Repair of PIG DURA In Vivo Using Temperature Controlled CO₂ Laser Soldering

Boaz Forer, MD,1 Tamar Vasilyev, MSc,2 Tamar Brosh, PhD,3 Noam Kariv, DMV,4 Ziv Gil, MD, PhD,1 Dan M. Fliss, MD,1,4 and Abraham Katzir, PhD2*

1Department of Otolaryngology Head and Neck Surgery, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel
2Goldschlager School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel
3School of Physics and Astronomy, Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, Israel
4Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background and Objectives: The purpose of this study was to demonstrate that laser soldering might be successfully used for closing holes or cuts in the dura layer, which encapsulates the brain.

Study Design/Materials and Methods: A temperature controlled fiberoptic CO₂ laser system and albumin solder were used for spot soldering of fascia patches to holes in the dura of farm pigs, in vitro and in vivo.

Results: The mean burst pressure of the soldered patches in the in vitro experiments was 190 ± 88 mm Hg—significantly higher than typical maximum CSF pressure of 15 mm Hg. In the in vivo experiments the pigs showed no postoperative complications. Histopathological studies exhibited an accepted level of inflammatory reaction and showed no thermal damage to the underlying brain tissue.

Conclusions: It has been clearly demonstrated that temperature controlled laser soldering is a very useful technique for the repair of the dura. It provides significant advantages over standard closure techniques: it is easy to apply, the bond is strong and watertight and the procedure is likely to be much faster than suturing. This research work will lead to clinical trials. Lasers Surg. Med. 00:1–7, 2005. © 2005 Wiley-Liss, Inc.

Key words: albumin solder; burst pressure; dural closure; infrared fibers; fascia patch; laser welding

INTRODUCTION

The dura is a structure that encases, protects, and maintains the sterility of the central nervous system. This vital structure could be damaged by many events, such as trauma, tumor in-growth, and surgical procedures [1]. Extirpation of large intracranial tumors frequently requires excision of the dura overlaying the tumor. In such cases, primary closure of the dura is inadequate, and the dural reconstruction requires placement of a fascial patch over the excised area, in order to form a watertight seal. In skull base procedures, the aim of reconstruction is to provide a barrier between the contaminated nasosinusoidal space and the sterile subdural compartment, thus preventing leakage of cerebrospinal fluid (CSF) from the intracranial space, or airflow into this space. In order to avoid these complications, the dura must be closed quickly, so that it would support the instantaneous CSF pressure and withstand considerable shearing forces during the postoperative period.

Conventional repair of large dural defects utilizes fascial grafts [2–5], which are sutured to the edges of the intact dura. The suture repair, however, is not watertight, and CSF leakage through gaps between the sutures and through the needle holes is not uncommon. Moreover, the reconstructed dura has an increased risk of tears and breaks during the immediate postoperative period, especially in elderly patients or in patients who underwent radiotherapy.

Surgical cuts may be bonded if heated by a laser beam [6]. Laser welding is based on heating of the approximated edges of an incision. Laser soldering is based on applying some soldering material (such as albumin) onto the approximated edges of the cut and heating the solder (and the underlying tissues) by a laser beam. Both laser welding and laser soldering are inherently sterile and non-tactile techniques, which do not involve a foreign body (i.e., sutures, clips, staples, or synthetic glues). Both procedures offer, in principle, many advantages, with respect to standard techniques: (1) a watertight bond; (2) a faster wound healing process; (3) potentially little scar formation; and (4) a procedure that is both faster to apply and easier to master. Researchers have studied laser bonding of numerous types of tissues for many years, but the method has not yet been widely accepted by surgeons [7].

Several earlier studies had discussed the use of laser bonding for primary dural closure. Foyt et al. [8] published a report on the use of a diode laser with albumin+ indocyanine green (ICG) solder for the closure of dural cuts. Desiccation of brain tissue was observed in this report, possibly because no temperature control was used, and because of the deep penetration of diode laser radiation into the brain tissue. Hadley et al. [9] reported on their laser...
welding experiments on the dura, but they did not obtain complete laser dural closure. As a result, CSF leakage was observed at the time of the operation. In other words, an immediate watertight seal had not been achieved. Menovski et al. [10] used a relatively low energy CO₂ laser and applied egg white as a solder. They were mostly interested in understanding the mechanism of the structural changes in the irradiated dura matter and the role of the solder in the process. These three studies did not prove that laser soldering had advantages over the standard dura closure methods.

Researchers associated with the Applied Physics Group at Tel Aviv University hypothesized that the crucial issue is to carry out laser bonding under temperature control, thus preventing overheating of the bonded tissues and the underlying layers. Extensive theoretical [11] and experimental [12–16] research work on laser soldering of many types of tissues, in vitro and in vivo, has already been done. In particular, the laser soldering of skin cuts in small (e.g., rats) and large (e.g., pigs) animals, using albumin as a solder, was studied very carefully. In these experiments it was clearly proved that laser soldering was a fast and reliable procedure, which generated strong bonding. The procedure provided a watertight seal, there were less inflammatory reactions, a better wound healing was observed, and there was very little scarring. Based on this work, it was thought that it would be possible to bond cuts and close holes in the dura, generating a watertight seal, without causing thermal damage to the underlying brain tissue.

The novel idea in this work was to place a segment of fascia on a hole (or a cut) in the dura, and to use laser soldering for bonding it to the dura matter. The animal model selected for this study was the farm pig—a model that has not yet been used for dura soldering. In this paper we discuss the in vitro and the in vivo experiments, which may eventually lead to the use of this technique on human patients.

MATERIALS AND METHODS

Laser Soldering System

The Applied Physics Group has developed, over the years, a fiberoptic CO₂ laser system for temperature controlled soldering of tissues. The basic idea was to apply a biological solder on the approximated edges of a cut and to use a CO₂ laser beam to bond the fascial patch to the dura at a controlled solder/tissue interface temperature of \(T \approx 65 \pm 3\) °C. The CO₂ laser was chosen, because its radiation is highly absorbed by the solder, and it has a shallow penetration depth in tissue [10,17,18].

The laser soldering system was fully described in our previous publications [12–17]. In brief, the system is based on a CO₂ laser, emitting at \(\lambda = 10.6 \mu m\), an infrared (IR) detector, two IR transmitting fibers, and a personal computer (PC). Fibers of diameter 0.7 mm and lengths of roughly 2 m were prepared in our laboratory from silver halide crystals of composition AgCl₄Br₁₋₅ (0 < x < 1). These fibers are highly transparent in the IR, flexible, they are non-toxic and biocompatible, and they are well suited for laser soldering [19]. Two fibers were placed in a flexible tube, so that the distal ends of the fibers were placed a few millimeters from the surface of the dura. The proximal end of the power fiber was held by a fixed holder, and the CO₂ laser radiation was focused into the end of this fiber. The laser beam emitted from the distal end of the fiber heated a spot of 2–3 mm in diameter on the surface of the dura. The heated spot emitted infrared radiation whose intensity \(I\) was determined by the temperature \(T\). The emitted IR radiation was collected by the distal end of the second IR fiber (“sensor” fiber). This radiation was transmitted to the proximal end of this fiber and then focused onto an IR detector. The PC measured the voltage signal \(V\) from the detector and used \(V\) to determine the temperature of the heated spot. A computer program was used for feedback control, so that if the temperature \(T\) was too low—the laser power increased and vice versa [20]. We have already used such a system for heating spots on various tissues to some preset temperature (e.g., 65 °C) with accuracy of few degrees centigrade [12–15].

In this work a sealed-off CO₂ laser (Sharplan 40C, Lumenis, Yokneam, Israel) was used, which operated in a DC mode. The output beam was modulated by a chopper, which generated pulses at a rate of 5 Hz. The duty cycle of the chopper was controlled by the PC, and this made it possible to control the average power, which was focused into the “power” fiber. The average power measured at the distal end of the “power” fiber was 0.7 W and the average power density at the irradiated surface was roughly 3 W/ cm².

Using the thermometer, based on the “sensor” fiber, we were able to measure the surface temperature of a heated spot with an accuracy of 0.1 °C and with a high spatial resolution.

In this experiment an aqueous 47% bovine albumin (Sigma Chemical Co., St. Louis, MO) served as a solder, which was spread on the edges of a cut or a hole. The solder layer was heated by the laser soldering system, and heat propagation into tissue was responsible for the heating of the underlying layers [21]. Since the albumin layer was very thin, the temperature gradient across it was low (probably around 5 °C). Therefore the surface temperature sampled by the IR fiber was approximately equal to the temperature of the albumin–tissue interface.

Laser Soldering of Fascia to Dura—In Vitro Studies on Pig Corpses

For the in vitro experiments we used juvenile farm pigs, each weighing 7–10.5 kg, which died of natural causes in the farm (The Institute of Animal Research, Kibbutz Lahav, Israel). The corpses were collected a short time after death and cooled to a temperature of 4 °C until the surgical procedure. A circular incision around the superior aspect of the skull was made and the skin and pericranium of the superior skull was excised. A wide craniotomy of the superior skull was performed. The dura was kept intact and separated from the cranium (Fig. 1). A circular 8 mm wide dural patch overlying the parietal lobe was excised, thus
forming a hole. The excised dura was positioned about 10 mm off the midline (which contains the superior sagittal sinus) and midway between the anterior and posterior ends of the brain, thus the dura was excised in the same place in all animals. The fascia covering the longissimus et lumborum muscle on the back of the animal was excised through a skin incision. A circular fascial patch of diameter 15 mm was made and inserted between the dura and pia matter (Fig. 2—the fascia is shown partially inserted). As a result, an overlap area of dura and fascia was created, measuring about 3 mm. The albumin solder was applied over the dura—fascia junction line and heated to 65 ± 3°C. Each spot along the line was irradiated for approximately 3–6 seconds. These values of temperature and time had already been studied in other tissues and they were found to provide optimal strength, without causing thermal damage [14]. The bonding technique used was end-to-side soldering, which was performed using an operating microscope (Zeiss, West Germany). A second layer of albumin was then applied and a second soldering procedure was performed in order to ensure a watertight seal. After heating, the tissue was allowed to cool back to room temperature for 5 minutes. A wide dural patch of diameter larger than 5 cm was then excised, which contained in its middle the soldered area. The dura was meticulously separated from the arachnoid and immediately taken for burst pressure measurement, using the system mentioned in the next section.

**Laser Soldering of Fascia to Dura—Burst Pressure Measurements on Excised Tissues**

A cylindrical metal container, shaped like a drinking glass, was specially designed and constructed for measuring the burst pressure of the bonded fascia (Fig. 3). The dura and bonded fascia were attached to the rim of the container, using sealing rings. The container was filled with water, and then connected to a mechanical system that gradually increased the water pressure in the container (Instron, Model 4502, Buckingamshire, UK). The burst pressure of the laser-soldered fascia was defined as the point at which water started leaking out of the dura/fascia interface.

**Laser Soldering of Fascia to Dura—In Vivo Studies on Farm Pigs**

Following the completion of the in vitro studies, we started the in vivo experiments. All procedures were in
accordance to protocols approved by the Animal Care and Use Committee of Tel Aviv University. Five female farm pigs (from the Institute of Animal Research, Kibbutz Lahav, Israel), each weighting 12 kg, served as animal models in this experiment. Rocephin (Ceftriaxone 50 mg/kg) antibiotic treatment was given perioperatively for 2 days (50 mg/kg once a day). Pre-medication of Ketamin (10 mg/kg, Fort Dodge, Iowa) and Xylazine (4 mg/kg) was administered intramuscularly 10 minutes before the general anesthesia. The anesthesia included I.V. injection of Nembutal (Pentobarbital 30 mg/kg). The animal cranium and back were scrubbed and covered with sterile drapes. Sterilized equipment was used for the surgical procedure. A longitudinal paramedian incision was made at the superior aspect of the skull. The pericranium underlying the incision was then cut. The skin and pericranium were retracted sideways. A small craniotomy of the superior skull over the parietal lobe was performed. The dura was kept intact and separated from the cranium. A circular 8 mm wide dural patch overlying the parietal lobe was excised, as was done in the in vitro model. The fascia harvesting and placement and the laser soldering procedure were done exactly as was described for the in vitro model (Fig. 4—the reconstructed dura is shown after laser soldering). Again, the soldering was repeated twice, to form a “two-layer” soldered area. After the procedure, the soldered area was examined for CSF leak using the operating microscope. The excised craniotomy bone was put back in place. Both back and skull skin incisions were closed, using Vicryl 3-0 sutures for the subcutaneous tissue and Nylon 3-0 sutures for the skin. The animal was kept for observation in the Institute of Animal Research for 10 days. During this period it was observed daily for behavior, food intake, and neurological complications. The surgical wound was checked daily for signs of infection or fluid collection. At the end of the observation period each animal was weighed and a second anesthesia was performed, exactly as described above. The surgical cranial wound was opened, preserving the soldered area. This area was observed under the microscope for regions of mal-union of the dura to the fascia and for CSF leak. Then, the craniotomy was enlarged and a wide dural patch, including the intact underlying brain tissue, was excised, so that the specimen contained the soldered area in the middle. After excision, the animal was sacrificed.

The fixation of the surgical specimen was done using 4% formaldehyde solution. The specimen was then embedded in paraffin, and serial sections, 2 mm wide, were made and microscopically examined by a pathologist. The fascia, dura, and underlying brain tissue were examined for coagulative necrosis, presence of inflammation, and thermal damage.

RESULTS

In Vitro Animal Model

Burst pressure measurements. In the preliminary set of measurements on excised tissues, we proved that laser soldering provided a useful means for bonding fascia to dura. We also tested the burst pressure system and proved that it provided reliable results. Burst pressure measurements were then carried out in vitro, on pig corpses. The burst pressure values and the animal weights are presented in Table 1. The mean burst pressure for this group was 194 mm Hg (range 78–359 mm Hg) and the standard deviation was 88 mm Hg.

<table>
<thead>
<tr>
<th>Animal serial no.</th>
<th>Pig weight (kg)</th>
<th>Burst pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>144</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>124</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>10.5</td>
<td>283</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>194</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>203</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>92</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>218</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>245</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>359</td>
</tr>
</tbody>
</table>

In Vivo Animal Model

Histology. Granulation tissue was present between the soldered area and the calvarium, representing a superficial inflammatory process. There were no signs of CSF leakage.
from the soldered area and no macroscopic signs of necrosis.
The bonding of the fascia to the dura was strong and the tissues could not be separated by manipulation of the soldered site.

Histological analysis (Fig. 5) showed plump fibroblasts infiltration of the longissimus et lumborum fascia, with no signs of necrosis. Small foci of calcium deposits as well as lymphocyte infiltrate of the fascia were also seen. The outer surface of the fascia and the dura—fascia junction contained foreign body reaction giant cells and inflammatory infiltrate. Small bone fragments from the previous craniotomy drilling were also seen in this area. The dura—fascia interface was sealed. The dura contained mild lymphocytic infiltrate. The arachnoid and pia were intact, with no signs of damage. The brain tissue (B) had a normal appearance, with no signs of bleeding or destruction. The neurons and glial cells had a normal histological appearance.

from the soldered area and no macroscopic signs of necrosis. The bonding of the fascia to the dura was strong and the tissues could not be separated by manipulation of the soldered site.

DISCUSSION

Dural reconstruction after craniotomy for trauma or tumor resection has been traditionally performed using sutures and synthetic materials. The use of these materials carries the risk of local inflammation and CSF leak. Laser soldering offers an alternative method, which could generate a watertight seal and a good tensile strength, as well as minimal inflammation during the healing process.

Foyt et al. [8] used laser soldering for the reconstruction of dura. In that study the authors used a diode laser heating system and albumin + ICG solder to perform end-to-end bonding of dural cuts in Lewis rats (in vivo). These authors found that dura soldering was superior to conventional suturing, giving a mean leak pressure of 26 mm Hg for soldering and 9.4 mm Hg for suturing. However, there were some inherent drawbacks in this method: (1) the laser radiation may have penetrated deep into the tissue layers and caused thermal damage to brain tissue and (2) the lack of temperature control might have enhanced tissue damage. Hadley et al. [9] used a canine model for investigating the use of CO$_2$ laser welding and fascial patches for dural closure. These researchers observed CSF leakage immediately after welding. They claimed that fibrin glue was superior to laser bonding or to standard suturing, and that it supported more than 40 mm Hg of CSF pressure, immediately after the reconstruction. As mentioned, both of these studies did not show that laser soldering offered advantages over the standard techniques used for dural reconstruction.

In the past we carried out a large number of laser soldering experiments, using the temperature controlled laser-soldering system, which was discussed above. In all these experiments we used a CO$_2$ laser as the heating source. The reason was that the radiation of this laser was highly absorbed in water, and was therefore highly absorbed both by soft tissues and by albumin. In the laser soldering experiments, the laser energy heated only the top layer (roughly 20 µm) of the albumin layer whose thickness was roughly 200–300 µ and the underlying tissue layers were heated by conduction. This procedure highly reduced the risk of thermal damage to the underlying tissues [18,22]. In all these experiments we applied the laser soldering technique for bonding cuts in tissues. We always approximated the edges of the cuts, applied an albumin layer and used the laser to heat a spot on the albumin.

In this work we tried to modify the technique, and bonded a patch of fascia to the dura, in order to close a dural hole. The reconstruction of excised dura, using laser bonding of fascia patch, has not been reported previously. One of the goals of our study was to find a suitable animal model for the dural reconstruction. The selection of the pig as a model was based on two reasons: (1) the pig’s brain is relatively big, so that a large piece of dura can be used for the soldering experiments (in vitro and in vivo); (2) the dura and the fascia of the pig are relatively thick (in comparison to those in small animals) and easier to work with. In our preliminary experiments we decided to excise a dural patch, thus forming a hole, and then to excise a patch of tissue.
whose size was larger than that of the hole and bond this patch to the dura. The process of tissue soldering is not fully understood, although the protein denaturation–renaturation process seems to be the mechanism [10, 23]. The use of protein-rich tissue as a patch seems to be a natural choice for laser soldering. Unfortunately no previous reports described the denaturation of dura in pigs. A temperature of 65°C was used since a major protein component of both the dura and the fascia is collagen which is denatured at this temperature. This proved to be very successful, and the patch closed the hole well, forming a watertight seal, and in some cases the patch was able to withstand pressures higher than 350 mm Hg. It should be mentioned that even the lowest value reported here (78 mm Hg) was sufficient for all practical applications. Soldering was performed in a double layer manner. It was assumed that the potentially weak points of the first layer are reinforced by the second layer, thus creating maximum bonding strength.

The standard deviation was high (88 mm Hg). The reason for this deviation was that the pressure measurement was dependent on the weakest point of the soldered line. Although the bonding was generally strong, there may have been areas along the soldered line that had been bonded under less than optimal soldering conditions. These weaker areas may have determined the burst pressure, so that one small area of weak bond may have significantly altered the results. Collagen fibers are oriented in domains with strong directionality, but it is not likely that this influenced the bonding strength, because a standard hole in a specific area of the dura was created in all pigs, both in vitro and in vivo. Although not uniform, the bonding was far stronger than needed for withstanding the intracranial pressure and therefore the laser soldering technique proved to be an effective technique for dural reconstruction. The in vitro experiments paved the way for the in vivo experiments on farm pigs in which the preliminary results showed a watertight seal with no injury to the brain tissue. The animals were observed for 10 days and no complications were noted. The histological report showed bonding of the fascia to the dura without necrosis or excessive inflammation. The inflammatory reaction observed on the outer surface of the fascia and the dura-fascia junction line was probably a reaction to the albumin solder. This material was recognized as a foreign body by the pig’s immune system and therefore produced a foreign body response. Moreover, the tertiary configuration of the albumin after the denaturation–renaturation process during soldering was significantly changed. This caused the “new” albumin to be even more stimulating to the immune system. The pia matter and the underlying brain tissue were intact and showed no signs of thermal damage. The neurons which are highly sensitive to damage and which do not regenerate if severed, appeared normal. No glial cell changes were observed. This normal appearance of the brain tissue supports the assumption that the use of the temperature controlled laser system was safe and that the CO₂ laser energy did not penetrate structures beyond the intended soldered area.

Our preliminary results demonstrated that laser tissue soldering has a good potential to replace conventional (suturing or adhesion) techniques in dural repair surgery, because it is a non-contact method that involves no foreign body and because it forms a very strong and watertight closure. Further in vivo studies are needed in order to investigate the brain tissue and the dural reaction to laser soldering.

CONCLUSIONS

We have developed a novel technique for dural reconstruction, using a temperature controlled fiberoptic laser soldering system. This technique involves the soldering of a fascia patch to dura, and it would be useful for closing cuts or holes in the dura. During the fascia bonding, the surface temperature was precisely controlled by the system, thus producing both optimal temperature for soldering and protection from thermal damage. A series of measurements was first carried out in vitro and it was found that the immediate strength was high. The mean burst pressure was roughly 190 mm Hg, in comparison to the maximal pressure of CSF liquid in the brain, which is 15 mm Hg. A series of experiments on pig corpses clearly demonstrated that the method was very suitable for dural reconstruction. Long-term in vivo experiments were then successfully carried out on several farm pigs. The animals were observed for a period of 10 days and no complications were noted. The histopathological results did not show any sign of thermal damage to the soldered tissue or to the underlying brain.

In this work we demonstrated the real potential of the temperature controlled CO₂ laser system in dural surgery. The laser tissue soldering procedure promises to be a safe and reliable technique, which may find numerous applications in neurosurgery and in many other fields of surgery.

ACKNOWLEDGMENTS

This work was partially supported by the Israeli Cancer Foundation. The authors thank Prof. Jose J. Bubis, MD, of the Department of Pathology, School of Medicine, Tel Aviv University, for his invaluable advice and his help with the analysis of the results.

REFERENCES


