue bonding.
*Although mean axon counts were considerably greater in immediate repair groups, because of the large standard deviation, these values were not significantly different. All histomorphometric measurements were greater in the immediate photochemical tissue bonding group compared with immediate suture.
†Statistically significant improvement compared with immediate suture. Table 2 provides detailed cross-pairs comparison.

photochemical sealing may have the ability to ameliorate the poorer outcomes expected following a surgical delay using standard microsurgical repair.

Outcomes following suture repair depend on surgical technique and microsurgical experience. Rodent operations in this study were performed by "nonexpert" microsurgeons. It is possible that the involvement of an "expert" microsurgeon may have improved outcomes following standard suture repair, ameliorating the observed superiority of light-activated sealing. However, the technical demands of microsurgery are its primary limitation. Expertise and equipment may not be available clinically. An advantage of light-activated sealing is that surgeons without microsurgery training can readily adopt the technique.

The delay of 30 days in this study had a deleterious impact on recovery, regardless of whether isografts were secured using conventional suture or photochemical sealing. This is consistent with

the tenet that increasing periods of axotomy and denervation result in a reduction in regenerating axons, reduced regenerative support in the distal fiber, and reduced motor unit reinnervation in muscle targets. Curiously, this effect was not evident from the analysis of muscle mass retention. Gordon et al. showed that freshly axotomized axons were able to recover full muscle mass and force of contraction when periods of distal fiber and muscle denervation were less than 50 days.[5] This was also observed when chronically axotomized nerve regenerated down freshly denervated distal nerve and muscle, reflecting compensatory increases in motor unit size.[5] A lack of significant difference in regenerative outcomes with shorter periods of delay has been reported by others.[22] Although mean muscle mass retention was greater in immediate repair groups in this study, it is possible that the lack of statistical significance reflects an element of compensation through an increased innervation ratio. Significant differences in Sciatic Function Index between immediate and delayed groups suggests that compensatory increases in innervation ratio, although sufficient to maintain muscle mass of the lower limb, may be unable to compensate for poor reinnervation of intrinsic musculature of the foot and sensory loss, both essential components for coordinated motor control.

The beneficial effects of photochemical sealing may be related to watertight isolation of the repair site. The avoidance of suture, prevention of axonal escape, protection from infiltrating scar tissue, and containment of neurotrophic rich fluid that is liberated from transected axons may all play a role. The absence of suture and therefore a reduction in foreign body reaction may lead to a less tumultuous repair environment, expediting the regeneration of axons across nerve graft coaptation sites. In a related set of experiments, the previously demonstrated beneficial effect of photochemical sealing of isografts, in comparison with conventional suture, was abrogated when applied to acellular nerve allografts (unpublished data). This suggests that the mechanism of effect may involve Schwann cells and the neurotrophic factors they release.

Following denervation, Schwann cells in the distal nerve up-regulate regeneration-associated genes and the expression of neurotrophic factors. With increasing denervation time, this expression progressively declines and Schwann cells become dormant, diminishing their ability to support regeneration.[13] The supplementation of exogenous Schwann cells and neurotrophic factors at the repair site has the potential to improve rates of regeneration. However, the delivery of these factors and their temporal and spatial regulation is far from being realized clinically. Inappropriately high concentrations of some neurotrophic factors can be inhibitory to regeneration and can even promote cell death.[11,12] Electrical stimulation of nerve repair sites may up-regulate regeneration-associated gene expression and the production of neurotrophic factors.[6,7] Improved rates of regeneration have been demonstrated in animal models of immediate and delayed repair[9,10] and, most recently, has facilitated full reinnervation of thenar muscles in humans with severe carpal tunnel syndrome.[8] This represents one of the few clinically translatable solutions to date. Light-activated sealing of nerve graft coaptation sites offers an alternative translatable approach that may help maintain neurotrophic levels and also retard Schwann cell dormancy by preventing the loss of mitogenic stimuli released from fresh nerve grafts. The uncertainty surrounding these mechanisms will provide impetus for further study.

The loss of two animals in the delayed plus photochemical tissue bonding group and the unsuccessful regeneration in one of these rodents was a concern. It is uncertain what caused this, although the delivery of excessive energy from the light source is most likely. The light used in this study was delivered by means of a divergent beam. As a result, small, inadvertent reductions in the distance between nerve and light source during bonding may have resulted in the delivery of excessive energy. Although not apparent at the time of bonding, subtle thermal damage to the internal architecture of nerve stumps and grafts may have compromised axonal regeneration and revascularization. Collimation of the beam would be a simple, easily achievable solution that would standardize spot size and energy delivery and improve safety for clinical translation.

A criticism of this study is that the period of delay may have been insufficient. Gordon et al. showed that the deleterious effects of prolonged axotomy and denervation were maximal following considerably longer surgical delays than found in this study. However, the exponential decline of motor unit reinnervation was evident with delays of less than 50 days.[5] In addition to surgical delay, denervation time is further extended by slow rates of regeneration. Although rates are commonly quoted as 1 to 3 mm/day, Brushart et al. explained that this referred to only the fastest growing sensory axons.[7] Large numbers of axons can take many weeks to traverse coaptation sites. This "staggered regeneration" is the result of the physical obstacle

presented by suture repairs, the arborization of daughter axons and the rationing of raw materials from the cell body, and sensory-motor mismatching leading to pruning.[7] These effects are exacerbated following nerve grafting when axons must traverse two coaptation sites. Clinically, denervation time may typically exceed that which has been tested in this study. Nevertheless, the 30-day delay led to a deleterious and detectable impact on regeneration and has allowed significant differences to be detected between treatment groups.

CONCLUSIONS

Importantly, this study confirms that light-activated sealing of isograft coaptation sites remains efficacious following a clinically relevant surgical delay and, as demonstrated in recent studies, results in significant improvements in outcome in comparison with conventional suture fixation. The use of light-activated sealing following delayed repair results in outcomes that are statistically comparable to those achieved with immediate suture. This finding is analogous to a recent study investigating efficacy of technique when applied to acellular nerve allografts[19] and, together, these findings may have potentially important clinical implications for the future repair of nerve gaps following periods of delay, particularly when the nature of the injury precludes the use of autologous tissue.

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