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Original Investigation

CO_2 Laser Soldering for the Reconstruction of Dural Defects in the Minipig Model

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ABSTRACT

AIM: To explore the feasibility and reliability of CO_2 laser soldering on the reconstruction of dura mater in the minipig model.

MATERIAL and METHODS: Ten minipigs were divided into 2 groups as Group A (n=5) and Group B (n=5). Bilateral fronto-parietal craniotomy was performed and 2cm×1cm dural defect created under general anesthesia. Then, the defect was repaired with autologous temporalis fascia by CO_2 laser soldering. After pressure and watertightness testing, the minipigs of group A were sacrificed immediately. Minipigs in Group B were followed for 4 weeks, with daily monitoring of behavior, food intake, skin incision and neurological condition. Animals of Group B were also subjected to the same tests as group A. Then, they were also sacrificed. The reconstructed area and underlying brain tissue were fixed in paraformaldehyde and submitted for histological analysis.

RESULTS: No neural impairment, hydrops or empyema, and no cerebrospinal fluid leak in the dura-fascia interface were observed in Group B. The mean burst pressures were higher than the mean intracranial crest pressure in groups A and B. This difference was significant (P=0.010, P=0.000, respectively). The physiological intracranial pressure of ten minipigs ranged between 4.53 and 6.47 mmHg. No thermal injury was observed in either group.

CONCLUSION: CO_2 laser soldering for dural defect reconstruction was feasible and reliable.

KEYWORDS: CO_2 lasers, Dura mater, Repair, Intracranial pressure, Minipig

INTRODUCTION

In 1960, the first laser device was designed by Maiman in the USA. Since that time, many different types of laser have been developed and used among the medical specialties such as ophthalmology, dermatology, gynecology, oncology and surgery (2,14,16,17,19,24,26). The radiation of the CO_2 laser is highly absorbed in water and it has a shallow penetration depth (<20μm) (7,8). The heat is propagated into the tissues by conduction (8). It therefore does not cause any secondary damage to the surrounding tissue. In time, the CO_2 laser was liked and used by more physicians and clinics.

Dural defects may be secondary to trauma, tumor erosion, surgical procedures, inflammatory destruction and some congenital diseases. It is a common clinical phenomenon, but successful repair of the defect is always a difficulty for neurosurgeons. The current closure techniques cannot be fully satisfactory (8,10,12,22,27). However, soldering with CO_2 laser has significant advantages and this technique is an alternative method for the reconstruction of dural defects (7). There are many studies on dural repair with the CO_2 laser, but the survival time of the experimental animals was not longer than 10 days in these studies.

The aim of this study was to create a minipig model to explore the feasibility and reliability of the CO_2 laser system for the reconstruction of dura mater in the early postoperative and late follow-up periods.

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MATERIAL and METHODS

The animal research committee of our institution approved the experimental protocol. The care and use of the animals in this study were performed according to the Helsinki Convention.

Ten mature minipigs (aged 6-8 months, 6 males and 4 females) were obtained from Kexing Breed Centre (Beijing, China). The mean weight was 19.46 ± 1.70 kg (ranged between 17.30 and 22.10 kg). They were randomly divided into 2 groups as Group A (5-minute group, n=5) and Group B (4-week group, n=5).

All animals fasted for 12 hours before operation. All experiments were performed under general anesthesia induced with ketamine (20 mg/kg, Gutian Pharmaceutical Co., China), midazolam (0.5 mg/kg, Nhwa pharmaceutical Co., China) and atropine (0.25 mg/kg, Kingyork pharmaceutical Co., China) intramuscular injection. The anesthesia was maintained with isoflurane (2%, Kingyork pharmaceutical Co., China). All animals received prophylactic intramuscular Ceftriaxone (25 mg/kg, Roche, Shanghai, China) and prophylactic dexamethasone (5 mg, Kingyork pharmaceutical Co., China) 30 minutes before skin incision to minimize bacterial contamination and brain swelling, respectively.

A horseshoe-shaped anteriorly based scalp flap and separate pericranial layer were raised in succession. Temporalis muscle fascia (1.5×2.5 cm) was harvested and stored as a graft in physiological saline, with the inner muscular side marked by a stitch. Bilateral fronto-parietal craniotomy was performed. A dural defect (2×1 cm) was created in the frontal area. Then, we cut the arachnoid membrane by a micro-scissors under microscope magnification. After a cerebrospinal fluid (CSF) leakage was confirmed, the temporalis fascia was then used to patch the defect. It was inserted under dura, with the marking stitch side faced to the dura.

An overlap area of dura and fascia was created as the fascia patch was larger than the defect of the dura mater (8). The fibrin glue was applied over the dura-fascia junction and heated by CO_2 laser in a serrated form (2.25w,3s/cm). After this procedure, the bone was inserted on the cranium defect and the pericranium, galea aponeurotica, subcutaneous tissue and skin were closed (8). After the measurements of physiological intracranial pressure (ICP), intracranial crest pressure and burst pressure, the soldered area with underlying brain tissue was resected en bloc. Minipigs of group A were sacrificed 5 minutes after the dural reconstruction. In group B, the animals were observed for 4 weeks, with daily monitoring of behavior, food intake, skin incision and neurological condition. The improved Tarlov scoring system was used to assess the neurological condition (8,11). At the end of 4-week follow-up period, animals in Group B were re-anesthetized and subjected to craniotomy. The same tests as group A were performed, and then they were sacrified.

An ICP monitoring probe (Parenchymal ICP/TEMP Sonde, RAUMEDIC, Germany) was inserted into the contra-lateral lateral ventricle of the repaired side and it was used for the records of physiological ICP when the value was stable. Then, we increased the airway pressure using the anaesthesia machine for 40 seconds, and the apex of the ICP value was recorded as intracranial crest pressure. Under a microscope's magnification, a syringe needle was inserted into the subdural space. 0.1% gentian violet solution was injected through the needle and the CSF pressure was raised to 10 mmHg by this injection. This pressure was maintained for one minute to verify the watertight seal of the reconstructed dura mater. Then, we continued to inject 0.1% gentian violet solution and the pressure was recorded as the burst pressure when the CSF leak was observed at the dura-fascia junction line.

After burst pressure measurement, the reconstructed area with underlying brain tissue was resected en bloc and fixed in 4% paraformaldehyde. Then, it was submitted for histological analysis. Specimens were processed and embedded in paraffin blocks. Five μm sections from each block were stained with hematoxylin-eosin and immunohistochemical staining was also performed for a random analysis.

Statistical comparisons were performed using SPSS (v 17.0; SPSS Inc, Chicago, IL). Measurement data was listed as means ± standard deviation. Paired sample T test was used to compare physiological ICP with intracranial crest pressure or burst pressure ($p< 0.05$ for significance).

RESULTS

The standard surgical procedure was performed for all animals. The mean duration of the surgery was 3.20 ± 1.03 hours (range 1.5 to 4.5 hours). In group B, all animals recovered very soon. No neural impairment was detected in the follow-up period. Four weeks later, the craniotomy was re-performed. All the incisions were healed well and no hydrops or empyema was found in any of the five animals. The soldered area was seen to be covered by a very thin layer of proliferous connective tissue. The dura-fascia junction was healed and no CSF leak was detected under the microscope. It was difficult to separate the soldered area by manipulation. The appearance of the soldered area after burst pressure testing is presented in Figure 1A, B. The mean physiological intracranial pressure, intracranial crest pressure and burst pressure for dural reconstruction with laser soldering are listed in Table I.

The mean physiological ICP for 10 minipigs was 5.5±1.35 mmHg, and its 95% confidence interval ranged 4.53 to 6.47 mmHg. And the mean intracranial crest pressure was 8.90±1.20 mmHg, 95% confidence interval ranged 8.04 to 9.77 mmHg. Histological observation showed fibroblast infiltration of dura-fascia bonding, with no signs of necrosis in Group B. The two tissues have been found adherent after CO_2 laser soldering, and partly cross-linking in the specimens of Group A (Figure 2A, B). The dura-fascia interface was healed without architectural changes or signs of damage.

The arachnoid and pia mater were intact with normal features. The appearance of the brain tissue was normal without any sign of thermal damage, bleeding or liquefaction (Figure 3A, B). The glial cells and neurons showed normal histological characteristics, and the expression of glial fibrillary acidic protein (GFAP) was not increased when compared with the normal brain tissue (Figure 4A-C).

DISCUSSION

The dura mater is a vital structure for the central nervous system (CNS). It protects the CNS and maintains its sterility. The dura mater may be damaged by trauma, tumor or surgical procedures. When the dura is damaged for any reason, dural repair is necessary in order to protect the CNS from the contamination and infection.

Unfortunately, primary dural closure could not be adequately performed in some cases, and dural reconstruction is required with a patch over the dural defect in a watertight fashion. Various dural substitutes have been used over the years (15). Fascia is a natural material for dural reconstruction. It is rich in collagen and can produce adequate strength (8). This is also in accordance with our earlier work on the selection of optimal material from temporal fascia, fascia lata, periosteum, muscle and small intestinal submucosa, and the temporal fascia was deemed the best choice. In addition, we can get enough temporal fascia of both sides directly from the only incision. This is the reason why autografts are more commonly used (13,15,28,32).

Table I: Pressure Testing Results (Mean ± SD in mmHg) in Group A and B

Group	MPP	MCP	MBP
A	5.80 ± 1.30	8.40 ± 1.14**	25.20 ± 9.58*
B	5.20 ± 1.48	9.40 ± 1.14**	249.40 ± 23.36**

MPP: *Mean physiological intracranial pressure,* ***MCP:*** *Mean intracranial crest pressure,* ***MBP:*** *Mean burst pressure.*
*The MCPs were significantly higher than MPPs in Group A and B (P<0.01, marked with **). The MBPs were significantly higher than MCPs in Group A and B (P<0.05, marked with * and P<0.01, marked with ** respectively).*

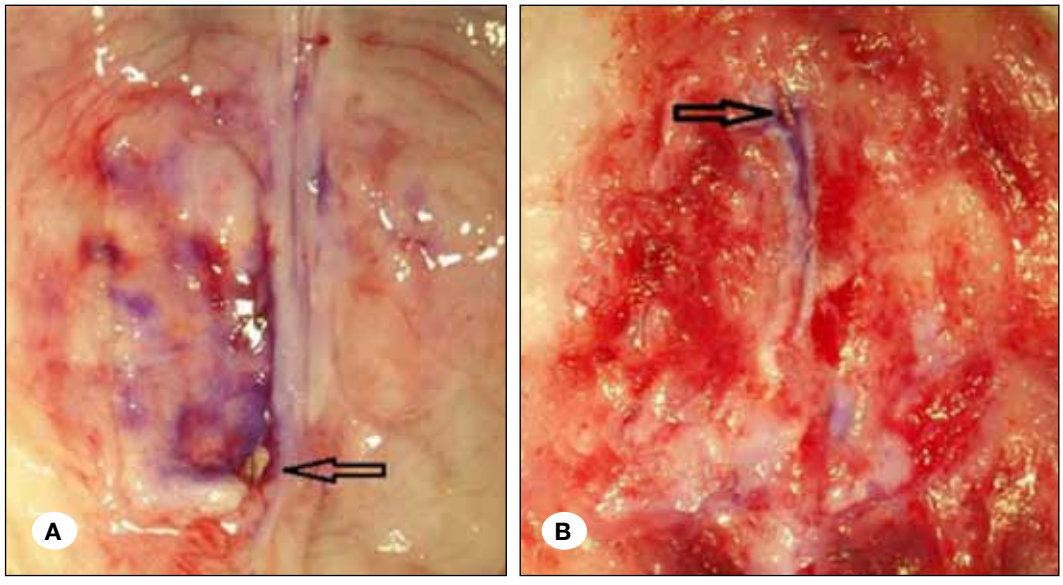

Figure 1: The pictures of a repaired dura mater 4 weeks after burst pressure testing. The arrow shows the leakage area. The left **(A)** shows the brain side, the right **(B)** shows the skull bone side. Compared with the around area, the CO_2 laser soldering area cannot be seen significant thickening, with a thin fibrous tissue.

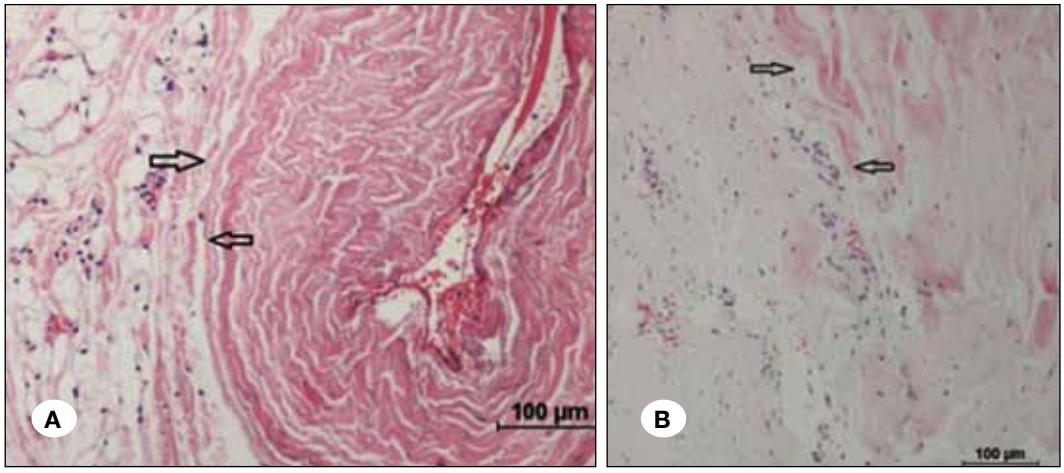

Figure 2: These two pictures are reconstructed dura mater with hematoxylin-eosin staining (magnification,×100). The **(A)** and **(B)** are belong to group A and B, respectively. The arrows points to the conjunctive line. The two parts are adherent together after CO_2 laser welding, partly cross-linking. It seems that there are more fibroblasts and blood vessels around the line than the other area in the right specimen.

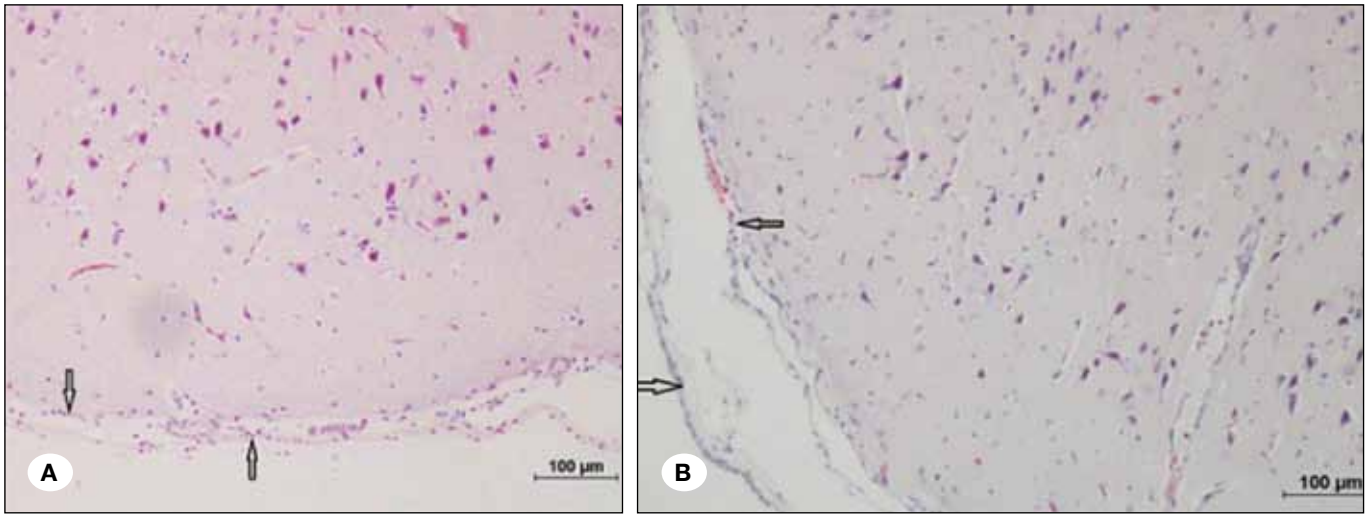

Figure 3: The brain tissue beneath the repaired dural mater of group A **(picture A)**, group B **(picture B)**, with hematoxylin-eosin staining (magnification ×100). The arrows point to the arachnoid and pia mater.

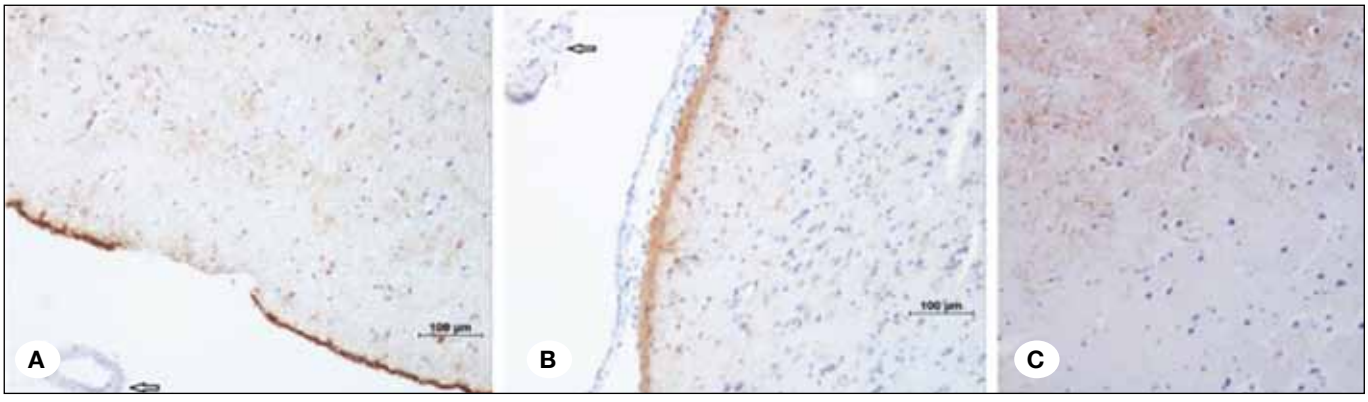

Figure 4: Immunohistochemical stained with antibodies against GFAP for brain tissues of group A **(picture A)**, group B **(picture B)** and a normal pig **(picture C)** (magnification ×100). The arrow points to the reconstructed dura mater. The brain tissues of A and B are just beneath it. During the process of fixation and staining, the dura mater and brain tissue have been detached. Under microscopy, few sporadic positive cells can be seen.

Dural reconstruction is classically performed with sutures and fibrin glue (5,6,8,18,30). The repair with suture is not always watertight, and CSF leakage may occur through the needle holes. This leakage increases the risk of meningitis and cerebral abscess in the postoperative period (10,22). Moreover, the suture materials may cause local inflammatory reaction and slow down the healing process. In some skull base surgery, the operation site is so small that suturing is not possible (12). Fibrin glue is better than suturing. It can provide watertight dural closure and good tissue compatibility (9). However, fibrin glue has also some limitations. When the defect is multiple or irregular, the method would not be sound, and the risk for postoperative or delayed CSF leakage and intracranial infection is high. In experimental animal studies, it was found that the ability of the repaired area to resist intracranial hypertension was limited, and relevant complications occurred in 10% cases (8). Papers on endoscopic dural reconstruction reported success rates about 90% (3,20,21,25).

CO_2 laser is an alternative technique for dural defect closure. It has significant advantages over the sutures and fibrin glue. Soldering with laser was performed endoscopically in some skull base surgeries when sutures are not feasible and it provides a watertight seal. It was also reported that the wound healing process was fast (31) with little scar tissue formation (4, 8, 31). This is also supported by our study. Four weeks later, when craniotomy re-performed, we found that the dura was covered by a thin layer of connective tissue. The soldered area was not thicker than the dura mater.

Burst pressure is a vital factor for a dural reconstruction technique. In contrast to previous studies, we also tested the physiological intracranial pressure and intracranial crest pressures. The mean immediate and 4-week postoperative burst pressures in our study were higher than the mean intracranial crest pressures. The mean 4-week burst pressure was even higher than the previously reported values (8).

These results showed that the reconstructed dura mater can withstand the intracranial pressure fluctuations in some conditions such as Valsalva maneuver, coughing and laughing.

The mechanism of the CO_2 laser is not fully understood, but the heating-induced protein denaturation-renaturation seems the reasonable explanation (1,23,29). In this study, we chose the serrated CO_2 laser power of 2.25 w, 3s/cm. The temperature of the welding area can reach approximately 65°C and provide strong bonding at this power. There was also a concern about the thermal damage with the use of laser in medicine. If a laser caused too much associated injury, it would never be used in clinical practice.

In our study, the postoperative histological observation showed that the pia mater and the underlying brain tissue were intact without any sign of thermal damage. The highly sensitive neurons appeared in a normal state and no regeneration evidence has been collected. There was no staining with GFAP. Therefore, no change in glial cells was observed. This normal appearance of the brain tissue showed that the use CO_2 laser is safe for dural reconstruction.

CONCLUSION

As an alternative choice, CO_2 laser soldering for dural reconstruction was feasible and reliable. Thus it offers a novel technique for dural defects closure, especially in skull base and endoscopic surgery. We also tested the ICP of the minipigs which offer a reference for further studies.

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