Laser-assisted nerve repair. An experimental study
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Since several years, there has been considerable interest in using lasers to repair peripheral nerves. Several studies attempted to define the role of the laser in nerve repair, producing both encouraging and conflicting results. The purpose of the studies reported in this thesis was to consequently develop, improve, and refine the CO$_2$ laser repair techniques in animals and to finally compare the technique with the established repair techniques.

After general introduction and outline in chapter I, the biology of nerve regeneration and current techniques of microsurgical suture nerve repair are reviewed in chapter II. In order to understand the need for reduction of sutures, the disadvantages of nerve repair by sutures are outlined, which include a foreign body reaction and connective tissue proliferation which is detrimental to nerve regeneration.

Chapter III summarises the nature of lasers, the types of interactions they produce with tissue, and their application in nerve repair. Possible mechanisms of tissue welding are presented, together with the benefits, limitations, and future implications of several techniques used in laser nerve repair. The current consensus of the reviewed literature is that although nerve welding has some advantages over standard suture nerve repair, such as less neuroma- and scar formation and shorter repair time, numerous factors which may lead to further improvements in the laser technique still need to be explored. A beginning of this exploration.

In chapter IV, optimal laser parameters to obtain the strongest bond between nerves are determined in an in vitro study using rabbit tibial nerves. Furthermore, several protein solders are examined for their ability to reinforce the repair site. Fifteen different combinations of laser power (50, 100, and 150 mW) and pulse duration (0.1, 0.5, 1.0, 2.0, and to 3.0 s) are used to repair the nerves using a spot size of 320 μm. Bonding strength measurements of LANR are compared to suture and fibrin glue nerve repair. In the LANR groups, the strongest welds (associated with whitening of tissue) are produced at 100 mW with pulses of 1.0 s and at 50 mW with pulses of 3.0 s. The use of a dried albumin solution as a protein solder at 100 mW with pulses of 1.0 s increases the bonding strength nine fold as compared to laser welding alone (bonding strength 21.0 ± 8.6 g and 2.4 ± 0.9 g, respectively). The use of albumin 20% solution and egg white, both at 50 mW with pulses of 3 s, results in a bonding strength of respectively 5.7 ± 2.1 g and 7.7 ± 2.4 g. Sutured nerves has the highest bonding strength. For consecutive studies, it is recommended to use laser parameters of 100 mW power with pulses of 1.0 s together with concentrated albumin solder for the optimal bonding strength.
The aim of chapter V is to elucidate the mechanism of CO₂ laser tissue welding in vitro of peripheral nerves, with emphasis on the alterations in tissue morphology and ultrastructure which occur during laser welding. Dura mater and the epineurium of tibial nerves of rabbits are welded with a CO₂ laser at 100 mW with pulses of 1.0 s, both with and without the additional use of a protein solder (egg white). Both tissues are investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). It appears that the tissues undergo specific alterations following laser welding. The collagen fibrils became swollen, densely packed, and fused together. When a protein solder is used, the coagulated solder forms a solid bridge between the tissue edges, which is melted on and between the collagen fibrils. In summary, three different types of welds can be distinguished: (i) welds in which collagen-to-collagen bonding occurs, (ii) welds in which tissue debris forms a coagulated mass between the tissue interfaces, and (iii) welds in which coagulated solder is melted on and between the collagen fibrils, forming a solid bridge between the tissue edges. These three different mechanisms have their own implications under in vivo conditions.

In chapter VI, the effects of CO₂ laser irradiation on intact rat sciatic nerves are investigated in vivo. In the first part of the study, 40 rat sciatic nerves are exposed to 12 different combinations of laser power (50, 100, and 150 mW) and pulse duration (0.5, 1.0, 2.0, and 3.0 s), normally used for CO₂ laser repair. The results are evaluated 24 h after surgery with functional toe-spreading test and light microscopy. Irradiations of 50 mW and 100 mW for up to 1.0 s exposure time per pulse results in almost no deficit in motor function, while 100 mW power with prolonged exposure times and 150 mW power results in significant decrease in motor function. Light microscopy shows significant focal injury to the epi- and perineurium and the subepineurial nerve fibres, proportional to the laser energy applied to the nerve, consisting of Wallerian degeneration and thrombosis of blood vessels. In conclusion, a power of 50-100 mW in combination with a pulse duration of 0.1-1.0 s produces no or minimal thermal damage with no or a negligible loss of motor function. Therefore, combinations of power and pulse duration above these thresholds are considered less suitable for CO₂ laser nerve repair. In the second part of this study, 48 rat sciatic nerves are irradiated with 100 mW for 1.0 s exposure time per pulse (both with and without a bovine albumin solder) and the effects on motor function and nerve morphology are studied up to 12 weeks after irradiation using toe-spreading test and light and transmission electron microscopy. A subperineurial degeneration of myelinated and unmyelinated axons is observed in the first two weeks after laser irradiation, while the central part of the nerve remains undamaged. The degeneration is followed by axonal regeneration with subsequent maturation of nerve fibres in time. No excessive intraneurial or extraneurial scarring was seen. In the soldered nerves, the solder elicits a inflammatory reaction upon the epineurium in the first week after irradiation. By week one, the solder is completely absorbed. After two weeks, the inflammatory reaction ceases and by week four no residual reaction is seen. In conclusion, CO₂ laser irradiation...
Summary

tion at 100 mW with pulses of 1.0 s has no long term negative effects on nerve function and morphology.

Chapter VII is designed to investigate peripheral nerve regeneration of sharply transected nerves, repaired with CO₂ laser welding in combination with three different suture materials and a bovine albumin protein solder as an adjunct to the welding process. Unilateral sciatic nerve repair was performed in 44 rats. In the laser group, the nerves are gently apposed and two stay sutures (10-0 nylon, 10-0 polyglycolic acid, or 25 μm stainless steel) are placed epi/perineurally. Thereafter, the repair site was fused at 100 mW with pulses of 1.0 s. In a subgroup of laser repair, albumen was used as a soldering agent to further reinforce the repair site. The control group consisted of nerves repaired by conventional microsurgical suture repair (CMSR) using four to six 10-0 nylon sutures. Evaluation is performed at one and six weeks after surgery and included light and transmission electron microscopy. Laser repair performed with a protein solder results in a good early peripheral nerve regeneration with an optimal alignment of nerve fibres and minimal connective tissue proliferation at the repair site. All three suture materials produce a foreign body reaction, the least severe with polyglycolic acid sutures. CMSR results in more pronounced foreign body granulomas at the repair site with more connective tissue proliferation and axonal misalignment. Furthermore, axonal regeneration in the distal nerve segment is better in the laser groups. Based on these results, laser soldering technique in combination of absorbable sutures has the potential of allowing healing to occur with the least foreign body reaction at the repair site.

In chapter VIII, the experimental work is concluded with a study that compares CO₂ laser assisted nerve repair with suture and fibrin glue repair. Unilateral sciatic nerve repair was performed in 24 rats. In the laser repair group, two 10-0 PGA absorbable sutures were used and welding was performed at 100 mW with pulses of 1.0 s with addition of a bovine albumin protein solder. The control groups consisted of fibrin glue nerve repair in combination with two 10-0 PGA sutures and conventional microsurgical suture repair using four to six peri/epineurial 10-0 PGA sutures. Evaluation was performed 16 weeks after surgery and included toe-spreading test, light microscopy, and morphometric assessment. The motor function of the nerves showed gradual improvement with time in all groups. At 16 weeks, the motor function was about 60% of the normal function, while no significant differences existed between the groups. Histologically, all nerves reveal various degrees of axonal regeneration with myelinated nerve fibres in the distal nerve segments. Slight differences in favour of the laser group exist in terms of wound healing at the repair site. In all groups, the number of axons distal to the repair site is higher compared to proximal, but axon diameter was significantly less than that of control nerves (p<0.05). No significant differences existed between the number, density, or diameter of the axons in the proximal or distal nerve segments of the three groups of nerve repair (p<0.05), although there was a trend to more and thicker myelinated axons in the distal segments of laser repaired nerves. It is concluded that CO₂ laser
soldering technique for peripheral nerve repair is at least equal to fibrin glue and suture repair in a rodent model of sciatic nerve repair.

Because no data were available on the effect of CO$_2$ laser irradiation on microsurgical suture material, chapter IX is designed to investigate the tensile strength of different suture materials. This study is performed before the work presented in chapter VIII, and is focused on a comparison of a tensile strength of 10-0 nylon and 25 µm stainless steel thread irradiated by a CO$_2$ laser. The suture threads are exposed to 12 combinations of power densities and pulse durations and tested on a tensometer for its tensile strength. At powers densities of 186 W/cm$^2$, the 10-0 nylon thread disrupts during laser irradiation, regardless of the pulse duration. This was also the case at a power densities of 124 W/cm$^2$ for 2.0 and 3.0 s pulse durations. At 124 W/cm$^2$ for 0.5 and 1.0 s, the tensile strength decreases with 70% relative to the control. At 62 W/cm$^2$, the tensile strength gradually decreased from 100% (0.5 s pulse duration) to 50% (3.0 s pulse duration) relative to the control. Stainless steel thread is resistant to all laser irradiation’s. The 10-0 nylon thread is significantly compromised by irradiation with the CO$_2$ laser and therefore irradiation of the sutures should be avoided during laser tissue welding, as was done in all previous chapters.

Discussion and conclusions of this thesis and future perspectives of LANR are provided in chapter X. Despite studies by us and others, laser repair has not convincingly shown to be significant inferior or superior to microsurgical suture repair. Laser repair seems a worthwhile alternative to sutures, but the histological advantages at the repair site do not result in better functional regeneration of the nerves which would be the most important clinical improvement the patient benefits from. The availability and financial cost are the strongest obstacles for the wide use of lasers in nerve surgery, while sutures on the contrary are cheap, reliable, and always readily available. In the future, use of the CO$_2$ laser may be justified for human nerve repair during skull base surgery or during repair of spinal nerve roots, situations in which limited surgical access makes suturing difficult. In these cases, the CO$_2$ laser permits a non-tactile repair of the fragile and vulnerable nerves.