Laser-assisted nerve repair. An experimental study
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OPTIMAL LASER PARAMETERS FOR LASER-ASSISTED NERVE REPAIR*

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As outlined earlier, LANR has great potential by avoiding foreign body reaction, minimising scar tissue formation and less traumatic manipulation of the nerve. Nevertheless, the clinical application of LANR is limited by the high dehiscence rate which can be 12% to 60% for a CO₂ laser (Bailes, 1986; Richmond, 1986; Maragh, 1988; Korff, 1992). To overcome this problem, one or more stay sutures for supporting the welds or nerve grafts to reduce the tension at the repair site are used (Fischer, 1985; Bailes, 1989; Benke, 1989; Eppley, 1989).

Many factors may influence the tensile strength of LANR, such as the amount of tissue available for fusion, and the LANR technique that is used. Although many investigators have studied tissue welding by means of histology and wound healing, no one has determined the laser settings and end points which produce the greatest tensile strength. Experimental studies on welding of other tissues have shown that for each tissue there is an optimal range of laser exposures that produces the strongest welds (Neblett, 1986; Nijima, 1987; Poppas, 1992). For nerves, the optimum exposure must still be determined.

To enhance the tensile strength of tissue welding by the CO₂ laser, various solders have been used in vessel and urethra welding (Cirrit, 1990; Poppas, 1992). These solders, which provide extra protein for the fusion process, are coagulated on the outer surface of the repair site to obtain stronger welds and therefore lower dehiscence rates.

This study was designed to investigate the in vitro tensile strength of nerves welded by a CO₂ milliwatt laser at different radiant exposures and exposure times. The effect of different solders on the tensile strength was investigated and compared to conventional microsurgical suture repair (CMSR), LANR, and FGNR. As a solder, albumin solution, dried albumin solution, egg white, fibrinogen solution, fibrin glue, and red blood cells were used.

Materials and methods

The study was approved by the local Animal Welfare Committee. A total of 333 tibial nerves of New Zealand White rabbits (weighing 1.9-2.5 kg) of both sexes were used for this study. All animals were used in other experiments and nerves were collected within ten minutes after the sacrifice of the animals. The diameter of the nerves ranged between 1.2 ± 0.1 mm (mean ± SD),

and the length of each nerve ranged between 3.5 to 4 cm. Before repair, each nerve was transected with a scalpel in two sections of approximately the same length.

In the first group, LANR was performed at 15 different laser settings (power outputs of 50, 100, and 150 mW; pulse duration of 0.1, 0.5, 1.0, 2.0, and 3.0 s). In this group, the opposite nerve ends were closely approximated, and the epineurium of one of the nerve sections was pulled over the nerve end of the other nerve section and welded around its circumference with repeated single spots of laser energy (Figs. 3.1a). Six repairs were performed for each of the 15 groups of laser settings, giving a total of 90 LANR procedures.

In the second group of laser welding, a 20% albumin solution that was left to dry up on a glass slide for 20 minutes, was used as a solder (LANR plus dried albumin solution). Twelve repairs were performed at 100 mW with pulses of 1.0 s. In this group, the opposite nerve ends were closely approximated, the epineurium of one of the nerve section was pulled over the nerve section of the other nerve and welded around its circumference with three to five laser pulses. The slab of dried albumin was positioned on the repair site and welded with the same parameters to weld the slab of dried albumin solution and nerve together (Figs. 3.1b).

In a third group of laser welding, a procedure identical to that in the second group was performed. Ten repairs were carried out. After the procedure, the nerves were positioned between cotton soaked in saline for 20 minutes to investigate the influence of rehydration of the slab of albumin on the tensile strength (LANR plus dried albumin solution plus rehydration).

In the fourth group of laser welding, egg white was used as a solder (LANR plus egg white). The same 15 laser settings were used as in the first group. In this group, the opposite nerve ends were closely approximated, the epineurium of one of the nerve section was pulled over the nerve section of the other nerve and welded around its circumference with three to five laser pulses. The egg white was applied using a small spatula. With the egg white covering the repair site, the area was welded again with the same parameters to coagulate the egg white on and to the nerve (Figs. 3.1c). Six repairs were performed for each group of laser settings, giving a total of ninety procedures.

In the remaining groups, LANR in combination with different solders was used in a similar procedure as described for egg white (fourth group of laser welding). As solders, two different albumin solutions (5% and 20%), fibrinogen solution, autologous fibrin glue, red blood cells, and a commercial fibrin glue (Tissucol, Immuno AG, Vienna, Austria) were investigated in a varying numbers of repairs. Fibrinogen solution and autologous fibrin glue were prepared according to SLEDENTOP (1985). Although a double irradiated LANR group would be more accurate as a control group to the LANR plus solder groups (as the LANR group had one set of exposures and the LANR plus solder groups had two sets of exposures), singly irradiated LANR group had greater tensile strengths and would probable have less thermal damage.
Optimal Laser Parameters for Laser-assisted Nerve Repair under in vivo settings.

The end point of the welding procedure was defined as the visible fusion of the epineurium. In the LANR group, ten to 30 laser pulses were needed for each repair. In the LANR plus solder groups, 15 to 50 laser pulses were used for each repair. Laser repairs at low powers (50 mW) and short exposure times (0.1-1.0 s) needed more laser pulses (>25 laser pulses) than repairs at high powers and long exposure times (<30 laser pulses).

The three control groups consisted of: i) nerves (n=6) repaired by LANR (100 mW power, 1.0 s pulse duration; one epineurial 10-0 monofilament nylon suture (Dermalon, Davis-Geck, Hampshire, United Kingdom)), ii) nerves (n=9) repaired by FGNR alone and, iii) nerves (n=8) repaired by CMSR (four epineurial 10-0 nylon sutures).

For all LANR procedures a CO₂ laser (Cooper LS 860, Cooper LaserSonics Inc., Santa Clara, California, U.S.A.) was used in conjunction with an operating microscope (OpMi-1, Zeiss GmBH, Jena, Germany) at 40 fold magnification and a joystick micromanipulator. The laser was operated in a CW mode using an electrical shutter (T 132, Optilas, Alphen aan de Rijn, The Netherlands) with a foot-switch to control the pulse duration. A spot size of 320 μm was used.

All other nerve repairs were performed using microsurgical instruments and the same operating microscope at 40 fold magnification. The mean time for performing LANR (without solder) was 4 ± 1.5 min, for LANR with a solder 7 ± 2 min, for FGNR 5 ± 1 min, and for CMSR 17 ± 4 min. In the laser groups, each repair site was observed meticulously with a 40 fold magnification and the visible effects of laser radiation on the epineurium were scored quantitatively with values varying from 1 to 6, where 0 indicated no visible effect, 1 drying of the epineurium, 2 shrinkage, 3 whitening, 4 caramelisation (slight browning), 5 carbonisation, and 6 perforation of the epineurium.

The relative tensile strength of the nerves was measured directly after the repair using a gradual weighing system. The distal end of the nerve was fixed with a clamp, while the proximal end was attached to a plastic container through a fixed pulley mechanism (Fig. 4.1). If the container was empty, the system was in equilibrium. The container was filled with water until the weight caused dehiscence of the nerve ends. The rate of water flow into the container was constant for all experiments (16.4 ml/min) using a 25 gauge needle. The tensile strength was defined as the weight of water (in g) that caused dehiscence of the nerve ends.

Dehiscence was defined as a total rupture of the nerves, which was the case for all laser and fibrin glue repaired nerves. For nerves which did gradually tear apart, which was the case for some sutured nerves, the dehiscence was defined as a minimal distance of 0.5 cm between the nerve ends. The tensile strength was measured by measuring the weight of the container, partially filled with water, with a balance, and by subtracting the weight of the empty container. The data were statistically analysed using a non-parametric Mann-Whitney U test.
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Results

Figure 4.2 gives an overview of the tensile strength for eight different methods of nerve repair. For LANR, LANR plus albumin 20% solution, and LANR plus egg white, tensile strengths are shown which were produced at the optimal laser parameters (at 50 mW with pulses of 3.0 s or at 100 mW with pulses of 1.0 s). A Mann-Whitney U test showed no significant difference between LANR and FGNR (p > 0.1). However, the tensile strength of the LANR plus albumin 20% solution and LANR plus egg white was significantly greater than that of LANR and FGNR (p < 0.05). The tensile strength of LANR plus dried albumin solution was higher than those of any other sutureless technique used (p < 0.05). However, after rehydration the tensile strength decreased by 50%. Finally, the tensile strength of CMSR was significantly higher than in any other technique of nerve repair (p < 0.05).

The use of albumin 5% solution, fibrinogen solution, autologous fibrin glue, red blood cells, and fibrin glue (Tissucol) as solders did not increase the tensile strength in comparison to LANR alone. Therefore, these results are not included in the figures and further analysis. Figure 4.3 shows the tensile strength as a function of pulse duration of LANR, LANR plus albumin 20% solution, and LANR plus egg white at 50, 100, and 150 mW respectively. Both for LANR and LANR plus solder some combinations of laser energy and pulse duration resulted in repairs that could not resist gentle handling. In these repairs no tensile strength could be measured and thus these repairs were considered as unsuccessful. In LANR, optimum bonding was achieved at 100 mW with pulses of 1.0 s (tensile strength 2.4 ± 0.9 g). The use of albumin 20% solution and egg white as a solder, both at 50 mW with pulses of 3.0 s, resulted in a tensile strength of 5.7 ± 2.1 g and 7.7 ± 2.4 g respectively. The use of a dried albumin solution as a solder at 100 mW with pulses of 1.0 s increased the tensile strength nine fold as compared to LANR (tensile strength 21.0 ± 8.6 g). However, rehydration of the repairs resulted in a decrease of the tensile strength to 9.8 ± 4.5 g.

Observation of the tissue welding process showed gradual changes in appearance of the thermal laser-tissue interaction. Tissue changes for LANR and LANR with egg white as a solder are summarised in table 4.1. Little difference was found in the appearance of the thermal laser-tissue interaction between LANR and LANR with the use of albumin 20% solution or egg white as a solder. Perforation of the epineurium did not occur if LANR with a solder was used, as the solder protected the epineurium. Figure 4.4 shows the average tensile strength (mean ± SD) as a function of the scored tissue changes for all LANR and LANR with the use of albumin 20% solution and egg white as a solder. The area under the curves represents the results of the polynomial evaluation of the data.

Figure 4.5 shows the macroscopic view of a nerve after LANR with the use of egg white at 100 mW with pulses of 1.0 s.
Fig. 4.1. Schematic view of the set up for measurement of the tensile strength. In each measurement, the container was filled with water until the weight caused dehiscence of the repair.

Fig. 4.2. Average tensile strength (± SD) of nerve repairs achieved by different methods. A = albumin (20%); EW = egg white; drA = dried albumin; drA* = dried albumin after rehydration; S = one suture. The laser repairs were performed at optimal laser settings of 100 mW with pulses of 1.0 s.
Fig. 4.3. Average tensile strength (± SD) as a function of pulse duration of LANR, LANR plus albumin 20%, and LANR plus egg white at 50 mW (upper), 100 mW (middle), and 150 mW (lower). An asterix (*) signifies that no sufficient tensile strength was achieved. Two asterices (**) signify that no measurement of tensile strength was performed.
Discussion

Because of the high dehiscence rate observed in experimental studies LANR has not widely been used in clinical practice. To make laser welding an alternative to CMSR, the tensile strength has to be sufficient. This study investigated the acute in vitro tensile strength of CO\textsubscript{2} LANR performed under different laser settings and with application of various solders, and the acute in vitro tensile strength in relation to changes in tissue appearance. As this was an in vitro study, factors that can influence nerve regeneration were not taken into account, like effects of the laser and the solders on neural tissue and wound healing.

Few studies report on the acute tensile strength of end-to-end CO\textsubscript{2} laser welded nerves. MARAGH (1988) reported that LANR (90-95 mW, 200 \textmu m spot size diameter, 0.2 s exposure time) had a strength of 43.1 g at day four postoperatively. At day eight, LANR had a strength comparable to the epineurial suture control group (166.7 g). The dehiscence rate was 12%. Other studies reported only dehiscence rates or strengths expressed as a percentage of the strength of normal nerves. SEIFERT (1989 & 1990) performed LANR (80-90 mW, 150 \textmu m spot size, no exposure time reported) of the oculomotor nerve in cats with dehiscence rate of 0%. BAILES (1986) evaluated LANR (70-80 mW, no spot size diameter, and pulse duration reported) both in end-to-end repairs and interposition grafts. The dehiscence rate in laser end-to-end repairs was 25%, in laser interposition grafts 18%, and in CMSR 0%. In another study of nerve grafting, BAILES (1989) reported 100% repair patentcy. THUMFART (1990) studied the tensile strength of LANR (2 W or 5 W, no spot size diameter mentioned, 0.5 s pulse duration), LANR plus fibrin glue, and suture repairs in vitro. No bonding could be achieved in the LANR group, and LANR plus fibrin glue provided lower tensile strengths than FGNR alone. In all other studies, one or more stay sutures were used resulting in a dehiscence rate of 0%. Only FISCHER (1985) found in LANR a dehiscence rate of 13%, but in this study some of the stay suture were removed after the welding procedure.

In this study, the tensile strength of LANR performed at optimal laser settings was significantly lower than in CMSR (2.4 ± 0.9 versus 29.6 ± 10.4 g). Comparison between the FGNR (2.7 ± 1.2 g) and LANR without solder showed no differences. For LANR using one 10-0 nylon stay suture, the tensile strength was about 20% of that of the nerves sutured with four 10-0 nylon stay sutures and was independent of the laser settings used (Fig. 4.3). The strength of the LANR was dependent on the number of stay sutures used. This is consistent with the report of BENKE (1986). Despite the low tensile strength of LANR, it is likely that the strength of the weld will increase in time in in vivo studies. The critical period for dehiscence is the first week postoperatively before the fibroblasts have formed a definite closure of the wound (ABRAMS, 1998). Although fibrin glue reaches its maximum strength one hour after the glue has been applied, experimental studies in end-to-end repairs showed a tensile strength of less than 1 g, and a dehiscence rate of
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Fig. 4.4. Average tensile strength (± SD) for all LANR, LANR plus albumin (20%), and LANR plus egg white as a function of scored tissue changes. A polynomial fit (third order) is included. 0 no observable effect, 1 drying, 2 shrinkage, 3 whitening, 4 caramelisation (slightly browning), 5 carbonisation, 6 perforation of the epineurium.

Fig. 4.5. The repair site of a nerve after CO₂ laser welding (at 100 mW and a pulse duration of 1.0 s) using egg white. The distance between two bars represents 1 mm.
Optimal Laser Parameters for Laser-assisted Nerve Repair

Table 4.1. The effects of CO\textsubscript{2} laser light doses on the epineurium during LANR without the use of a solder, and on the epineurium and solder during LANR with the use of egg white (appearance of effects was approximately the same in the two groups).

<table>
<thead>
<tr>
<th>Technique</th>
<th>Power (mW)</th>
<th>Pulse duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>LANR</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>150</td>
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<tr>
<td>LANR plus</td>
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<td>egg white</td>
<td>100</td>
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<tr>
<td></td>
<td>150</td>
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0 no observable effect, 1 drying, 2 shrinkage, 3 whitening, 4 caramelisation (slightly browning), 5 carbonisation, 6 perforation of the epineurium

80\% (CRUZ, 1986; RICHMOND, 1986). These dehiscence rates are comparable to those of LANR and our results also confirm that LANR as well as FGNR have low tensile strengths. Therefore, these repairs may fail under in vivo conditions as tension on the repair site occurs by retraction of the nerve ends.

Several technical points were essential for effective welding with the CO\textsubscript{2} laser. Most importantly, bonding occurred only when the tissues were directly opposed with the epineurium overlying the repair site. Also, dry tissue surfaces were essential to obtain adequate welds. The dryer the tissue surface (epineurium), the greater is the effect of the CO\textsubscript{2} laser on the tissue.

During welding, it is important to determine an end point at which bonding has been achieved. In experimental and clinical tissue welding, this end point is normally based on visual changes of tissue. Although there may be other, more sophisticated methods to determine the end point like temperature measurements (SCHENFIELD, 1994; CILESIZ, 1997; POHL, 1998) or measurements of changes in reflectance (WELCH, 1984), visual changes currently remain the only practical end point for most surgeons. Drying and shrinkage, blanching, tanning, and browning of the tissue were indicated in previous reports as the end points for proper welds (NEBLETT, 1986; CIRKE, 1990; KRISCH, 1990; POPPAS, 1992). The results of this study show that the strongest welds were produced at powers and exposure times that gave whitening and a beginning of caramelisation of the epineurium (Fig. 4.4). Also, the whitening and caramelisation that occurred in the welded egg white suggest that these visual changes can be taken as the end points for welding. Whitening is related to the denaturation of proteins. The use of higher powers and longer pulse durations gave rapid carbonisation with weaker bonds. When the epineurium was perforated, the exposed fascicles (ensheathed by perineurium) reacted differently to the laser radiation. Curling of the fascicles and creating small excavations without bonding of the tissue occurred. As a result, no welding of the perineurium could be achieved. This is supported by the finding that the perineurium seems to react differently to thermal inju-
ry (LELE, 1963). The difference in the reaction of epineurium and of perineurium to laser radiation can be explained by their difference in composition. The epineurium is mainly composed of densely packed collagen bundles, while the perineurium is formed of several lamellae which consists of closely packed perineurial cells (ROHLING, 1961). Since collagen is believed to be the main chromophore in the fusion process, it is not surprising that the epineurial layer reacts differently to laser radiation and is more easily welded than the perineurium.

As proteins are believed to be the primary component of the welding process, topical applied proteins, used as solders, may provide the necessary amount of proteins for welding and result in a greater tensile strength (POPPAS, 1988 & 1992). Several authors have described the use of solder for tissue welding with encouraging results. In urethral tissue welding with the CO$_2$ laser, the use of egg albumin as a solder resulted in a significant increase of bursting pressure (POPPAS, 1992). In vessel welding, autologous fibrin glue (fibrinogen and thrombin) in combination with the CO$_2$ laser decreased the disruption rate by 32% (CIRRIT, 1990). There have been no reports, however, on the use of solders for CO$_2$ laser nerve welding. In this study, albumin suspension, autologous fibrin glue, fibrinogen suspension, and red blood cells used as a solder did not increase the acute tensile strength, and albumin 20% solution, egg white and dried albumin 20% solution gave significantly higher tensile strength than LANR. These solders were applied in a thin layer to the repair site and coagulated with a CO$_2$ laser. The results indicate that there is a correlation between the amount and concentration of structural proteins and the tensile strength of the union. The decrease in tensile strength of LANR plus dried albumin solution as a solder after the albumin has been rehydrated is explained by the fact that the slab of albumin that was not coagulated by the laser has been dissolved in the presence of water. Although we did not perform histological or electron microscopical studies of the welding process, our results support the theory that coagulation is probably the responsible process for the tissue fusion.

Although the tensile strength of LANR in combination with the use of albumin 20% solution and egg white as a solder was lower than in CMSR, improvement over LANR alone was substantial and encourages further in vivo research on the use of solders. Besides the use of protein solders, extra tissue for improving the tensile strength have been used. KIM (1990) used perineurial and epineurial tissue to serve as a supplement for the welding procedure (30-85 mW, no pulse duration reported, 150 µm spot size diameter), resulting in 100% patency rate. KORFF (1992) used LANR in combination with subcutaneous tissue wrapped around the nerve (1 W, exposure time 0.05 s, 360 µm spot diameter). Although the acute tensile strength was relatively high (6.1 ± 5.0 g), two months post-operatively nine of 15 nerves had separated (60% dehiscence rate). OCHI (1995) used a fibrin film to reinforce the repair site and achieved a tensile strength of 10.1 ± 1.3 g directly after repair, increasing to 30.1 ± 1.6 g after 24 hours.

Despite the optimal results achieved with egg white, we realise that the in
vivo use of egg white as a solder would probably initiate an immunological reaction of the host. Furthermore, as egg white is not sterile, the potential danger of viral or bacterial transmission is present. A possible solution to these problems is to analyse what constituents enhance the welding and to make such a solution with sterile components. The main constituents of egg white are 10.2% albumin, 0.05% fat, and 88.1% water (Long, 1961). The use of sterile albumin solution as a solder avoids such problems. Recently, Poppas (1993 & 1996) has significantly improved the preparation of the protein solder, making it suitable for clinical use. Another source of complications with the use of solders in general could be the persistence of solder between the nerve ends. If this happens, the solder could block the sprouting axons and could induce scar tissue formation between the nerve ends. Also premature absorption and disintegration of the solder is possible, which may result in early dehiscence of the union.

In conclusion, we have demonstrated that 

i) the operation time of LANR and LANR plus solder is short compared to CMSR, 

ii) the strongest welds are associated with specific changes in tissue appearance (whitening and beginning of caramelisation) which can be used to determine the end point of the welding, 

iii) LANR in combination with albumin 20% solution, dried albumin solution, and egg white as solders gives tensile strengths that may be sufficient for holding the nerve ends together under in vivo conditions, and 

iv) that the strongest welds in LANR and LANR plus solder were found at 100 mW with pulses of 1.0 s and at 50 mW with pulses of 3.0 s.