Laser-assisted nerve repair. An experimental study
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Chapter X

GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE PERSPECTIVES

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Developments in peripheral nerve repair have significantly advanced during the two World Wars, mainly due to the high incidence of traumatic nerve lesions. Since then, understanding the biology of nerve healing and regeneration, knowledge of anatomy, and the introduction of surgical microscope and microinstruments significantly improved the results of peripheral nerve repair. Nevertheless, research into new repair techniques continues as the results of current suture techniques are still far from satisfactory.

To study the possible role of the CO$_2$ milliwatt laser in peripheral nerve repair, a series of experiments were designed, first to optimise the various aspects of the technique which may influence the final results of LANR, and second, to compare LANR with current nerve repair techniques. The laser choice for nerve repair is usually determined by the available resources. Our choice of the CO$_2$ laser has been based on literature data and the technical requirements for microsurgery of peripheral nerves. Due to the shallow tissue penetration at 10,600 nm (Welch, 1984), the CO$_2$ milliwatt laser was expected to fulfil the criteria for successful LANR, i.e. superficial coagulation of the epineurium without excessive damage to the underlying axons. An important technical point, the application of a precise amount of energy to the irradiated spot for a controlled period of time, was easily met by the use of a micromanipulator attached to the surgical microscope and the use of an electrical time shutter.

To obtain the best possible results of LANR, we have investigated chronologically several issues for optimisation of LANR. The first question was how to make LANR reliable and how to obtain an adequate acute tensile strength. In the literature, dehiscence rates of 12% to 60% of CO$_2$ laser repaired nerves were reported. Several factors influence the acute tensile strength, such as the duration of laser exposure and the amount of tissue available for fusion. In practice, incorrect laser exposures can influence clinical results significantly. A good example is the formation of aneurysm in overexposed laser-assisted vessel anastomoses (Quigley, 1986b; Tang, 1997a). Thus, it seemed logical to test different laser parameters (irradiance and exposure time) to optimise the tensile strength in vitro and to determine a visual end point at which bonding has been achieved. It became clear that the tensile strength of LANR performed at optimal laser settings (100 mW power, 1.0 s pulse duration, 320 µm spot size) was low (2.4 g) but comparable to FGNR (2.7 g). Sutured nerves were significantly stronger (29.6 g).
When using additional sutures in LANR, the tensile strength increased and was depended on the number of sutures used.

As denatured proteins are believed to be responsible for the acute tensile strength of the welds (Poppas, 1988) application of exogenous proteins at the repair site provided an alternative to increase the tensile strength. The protein, acting as solder, is applied in a thin layer to the repair site and the laser light is painted over the solder until a solid bond has been created. We have tested several solder substances. Especially the use of egg white and albumin resulted in a substantial increase of the strength from 2.4 g to 21.0 g. Moreover, the results indicated that there is a correlation between the amount and concentration of proteins and the tensile strength. Furthermore, it was observed that the strongest welds, regardless whether performed with or without a solder, were associated with a specific change in tissue appearance, namely whitening and very slight caramelisation. As a consequence, these tissue changes were used in our experimental studies and we suggest them as the end point for tissue welding in clinical practice. However, physical end points, like measurement of temperature or reflectance, would provide a more accurate and objective control (Barak, 1997; Cilezik, 1997, Pohl, 1998). Although we performed one pilot study to investigate such feedback (Menovsky, 1995e), further investigations in this field were considered to be beyond the scope of this thesis. Several other technical points became apparent during the studies of this thesis which were essential for the reliability of CO2 laser welding. Most importantly, bonding occurred only when the nerve ends were directly opposed and when the tissue surface was dry. This is logical, taking into account the physical properties of the CO2 laser such as high absorption by water.

The next step was to gain insight into the tissue welding mechanisms and subsequent wound healing after laser irradiation. The exact mechanism of tissue welding is still not fully understood, in part because many alterations observed in tissue after laser welding are not necessary responsible for fusion. Some authors speculated that the mechanism may be in part wavelength dependent or photochemical of origin, but it is now clear that it is a thermal rather than a photochemical effect. Many mechanisms of laser tissue welding have been proposed such as denaturation of structural proteins (Poppas, 1992; Dew, 1993; Tang, 1997b & 1998), dehydration of the proteins (Fenner, 1992a & 1992b), acceleration of natural fibrinogen polymerisation (Vale, 1986), collagen-to-collagen fusion (Godlewski, 1987; White, 1988), crosslinking of proteins (White, 1983), formation of noncovalent bonding between collagen (Bass, 1992), and interdigitation of collagen fibres (Schober, 1986). Our results in epineurium and dura mater revealed that collagen undergoes specific changes like swelling and reorganisation of the fibrils in one direction. Although the fusion area of the tissue specimens consisted of collagen-to-collagen bonding, the nature of these connections was not elucidated. In the soldered specimens, the coagulated solder acted as a dense homogeneous cuff in which the collagen fibrils are embedded. In other words, the solder behaved both as an internal and external glue.
During the histological examinations of the tissue, three important technical points became apparent. First, depending on the precision of tissue apposition, two different welds could be distinguished. When the tissue was very closely opposed, the welds consisted of collagen-to-collagen bonds. However, if the tissue apposition was poor, the welds were composed of coagulated red blood cells, fibrin plug, and cell debris. Second, in experiments investigating under- and overexposed specimens, significant differences were noted in the structure of the coagulated solder, which depended on the irradiance applied and the thickness of the solder. High radiation energies either vaporise the solder or, especially in combination with a thick layer of the solder, create air bubbles within the solder. Low radiation energies only coagulate the superficial solder layer, leaving deeper parts undisturbed. The thickness of the solder affects the quality of the coagulation process in the same way. Logically, such alterations do not contribute to optimal tensile strength and therefore optimal laser parameters must be used for a relatively homogeneous solder coagulation. The selected laser parameters of 100 mW with pulses of 1.0 s were found to be suitable for this purpose.

Concerning the mechanisms of laser tissue fusion, it seems likely that most of the observations reported in the literature are an expression of the same phenomenon, namely that tissue structure after laser welding is dramatically altered due to denaturation. Based on our observations and a review of the literature, we postulated that tissue welds result from a thermal degradation of tissue proteins which form new bondings after denaturation, in the same fashion as boiling of an egg. Both extracellular proteins such as collagen and intracellular proteins are involved in the welding process. It is logical that the new molecular bonds do not have the tensile strength of the original structure (GORISH, 1982).

In the wound healing investigations, several aspects of laser irradiation on intact peripheral nerves were addressed. An important question was whether milliwatt CO2 laser irradiation, eventually in combination with a solder, results in any unfavourable effects, particularly with respect to axonal regeneration, scar tissue formation, and loss of function. Therefore, laser irradiation was performed on intact peripheral nerves as transection itself would result in pathological changes not discernible from the laser effects. Acute histopathological changes consisted of a small zone of subepineurial damage to nerve fibres with oedema and vascular thrombosis. The acute alterations were followed by classical Wallerian degeneration in the same area of the nerve, while the central part of the nerve including the vascularisation was preserved. This degeneration had hardly any negative effect on the sensor and motor nerve function. In the extraneural parts of the nerve (epi- and perineurium), minimal inflammatory reaction was seen around the irradiated area which disappeared in several days. New collagen was formed and thereafter continued healing occurred which resulted in nearly complete repair of the epineurium. No extraneural scar formation, or adhesions were noted. This finding is important because such pathological changes may interfere with the physiological longitudinal sliding of the nerves during limb move-
ments (Hunter, 1991). Carbonisation of the tissue as a result of excessive laser irradiation should be avoided. In several studies, carbon particles within the tissue have shown to cause a chronic foreign body reaction that complicates wound healing (Fischer, 1985; Filmar, 1989a & 1989b). Using the laser parameters selected in our studies, no carbonisation was found.

As the CO\textsubscript{2} laser at higher powers has been experimentally used to transsect peripheral nerves in order to prevent traumatic neuroma formation by sealing the nerve fibres (Fischer, 1983; Hurst, 1984), concern raised whether the regenerative potential of peripheral nerves was not impaired after laser irradiation. This was not the case, as many regenerating myelinated and unmyelinated axons were observed across the irradiation site with subsequent maturation of the axons in time. At twelve weeks, the nerve morphology had returned almost to normal. Also when using protein solder, the wound healing proceeded favourably with no adverse effects on peripheral nerve regeneration or intra- or extraneural scar formation. Although the solder provoked an acute inflammatory reaction and some minor adhesions in the first two weeks, absorption of the solder was completed within one week with no residual reaction. Several studies have stressed the importance of morphological integrity of the perineurium in relation to uncomplicated nerve healing (Sunderland, 1965; Morris, 1972). In our opinion, one of the key factors leading to undisturbed wound healing in laser irradiated nerves is the relative preservation of the extracellular matrix components and the perineurium. By preserving this important layer, inflammatory and fibroblastic cell invasion into the intraneural parts, that can interfere with axonal growth, is prevented. The absence of clear macroscopic changes during laser irradiation does not mean that the nerve is not injured as some histological damage was found without macroscopic visual changes of the nerve. Vice versa, significant changes such as whitening of the nerve is not leading to extensive histological damage or severe functional deficit.

The next step of the investigations was to define and refine optimal LANR technique which would result in most favourable healing after nerve transsection. Regardless of any technique, the ideal surgical repair technique should be aimed to direct the nerve sprouts into their correct targets, thereby preserving the original microskeleton of the nerve as much as possible. Hereby, connective tissue proliferation at the repair site should be kept to a minimum by causing as little additional trauma to the nerve as possible. It was felt that two sutures placed equidistantly were essential to facilitate the initial coaptation and subsequent handling of the nerve during LANR. It is important to use sutures which cause the least tissue reaction in combination with LANR and therefore both absorbable (PGA) and nonabsorbable sutures (nylon) were included. In CO\textsubscript{2} laser irradiation of nylon sutures, significant decrease of the tensile strength was observed, even without distinct macroscopic changes of the suture material. The consequences of this may be early dehiscence of the nerves in the postoperative period and thus meticulous care should be taken not to irradiate the surgical thread during laser welding. As an alternative to nylon, we have also investigated stainless steel thread in
laser repaired nerves (with a diameter of 25 μm) for two reasons. First, studies in the 1960's showed that stainless steel sutures resulted in least foreign body reaction and scarring compared with other suture materials when implanted in peripheral nerves (EDSHAGE, 1964). Second, the tensile strength of the steel thread was not diminished after irradiation, regardless of the power density or pulse duration used. Histological examination of the nerves at one and six weeks after repair revealed that the CO2 laser repaired nerves were healed with less cellular response and less scar tissue at the repair site than conventionally sutured nerves. In the soldered nerves, an increased inflammatory reaction was seen around the solder at one week, but at six weeks a relatively well defined epi- and perineurium without excessive fibrosis was present. The alignment of axons and intraneural scar was most favourable in the soldered nerves and regeneration at six weeks was also most advanced in the soldered nerves. Cellular and fibroblastic reaction was found around all sutures, surprisingly including the stainless steel sutures. However, at six weeks the absorbable sutures had begun to dissolve and the tissue reaction was less than that of other sutures.

These data suggest that a combination of absorbable sutures and a solder may prove to be the most effective technique of LANR. Although some histological advantages were gained by using PGA sutures, it is still doubtful whether their use can be translated into better functional results. In addition, the results confirmed that the sutures must be placed with caution because they may shift towards the centre of the nerve during the healing process and that a considerable space is occupied by the fibroblastic reaction around the sutures.

Finally, LANR using a solder and absorbable sutures was compared to CMSR, the current standard technique of nerve repair, in a long term in vivo study. A second control group consisting of FGNR was also included as this technique has gained popularity in the last years. Both LANR with soldering and FGNR use an exogenous substance to seal the nerves together, and in both techniques manipulation of the nerve ends can be kept to a minimum. However, in LANR with soldering, the epineurium is fused together which seems to be essential to prevent axonal escape out of the nerve and growth of fibrous tissue into the nerve. In fibrin glued nerves, the fibrin complex is not a barrier for growing axons or connective tissue (ZENG, 1995; PALAZZI, 1995). Whether fibrin glue results in fibrosis is still a subject of investigation (HERTER, 1989), although our study does not support this.

LANR with soldering, FGNR, and CMSR resulted in good axonal regeneration. Technically, FGNR was technically easiest and fastest to perform. No differences in surgical time between LANR with soldering and CMSR were noted. The extra time spend on placing two to four additional sutures in the CMSR group corresponded with the time spend for the preparation and application of the solder. The soldering application costs only several seconds of time. Laser soldered nerves had somewhat better histological architecture at the repair site in regard to epi- and perineurial proliferation, intraneural scar tissue, and neural alignment. In spite of the more favourable
histology at the repair site of the LANR with soldering and the acquired experience in LANR, there were no significant differences in functional or morphometrical outcome of the three techniques. There was only a trend to more and thicker myelinated axons in the distal nerve segments of laser soldered nerves, followed by sutured and then by fibrin glued nerves. Each of three groups showed some degree of variation in histology, probably reflecting differences in the execution of the surgical method. Whether the lack of statistical significance can be attributed to the inherent superior nerve regeneration potential of rats remains unproved. Therefore, similar experiments performed in higher mammals may provide additional data regarding this point.

Future perspectives of LANR

Despite studies by us and others and despite theoretical advantages, LANR has not convincingly shown to be significantly inferior or superior to CMSR in animal models. An important question remains whether the laser should be used for human nerve repair. Unfortunately, the histological advantages observed at the repair site in rat do not result in better functional regeneration of the nerves which would be the most important benefit for the patient.

Availability and cost are the most important obstacles for the wide use of CO_2 lasers in nerve surgery. Most of the CO_2 lasers are not equipped with a stable milliwatt mode and/or a micromanipulator. Sutures on the other hand are cheap, reliable, and always readily available. From a technical point of view, elements of laser welding technique are unfamiliar to most surgeons involved in peripheral nerve repair. Therefore, acquisition of technical skills and handling of the laser is essential in a laboratory set up before proceeding to the clinic. For end-to-end repair of nerves in the extremities, placement of sutures seems to be mandatory to facilitate manipulation of the nerve during laser irradiation and to lower the risk of postoperative dehiscence of the nerves due to limb movements. However, to use the laser only to avoid placement of extra sutures is to our opinion not justified. Moreover, we noted that multifascicular repair, where more than four individual fascicles are coapted one by one, seems to be much easier performed with sutures. The individual alignment and coaptation is more accurately accomplished using sutures than by LANR. In contrary, bi- and monofascicular nerves are more easily coapted by LANR with soldering where the epi- and perineurium can be more easily fused.

Specific circumstances may justify using the laser for nerve repair. A non-tactile repair in areas of limited surgical access where suturing cannot be easily performed is possible because the CO_2 laser has the advantage of being controlled, focused, and activated with a micromanipulator attached to the surgical microscope. Such situations arise during skull base surgery or during repair of spinal nerve roots. This was clearly demonstrated by SEIFERT (1989 & 1990) who repaired the oculomotor nerve in cats and found that suture repair was not feasible because of the limited operating field. We con-
firmed this observation in a pilot study on the oculomotor nerve in rats (unpublished results). Clinically, laser-assisted cranial nerve repair has been performed in few cases by Powers (1994). An other advantage of using the CO₂ laser in these situations is the fact that the intracranial and intraspinal nerves do not possess a firm epi- and perineurium making them very vulnerable to damage induced by suture insertion (MENOVSKY, 1996B & 1998A). Also, the nerves are not subjected to such stretching forces like in the extremities and, therefore, stay sutures may be omitted without increasing the risk of dehiscence (unpublished observation). In such situations, both LANR and FGNR may be more suitable than suture repair.

Can some significant improvements in laser welding be expected in the near future? The end point in tissue welding is at this moment based on visual changes of the tissue and the surgeon must 'see and feel' whether the welding is completed. Several investigations are aimed at developing a temperature feedback system to control the temperature at the repair site, which should result in a more reproducible and reliable tissue welding (MENOVSKY, 1995E; BARAK, 1997; CELESIZ, 1997, POHL, 1998). By monitoring the surface temperature during the laser procedure, the optimal temperature range for tissue welding can be determined. Once the optimal range is known, which is believed to be between 70 °C and 90 °C, a computer-assisted feedback system can be employed to maintain the surface temperature within this range by altering the laser power output. Temperature measurements using infrared cameras, radiometers, and changes in reflectance have extensively been tested, but so far the limitation of these systems for nerve repair is the relatively large spatial resolution for a small spot size resulting in incorrect temperature measurements (TORRES, 1990; SHENFELD, 1994). In addition, the feedback system requires several technical adaptations to the laser set up which ultimately makes its use more cumbersome.

We do not expect that further refinements in the solder material will result in significant improvements. Modifications in concentration and viscosity of the solder might improve factors such as the tensile strength, but significant improvement of histological or functional regeneration of the nerves cannot be expected (LAUTO, 1997, 1998A & 1998B). Eventually, nerve growth factors may be mixed with the solder providing an extra stimulus for nerve regeneration, as has been shown by VANDERTOP (1994) using fibrin glue.

Probably, the best results of LANR are achieved when performed by someone with a longstanding experience with the laser system and the execution of the technique. In other words, the statement of TARLOV (1942A) on the different peripheral nerve repair techniques also holds true for LANR.