

ONLINE FIRST

Laser Facial Nerve Welding in a Rabbit Model

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Objective: To assess the feasibility of laser tissue welding for repair of facial nerve injury.

Methods: In a prospective in vivo animal survival surgery model, rabbit facial nerve injury was followed by either standard suture neuroorrhaphy or laser tissue welding using a diode laser (808 ± 1 nm) to weld biological solder. Rabbits were evaluated at 4, 8, 12, and 16 weeks by facial videography and electromyography. Histopathological analysis of the repair was performed at 4 and 16 weeks.

Results: Videographic analysis demonstrated the laser tissue welding repair trended toward superior outcomes compared with suture neuroorrhaphy at all 4 time points. Electrophysiological analysis demonstrated similar or better results, with statistically significant improvement at week 16 ($P < .05$). Histologic analysis demon-

strated no difference in axon organization or extravasation between groups; however, the laser nerve repair created a greater initial inflammatory reaction. An analysis of operative time demonstrated significantly decreased time and ease of use for laser tissue welding.

Conclusions: This pilot study demonstrates that laser nerve welding may be an expedient, feasible, and safe method for facial nerve repair in a rabbit model. Further experiments with larger numbers are needed to provide additional evidence that laser tissue welding produces a neuroorrhaphy that has functional, electrophysiological, and histological results that could rival traditional suture neuroorrhaphy.

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A S MICROSCOPIC RECONSTRUCTIVE surgery for nerve repair continues to evolve, the overall goal of decreasing morbidity and improving functional outcomes must constantly be stressed, and thus the current techniques challenged. The gold standard neuroorrhaphy technique involves suture repair of the interrupted nerve ends with 8-0 or 9-0 monofilament suture, allowing the axons to regrow across the neuroorrhaphy and regenerate function. Unfortunately, this repair technique exposes patients to potential complications with scar tissue formation, foreign body reaction from the suture material, neuroma formation, axon escapement around the sutures, and mismatching of the fascicles. These problems have catalyzed an active investigation into laser nerve welding (LNW) as an alternative method of nerve repair. A novel technique to produce a strong bond and coapt nerve ends, without injury to the nerve itself, would contribute to preventing these aforementioned obstacles, which may inhibit functional nerve recovery.

The potential of using lasers in tissue adhesion was initially described in 1966 by Yahr and Strully,¹ who used laser tissue welding in blood vessel anastomosis. While the earlier studies looking at laser tissue welding models primarily used Nd:YAG lasers,² multiple different laser systems were introduced to the clinical armamentarium including the argon, carbon dioxide, and diode laser.³ In 1984, Almquist et al⁴ first reported the use of an argon laser in peripheral nerve repair in both rats and monkeys. The following year, Fischer et al⁵ published similarly positive reports with the use of the carbon dioxide laser for nerve repair in rats.

In addition to the first studies of LNW, an important advance was made with the introduction of biological solders that were coupled to wavelength specific chromophores, such as indocyanine green and fluorescein.^{3,6} By doping the biological solder with a chromophore, the laser energy absorption can be exquisitely controlled and confined specifically to the solder, offering sufficient thermal energy for the welding process while allowing for a reduction in the power of the laser to mini-

mize collateral damage. A review of the literature shows that the use of protein solders, as adjuncts to laser tissue welding, is an improvement over laser welding alone. The use of these biological solders has been shown to create a watertight seal, decrease thermal damage, improve the consistency of welds, and reduce operative times compared with standard suture repairs.³ Furthermore, the use of chromophores also provides an objective means of determining the adequacy of the laser weld by providing a predictable color change.⁷

Not only were there improvements seen with the weld itself, but the use of biological solders has shown benefit in promoting native wound healing processes. In contrast to the normal granulomatous inflammatory response seen with suture material, the lasered solder coagulum provides a nonimmunogenic scaffold⁷ and is gradually absorbed during the normal wound healing process.^{8,9}

While LNW has been studied using different nerves and a combination of lasers, to our knowledge, no group has looked at a rabbit facial nerve model using a combination of the 810-nm diode laser and a 42% albumin solder doped with an indocyanine green chromophore.¹⁰⁻¹² We hypothesize that this solder and laser combination will produce minimal collateral thermal damage to the facial nerve itself and surrounding tissues, while serving as a scaffold for axonal regrowth, resulting in superior functional outcome compared with suture neuroorrhaphy. To test this hypothesis, we devised a pilot feasibility study to perform LNW in a rabbit facial nerve model and evaluate the repair with functional, electrophysiological, and histological parameters and compare this with suture neuroorrhaphy over a 4-month period.

METHODS

This study received approval from the Institutional Animal Care and Use Committee (IACUC) at both the University of Pennsylvania and the Philadelphia Veterans Affairs Medical Center. Fourteen New Zealand white rabbits (3-4 kg) were used for the pilot study and were divided into 1 negative control group of 2 rabbits and 2 experimental groups consisting of 6 rabbits each.

RABBIT SURVIVAL SURGERY

Each animal's face was unilaterally shaved and the overlying skin was tattooed with a dot over the quadratus labii superioris muscle. The procedures were then performed under sterile conditions by a single surgeon (J.D.B.) with $\times 2.5$ magnification surgical loupes. A 2.0-cm oblique incision was made over the rabbit zygoma, from posterosuperior to anteroinferior. The platysma and the rabbit facial muscles were divided sharply with a No. 15 scalpel, and a microdissecting tool was used to clear overlying tissue from the rabbit facial nerve. At this point, the horizontally running buccal division of the facial nerve was identified running over the masseter muscle (**Figure 1** A). A handheld electric nerve stimulator set at 1.0 mA was used to assist in identifying the facial nerve. This buccal branch innervates the rabbit's quadratus labii superioris muscle, which is important in movement of the upper lip and whiskers.¹³ The facial nerve was then transected sharply on one side with a microscissors. The 2 rabbits in the negative control group had a 1-cm piece of nerve excised without any reapproximation to examine the rabbit's innate regenerative processes. Six of the rab-

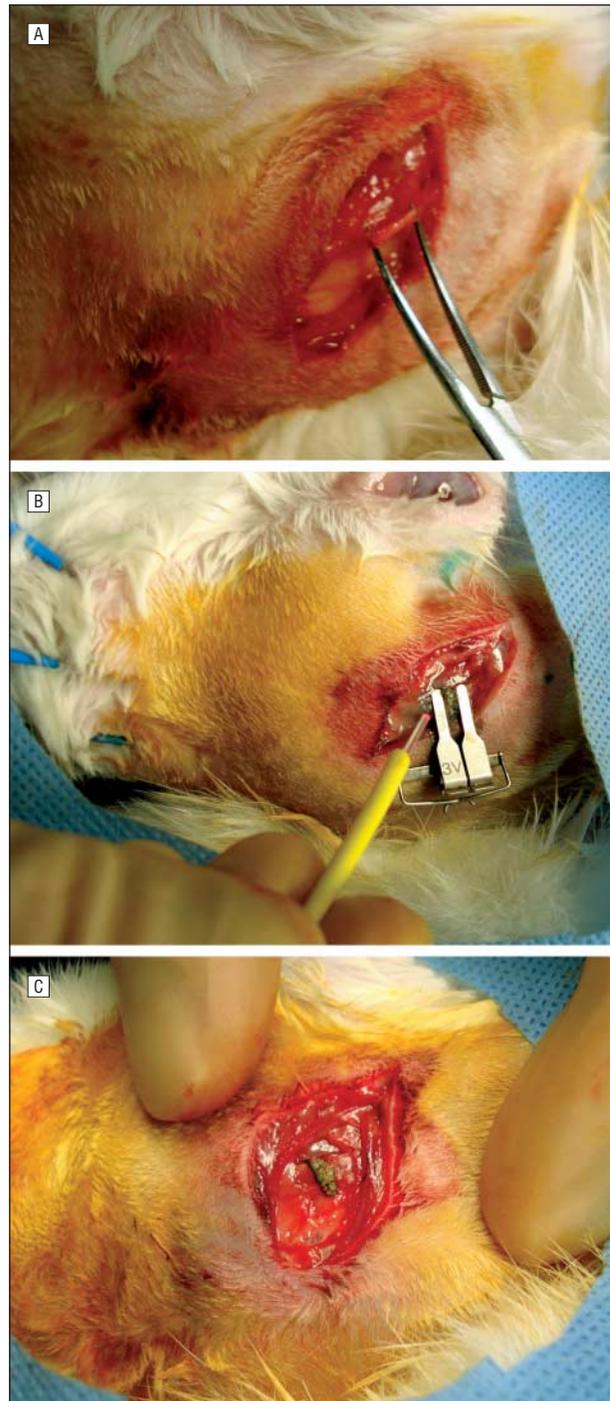


Figure 1. Rabbit survival surgery and laser welding. A, Microdissection tool displaying the buccal branch of the facial nerve as it courses obliquely across the rabbit's face. B, Microvascular clamp in place, approximating the nerve ends, and the end of the fiberoptic laser cable seen after laser nerve welding. C, View of the completed laser nerve weld. Note the tan color change to the green solder.

bits had their facial nerves directly suture repaired using a Carl Zeiss OPMI CS-1 operating microscope (Carl Zeiss Surgical Inc, Dublin, California), and epineural technique with three 9-0 monofilament polypropylene (Prolene) sutures (Ethicon Inc, Somerville, New Jersey) on a taper needle. Finally, in the remaining 6 rabbits, the facial nerves were laser welded using an 810-nm diode laser and a "ribbon" of 42% albumin-based biological solder coupled with an indocyanine green dye chromo-

phore, applied with a 27G needle to the approximated nerve ends. A modified microvascular clip and microforceps were used to realign and hold the ends of the severed nerve together during laser welding (Figure 1B). Once the nerve repair had been performed, the platysma muscle and subcutaneous tissue layer were closed with interrupted, buried 5-0 braided polyglycolic acid (Vicryl) sutures (Ethicon Inc). The skin overlying the surgical site was then closed in all groups with tissue glue to reduce irritation from sutures.

LASER WELDING SET-UP

A diode laser (Iridex, Mountain View, California) with a 600- μ m core diameter quartz silica fiberoptic cable was used, with the following specifications: power, 0.5 W; pulse duration, 0.5 seconds; pulse interval, 0.1 second; power density, 15.9 W/cm²; fluency, 8.0 J/cm²; and major wavelength output, 810 \pm 1 nm. The biological solder comprised a 2:1:2 mixture of 42% albumin solution (Thermo Fisher Scientific Inc, Waltham, Massachusetts), indocyanine green dye (Sigma-Aldrich, St Louis, Missouri), and hyaluronic acid sodium (Sigma-Aldrich), respectively. Welding of each nerve was performed by a single surgeon (J.D.B.) and used a 0.5-cm³ aliquot of solder. The end point of the laser welding was determined by a characteristic solder color change from green to tan (Figure 1C).

ELECTROPHYSIOLOGICAL TESTING

The rabbits underwent electromyography (EMG) and electrophysical testing of their facial nerves preoperatively, immediately postoperatively, and then postoperatively at weeks 4, 8, 12, and 16. Each rabbit had an intact facial nerve side to compare the EMG/electrophysical results and clinical facial nerve responses. This intact facial nerve served as an internal control for each animal and group. The EMG (Nicolet Viking IV Electromyography Unit) electrodes were placed superficially in each rabbit's facial musculature at the site of the facial tattoo and the EMG system (VIASYS Healthcare, San Diego, California) was used to record compound action potentials bilaterally, comparing the normal side with the side of the nerve injury and repair.

CLINICAL AND FUNCTIONAL TESTING

On each testing day, the rabbits were moved into a preparation room that allowed for standardized video recording of facial movements for noninvasive functional testing. Gentle nasal and facial taps were performed in an attempt to elicit the rabbits' facial nerve motion. The video tapes were then analyzed to objectively grade facial nerve recovery by 2 different, blinded observers (S.A.G. and B.S.B.). The precise video clips shown to the 2 blinded observers were chosen for evaluation by another surgeon who was blinded to the 2 groups of rabbits. In this study, we assessed movement of the rabbits' upper lip and whiskers. A scale of facial nerve movement from 0 (no movement) to 3 (normal facial movement) was used by the blinded observers to record this functional testing.¹⁴ The results for the operated side were compared with the rabbit's internal control on the unoperated and normal side, as well as to each rabbit's preoperative baseline video.

HISTOPATHOLOGICAL EVALUATION

Harvested facial nerve specimens from both experimental groups were prepared and analyzed by our staff histopathologist. The nerve repair was dissected out by reopening the skin incision, finding the site of nerve repair, and resecting the nerve with a

microscissor. Six neurotaphies from each experimental group were harvested and stained with hematoxylin-eosin for qualitative analysis of immune response to the solder, foreign body reaction, native wound healing progression, extent of residual laser solder material, and extent of collateral nerve thermal injury. The nerve repairs were also stained with Masson trichrome to view the arrangement of nerve axons across the repair sites, amount of neuroma formation, and nerve axon extravasation.

EXPERIMENTAL GROUPS AND STATISTICAL ANALYSIS

Four rabbits (2 from the laser weld group and 2 from the suture group) were humanely killed at postoperative week 4 and 10 rabbits (4 from the laser weld group, 4 from the suture group, and 2 negative controls) were humanely killed at postoperative week 16, for a total of 14 rabbits. Each rabbit had nerve repair on only one side, and the opposite facial nerve was untouched and served as an internal control. There were 6 wounds per experimental group (6 laser welded and 6 suture neurotaphy), and the 2 negative control rabbits were subjected to clinical and EMG analysis. Then, all of the nerve repairs were harvested for histopathological analysis.

These study populations were derived from the following standard equation considering α error with z_{α} as specified. The values for all variables are derived from the results of our aforementioned pilot studies.¹⁰⁻¹²

$$N = \frac{(z_{\alpha})^2 \times 2 \times s^2}{d^2},$$

where z_{α} =value for α error (1.96); s^2 =variance (8.1225); d =difference to be detected (3.5); and N =number of subjects per study group. A minimum of 6 subjects per study group were used because our sample size calculation demonstrated a need for 5 or more subjects for each study group for adequate power. In addition, these populations are in agreement with previous LNW studies in the literature.¹⁴⁻¹⁸

All statistical analyses were performed using SigmaStat v3.1 (Systat Software Inc, San Jose, California). The differences between operative time results, the mean EMG response, functional nerve recovery, and time to clinical recovery were analyzed using a 2-tailed unpaired t test, with a significance level set at $P < .05$.

RESULTS

OPERATIVE TIME AND LEARNING CURVE

The mean (SD) time to perform the actual neurotaphy portion of the procedure in this feasibility study was significantly shorter with LNW vs the standard suture repair (3.50 [1.64] minutes vs 13.17 [3.60] minutes; $P < .001$) (Figure 2). Not only did it take less than half of the time to perform the laser-welded neurotaphy vs the standard suture nerve repair, but a small learning curve was observed for the operating surgeon with the traditional suture neurotaphy. This was not observed in the laser welding group (Figure 3). In this study, LNW was as fast to perform from the first attempt to the last, unlike that of the suture neurotaphy.

ELECTROPHYSIOLOGICAL RECOVERY

It is apparent from the pilot study that the laser nerve repair group outperformed the traditional suture nerve

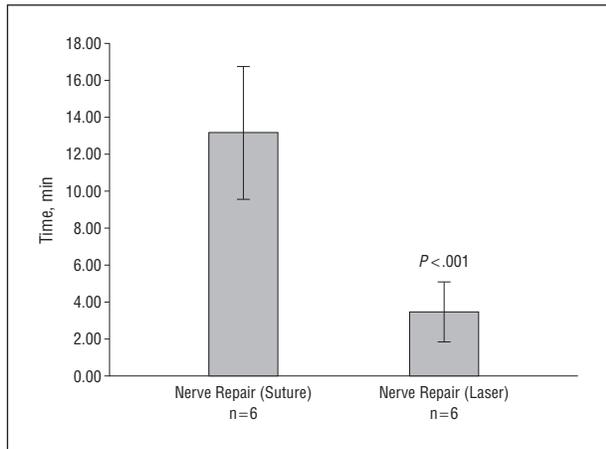


Figure 2. Operative neuroorrhaphy time. This graph shows that the mean time to complete the actual nerve repair portion of the procedure was significantly shorter with the laser-welded nerve group compared with the suture nerve repair group. The error bars indicate SD.

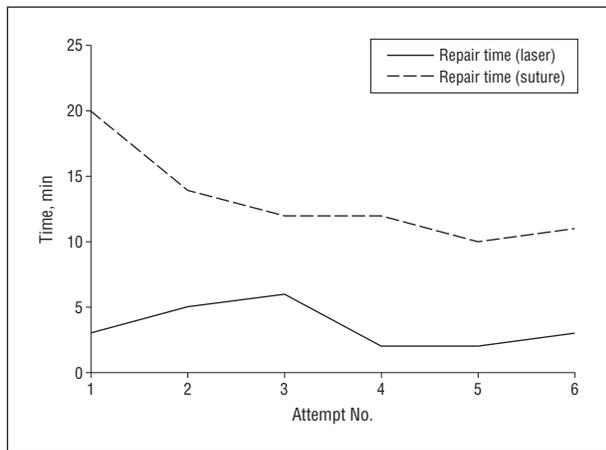


Figure 3. A learning curve is witnessed for the suture neuroorrhaphy, eventually shortening 20 minutes down to 10 to 12 minutes by the sixth attempt. For the laser, depending on the difficulty of aligning the cut nerve ends, the procedure took anywhere from 2 to 6 minutes.

repair group at all time points during the rabbits' facial nerve recovery. **Figure 4** illustrates the mean EMG amplitude (measured baseline to peak) at each time point, as a percentage of the mean baseline amplitude vs time. Over the course of the study, although the laser welding group had better EMG amplitudes at every time point, only the week 16 data point was statistically significant (2.02 [0.66] vs 1.11 [0.40]; $P = .02$). The week 4 time point (0.70 [0.35] vs 0.43 [0.28]) did not reach significance ($P = .09$).

FUNCTIONAL RECOVERY

Two blinded observers reviewed the video recordings of the rabbits' facial movement and scored the nerve function based on the 0 to 3 scale of facial nerve recovery, previously used in other rabbit facial nerve studies.¹⁴ The mean (SD) scores for the laser-welded group and suture neuroorrhaphy were as follows: postoperative week 4: suture, 1.29 (0.98), and laser, 1.47 (0.87); postoperative week 8: suture, 1.85 (0.55), and laser, 1.90 (0.60); post-

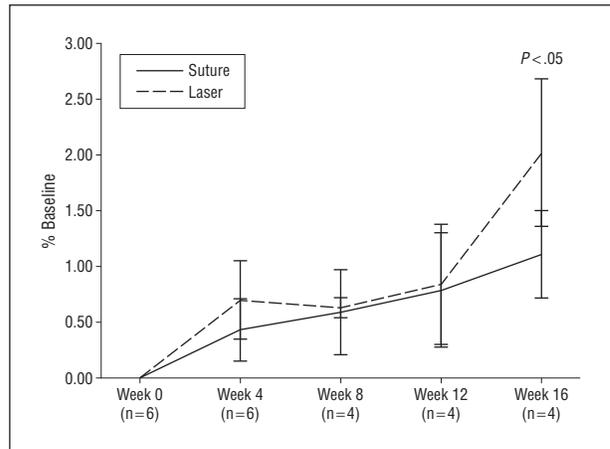


Figure 4. Clinical facial nerve recovery. This graph shows the mean electromyography amplitude (measured baseline to peak) at each time point, as a percentage of the mean baseline amplitude vs time. Only the week 16 data point was statistically significant ($P = .02$). The week 4 time point was nonsignificant at $P = .09$. The error bars indicate SD.

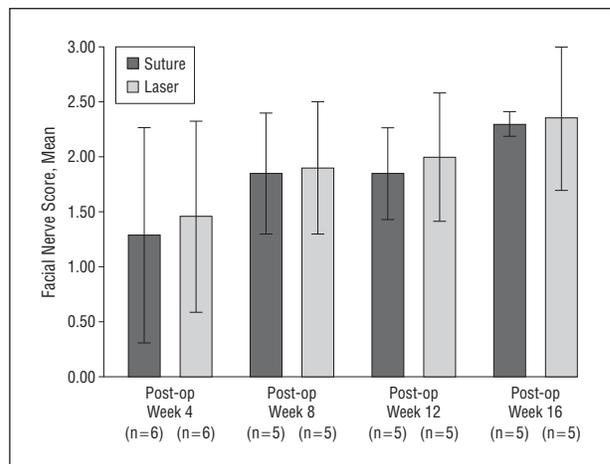


Figure 5. The clinical facial nerve recovery scores (0-3 scale) for the experimental groups, averaged between the 2 blinded observers. As seen, the laser group performs better than the suture group at each time point, but these results were not significant. The error bars indicate SD.

operative week 12: suture, 1.85 (0.42), and laser, 2.00 (0.59); and postoperative week 16: suture, 2.30 (0.11), and laser, 2.35 (0.65) (**Figure 5**). At all time points, the LNW group outperformed the traditional suture repair group; however, none of these results were statistically significant (Figure 5).

HISTOPATHOLOGICAL ANALYSIS

No destruction of the axons or collateral nerve damage was evident at 4 weeks in the laser repair group with the hematoxylin-eosin stain. In addition, a moderate amount of the albumin solder remained around the weld site at 4 weeks with a robust inflammatory reaction around the solder, not seen with the suture repair group (**Figure 6**). At 16 weeks, the solder material had been absorbed and was replaced by collagen, connective tissue and fibrosis, as seen with the Masson trichrome stain (**Figure 7**). Histological analysis revealed no difference in axon organization between the laser and suture repair groups, and

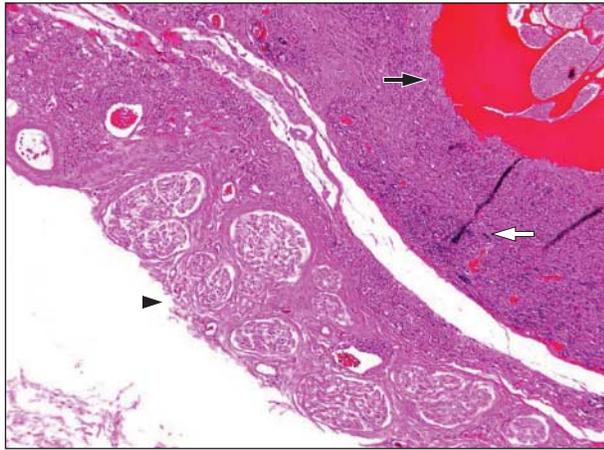


Figure 6. Sagittal cut through the nerve anastomosis in the laser group. A considerable amount of solder remains over the nerve (closed arrow) and is surrounded by an inflammatory reaction (open arrow). Normal nerve fascicles are seen (arrowhead) (hematoxylin-eosin; original magnification $\times 10$).

no neuroma formation was seen in either of the experimental groups. Furthermore, there was no difference seen between groups in the amount of extravasation outside of the neurorrhaphy site. Finally, as previously mentioned, the laser weld group initially showed a larger amount of acute inflammation around the repair site; however, this difference was not seen at the end of the study.

COMMENT

Since the early 1960s, when lasers were first reported to be used for the purpose of tissue adhesion, laser tissue welding has been studied in a myriad of tissues including blood vessels, gut, nerves, skin, dura, urethra, and bladder.¹⁹ Initial studies looking at the use of the laser for facial nerve repair have been reported. Eppley et al¹⁵ first demonstrated that repair of the rabbit facial nerve with a carbon dioxide laser reduced neuroma formation, connective tissue invasion, and axonal proliferation or extravasation. While the use of the carbon dioxide laser in that study reduced the problems of suture nerve repair by producing a “seal” around the nerve through protein denaturation and subsequent fusion of the collagenous portion of the epineurium,¹⁵ the use of the carbon dioxide laser in this study did have some problems. Furthermore, histopathological analysis in that study demonstrated deleterious effects of laser induced thermal injury including, destruction of myelin and loss of axons immediately adjacent to the anastomotic site, as a result of direct laser heating of the epineurium without a biological solder.²⁰ While there was thermal nerve injury in that study, all of the rabbit facial nerves exhibited electrophysiological recordings at 3 months that were not statistically different from the normal suture repaired nerves.¹⁵ In addition, 2 studies by Hwang et al^{16,17} showed that carbon dioxide laser welding of a rat facial nerve, again without the use of a biological solder, affected regeneration of the repaired nerve equally or more effectively than microsurgical suturing. They showed that nerve regeneration in laser-welded nerves occurred in a manner similar to microsurgical repair, through the immunohistochemical detection of a retro-

grade nerve tracer. Those studies also demonstrated that carbon dioxide laser repaired nerves healed with less cellular and fibroblastic response, less scar, and less neuroma formation.^{16,17} The use of laser targeted chromophores in a biological solder was seen when Trickett et al¹⁸ evaluated the efficacy of an albumin-based biological solder containing an indocyanine green chromophore for laser welding rat sciatic nerves with an 800-nm diode laser. That study proved that laser-activated solders had denatured, eliminating underlying nerve or collateral tissue thermal injury through selective absorption of laser energy by the chromophore.¹⁸

With knowledge from these studies, we used a rabbit facial nerve model to devise a pilot study that would assist in demonstrating any operative, electrophysiologic, histologic, or functional benefit from using LNW, in contrast to traditional suture neurorrhaphy. After investigating the operative results, it is clear that the time to complete the nerve repair was significantly shorter with the laser-welded nerves (mean [SD], 3.50 [1.64] minutes vs 13.17 [3.60] minutes). Moreover, LNW appears to be both feasible and quick to learn. As demonstrated in Figure 3, suture nerve repair was originally taking approximately 20 minutes to perform, but by the sixth attempt, the procedure time was down to 10 to 12 minutes. The laser, however, was just as fast to use from the first to the last attempt. Depending on the difficulty of aligning the cut nerve ends, the procedure required 2 to 6 minutes to perform.

Furthermore, LNW has the additional benefit of not requiring the operating microscope. Confirmatory studies could reveal that the ease and speed at which this procedure can be performed with the laser would be advantageous for any surgeon performing nerve repair. These benefits may translate to shorter times under anesthesia for the patients, additional nerve branches anastomosed, and ability of surgeons not trained in microvascular technique to perform laser neurorrhaphy.

After evaluating the electrophysiological results, we found what could be an additional advantage of laser nerve repair. Throughout the study, only the week 16 data showed a statistically significant improved EMG result ($P = .02$); the week 4 data showed an EMG result that did not reach significance ($P = .09$). This may be related to a more rapid initial improvement in the electrophysiologic recovery of the laser-welded nerve repair and then a longer-term improved nerve recovery seen with the laser-welded nerves. The reason that the initial EMG result was better with the laser-welded nerves could have been because there was less nerve injury from the laser than from the trauma of the suture repair. In addition, the EMG results are interesting in that by the 16th week, both the repaired nerve groups were performing at levels above the baseline. This is possibly because of an electrophysiologic phenomenon known as collateral axon sprouting. In the rabbit model, the buccal branch of the facial nerve is composed of multiple small branches, all taking collateral courses to the same end muscle. With a single injured facial nerve branch, the collateral branches around the injured branch will help to overcome the function of that branch and often become hyperactive. Electromyography will confirm the cumulative recovery of the injured nerve plus the additional hyper-

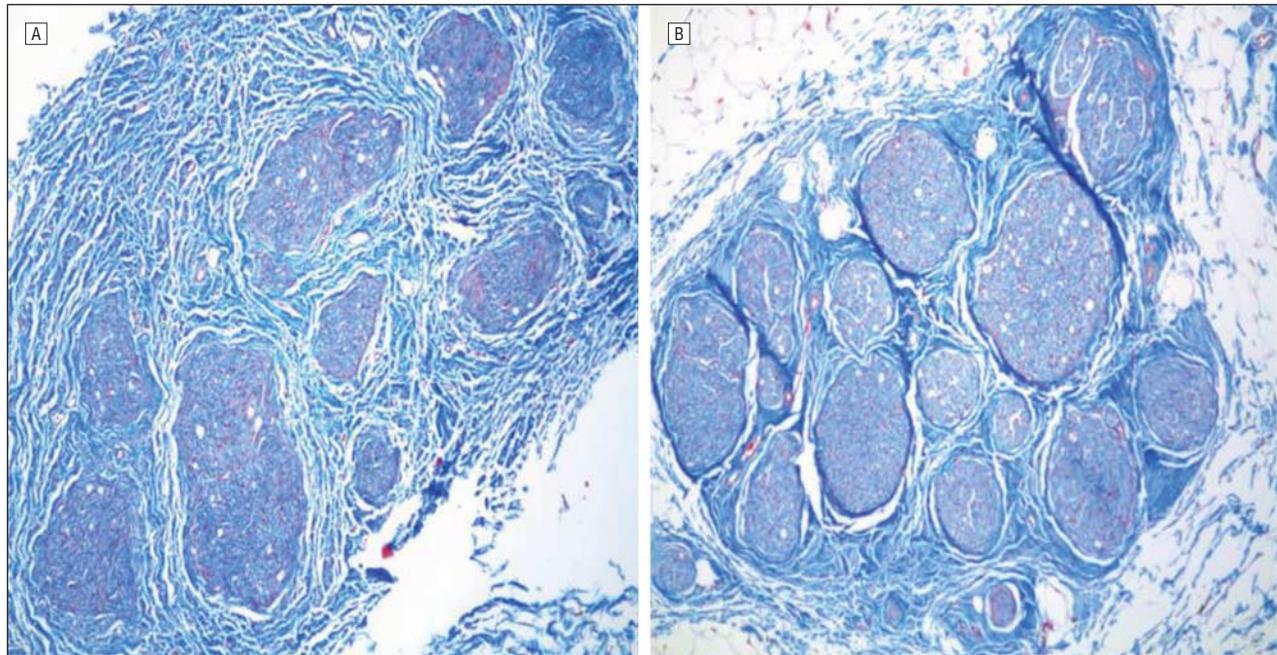


Figure 7. Two different specimens (A and B) of normal nerve fascicles seen at week 16 in the laser nerve weld group. Initial inflammation is now replaced by connective tissue and fibrosis surrounding the nerves (Masson trichrome; original magnification $\times 10$).

active help from the collateral branches. We tried to spare the facial nerve at the division of the zygomatic and buccal branches so that the rabbits would not have problems with corneal irritation and exposure; however, using the buccal branch, which may be arborized into multiple branches, could have potentially confounded some of these results. Thus, additional studies could confirm the potential for faster recovery.

The histopathological results demonstrate, similar to prior LNW studies, that laser welding with a biological solder does not have a deleterious effect on the nerve axons or fascicles. This was seen in the welds examined at 4 weeks, which showed no difference in nerve injury between the 2 groups. What was interesting, however, was that there was more acute inflammation created around the solder in the laser-welded nerves than there was around each of the sutures in the standard repair group. This inflammation did not remain in the laser-welded specimens examined at the end of the study and in the areas where the solder had been replaced by connective tissue and fibrosis. Furthermore, the results displayed that the albumin solder's foundation was still present at 4 weeks, as we had originally postulated, but had completely regressed by the end of the study. This is important because this solder material is not meant to be permanently surrounding the nerve but serves only as a temporary scaffold that remains long enough for nerve recovery to begin and normal wound healing to take place. Lastly, both groups were found to be equivalent in terms of neuroma formation, nerve arrangement, and axon escapement. This was determined to be minimal in both of the experimental groups.

An analysis of the results from the videographic portion of the experiment, which looked at the functional recovery of the rabbits' facial nerve over time, demonstrated, though without statistical significance, that the

use of the laser for facial nerve repair could lead to improved clinical recovery. It was seen that the facial nerve recovery scores improved for both the laser welding and suture nerve repair groups, but the scores for the laser welding group were better than the suture neurotomy group at each time point and eventually reached a higher mean (SD) outcome by week 16 (suture = 2.30 [0.11] vs laser = 2.35 [0.65]). There appears to be some evidence from this study that the functional outcomes of the laser nerve repair rabbits did as well or better than the suture repair group. Not only were the mean recovery scores better at each week that was tested, but the long-term results showed a more improved recovery with the laser-welded repair. However, larger experimental groups need to be examined before this information can be extrapolated to clinical facial nerve recovery outcomes.

In conclusion, this pilot study demonstrates that LNW can be an expedient, feasible, and safe method for facial nerve repair in a rabbit model. Further experiments with larger numbers may provide additional evidence that laser tissue welding produces a nerve weld that has functional, electrophysiological, and histological results that may someday rival or surpass traditional suture neurotomy. Further areas of research into this laser welding technology include its use in a myriad of tissue adhesion applications. If the results of more extensive LNW studies prove efficacious, this procedure would be ideally transitioned to clinical trials in the future.

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