Peripheral nerve repair with microsutures is the gold standard against which any alternative technique must be compared before being accepted as worthwhile. Laser-assisted repair of peripheral nerves may offer several advantages over conventional suturing methods, such as less trauma to the tissue, less inflammatory reaction, and a faster surgical procedure. Nevertheless, the clinical application of laser-assisted nerve repair has been limited by the risk of dehiscence in the postoperative period and the inability to achieve consistently successful laser welds. In previous studies conducted by us and others, the high risk of nerve dehiscence has been overcome by placing one or two stay sutures before laser welding or, more recently, by the use of protein solders, which are melted onto the outer surface of the repair site, resulting in stronger welds. Moreover, consistent achievement of successful laser welds can be increased by the aforementioned use of solders and by careful selection of the laser parameters.

Fibrin glue can be used as an alternative to laser repair to reconnect peripheral nerves, although this method’s disadvantage is similar to that seen with laser repair, that is, low bonding strength directly postoperatively. Fibrin glue has been used mainly in Europe, because it was only recently approved by the Food and Drug Administration for use in the United States. Although there are several reports on the use of fibrin glue in peripheral nerve surgery, most of them are controversial, and an experimental comparative study of nerve repair with lasers, fibrin glue, and sutures has not yet been performed.

Therefore, this study was designed to investigate peripheral nerve regeneration after CO₂ laser-assisted nerve repair in comparison with fibrin glue repair and conventional microsurgical suture repair. Evaluation of the results was performed at 16 weeks postsurgery and included the functional toe-spreading test, along with light microscopy and morphometric analysis.

Materials and Methods

This study was approved by the local Animal Welfare Committee.

Laser, fibrin glue, or suture repair of peripheral nerves: a comparative functional, histological, and morphometric study in the rat sciatic nerve

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Object. This study was undertaken to evaluate CO₂ laser–assisted nerve repair and compare it with nerve repair performed with fibrin glue or absorbable sutures.

Methods. In eight rats, the sciatic nerve was sharply transected and approximated using two 10-0 absorbable sutures and then fused by means of CO₂ milliwatt laser welding (power 100 mW, exposure time 1 second per pulse, spot size 320 μm), with the addition of a protein solder (bovine albumin) to reinforce the repair site. The control groups consisted of eight rats in which the nerves were approximated with two 10-0 absorbable sutures and subsequently glued using a fibrin sealant (Tissucol), and eight rats in which the nerves were repaired using conventional microsurgical sutures (four to six 10-0 sutures in the perineurium or epineurium). Evaluation was performed 16 weeks postsurgery and included the toe-spreading test and light microscopy and morphometric assessment. The motor function of the nerves in all groups showed gradial improvement with time. At 16 weeks, the motor function was approximately 60% of the normal function, and there were no significant differences among the groups. On histological studies, all nerves revealed various degrees of axonal regeneration, with myelinated fibers in the distal nerve segments. There were slight differences in favor of the group treated with laser repair, in terms of wound healing at the repair site. In all groups, the number of axons distal to the repair site was higher compared with those proximal, but the axon diameter was significantly less than that in control nerves (p < 0.05). There were no significant differences in the number, density, or diameter of the axons in the proximal or distal nerve segments among the three nerve repair groups (p < 0.05), although there was a trend toward more and thicker myelinated axons in the distal segments of the laser-repaired nerves.

Conclusions. It was found that CO₂ laser–assisted nerve repair with soldering is at least equal to fibrin glue and suture repair in effectiveness in a rodent model of sciatic nerve repair.

Key Words • fibrin glue • laser microsurgery • nerve regeneration • suture • tissue welding • rat
Comparative study of nerve repair with laser, fibrin glue, or sutures

Animal Preparation

A total of 24 male rats of an inbred Wistar strain, each weighing 270 to 350 g, was used in the experiments. The rats were housed no more than six in a cage and were kept under conventional laboratory conditions before the experiments. Before surgery, general anesthesia was induced by intraperitoneal injection of a mixture of 90 mg/kg ketamine, 10 mg/kg xylazine, and 0.05 mg/kg atropine.

In each rat, the sciatic nerve was exposed through a dorsolateral incision and by dissecting the hamstring muscles. With the aid of an operating microscope, the nerve was dissected from its surrounding tissue and isolated with a plastic sheet. The nerve was then transected using microscissors, and the ends were trimmed with a razor blade; a wooden spatula served as a cutting board.

Laser Repair Group

In the laser repair group (eight rats), the nerve ends were approximated with two monofilament 10-0 polyglycolic acid stay sutures placed in the epineurium and perineurium, and the external epineurium was coated with a small amount of protein solder (bovine albumin dissolved in saline). Subsequently, the nerve was fused with repeated pulses of CO₂ laser energy (power 100 mW, exposure time 1 second per pulse, spot size 320 μm). These laser settings have been evaluated previously for their tensile strength and early thermal damage in an in vitro study. Meticulous care was taken to avoid exposing the suture thread to laser energy, because the tensile strength of sutures is adversely affected by the laser’s heat. After the repair, the wound bed was carefully flushed with saline. For the welding procedures a CO₂ laser with a joystick micromanipulator was used in conjunction with an operating microscope at 40-fold magnification. The laser was operated in continuous-wave mode by using an electric shutter; a foot switch was used to control the pulse duration. A spot size of 320 μm was used.

Fibrin Glue Repair Group

In the group treated with fibrin glue (eight rats), the nerves were also approximated with two 10-0 polyglycolic acid stay sutures placed in the epineurium and perineurium, and the repair area was covered using a biological two-component fibrin glue. This glue consists of fibrinogen solution and thrombin; 1 ml of the fibrinogen solution contains 70 to 110 mg fibrinogen, 10 to 50 U factor XIII, 40 to 120 μg plasminogen, and 3000 kallidinogenase inactivator units, and 1 ml of thrombin solution contains 500 IU thrombin and 40 μmol calcium chloride. These components come deep frozen in two preloaded syringes, and after thawing they are mixed during application; the glue is delivered to the repair site by using a double-lumen syringe. After the two components are mixed at the repair site, the glue becomes a white elastic mass that mimics a natural blood clot.

Suture Repair Group

In the suture group (eight rats), the nerves were repaired with four to six 10-0 polyglycolic acid sutures placed equidistantly around the nerve in the epineurium and perineurium by using standard microsurgical methods. After repair, the fascia of the hamstring muscles was closed with two 6-0 polyglycolic acid sutures and the skin was closed with 4-0 polyglycolic acid sutures. The rats were not immobilized and were permitted to engage in unlimited activity in their cages.

Functional Recovery

The functional recovery of the rats was examined 8 and 16 weeks postsurgery by using a modified version of the toe-spreading test. Briefly, toe spreading, defined as the distance from the first to the fifth digit, was measured in both of the animals’ hind legs from tracks made while walking. The relative toe spreading of the right foot was calculated using the untreated left foot as a control. A 100% motor function loss will result in a relative toe spreading of 30%, whereas a 0% motor function loss will result in a relative toe spreading of 100%. A motor function loss of 50% correlates with a relative toe spreading of 65%. For each measurement, at least four footsteps were recorded.

Neuropathological Examination

Sixteen weeks postsurgery, the rats were killed by an overdose of phenobarbital administered intraperitoneally, and the nerves were exposed, inspected, and carefully removed for neuropathological examination. The nerves were fixed with the Karnovsky fixative, postfixed in 1% osmium tetroxide, dehydrated in acidified 2,2-dimethoxypropane, and embedded in Epon. After hardening, semithin sections (1.25 μm) were stained with 1% toluidine blue for light microscopy. Transverse sections were obtained from the proximal and distal segment of the nerve, whereas longitudinal sections were cut from the repair zones. The sections were examined with light microscopy and semiquantitatively scored for the following: 1) epineurial fasciculation (that is, the presence of a few nerve fibers ensheathed by epineurium and perineurium); 2) neurona formation; 3) extraneural nerve fibers at the repair site; 4) intraneural scarring; 5) axonal alignment; and 6) extraneural nerve fibers in the distal nerve segment.

Morphometric and Statistical Analyses

Morphometric analysis of the nerves was performed using light micrographs at a 400-fold magnification. Nerve tissue area, axon count, diameter of myelinated nerve fibers, and nerve fiber density were determined for the proximal and distal nerve segments. The results are presented as the means ± standard deviation. The data were statistically analyzed using the Mann–Whitney U-test.

Sources of Supplies and Equipment

The Vicryl polyglycolic acid sutures were purchased from Ethicon, Inc., Norderstedt, Germany. The CO₂ laser (model LS 860) and micromanipulator (model LS-11) were acquired from Cooper LaserSonics, Inc., Santa Clara, CA. The operating microscope (model OpMi-1) was purchased from Zeiss, Inc., Oberkochen, Germany. The laser’s electric shutter (model T 132) was purchased from Optilas, Inc., Amsterdam, The Netherlands. The Tissucol fibrin glue was supplied by Immuno AG, Vienna, Austria.

Results

There were no postoperative deaths, and no clinical evidence of wound infections was observed. Of the eight rats in the laser group, three had some heel ulcerations on their right foot. This was statistically comparable with the ulcerations in one rat and three rats from the fibrin glue and suture groups, respectively. The ulcerated heels did not affect the measurements in the toe-spreading test.

Toe-Spreading Test

There were no significant differences in recovery of motor function among the three repair groups. All nerves exhibited significantly diminished motor function directly postoperatively, with a subsequent progressive increase to approximately 60% of the normal function at 16 weeks (Fig. 1).

Macroscopic Findings

On reexploration, dehiscence of the nerves was found in none of the repair groups. The gross appearance of the repaired nerves showed good coaptation in general, and healing at the repair site. Slight adhesions were found in four rats in the laser and fibrin glue repair groups, whereas moderate adhesions were found in six rats in the suture group. Some thickening at the repair site was observed in three, three, and five rats from the laser, fibrin glue, and suture repair groups, respectively; the thickening at the repair site was more pronounced in the suture group. No

J. Neurosurg. / Volume 95 / October, 2001
suture material, carbonaceous deposits, fibrin glue, or solder material were observed at the repair sites.

Light Microscopy Findings

All nerves demonstrated anatomical continuity and revealed various degrees of axonal regeneration across the repair site. The three repair methods had a number of morphological features in common, and the differences were mainly quantitative in origin. The intraneural organization in the transected and repaired nerves was altered when compared with normal nerves: the epineurium was thickened, and it revealed various degrees of fasciculation and proliferation. Normal epineurium and perineurium were only present beyond the repair site. In all repair groups, both the epineurium and perineurium contained regenerating axons. True neuroma formation was only present in one nerve in the laser repair and one in the suture repair group. Intraneural scar tissue was present in all nerves, but fibrosis was more abundant in the sutured nerves. In the

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of histological results for the three repair groups*</th>
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<tr>
<td></td>
<td>Type of Repair</td>
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<tr>
<td></td>
<td>(8 rats in each group)</td>
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<tr>
<td></td>
<td>epineurial or perineurial fasciculation</td>
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<tr>
<td></td>
<td>mild</td>
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<td></td>
<td>moderate</td>
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<td></td>
<td>severe</td>
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<tr>
<td></td>
<td>neuroma formation</td>
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<td>none/slight</td>
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<td></td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>severe</td>
</tr>
<tr>
<td></td>
<td>extraneural fibers at repair site</td>
</tr>
<tr>
<td></td>
<td>few</td>
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<tr>
<td></td>
<td>several</td>
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<tr>
<td></td>
<td>numerous</td>
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<tr>
<td></td>
<td>intraneural scarring</td>
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<td>axonal alignment</td>
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<td>moderate</td>
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<td>poor</td>
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<td></td>
<td>extraneural fibers in distal nerve</td>
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<td></td>
<td>none/few</td>
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<td></td>
<td>several</td>
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<td></td>
<td>numerous</td>
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</table>

* Values represent the number of nerves. — = none.
Comparative study of nerve repair with laser, fibrin glue, or sutures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Laser</th>
<th>Fibrin Glue</th>
<th>Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td>nerve tissue area (mm²)</td>
<td>0.57 ± 0.03</td>
<td>0.39 ± 0.08</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td>diameter of myelinated axons (µm)</td>
<td>6.02 ± 0.23</td>
<td>3.51 ± 0.21</td>
<td>6.30 ± 0.74</td>
</tr>
</tbody>
</table>

*Values are expressed as the means ± standard deviation.

laser repair group, the solder was completely absorbed at the repair site without residual reaction. Again, no carbonized particles were seen, and in the fibrin glue repair group, no signs of the repair material could be found.

Remnants of the sutures, which we observed only in some cases, were displaced more toward the center of the nerve, and were usually located intraneurally and covered by the epineurium and perineurium. In these nerves most of the suture material had been absorbed, and only some remaining cellular aggregate, which consisted of concentrically arranged mononuclear and giant cells, was detected. Minor fibrosis was observed around the remnants of the sutures, and a disorganized pattern of nerve fibers was observed adjacent to them.

In the midst of the repair site, a defined fascicular pattern was lost, and the axons formed numerous small fascicles, especially at the periphery of the nerve. In the longitudinal sections, many axons were cut transversely, indicating some disorientation of the nerve fibers. Axonal alignment was good to moderate in laser- and fibrin glue-repaired nerves, and good to poor in sutured nerves (Table 1).

More distal to the repair site, the neural architecture became more normal. The proximal section of all repaired nerves generally had a normal appearance that was comparable to intact sciatic nerves from the contralateral side. The nerves usually had one large fascicle, but in the distal nerve segment, two or three large fascicles with numerous myelinated nerve fibers were present. No fibrosis was found in the distal nerve segments. Extraneural fibers, which indicated escape of nerve fibers at the repair site, were present regardless of the repair method. The location and site of these extraneural fibers corresponded well with the repair site location at which the nerve fibers were intermingled within the epineurium, where the perineural layer was not clearly defined. A few degenerating axons were occasionally noted in the distal nerve segment.

**Morphometric Outcome**

In the proximal portions of the nerves there was a bi-modal distribution of the axonal diameter, and no significant difference in fiber counts or diameter among the groups (p > 0.05). The regenerated fibers in the distal nerve segment of the repair groups were significantly thinner (p < 0.001) than in the proximal segments of all groups. The normal bimodal distribution of the myelinated nerve fibers was lost due to loss of larger nerve fibers, and the total spectrum shifted to smaller nerve fibers (Fig. 2). The mean myelinated axonal diameter, axon count, and density was not significantly different among the three repair groups (p > 0.05), although there was an overall trend toward more and thicker nerve fibers in the laser repair group. The total number of fibers in the distal nerve segments was significantly increased compared with the proximal segments for all repair groups (p < 0.001). In Table 2 we summarize the morphometric data in all repair groups.

**Discussion**

A major focus of nerve repair research has been the development of procedures with which to avoid or minimize the use of sutures and prevent fibrous ingrowth at the repair site. Several sutureless methods have been developed, although none of them has been demonstrated to be consistently superior to sutures. Repair of peripheral nerves with sutures, whether epineurial or perineurial, is the gold standard against which any alternative method must be compared. Any experimental repair procedure must yield at least equal histological and functional results before being accepted as a worthwhile alternative.

Most importantly, sutureless methods must fulfill several criteria to have an advantage over suture repair. First, the procedure must result in a sufficient acute tensile strength. Second, it must not compress the nerve and must not involve increased severity of trauma compared with sutures. Third, the early and late tissue reaction of the nerve must be kept to a minimum and the axonal regeneration must not be impaired.

Before performing this comparative study, we have refined the surgical procedure of laser-assisted nerve repair, which is based on more than 400 previous manipulations of peripheral nerves in which the CO₂ milliwatt laser was used. By using a protein solder in combination with two absorbable 10-0 stay sutures, the risk of mechanical damage to the nerve and of scar formation are reduced (unpublished results). Soldering procedures rely on laser energy to produce fixation of the solder to the tissue. The protein behaves the same way in the welding process as does an inorganic solder used to join metal parts with the application of heat. In this way, a sleeve-type joint is formed by the solder, which is much stronger mechanically than a simple edge-to-edge joint. In addition to being mechanically stronger, laser soldering methods may be more technically forgiving than nonsoldering methods, because the solder may be able to bridge small gaps in coaptation that would otherwise produce a lead point for separation of the weld, and therefore it may reduce the need for stay sutures. Solder may also be beneficial in that it can protect the underlying tissue from the damaging thermal effects seen with nonsoldering methods. An enormous advantage...
is that the solder hardens immediately when hit by laser energy, and that there is control over the welding process. Also, when alignment has been achieved by micromanipulation, it can be maintained instantly by welding it.

In our study, an observation period of 16 weeks was chosen, because that is when nerve regeneration is thought to be completed. This is supported by the study published by Huang, et al., in which they demonstrated that at 4 months after laser and suture repair of the rat sciatic nerve, no further improvement of motor function (assessed by the toe-spreading test) was observed. In our study, two sutures were placed at 0˚ and 180˚ to obtain adequate tensile strength and to facilitate manipulation of the nerve during laser and fibrin glue repair. We believe that two sutures are essential because the nerve ends retract after transection and trimming, and some acute force is needed to approximate the nerve ends. Once covered by solder or glue, the sutures are not removed, because that would cause damage to the repair site.

Our results demonstrate that both laser, fibrin glue, and suture repair result in good axonal regeneration, although normal nerve architecture is not restored even at 16 weeks postoperatively. The CO₂ laser–assisted nerve repair resulted in an improved histological architecture at the repair site (less epineurial and perineurial proliferation, less intraneuronal scar tissue, and better neural alignment) compared with sutures, both for the early (unpublished results) and late period of wound healing (this study). These findings are related to each other and can be explained as follows. Laser repair in which solder is used produces a more complete epineurial seal than is achieved with sutures, which prevents scar tissue ingrowth from outside the nerve. The epineurium heals more favorably due to minimizing the foreign body reaction, and thus the axons are less blocked or misdirected at the repair site; consequently, the neural alignment is improved. Better alignment may also be achieved because the compressive forces and trauma to the epineurium resulting from sutures are reduced by simply using fewer of them. Intraneuronal scarring, which is mainly caused by collagen production by fibroblasts and Schwann cells inside the nerve, is less likely to be prevented by laser repair. Nevertheless, intraneuronal scarring was mild in the laser repair group, possibly because there was minimal manipulation and less distortion of the nerve ends during repair. The epineurial seal is also postulated to provide a more favorable microenvironment for axonal growth by holding neurotrophic factors, although this aspect was not investigated in our study. Our histological results with laser-repaired nerves compare favorably with findings in the literature. Al-Hussaini, et al., showed that in CO₂ and potassium titanyl phosphate laser–repaired rat sciatic nerves, fibroblasts, Schwann cells, and myelin sheaths had a normal appearance, whereas in sutured nerves the fibroblasts were altered and the myelin sheath was disorganized. In facial nerve grafting performed in rabbits, no neuromas, connective tissue invasion, axonal extension or proliferation outside the epineurium, and less entrapment of axons at the repair site were found in the CO₂ laser group 3 months postoperatively. The use of CO₂ laser repair for nerve grafting in primates led to less escape of axons outside the intraneuroscleral space, and less neurona formation than in a sutured group. Kim and Kline reported that CO₂ laser–repaired nerves with the addition of epineurial and peri-

neural tissue to the repair site had less proliferation of the epineurial tissue.

In all three types of nerve repair in our study extraneuronal nerve fibers were located in the distal segment, indicating that some axons must have escaped at the repair site. This study shows that the fibrin glue does not prevent the axons from escaping at the repair site. Based on our findings, one can conclude that neither the solder nor fibrin glue provided a good seal at the repair site, which has been confirmed for fibrin glue by Palazzi, et al. Axons may escape the nerve between the fascicles, however, and follow their course distally, while still being located extraneurally.

Despite the more favorable histological findings for the laser repair group, there were no significant differences in functional or morphometric outcome for the three methods. Assuming that an increased number and diameter of myelinated nerve fibers in the distal nerve segments correlate with better regeneration, however, there is a trend toward better results in the laser group, followed by the suture and then the fibrin glue group. This trend has been observed, albeit without statistical significance either, by others who used different animal models.

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J. Neurosurg. / Volume 95 / October, 2001
Comparative study of nerve repair with laser, fibrin glue, or sutures


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