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ORIGINAL ARTICLE

Tissue response to different incision tools in animal model

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Abstract

Purpose The aim of this study is to compare the repair of incisions performed with microdissection electrocautery tip, conventional electrocautery tip, high potency diode laser, and conventional scalpel blade in a in vivo model.

Methods Different incisions were performed in adults Holtzman rats using the four types of instruments: microdissection electrocautery tip, conventional electrocautery tip, high potency diode laser, and conventional scalpel blade, in different periods of healing process. Thirty rats were divided into 5 groups, according to the period of euthanasia—24 h, 48 h, 72 h, 7 days, and 14 days. All animals received four incisions, each by a different method. Quantitative histological and histomorphometric analyses were performed using hematoxylin and eosin (HE) and Picrosirius Red staining.

Results Inflammatory profile and tissue repair presented small statistically significance differences comparing conventional scalpel blade and microdissection tip; moreover, both presented quantitatively superior to the others.

Conclusion It is believed that the microdissection tip can perform a dynamic incision just as a common scalpel blade, but more effective. Furthermore, it can promote a better hemostatic control of the surgical field that is comparable to conventional electrocautery tip without affecting tissue repair.

Keywords Electrocoagulation · Microdissection · Surgical wound · Wound healing · Diode laser

Introduction

The incision is a fundamental surgical principle in any type of surgery in which the exposure of operative field is made [1]. The ideal instrument should be easy to use, precise, cause minimal damage to adjacent tissues, diminished transoperative bleeding, and be financially viable [2].

Conventional scalpel blade is the most used instrumental option. In order to achieve adequate hemostasis, electrocautery was added to surgeons' instrument catalog, and it can be used with different tips. Moreover, some tips are more indicated to oral and maxillofacial surgeries performing a satisfactory incision and with minimum amount of adjacent damage [3–5]. Some studies demonstrate that the heat and damage generated by electrocautery are influenced by the duration of electrode's contact to the tissue, current intensity, wave type, and size and shape of electrode tip. Consequently, a blunt or extended tip causes greater tissue damage due to large contact surface [6, 7]. Therefore, an electrode commonly used in oral and maxillofacial surgery is the needle type (microdissection tip) made of an extremely sharp tungsten tip, in which the shape allows to use at a very low intensity, resulting in less necrosed tissue, greater precision during cut, adequate cauterization, and less postoperative pain [4, 8–10].

Looking for optimize the procedure—mainly to reduce intraoperative bleeding—in 1960s, experiments and clinical trials with laser began [11]. Diode laser was the most used in cases of surgical treatments [12]. Its use leads to less bleeding, better intraoperative vision, and a reduction of bacteremia [13, 14].

Several experiments demonstrate advantages and disadvantages of each method. However, there is a lack in literature when it comes to direct comparative assessments regarding the evaluation in a cellular tissue repair level [15]. The objective of study was to evaluate histologically and histomorphometrically the tissue repair after the use of

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microdissection electrocautery tip, conventional electrocautery tip, high potency diode laser, and conventional scalpel blade in an in vivo model at different periods of tissue repair.

Materials and methods

This study was approved by local Animal Use Ethics Committee (CEUA) (Process nº 08/2017) and followed the protocol described for this type of experiment, according to ARRIVE (Animal Research: Reporting of In Vivo Experiments).

Thirty adult male rats (*Rattus norvegicus* albinus), variety Holtzman, with an average body weight of 350 g and varying between 3 and 4 months of life, were used. The animals were kept at the Bioterium of Araraquara Dental School—UNESP in a controlled environment, in cages with shavings, normal feed (pellets or crushed), and water ad libitum.

Surgical procedures followed the antisepsis and anesthesia protocols, using Ketamine Hydrochloride (Agener® Ketamine, Agener União Ltda, São Paulo/SP, Brazil) 0.08 ml/100 g of body weight and 2% Xylazine (Rompun® Bayer SA, São Paulo/SP, Brazil) 0.04 ml/100 g of body weight, both introduced by intraperitoneal injection. At the end, the animals received antibiotics (Pentabiotic®, Wyeth-Whitehall Ltda., São Paulo/SP, Brazil) and Ketoflex (1% ketoprofen, 0.03 ml/rat) intramuscularly. The incisions were made using four different methods: conventional scalpel blade, conventional electrocautery tip, microdissection electrocautery tip, and diode laser. The animals were separated into 5 groups with 6 animals each, according to euthanasia period: group 1 (24 h after surgery); group 2 (48 h after surgery); group 3 (72 h after surgery); group 4 (7 days after surgery); and group 5 (14 days after surgery).

Euthanasia was performed with pentobarbital (60 to 100 mg/kg) associated with lidocaine (10 mg/ml), intraperitoneally, after each experimental period. After euthanasia, the area of interest involving incisions was carefully removed for the sample preparation.

Surgical procedure

Four 20-mm length incisions were made in each animal through the cutaneous and subcutaneous planes using the following instruments:

Conventional scalpel blade (Sterile Carbon Steel Scalpel Blade, Lamedid, Barueri/SP, Brazil): attached to scalpel handle, the incisions made according to length and depth, previously explained.

Electrocautery (BP-100 Plus, Emai Transmai, São Paulo/SP, Brazil) equipped with a conventional tip (knife-type electrode) (67 mm).

Electrocautery (BP-100 Plus, Emai Transmai, São Paulo/SP, Brazil) equipped with a microdissection tip (needle-type electrode) (52 mm) (Traumec Health Technology, Rio Claro/SP, Brazil).

Diode laser (Gemini®, Ultradent, South Jordan/UT, USA): used in power of 2 W and application time of 8 s, continuously and applied in contact with an optical fiber of 300 μm and speed of 2.5 mm/s.

The incisions were made respecting a 10-mm distance between them (Fig. 1). At the end, the incisions were sutured by planes with nylon thread (Fig. 2).

Analysis methods

Histological evaluation was performed using a DIASTAR light microscope (Leica Reichert & Jung Products, Germany) with a magnification of 5 to 100 times, eyepieces with a tenfold magnification, in which images were observed, evaluated, captured, and sent to a microcomputer.

Quantitative analysis was made on all sections stained with hematoxylin and eosin (HE) considering the pattern of inflammatory infiltrate [1]. A panoramic image with

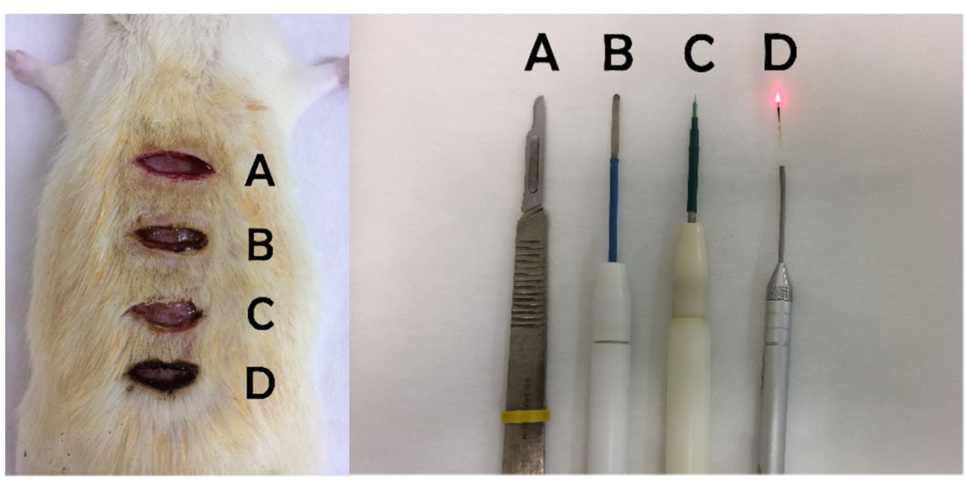

Fig. 1 Types of experiment incisions. (**A**) Conventional blade. (**B**) Electrocautery with a knife tip. (**C**) Electrocautery with microdissection tip. (**D**) Diode laser

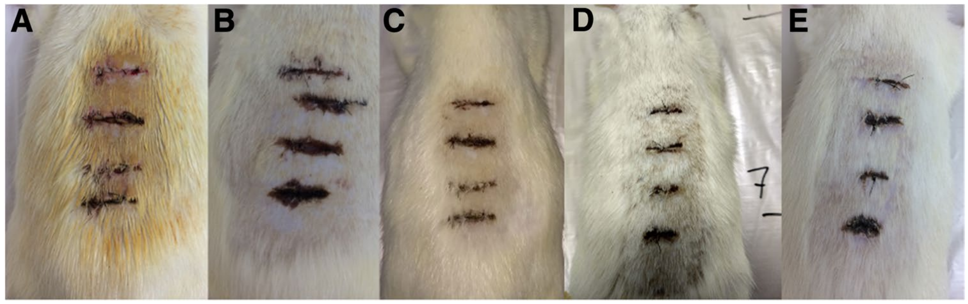

Fig. 2 Follow-up of euthanasia times. **A** 24 h. **B** 48 h. **C** 72 h. **D** 7 days. **E** 14 days

2.5 × objective was captured and recorded, covering two peripheral areas and the center of wound. Another image with 20 × objective was captured, in order to obtain an image for cell counting. Using ImageJ software (NIH, USA), in Plugins tab, Grid tool was used to make a lattice containing 768 stitches. Using the cell count tool, only the cells that coincided with the intersection of points were counted. The sum of points results in total number of cells in inflammatory infiltrate of each animal.

The samples with Picrosirius red were observed in an optical microscope with polarized light to determine the concentration of type I and type III collagen fibers [1]. The staining allows a qualitative analysis of the collagen fibers and connective tissue through different color interferences, intensity, and birefringence of the tissues by differentiating the type I and type III fibers. The references observed were area, density, and percentage of collagen types I and III [1, 2, 3]. Type I collagen fibers emit a red color and collagen III fibers emit a green color, so in overlapping colors, we can see a yellowish to orange color [4, 5]. The photographs were obtained with 2.5 × and 20 × magnifications and the analysis was made on 20 × magnification. The photomicrographs were analyzed by ImageJ software, measuring the area in µm and in percentage of type I and III collagen. This program lists the percentages by tissue area that were filled by type I and III collagen after staining with Picrosirius red. Initially, the software was calibrated, and, subsequently, images were subjected to individual analysis for type I and type III collagen, using Split Channel and Threshold adjusting images of each group in a single pattern.

Statistical analysis

All tabulated data were in continuous quantitative values for cell count, area of different types of collagen (µm), and percentage of the area containing different types of collagen.

The 24 samples were divided into 4 groups of 6 individuals according to the cutting material used, and for the respective period of time. For each group, Shapiro–Wilk normality and Levene's homoscedasticity tests were performed. The data obeyed the normal distribution and variances in all groups ($p > 0.05$). Multiple comparisons were performed to each variable using two-way ANOVA and Tukey's post-test.

Results

Histological and histomorphometric analysis

In the histological analysis of repair process, it was observed that there was light difference in results in groups of conventional scalpel blade and microdissection tip. In periods of 7 and 14 days, same phases of healing process were observed: proliferation and maturation. However, in groups of conventional electrocautery tip and diode laser, there was a great increase in the inflammatory infiltrate that remained until the period of 14 days. Therefore, the maturation process was not observed in any of these groups, evidencing the great delay in healing process of conventional electrocautery tip and diode laser in comparison to the others (Fig. 3 and Table 1). Inflammatory infiltrate did not present statistical difference between timepoints in any of the instruments evaluated; however, scalpel incisions presented a lower inflammatory cell count in all timepoitns, followed by microdissector that also with reduced cell count ($p < 0.05$). Both conventional electrocautery and laser incisions had higher values of inflammatory cell count ($p < 0.05$) (Table 1).

Through the Picrosirus red staining, it was noticed that remodeling of collagen using conventional scalpel blade was greater and earlier when compared to the other groups, followed by microdissection needle that presents type I collagen fibers in process of maturation on center of surgical wound, within 14 days, indicating satisfactory tissue repair. However, the minor percentage of collagen type I fibers was in 7 days timepoint for all the tested instruments ($p < 0.05$) (Table 2). The conventional electrocautery tip showed less favorable results but still close to microdissection tip. Diode laser showed type I collagen fibers for a longer time and when initial remodeling process occurred—after 14 days—the presence of type III fibers was noticed (Fig. 4).

Regarding the instrument used, there was no statistically significant difference in type I collagen. Moreover, there is a higher percentage of type III collagen in incisions made

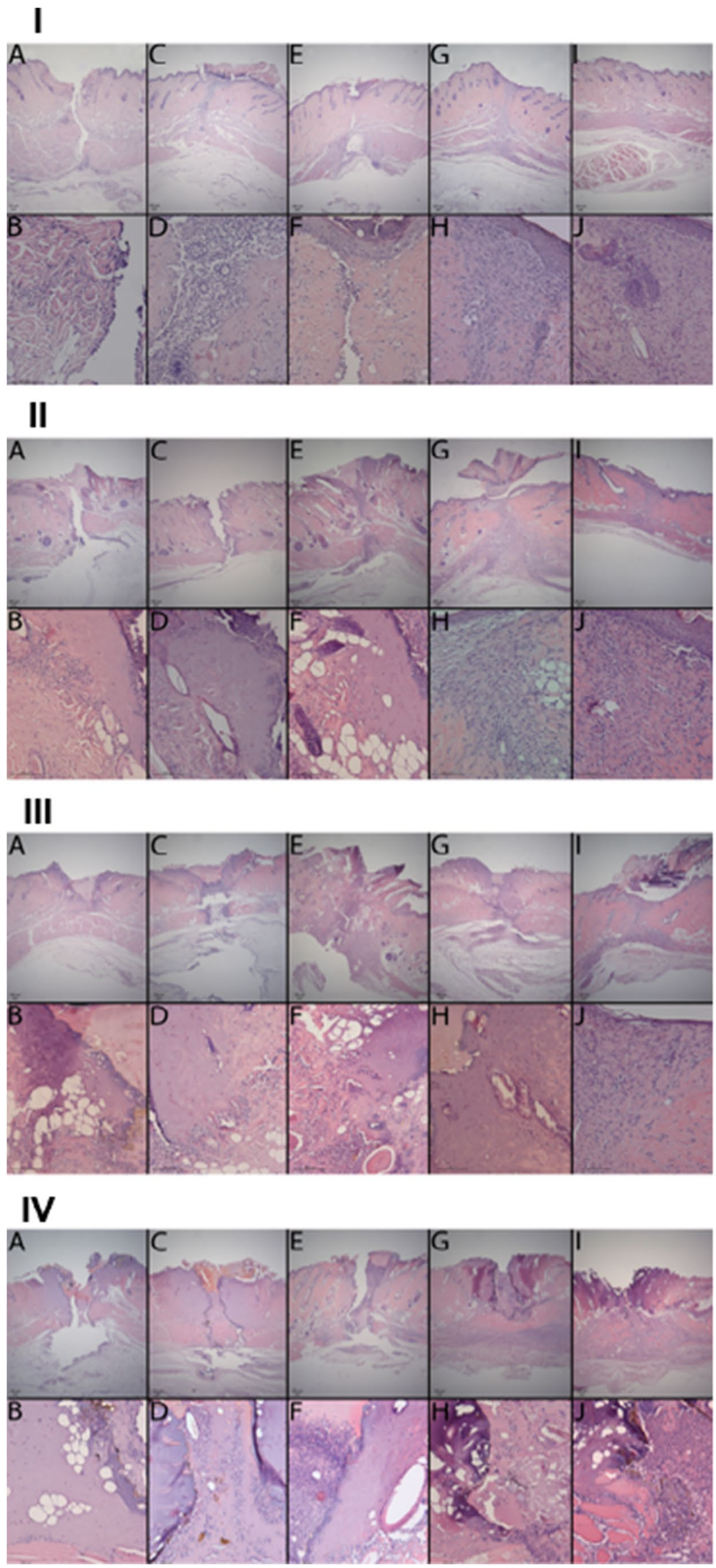

Fig. 3 Photomicrograph of HE. I—Scalpel blade; II—electrocautery with a microdissection tip; III—electrocautery with a knife tip; IV—laser-stained diode sections. **A** Cut the 24-h group in 2.5× magnification. **B** Cut the 24-h group in 20× magnification. **C** Group 48 h in 2.5× magnification. **D** Group 48 h in 20× magnification. **E** Group 72 h in 2.5× magnification. **F** Group 72 h in 20× magnification. **G** Group 7 days in 2.5× magnification. **H** Group 7 days in 20× magnification. **I** Group 14 days in 2.5× magnification. **J** Group 14 days in 20× magnification

Table 1 Inflammatory cell count according to time period and cutting material used

SPeriod						
Instrument	24 h	48 h	72 h	7 days	14 days	Incision
Blade	64.17 (7.31)^{Aa}	45.33 (5.24)^{Aa}	42 (9.82)^{Aa}	53.5 (12.97)^{Aa}	45.17 (12.12)^{Aa}	50.03 (12.31)
Microdissector	61 (18.38) ^{Ba}	69.83 (11.87)^{Ba}	59.5 (5.13)^{Ba}	67.33 (10.61)^{Ba}	59.67 (8.14)^{Ba}	63.47 (11.7)
Knife	71.17 (13.73)^{Ca}	74.17 (15.24)^{Ca}	86.33 (13.22)^{Ca}	69.83 (12.37)^{Ca}	66.33 (7.2)^{Ca}	73.57 (13.66)
Laser	73.5 (10.99)^{Ca}	74.33 (10.17)^{Ca}	77.5 (16.59)^{Ca}	85.17 (20.31)^{Da}	82.33 (7.2)^{Da}	78.57 (13.69)
Period	67.46 (13.38)	65.92 (16.14)	66.33 (20.69)	68.96 (17.77)^{a}	63.38 (15.97)	

*Different lowercase letters indicate statistical difference between columns; different uppercase letters indicate statistical difference between rows ($p < 0.05$)

Table 2 Percentage of type I collagen according to time period and cutting material used

Period						
Instrument	24 h	48 h	72 h	7 days	14 days	Incision
Blade	32.76 (2.55)	35.71 (1.66)	34.8 (2.98)	30.05 (2.31)	32.09 (2.05)	33.08 (2.99)^{A}
Microdissector	29.81 (4.18)	33.44 (2.25)	33.23 (2.13)	31.92 (3.37)	31.21 (3.67)	31.92 (3.29)^{A}
Knife	31.51 (2.84)	32 (1.83)	32.33 (4.2)	30.39 (2.09)	32.99 (2.76)	31.85 (2.8)^{A}
Laser	33.21 (2.21)	32.8 (1.62)	32.22 (1.31)	31.59 (3.33)	29.81 (2.9)	31.93 (2.53)^{A}
Period	31.82 (3.14)^{ab}	33.49 (2.23)^{b}	33.14 (2.87)^{b}	30.99 (2.76)^{a}	31.52 (2.96)^{ab}	

*Different lowercase letters indicate statistical difference between columns; different uppercase letters indicate statistical difference between rows ($p < 0.05$)

by conventional scalpel blade and microdissection tip, which did not present statistically significant differences between them. The percentage of type III collagen was lower in the diode laser when compared to microdissection tip ($p > 0.05$), but there were no statistically significant differences between diode laser and conventional electrocautery tip. The percentage of type III collagen presented no statistically significant differences in relation to the periods analyzed ($p > 0.05$) (Tables 2 and 3).

Discussion

The surgical trauma caused by incisions initiates a cascade of tissue repair events: inflammation, proliferation, and maturation [6]. In order to be able to specify the staging of the phases and the healing velocity, it was evaluated using histological analysis, the quantification of the number of local cells specific to each phase. Picrosirus red staining was used to assess the type of deposited extracellular matrix, in which type III collagen indicates the initial phase of proliferation, less organized tissue, and type I collagen indicates signs of cell migration and the end of local maturation [7, 8].

The precision of the microdissection tip was similar to that of the conventional scalpel blade, as well as the marginal damage caused when compared to the others [9]. Certainly, this difference is due to the smaller contact surface with the adjacent soft tissue, when evaluated with other thermal cutting devices. The finding that could validate this theory is the smaller number of acute inflammatory infiltrate cells, which generate a faster repair process of collagen fibers in a more organized tissue [10]. The same results had been observed when comparing the use of microdissection tip, conventional electrocautery tip, and conventional scalpel blade in periodontal surgeries. Yet, the hemostasis that the non-thermal instruments do not provide can be added to the list [11].

The thermal effects produced by the laser increase the area of tissue damage, the healing period of surgical wounds, and the signs of inflammation that are more exacerbated in this case [10, 12, 13]. On the other hand, its use leads to less bleeding, improvement of the operative field of view, decreasing of the time of the procedure, and bacteremia [9, 14, 15]. These two aspects were contemplated and verified in this proposition. It was found that the damage caused using the laser to the proximal tissues was greater, the inflammation was more exacerbated, and the healing period was much greater, when compared to the other instruments. The factor of visibility of the operative field was advantageous only when compared to the conventional scalpel blade. Also, considering the greater tissue damage caused, the use of electrocautery tips is better indicated than the laser, due to the precision and velocity, especially using the microdissection tip.

Studies have demonstrated an earlier or similar cicatricial repair when the laser is used, due to cell

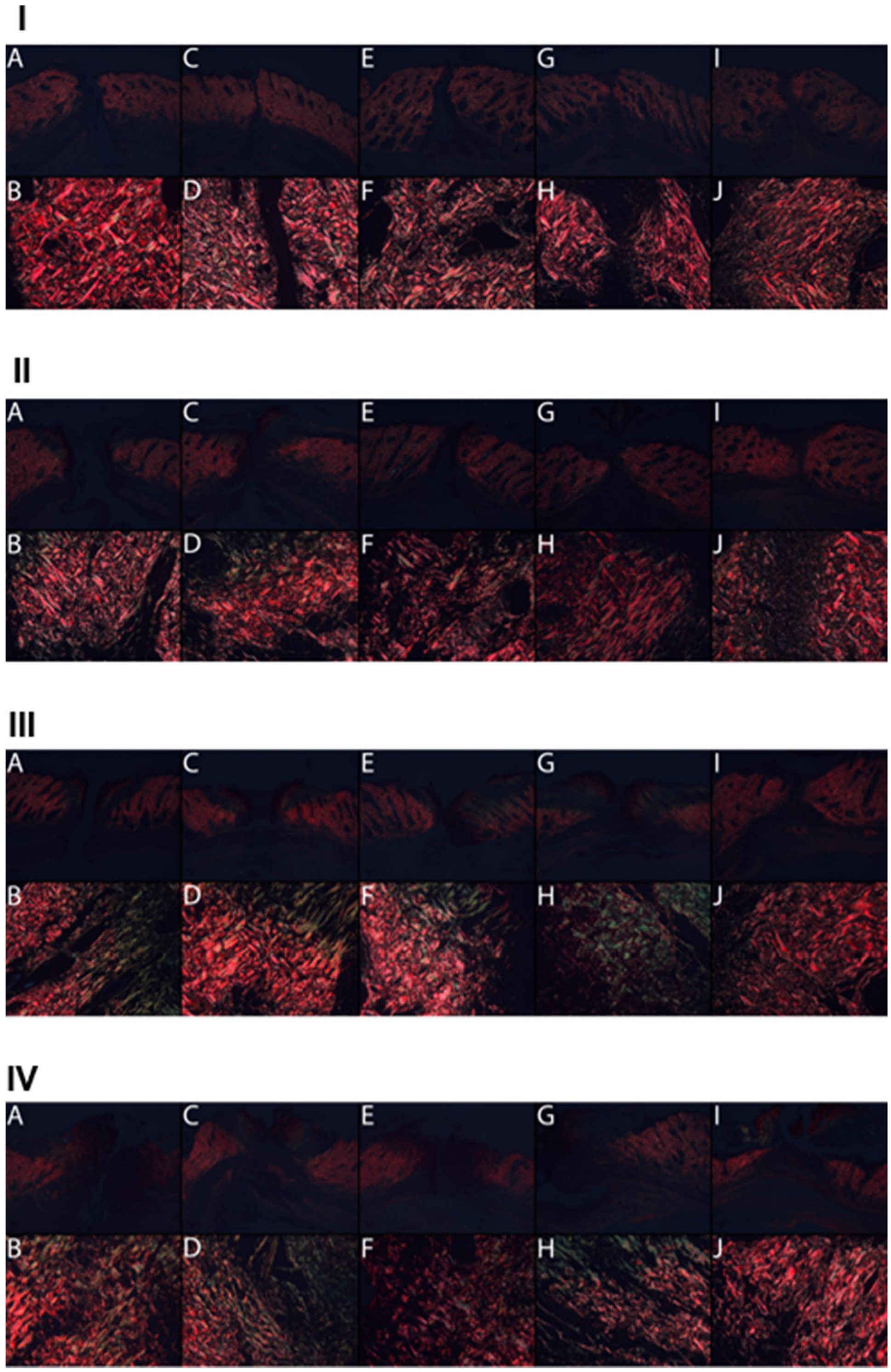

Fig. 4 Photomicrograph of Picrosirius red. I—Scalpel blade; II—electrocautery with a microdissection tip; III—electrocautery with a knife tip; IV—laser-stained diode sections. **A** Cut the 24-h group in 2.5 × magnification. **B** Cut the 24-h group in 20 × magnification. **C** Group 48 h in 2.5 × magnification. **D** Group 48 h in 20 × magnification. **E** Group 72 h in 2.5 × magnification. **F** Group 72 h in 20 × magnification. **G** Group 7 days in 2.5 × magnification. **H** Group 7 days in 20 × magnification. **I** Group 14 days in 2.5 × magnification. **J** Group 14 days in 20 × magnification

photomodulation [10, 16, 17]. Still, according to Bornstein [16], a rapid healing is promoted by the reduction of toxins as a result of increased lymphatic flow, consequently, accelerating the repair and inducing regeneration. On the other hand, the collateral damage caused to the tissues by the laser was evidenced by the presence of necrotic tissues and the exacerbated acute inflammatory reaction, presented even in the 14-day group. In this context, it is stated that the healing process was jeopardized, and even with the biomodulation, there is a considerable delay in tissue repair when compared to other instruments [8, 18, 19].

Table 3 Percentage of type III collagen according to time period and cutting material used

Period						
Instrument	24 h	48 h	72 h	7 days	14 days	Incision
Blade	31.85 (2.82)	35.83 (1.3)	35.66 (1.32)	32.8 (5.75)	32.74 (1.65)	33.78 (3.3)A
Microdissector	33.32 (3.85)	33.36 (3.69)	35.27 (2.84)	31.49 (2.13)	32.84 (4.29)	33.25 (3.43)AB
Knife	33.51 (2.64)	33.71 (1.78)	33.36 (4.27)	33.2 (2.03)	35.68 (2.86)	33.89 (2.81)A
Laser	31.35 (2.8)	30.86 (2.53)	30.39 (3.09)	33.39 (4.74)	31.9 (3.66)	31.58 (3.38)B
Period	32.51 (3.01)a	33.44 (2.94)a	33.67 (3.56)a	32.72 (3.81)a	33.29 (3.38)a	

*Different lowercase letters indicate statistical difference between columns; different uppercase letters indicate statistical difference between rows ($p < 0.05$)

Despite the scalpel blade having the best tissue response and an earlier cicatricial repair, there are no statistically significant differences that generate a lower clinical repercussion when using the microdissection tip. These results are similar to that found in the literature, opposing to the conventional electrocautery tip that demonstrates a considerable clinical difference, as well as the diode laser [20].

Histomorphometric analysis of tissue repair after conventional scalpel blade incisions, electrocautery tips, and diode laser showed different results, presenting a better performance of the microdissection tip, which presented low tissue damage and a repair process similar to the one seen by the scalpel blade, with the advantage of generating coagulation and hemostasis during the surgery, thus improving the view of the surgical field and shortening the procedure time. Although the scalpel blade presents minimal damage to the tissues, precision in the cut, and excellent capacity of tissue repair, it does not promote hemostasis and visibility is impaired. The results obtained here are similar to the systematic reviews and clinical trials that compared conventional or microdissection electrocautery tips in relation to scalpel blade, in which it is reported to lead to less time spent in the incision, better quality of the visual field, and improvement in hemostasis, overcoming the small statistical difference between periods of tissue repair [10, 20–23].

As for the conventional electrocautery tip, a high inflammatory reaction is caused by the extended contact surface, exposing the surroundings to greater heat damage, and a slower healing process. The laser had the worst performance in relation to tissue damage, with an extreme acute inflammatory reaction and longer healing time, suggesting that its use is not recommended under these conditions. It was probably due to the absorption that high-powered lasers have in soft tissues, leading to a necrotic extension, beyond the edges of the wound. However, its use in another type of tissue, such as mucosa, may be able to present a more adequate result [8, 10, 24].

It is believed that the use of a microdissection electrocautery tip leads to a faster and an effective incision, as it could be made by the scalpel blade, but adding a hemostatic effectiveness close to the conventional electrocautery tip. Improving the hemostatic conditions during surgery leads to a considerable reduction of the surgical procedure time.

In conclusion, the conventional scalpel blade showed a slight statistical superiority, in some evaluations, in relation to the microdissection tip. However, when the repair process was evaluated, there were no statistically significant differences in its time, which is associated with hemostasis of the operative field. Thus, its use can be recommended without prejudice or noticeable postoperative tissue repair alteration. The conventional electrocautery tip and the diode laser, on the other hand, should be avoided, as they considerably delay repair. At last, despite all efforts to mimic the tissue as closely as possible to the human oral mucosa, it is known that there are some limitations in this study, requiring clinical research on the human oral mucosa itself.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Renato Torres Augusto Neto, Cássio Amaro Comachio, Pedro Henrique de Azambuja Carvalho, Guilherme dos Santos Trento, and Valfrido Antônio Pereira-Filho. The first draft of the manuscript was written by Renato Torres Augusto Neto and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval All procedures performed during studies involving animals were in accordance to ARRIVE (Animal Research: Reporting of In Vivo Experiments) and with the ethical standards of the institution at which the studies were conducted—ethical approval was obtained by Animal Use Ethics Committee (CEUA) (Process nº 08/2017) from Araraquara Dental School, Sao Paulo State University – UNESP.

Consent to participate Not applicable for this study.

Consent for publication Not applicable for this study.

Competing interests The authors declare no competing interests.

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