



Posterior laryngofissure using a surgical contact diode laser: an experimental feasibility study

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Abstract

To evaluate the feasibility of a 980-nm contact diode laser (CDL) as a method for creating a posterior laryngofissure in live pigs. Twenty-eight Landrace pigs (15–20 kg) were anesthetized, intubated, ventilated, and submitted to a cervical tracheostomy. An anterior and posterior midline longitudinal laryngofissure incision was created according to randomization—control ($n = 4$), posterior laryngofissure with a scalpel blade; electrocautery ($n = 12$), posterior laryngofissure by electrocautery (10, 15, 20, 25 W powers); CDL ($n = 12$), posterior laryngofissure by the CDL (10, 15, 20, 25 W peak powers in pulsed mode). Larynx and proximal trachea were excised, prepared for histopathology, and digital morphometric analysis. Measurements in and within each group were analyzed (Kruskal-Wallis and Dunn test) with a level of significance of $p < 0.05$. Incision width was not different between the groups, as well as in the powers used in CDL ($p = 0.161$) and electrocautery group ($p = 0.319$). The depth of the incisions was smaller in the Laser group compared to control ($p = 0.007$), and in the electrocautery compared to control ($p = 0.026$). Incision area was smaller in CDL compared with the control ($p = 0.027$), and not different between laser and electrocautery groups ($p = 0.199$). The lateral thermal damage produced by electrocautery was the largest, with a significant difference between laser and electrocautery ($p = 0.018$), and between electrocautery and control ($p = 0.004$), whereas the comparison between laser and control showed no significant differences ($p = 0.588$). The posterior laryngofissure incision using a 980-nm CDL is feasible resulting in smaller incisional area and less lateral thermal damage.

Keywords Contact diode laser · Larynx · Pigs · Tracheal stenosis · Surgery

Introduction

Laryngotracheal stenosis secondary to prolonged orotracheal intubation or tracheostomy is a complex surgical problem presenting at all age groups [1]. The complex laryngotracheal stenosis involves both larynx and the subglottis, usually

including a dense fibrous scar tissue extending to the posterior cricoid area. These stenoses are difficult to solve and often require multiple procedures for its management [2].

Definitive surgical treatment of complex laryngotracheal stenosis requires a laryngeal split procedure. This is a major surgical undertaking that consists of splitting of the thyroid

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cartilage and the posterior cricoid plate in the midline. This is followed by a resection of the stenotic area, an enlargement procedure of the larynx using a rib cartilage graft interposed into the split posterior cricoid plate and a thyrotracheal anastomosis. The procedure also requires stenting of the laryngotracheal area for 6 to 8 weeks using a laryngeal silicone mold and a tracheostomy [3, 4]. Later, the laryngeal mold is withdrawn and the upper airway is stented for 1 to 3 years using a silicone Montgomery T tube [5].

The extensive laryngotracheal manipulation inherent to this surgical procedure can contribute to the complications and re-stenosis, as well as the deglutition and speech quality disorders. The development of a less invasive hybrid surgical and endoscopic technique can achieve the same results with less complications and better functional outcome.

The use of suspension laryngoscopy and CO₂ lasers has been reported as a way to perform the posterior split in children for the treatment of laryngotracheal disorders and subglottic stenosis [6]. Inglis et al. described a combination of endoscopic technique of treatment of glottic and subglottic stenosis with the use of laser and cartilage graft interposition with satisfactory results without major complications [7, 8].

The introduction of the contact diode laser carried by flexible fiberoptics enabled a better endoscopic instrumentation and, to the best of our knowledge, it has not yet been reported in the literature for the laryngeal split procedure.

Based on the hypothesis that the use of a 980-nm contact diode laser is feasible for endoscopic and surgical airway enlargement procedure for the treatment of laryngotracheal stenosis, we designed an experimental study focused on identifying the ideal power of the 980-nm contact diode laser capable of combining precision, high effectiveness, and low adjacent tissue damage.

Materials and methods

The research protocol was approved by the ethics committee (CEUA 153/14). Landrace pigs weighing between 25 and 30 kg received human care according to the Guide for the Care and Use of Laboratory Animal (Institute of Laboratory Animal Resources–National Academy of Sciences, Washington-USA 1996) and the ethical principles according to the Brazilian Legislation and to the Brazilian College of Animal experimentation.

The animals were anesthetized with midazolam (Midazolam, Teuto Brasileiro, Anapolis, Goias-Brazil) (0.5 mg/kg intramuscular), ketamine (Cetamina, Cristalia, Itapira, São Paulo, Brazil) (5 mg/kg intramuscular), and acepromazine (Acepran 1%, Univet, São Paulo, Brazil) (0.1 mg/kg intramuscular). All animals received intravenous saline solution (Baxter Hospitalar, Sao Paulo, Brasil) and lactate Ringer's solution (Baxter Hospitalar, Sao Paulo, Brasil)

(60 ml/h). Orotracheal intubation was proceeded with a #7 Fr oro-tracheal tube (Portex, Smiths Medical, São Paulo-Brazil), and pressure-controlled mechanical ventilation (Mini Ventilador 600, K. Takaoka, Sao Paulo-Brazil) was established (flow 5–6 L/min; 20 breaths/min; FiO₂ 0.21). Anesthesia was maintained by continuous venous infusion of ketamine (Cetamina, Cristália, Itapira, São Paulo, Brazil) (1.7 mg/kg/h), midazolam (Midazolam, Teuto Brasileiro, Anapolis, Goias, Brazil) (0.3 mg/kg/h), fentanyl (Fentanyl, Hipolabor, Sabara, Minas Gerais, Brazil) (0.02 mg/kg/h), and pancuronium (Pancuron, Cristalia, Itapira, Sao Paulo, Brazil) (0.4 mg/kg/h).

After a 10-min stabilization of anesthesia and ventilation, a vertical midline cervical incision was made with a WEM, model SS-200A electrocautery (WEM, Ribeirao Preto, Sao Paulo, Brazil) and a transverse tracheostomy was performed in the fourth tracheal ring using a #11 scalpel blade (Embramed, Sao Paulo, Brazil), a #7 Fr oro-tracheal tube (Portex, Smiths Medical, São Paulo, Brazil) was placed through the tracheostomy. Ventilation was then transferred to the tracheostomy and the oro-tracheal tube was withdrawn.

An anterior laryngofissure was done with a # 11 scalpel blade (Embramed, Sao Paulo-Brazil) in order to obtain a wide exposure of the luminal aspect of the posterior laryngotracheal region. A linear longitudinal incision was then performed manually by one researcher (AQM) in the posterior midline starting at the upper margin of the cricoid cartilage and ending at 2 cm below the inferior border of cricoid plate in the luminal aspect of the previously open laryngotracheal area (Fig. 1A, B). The researcher was blinded for the powers used in the electrocautery and laser groups which were sorted in a randomized fashion.

A diagram of the experiments is shown in Fig. 2. Pigs were randomized according to the method used for producing the incisions, as follows: control ($n = 4$) posterior laryngofissure incision performed by a #11 blade scalpel (Embramed, Sao Paulo, Brazil); electrocautery ($n = 12$) posterior laryngofissure incision performed by a surgical electrocautery (WEM, model SS-200A, Ribeirao Preto, Sao Paulo, Brazil) and a needle tip in cut mode at four different power levels (10, 15, 20, 25 W; $n = 3$ for each power); diode laser ($n = 12$) posterior laryngofissure incision performed by a 980-nm contact diode laser (MediLaser 980 nm, DMC São Carlos-SP, Brazil) at 4 different power levels (10, 15, 20, 25 W peak power; $n = 3$ for each power).

Pulsed mode was set up by 1 KHz repetition rate and 0.5 ms pulse width, yielding to mean power of 5, 7.5, 10, and 12.5 W). The Laser energy was delivered using bio-compatible 400 μm optical fibers (Model B med, DMC São Carlos, Sao Paulo, Brazil). Members of the research team exposed to laser light wore protection goggles with filter for 980 nm blocking (DMC-02, DMC São Carlos, Sao Paulo, Brazil).

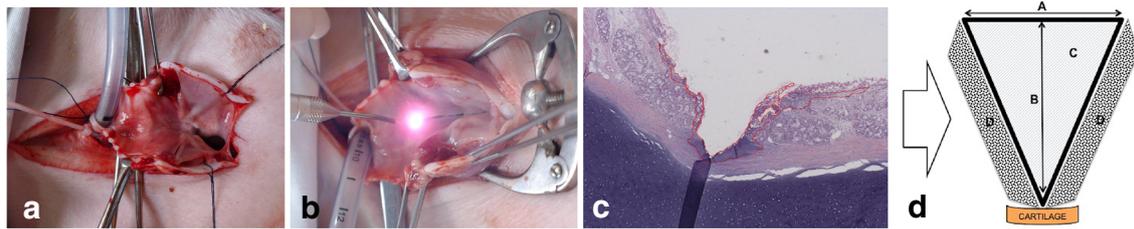


Fig. 1 (A) Anterior laryngeal split; (B) Posterior laryngofissure in the luminal aspect of the cricoid cartilage using a 980-nm contact diode laser; (C) Optical microscopy of a cross section of an incision produced by the 980-nm contact diode laser at 15 W (hematoxylin-eosin stain, 50

powers); (D) Schematic depiction of the measurements performed (A = distance between the cut margins of the incision; B = distance from midline of the cut margins to the bottom of the incision; C = area of the incision; D = area of the lateral thermal damage)

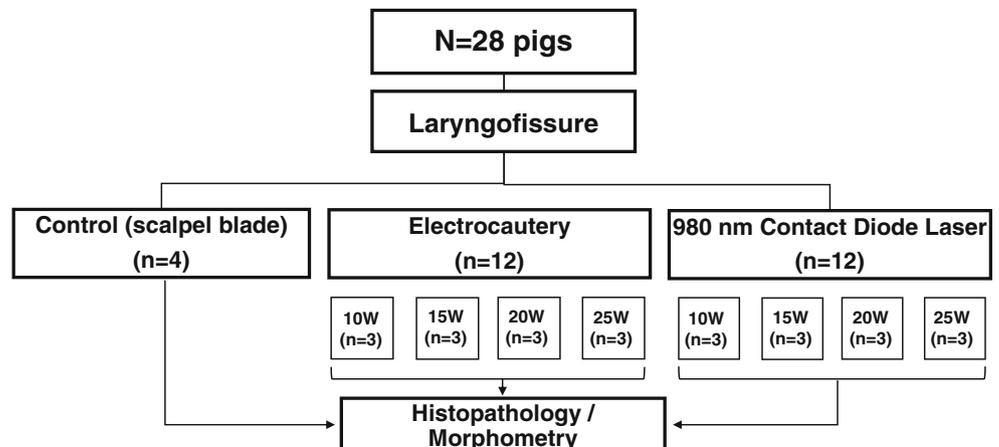
Immediately after the incisions were produced, the larynx and proximal trachea were resected en bloc, and fixated in 10% formaldehyde (Anidrol, Diadema, Sao Paulo, Brazil). Care was taken to ensure that both luminal and external sides were equally exposed to fixation media. The specimen preparation included transverse sections at three levels of the incisional area. Only the middle third of the incision containing the cricoid plate was used for paraffin embedding (Labsynth, Diadema, Sao Paulo, Brazil). The embedded specimens were then cut using a microtome (RM2145, Leica Biosystems, Nussloch, Germany), mounted on glass slides (EasyPath, Sao Paulo, Brazil) that were stained with hematoxylin (Sigma-Aldrich, St. Louis, Missouri, USA) and eosin (Labsynth, Diadema, Sao Paulo, Brazil). Each paraffin block produced 10 slides, which were analyzed by one of the authors (AQM) who selected a set of 3 or 4 slides that contained the best representation of the incisions. The slides were evaluated by optical light microscopy (Primo Star, Carl Zeiss, Oberkochen, Germany) and then scanned using a Panoramic Scan (3DHitech, Budapest, Hungary). Measurements were taken using Image-Pro Plus version 4.5 for Windows (Media Cybernetics, Silver Spring, Maryland, USA). Morphometry was carried out at one time by one researcher (AQM) under the supervision of a certified pathologist (MD and MC) using optical light microscopy at 50 and

100 powers. The microscopic measurements obtained in the incisions in all groups were: width (measure A, μm); depth (measure B, μm); area (measure C, μm^2); and lateral thermal damage area (measure D, μm^2) (Fig. 1C, D). The measurement of the lateral thermal damage area was obtained by marking the boundaries between the area of carbonization and necrosis of the edges of the incision [9]. The numerical data were collected and plotted in a MS Excel worksheet (MS Excel version 14.7.3, Microsoft Corporation, USA) for further analysis.

Statistical analysis

Normally distributed data in each group and homogeneity of the variances between groups were assessed using the Shapiro-Wilk and Levene tests. Morphometry data collected were submitted to a descriptive analysis and represented as median and interquartile range (IQ 25–75%). The measurement values in and within each group were analyzed using the Kruskal-Wallis test and multiple comparisons were carried out using the Dunn test. Sample power calculated for measurements A, B, C, and D between the groups in the Kruskal-Wallis test varied between 0.05 to 0.54 with Beta values varying between 0.46 to 0.95. Sample power calculated for

Fig. 2 Diagram showing the procedures, experimental groups, and energy powers used in the study



measurements A, B, C, and D between the groups in the Dunn test varied between 0.05 to 0.83 with Beta values varying between 0.17 to 0.95. Sample power calculated for measurements of powers L10, 15, 20, and 25 W in the Kruskal-Wallis test varied between 0.05 to 0.28 with beta values varying between 0.72 to 0.95. Sample power calculated for measurements of powers L10, 15, 20, and 25 W in the Dunn test varied between 0.05 to 0.99 with beta values varying between 0.01 to 0.95 (Minitab 14.12.0; Minitab Inc., State College, Pennsylvania, USA). All other analyses were performed using the software SPSS 21 for Windows (IBM, Armonk, New York, USA). The level of significance was set for values of $p < 0.05$.

Results

The measurements of the dimensions of the incisions and area of lateral thermal damage obtained in each group are described in Table 1 below.

The analysis of incision width showed no statistically significant differences between the three groups ($p = 0.074$) (Fig. 3A).

No differences were also observed in the comparison between the different powers in both the laser group ($p = 0.161$) and electrocautery group ($p = 0.319$), as well as in the comparison of the different powers between the two groups and the control group ($p = 0.121$ and $p = 0.388$, respectively).

The depth of the incisions was different between the groups ($p = 0.008$) (Fig. 3B). Multiple comparisons showed a significant difference between laser and control group ($p = 0.007$), with a smaller incision depth in the laser group. There was no difference between the groups laser and electrocautery ($p = 0.372$), and between electrocautery and control ($p = 0.145$).

No significant difference was found between the depth of the incisions in the different powers in both laser and electrocautery ($p = 0.789$ and $p = 0.101$, respectively), as well as between the laser and control ($p = 0.155$), whereas the depth of incisions obtained with the different powers in the electrocautery group were smaller compared to control ($p = 0.026$) (Fig. 3C). The incision areas were different between the groups and smaller in the laser group ($P = 0.020$) (Fig. 3D).

The incisional area in the laser group was smaller compared to the control group ($p = 0.027$). Nevertheless, no difference was found between laser and electrocautery ($p = 0.199$) as well as between electrocautery and control group ($p = 0.565$).

The incisional areas produced by the four powers tested both in the laser and electrocautery group were not different ($p = 0.172$ and $p = 0.05$, respectively), as well as between the powers in the laser and electrocautery compared to the control group ($p = 0.072$ and $p = 0.052$).

The lateral thermal damage was different between the groups. The electrocautery produced the largest lateral thermal damage area, whereas the comparison between the laser and the control group showed no significant difference (Fig. 4). Multiple comparisons showed a significant difference between laser and electrocautery ($p = 0.018$), and between electrocautery and control group ($p = 0.004$).

There was also a significant difference between lateral thermal damage produced by the different powers in the electrocautery group ($p = 0.033$), where the larger areas were measured in the 10 W and 25 W powers (Fig. 5). Conversely, the comparison between the laser powers showed great variability, oscillating from no thermal damage observed in the 10 W power, greatest measurements in the highest power (25 W), and lowest in the 20 W power. This variability prevented the accurate determination of which was the power in the contact diode laser related to the lowest lateral thermal damage.

Table 1 Dimensions of the incisions and area of lateral thermal damage

Group	Power watts	Incision width (measure A) μm	Incision depth (measure B) μm	Area of incision (measure C) μm^2	Area lateral thermal damage (measure D) μm^2
Laser	10	211 (141–242.5)	83 (69–98.5)	9747 (7928.5–11,678)	—*
	15	235 (194.5–707.5)	69 (48.5–306.5)	11,453 (7572.5–202,494)	2417 (1208.5–27,945.5)
	20	466 (438–598)	117 (91.5–136)	35,979 (25,545.5–53,222.5)	4483 (2241.5–11,206.5)
	25	425 (403.5–864)	259 (151–452)	75,071 (44,592.5–314,627)	33,446 (16723–94,756)
	10	1010 (688–1020.5)	138 (124.5–154)	120,664 (73830–122,990.5)	97,238 (93160–150,209.5)
Electrocautery	15	275 (192–684)	126 (108–172.5)	46,118 (27238–66,140.5)	14,033 (7735–21,294.5)
	20	1089 (916.5–1231)	300 (227.5–305)	171,160 (129558–203,037)	49,254 (45025–51,553)
	25	1194 (1028.5–1743.5)	336 (274–423.5)	349,415 (238,655.5–353,962)	53,367 (53367–118,240)
Control	—	843 (559–1288.5)	799.5 (390–1257)	287,714.5 (145,136.5–443,008)	—

Values are expressed in median (interquartile range 25–75%)

*No lateral thermal damage

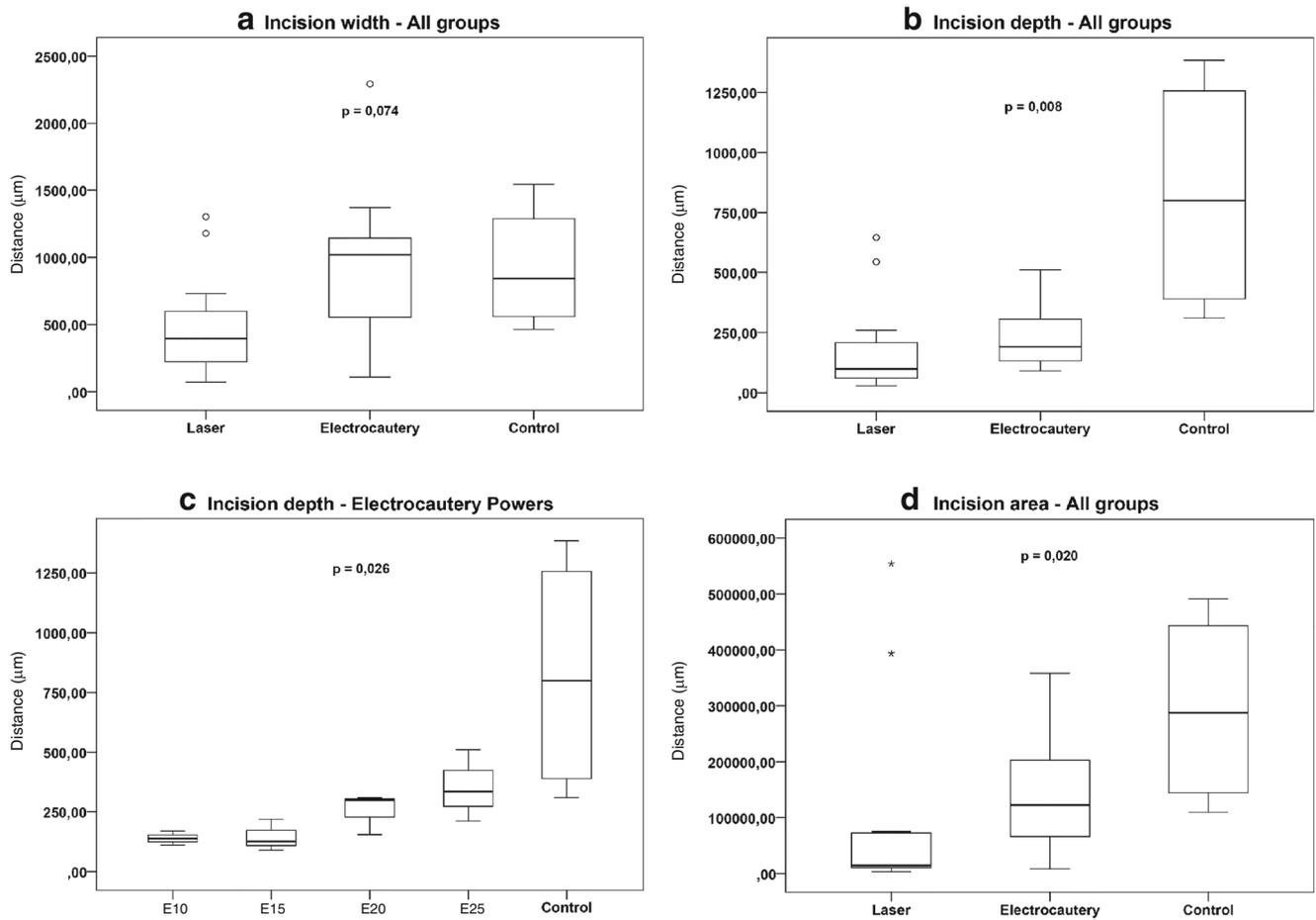
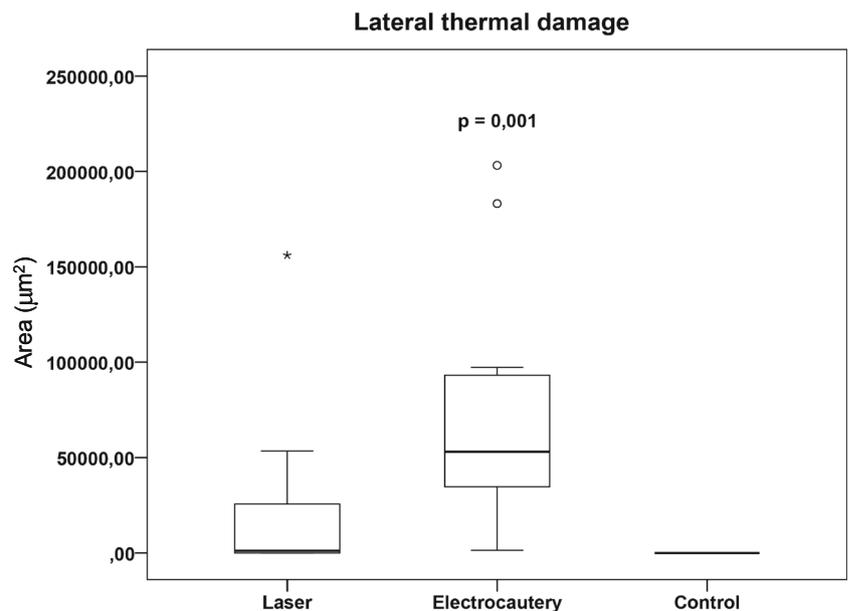


Fig. 3 Incision width, depth, and area in all groups: **(A)** incision width was not different between the groups; **(B)** different incision depth between the groups, smaller in the laser group, and largest in the

control group; **(C)** smaller incision depth in the different powers of the electrocautery compared to control; **(D)** the smaller incision area in the laser group was not significantly different from electrocautery

Fig. 4 Lateral thermal damage showing a greater area in the electrocautery group



Discussion

The present study shows that the posterior laryngofissure performed with a 980-nm contact diode laser is feasible, and capable of producing precise incisions with less lateral thermal damage compared to electrocautery.

The conventional open laryngeal split procedure requires a transcervical approach, an anterior laryngofissure to expose the posterior aspect of the cricoid cartilage. The posterior cricoid split requires the placement of a cartilaginous graft with or without stenting in addition to a tracheostomy [3, 10]. This is a lengthy surgical procedure that often requires an intensive care unit stay for 1 or 2 days [7]. The procedure leads to subsequent scarring and adhesences to adjacent structure results in functional derangements such as dysphagia and dysphonia. Our initial experience with laryngotracheal stenosis in adult patients included a standardized laryngeal split with rib cartilage interpositional grafting in the posterior cricoid. We reported a 30% early postoperative complication rate, represented mostly by infection at the surgical site, whereas dysphonia was the most frequent late complication (25%). Decannulation was possible in 80% of the patients after 2 years [3]. The magnitude of the laryngeal split procedure in addition to its increasing demand in our patient population and the incidence of complications in our series have led us to look into an alternative less invasive way to perform the procedure to minimize complications and dysfunction.

The use of laser energy in surgery for laryngotracheal stenosis is scarcely reported in the literature and most reports are focused on the use of CO₂ lasers in the pediatric population. The first report on the use of a CO₂ laser for the treatment of posterior glottic and subglottic stenosis was published in 1976 [11], followed by a review of the results in 10 children submitted to endoscopic posterior cricoid split with a CO₂ laser plus a cartilaginous grafting for the treatment of posterior glottic stenosis [12]. Both reports found the procedure to be

advantageous when compared to other traditional approaches such as cordotomy and arytenoidectomy. A multicenter study reported the results on the endoscopic posterior cricoid split with a CO₂ laser and cartilaginous graft interposition in pediatric patients with bilateral vocal fold paralysis and posterior glottic and subglottic stenosis. The post-operative results showed an absence of postoperative aspiration and a high decannulation rate [8].

A variety of surgical lasers are used as an alternative to conventional electrocautery in oral surgery [13]. The advantage of using of a surgical laser in soft tissues is its ability to provide both coagulation and precise cutting in addition to promote sterilization of the surgical site. The end result is minimal swelling, less scarring, and less post-surgical pain [14].

The conduction of energy in the 980-nm contact diode laser uses flexible fiberoptics that can bend up to a 60-degree angle. This allows for an easier approach and a better handling of the posterior aspect of the airway lumen through the rigid endoscopic instruments. However, the safety, effectiveness, and the ideal power utilized in the 980-nm contact diode laser in the upper airway remain unclear. The 980-nm contact diode laser has a wide range of wavelengths of clinical interest. When compared to other laser technologies (e.g., CO₂, Nd: YAG and KTP), the 980-nm contact diode laser is portable, has a high electrical-optical efficiency that enables to convey the energy with low loss, high stability, and long life of the emitters when compared to the other types of lasers.

We elected to use the 980 nm wavelength in this study because of its simultaneous absorption by tissue water and hemoglobin. At this wavelength, the water absorption coefficient is $\mu_a(\text{H}_2\text{O}) = 0.43 \text{ cm}^{-1}$. In the context of the intended application, this wavelength compares to the Nd: YAG laser at an emission wavelength of 1064 nm where the water absorption coefficient is $\mu_a(\text{H}_2\text{O}) = 0.12 \text{ cm}^{-1}$. At this wavelength, the contact diode laser is also advantageous over the range of

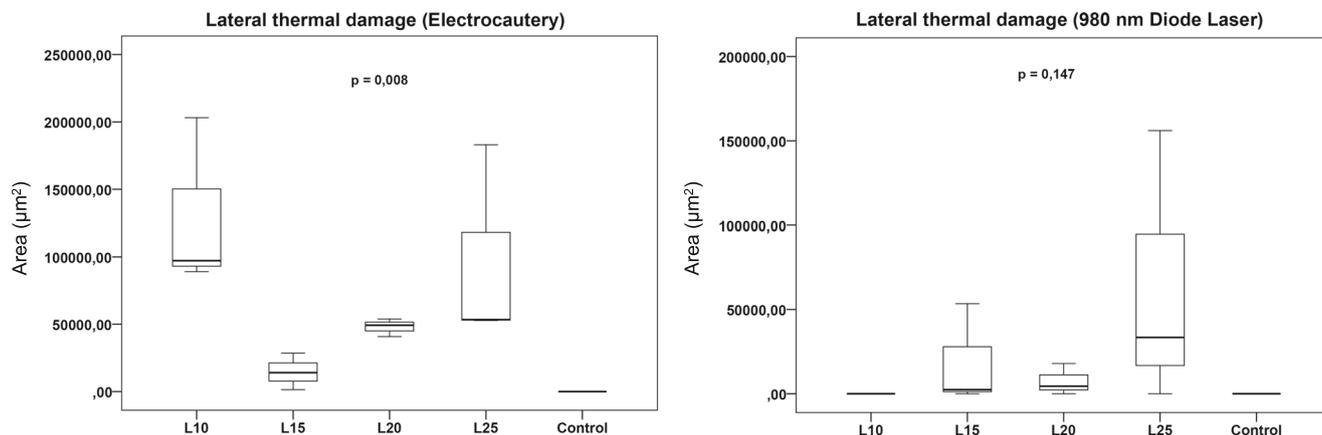


Fig. 5 (LEFT) Electrocautery produced the largest lateral thermal damage (measure D; μm^2) at 10 W and 25 W compared to control, but no differences were found in multiple comparisons between the powers

tested; (RIGHT) No significant difference in the comparison between lateral thermal damage (μm^2) produced by the different powers in the contact diode laser group versus control

805–810 nm, which exhibits a very poor absorption by water ($\mu_a(\text{H}_2\text{O}) = 0.023 \text{ cm}^{-1}$). The 980 nm contact diode laser is better absorbed by water and hemoglobin ($\mu_a(\text{Hb}) = 2.0 \text{ cm}^{-1}$) if compared to the Nd:YAG (1.64 mm), which is also poorly absorbed by blood ($\mu_a(\text{Hb}) = 0.4 \text{ cm}^{-1}$) [15, 16].

The heat generated by a laser light absorption through photo-thermal interaction in the tissues is difficult to assess [17, 18]. The temperature increase is proportional to the denaturalization and hyalinization of the tissue adjacent to the irradiated area. This can be assessed using conventional microscopy techniques by means of the standard hematoxylin-eosin staining that accurately shows the area of eosinophilic coagulation [19].

In the present study, we elected the lateral thermal damage as an indicator for the safety of the 980 nm contact diode laser. Therefore, the lower the lateral thermal damage, the lesser is the heat generated and dispersed which can ultimately cause damage to adjacent tissues. The choice for a pulsed mode in the 980-nm contact diode laser was based on previous clinical reports of the reduction of the lateral thermal damage resulting in a smaller peripheral zone of carbonization [9, 20].

We elected to use the powers of the contact diode laser in pulsed mode at peak 10, 15, 20, and 25 W based on the same nominal powers often used in electrocautery for both surgical and endoscopic procedures. Nevertheless, we acknowledge that the power in Watts of the electrocautery, and the photonic energy of the contact diode laser may have differences inherent to the sources of energy and the method of delivery.

An ideal cutting method must produce the smallest distance between the edges of the incision with the smallest lateral thermal damage that is what ultimately triggers a lesser local inflammatory response. This is a potential advantage of the 980-nm contact diode laser in laryngotracheal surgery where the focus is on minimizing the injury to the deeper tissue layers.

The results of the present study shows that the 980-nm contact diode laser produced the smallest incision depth and area compared to the other methods tested. This can also be explained by the use of energy and the non-mechanized production of the incisions. In fact, the conventional scalpel blade (control group) resulted in the largest incisional area and depth, which were equivalent to the area produced by the electrocautery incisions. The absence of energy intuitively creates the need to impose a greater force to the scalpel blade in order to produce the incision. On the other hand, Beer et al. [9] found no differences in the width of the incisions in bovine liver performed by the 980 nm contact diode laser in the presence of smaller powers (2.5 W to 4.5 W). This is probably a result of the homogeneous, low-density and high-water contents of the liver parenchyma as opposed to the heterogeneous character of the posterior cricoid region. A similar study carried out in porcine myometrium concluded that monopolar

electrosurgery compared with CO₂ laser energy delivered via flexible fiber system showed higher incising efficiency, a greater incision depth than width as the power increased, and lower variability in the incision [21].

A recent systematic review compiled clinical data from 11 studies between 2003 and 2014 on the use of the diode laser in laryngeal surgery [22]. In this review, five studies reported no complications whereas the major complications reported by the other six studies were related to extensive surgical procedures with anterior commissure involvement. The review suggested an improvement in diode laser technology, particularly in the 980 nm wavelength.

This study has limitations. The manual production of the incisions may have an influence in the results, particularly in regard to the depth of the incisions in the control group. A similar situation was observed in the higher values and greater variability obtained in the group electrocautery at 10 W (97,238 μm^2 ; IQ range 93,160–150,209.5), where the low power induced the surgeon to prolong the exposure of the energy to the tissue. The lack of a continuous monitoring of perfusion by means of either systemic or local arterial pressure in this model overlooked the possible variations in tissue perfusion that can potentially interfere in laser energy absorption by the tissues. Lastly, we acknowledge that the use of similar nominal powers in different sources and delivery of energy is not ideal.

In conclusion, the present study shows that 980-nm contact diode laser was safe and effective for the production of a posterior laryngofissure incision in a pig model. The safest powers of the 980-nm contact diode laser were within the range of 10 W and 20 W that produced a smaller lateral thermal damage and variability, as opposed to electrocautery where a greater lateral thermal damage was found. The forthcoming steps in this research will focus on the evaluation of other wavelengths as well as the development of dedicated instrumentation to perform a posterior laryngofissure using suspension laryngoscopy. The immediate future clinical implications of the use of a contact diode laser are the possibility of performing an endoscopic laryngofissure for the treatment of laryngotracheal stenosis.

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Compliance with ethical standards

Conflict of interest statement The author Marilia Wellichan Mancini is a physicist working at the company DMC at the department of research and development of the laser equipment used in this study. The author Paulo Francisco Guerreiro Cardoso was the recipient of the FAPESP grants 2015/17847-1 and 2016/25437-0. The remaining authors have no conflict of interest to disclose.

Ethical approval The research protocol was approved on by the ethics committee (CEUA 153/14) of the Faculty of Medicine of the University of Sao Paulo, Brazil.

Informed consent Not applicable in the current animal experimental protocol.

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