Original Article

Clinical and Histological Comparison of Healing by Steel Scalpel, Diode Laser, and Radiofrequency in Palatal Wound: An Animal Study

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Received: 30-Aug-2022; **Revision:** 08-May-2023; **Accepted:** 05-Aug-2023; **Published:** 21-Sep-2023

Abstract

Background: Wound healing following periodontal soft tissue procedures can differ owing to different techniques, the feasibility of which can be determined through detailed macroscopic and microscopic observations. **Aims:** This study aimed to clinically and histologically evaluate palatal wound healing in rats by secondary intention after excision using a steel scalpel, diode laser, and radiofrequency. **Materials and Methods:** An excision was made in the edentulous anterior maxilla of 42 4-month-old male Wistar rats weighing 289–428 g. Part of the connective tissue was left in the surgical area to observe the dynamics of secondary intention wound healing. Three experimental groups were established: the steel scalpel, an 810-nm diode laser at a power output of 1.5 W in continuous mode, and a monopolar radiofrequency in a fully rectified waveform at 15 W. Clinical and histological analyses were performed on days 2, 4, and 7. Hemostasis, changes in body weight, defect size, epithelial gap, and inflammatory infiltration were evaluated. **Results:** The epithelial gap closed completely in all groups on day 7. Bleeding occurred significantly more in the scalpel group ($P < 0.001$). No significant changes were observed in body weight between the groups. Macroscopically, the mean wound area decreased over time in all groups. Wound healing was significantly slower in the laser group on day 2 and in the radiofrequency group on days 4 and 7 ($P < 0.001$). Microscopically, the laser created the cleanest wound area, with minimal inflammatory infiltration and no thermal injury. More damage occurred in the connective tissue of the radiofrequency group. Wound healing was observed on day 7 in all groups. **Conclusions:** Palatal wound healing with secondary intention yielded different outcomes in a rat model when different techniques were used. However, almost complete healing was observed in all wounds, which highlights the importance of the soft tissue left in the surgical area. Wound healing in periodontal soft tissue procedures is not compromised by different techniques, as long as the clinician has sufficient knowledge and experience.

Keywords: *Diode laser, radiofrequency, secondary intention, wound healing*

Introduction

Wound healing is a complex and dynamic process that involves highly organized cellular, humoral, and molecular mechanisms. The complete process comprises three overlapping phases: inflammation, proliferation, and remodeling.[1] Oral wound healing differs according to age, the presence of systemic diseases, such as diabetes mellitus, location (keratinized or non-keratinized mucosa), and type of technique or instrument applied.[2,3]

Periodontal wound healing has been clinically and histologically investigated for several years.[1,4] However, few studies have compared different techniques with

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Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_578_22

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How to cite this article: Elemek E, Gelmez YM, Hekimoğlu ER, Eşrefoğlu M. Clinical and histological comparison of healing by steel scalpel, diode laser, and radiofrequency in palatal wound: An animal study. Niger J Clin Pract 2023;26:1264-72.

a focus on secondary healing. Periodontal soft tissue surgical procedures that create an open wound, including harvesting connective tissue graft, gingivectomy, gingivoplasty, crown lengthening, depigmentation, and frenectomy, can be performed using several techniques. Steel scalpels are widely used. However, side effects such as bleeding, need for sutures, and postoperative patient discomfort, have led dental professionals to choose alternative instruments. Radiosurgery eliminates soft tissue by radio waves generated by a radiofrequency unit.[5] Radio waves pass through the cells and generate heat. Increasing the temperature to varying degrees has different biological effects on tissues resulting in incision, excision, or coagulation. Radiosurgery has the advantages of minimal bleeding, decreased chair time, and reduced or no postoperative discomfort.[6] Diode lasers are alternatives to excisional periodontal soft tissue procedures based on the principle of emission of light energy by an atom.[7] Diode laser waves are absorbed by hemoglobin and melanin, but not by hard tissues such as dentin and bone. Thus, diode lasers can be used safely on soft tissues around teeth and bones. Similar to radiosurgery, diode lasers have the advantages of less or minimal bleeding, no need for sutures, sterilization of the surgical site, and increased healing time.

Nevertheless, these lasers have disadvantages. Lateral thermal injury (LTI) created by the heat produced during radiosurgery and diode laser treatment can impair wound healing[8] and has been associated with intra- and postoperative discomfort and pain. As a result of thermal injury, lasers tend to produce more pronounced tissue changes, including an increased inflammatory response and delayed healing.[7,9]

The superiority of different instruments in soft tissue wound healing by secondary intention is still unclear. Therefore, this study aimed to clinically and histologically compare palatal secondary wound healing following the use of scalpel, diode laser, and radiofrequency in a rat model.

Materials and Methods

Animals and preparation of experimental model

Forty-two 4-month-old male Wistar rats, weighing 289–428 g, were male used in this study. The Ethics Committee of Animal Experiments of Bezmialem University approved the study protocol (approval number: 2020/232). All animals were housed in metal cages at room temperature and fed a standard laboratory diet. General anesthesia was achieved by the intraperitoneal injection of 10% ketamine (35 mg/kg; Ketasol, Wels, Austria) and 2% xylazine (15 mg/kg; Rompur, Istanbul, Turkey).

Before the experimental procedure, the rats were randomly divided into three groups of 14 rats each. An excisional wound of 3 mm in length and 1.5 mm in width was created on the hard palate of each rat between the incisors and molar teeth. Special attention was paid to perform the split-thickness dissection, leaving a part of the connective tissue on the palatal bone. No local anesthesia was administered to the hard palate. An endodontic file was used as a depth marker to create a wound depth of approximately 1 mm [Figure 1].

Experimental Groups

Three experimental groups were created [Figure 2]:

S: Excision by a steel scalpel (Swann Morton No. 15C, Sheffield, UK)

L: Excision using an 810-nm diode laser at an output power of 1.5 W in continuous mode (Cheese™, GIGAA Laser, Wuhan, China).

R: Excision using a radiofrequency instrument at 15 W in a fully rectified waveform (Surtron 50D; LED SpA, Aprilia, Italy).

Laser radiation was applied to the palatal mucosa of rats. The system comprised a fiber-optic tube with a tip diameter of 400 µm. The laser tip beam was kept perpendicular to the irradiated area and constantly moved to prevent LTI.[10] A monopolar radiofrequency device was used in the other group owing to its continuous circuit, which has less of a thermal effect than that associated with bipolar devices or electrocautery.[11]

As controls, two animals per group were sacrificed 15 min after each procedure. The remaining animals were sacrificed 2, 4, and 7 days postoperatively as day 7 corresponded to the end of the inflammatory phase of soft tissue wound healing.[4,12] The weights of the animals were measured before each procedure and sacrifice. Hemorrhage was also evaluated after different excisional methods (1, no; 2, limited; 3, needs gentle pressure; 4, needs coagulation; and 5, needs suturing). Gentle pressure with gauze was applied for hemostasis, when needed. No sutures were placed after any of the procedures. An experienced periodontist (EE) performed all procedures. The animals received no medications not to affect the natural healing process. To prevent mechanical injury to the wound area, no food was administered until 2 h postoperatively.

Tissue harvesting and histological evaluation

After sacrificing by CO_2 inhalation, the maxillae were separated and fixed in 10% buffered formalin for one day at room temperature. The specimens were decalcified in formic acid for 40 days until the bone had undergone sufficient decalcification for sectioning. The specimens were embedded in paraffin, and 3–4 µm sections were cut in the coronal plane using a microtome. Staining with hematoxylin-eosin and Masson's trichrome was performed. The sections were examined under a light microscope.

Wound healing measurement

To observe healing clinically, photographs were taken with a Canon EOS 50D camera (Canon Inc., Tokyo, Japan) immediately after sacrifice using a 15-mm University of North Carolina color-coded periodontal probe with markings (Hu-Friedy Manufacturing Inc., Chicago, IL, USA).

The images were subsequently analyzed using the Image J program (Wayne Rasband, National Institute of Health, USA) and calibrated with markings on a periodontal probe. On digital photographs, the boundaries of the wounds were determined, and in each group, the total wound area (mm^2) and the maximum laterolateral (L-L) and anteroposterior (A-P) distances (mm) were measured on days 2, 4, and 7 [Figure 3a-c]. Thereafter, the mean values were calculated, and the degree of wound healing was expressed as a percentage of the initial value at different time points.

Epithelial gap measurement

The epithelial gap, defined as the absence of wound tissue in the excision area, was measured using a x 4 Nikon light microscope (Eclipse 920248, USA) [Figure 4].

Statistical analysis

The sample size was calculated using the G-power software (type 1 error of 5% and statistical power of 80%). Data were analyzed using SPSS (SPSS Inc., Release 24.0 for Windows, Chicago, IL, USA). Descriptive statistics were produced, and means and standard deviations were calculated for all continuous measurements. To examine the changes in the wound area, A-P, and L-L dimensions between groups on different days, the Kruskal–Wallis test was used. For statistical significance, the Mann–Whitney U test was conducted between both groups. The significance level was considered as $P < 0.05$.

Results

All animals survived until the end of the study and were included in the statistical analyses (n = 42). No complications such as wound infection and swelling were observed. No significant differences in body weight between groups were observed during the seven days of observation (P = 0.98) [Figure 5].

Clinical evaluation

The mean score of hemostasis was 2.07 ± 0.82 for the scalpel group, 1.00 ± 0.00 for the laser group, and 1.14 ± 0.36 for the radiofrequency group. Gentle pressure was applied to only four animals in the scalpel group. No hemorrhage was observed in the laser group, but hemorrhage was limited in the radiofrequency

Figure 1: Endodontic file used as a depth marker (1 mm)

Table 1: Macroscopic wound area measurements, mean and standard deviation in scalpel, laser, and radiofrequency group

Measured parameter	Scalpel group	Laser group	Radiofrequency group	P
L-L distance (mm)				
Day 2 (n=4)	2.01±0.25	3.53±0.56	2.37±0.54	0.03*
Day 4 (n=4)	0.74±1.01	1.64±0.49	1.86±0.43	0.14
Day 7 (n=4)	0.19±0.38	0.35±0.41	0.46±0.32	0.69
A-P distance (mm)				
Day 2 (n=4)	1.14±0.15	1.41±0.28	1.61±0.50	0.11
Day 4 (n=4)	0.46±0.57	0.86±0.14	1.47±0.55	0.07
Day 7 (n=4)	0.12±0.25	0.22±0.26	0.54±0.40	0.14
Total area (mm^2)				
Day 2 (n=4)	2.19±0.87	4.37±0.56	4.21±0.88	0.00*
Day 4 (n=4)	0.28±0.36	1.81±0.96	2.93±0.57	0.00*
Day 7 (n=4)	0.02±0.03	0.05±0.12	0.44±0.78	0.00*

*Kruskal–Wallis test

Table 2: Changes between initial and final area, mean and standard deviation (SD) in scalpel, laser, and radiofrequency group relative to day 0 (4.5 mm diameter)

Measured parameter	Scalpel group	Laser group	Radiofrequency group	P
Total area fraction relative to day 0 (%)				
Day 2 (n=4)	53.77±8.14	101.66±20.07	86.47±36.85	0.00*
Day 4 (n=4)	18.75±26.61	31.94±13.53	63.39±32.83	0.00*
Day 7 (n=4)	2.00±4.00	3.44±4.29	6.83±4.56	0.00*

*Kruskal-Wallis test

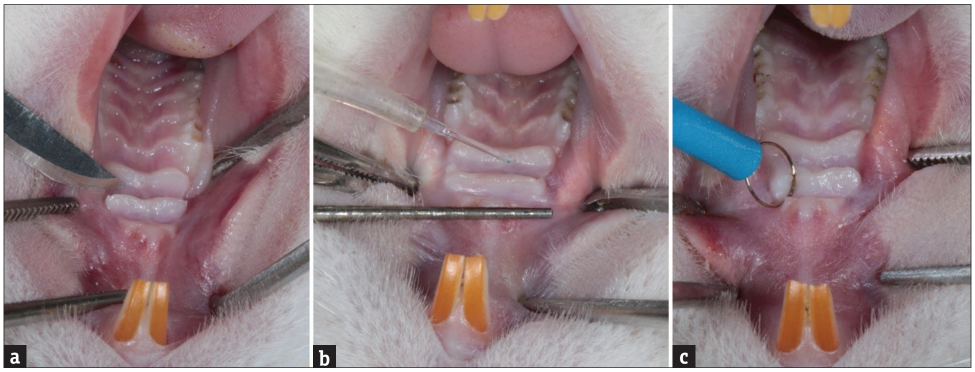

Figure 2: Excision by a scalpel (a), diode laser (b), and radiofrequency (c)

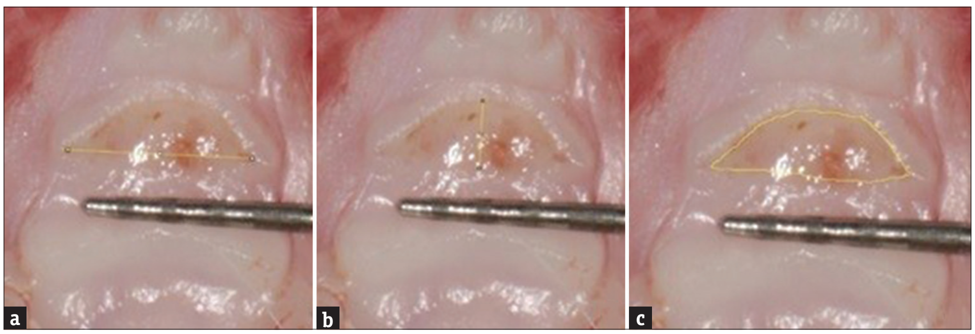

Figure 3: Measurement of wound area; the final L-L distance (a), the final A-P distance (b), and the final wound area (c)

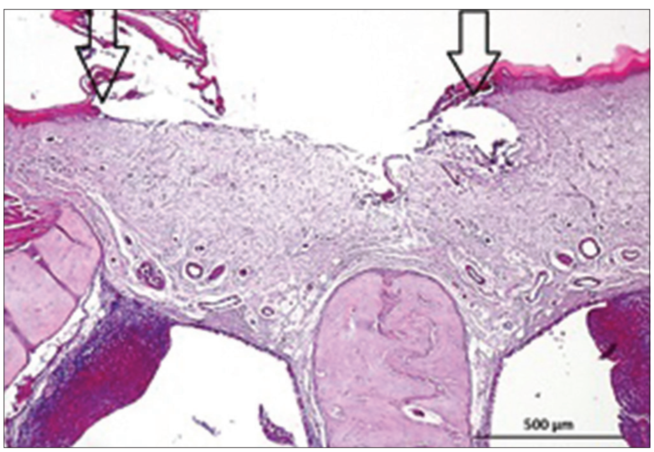

Figure 4: Hematoxylin and eosin staining (x4) for epithelial gap measurement

group. The laser and radiofrequency groups differed significantly from the scalpel group ($P < 0.001$).

The wound area measurements of all groups upon clinical examination are given in Table 1. The L-L distance was significantly greater in the laser group on day 2 than in the other groups ($P < 0.05$). It decreased on day 4, with no significant association between groups at the end of day 7 ($P = 0.69$). The A-P distance was slightly shorter in the scalpel group than in the other groups on day 2. A small decreasing trend occurred on day 4 in the radiofrequency group. On day 7, the wound area continued to decrease, but the difference between groups was not significant ($P = 0.14$). The total wound area was significantly smaller in the scalpel group than in the other groups on days 2 and 4 ($P < 0.01$). The decrease was significantly slower in the radiofrequency group on day 4 ($P < 0.01$). Almost complete healing was observed in the scalpel and laser groups but not in the radiofrequency group on day 7. This difference was statistically significant ($P < 0.01$) [Figure 6].

Further analyses were conducted to observe healing rates relative to day 0 [Table 2]. Healing was significantly slower in the laser group than in the scalpel group on day 2 ($P < 0.01$). In the radiofrequency group, the total wound area continued to heal significantly less on day 4 ($P < 0.01$). The total area fraction change was still significantly slower in the radiofrequency group than those in the other groups at the end of day 7 ($P < 0.01$).

Microscopic evaluation

The control samples were evaluated in relation to the different excisional procedures. In all groups, the epithelium was completely lost. Connective tissue was highly damaged in the radiofrequency group. It was partially lost in the scalpel group [Figure 7a-c], whereas a minimum loss was observed in the laser group. Hemorrhage to the surface and into the damaged tissue was evident in the scalpel group. The wound surface was highly even in the laser group [Figure 7d-f]. In the radiofrequency group, connective tissue next to the surface was condensed, as in the burns [Figure 7g,i].

On day 2, the epithelium was still absent in all groups. The wound area was highly even and clean in the laser group. In the scalpel group, a prominent inflammatory reaction and hemorrhage were observed in the connective tissue, especially next to the surface. Many pieces of debris were observed on the wound surface [Figure 8a-c]. In the laser group, a mild inflammatory reaction was observed near the surface. Tissue exfoliation and hemorrhage were not detected [Figure 8d-f]. Inflammation was much lower than that observed in the scalpel group. Mild-to-moderate

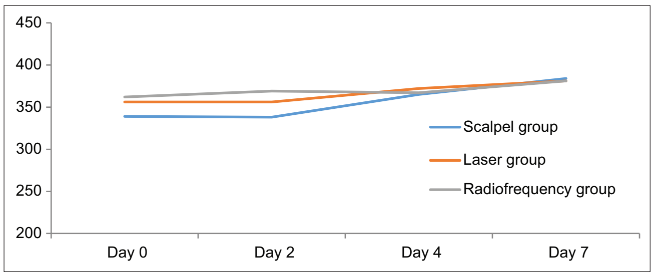

Figure 5: Changes in body weight with time

inflammation and occasionally a small area of hemorrhage were observed in the radiofrequency group. Tissue condensation was no longer obvious [Figure 8g,i]. Instead, mild edema was detected in some of the samples.

On day 4, the wound area had not completely healed in any group. In the scalpel group, the keratinized stratified squamous epithelium was irregular and sometimes thicker than expected. Occasionally, it contained large vacuoles [Figure 9a-c]. Moreover, it was remodeled and nearly completely continuous in the laser-treated group. It was thin and not fully keratinized. Papillae were not obvious [Figure 9d-f]. In the radiofrequency group, the wound lips remained separated. Mild-to-moderate inflammatory reactions were observed [Figure 9g,i].

On day 7, all wounds had healed. No inflammatory reactions or hemorrhages were observed. The epithelium and connection between the epithelium and lamina propria were intact in the scalpel group [Figure 10a-c]. The keratinized stratified squamous epithelium was highly thick and keratinized in the laser group [Figure 10d-f]. The density of connective tissue was higher than that of healthy tissue in the radiofrequency group [Figure 10g,i].

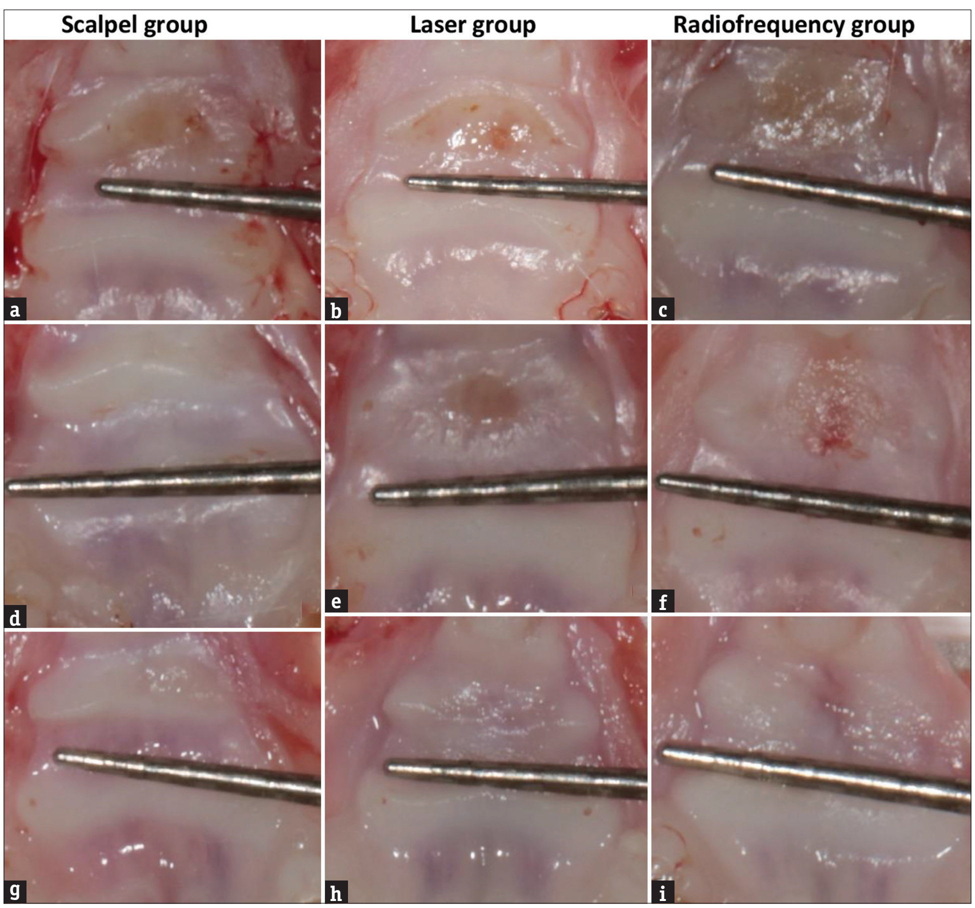

Figure 6: Clinical photographs of the palatal wounds showing gradual healing at day 2 (a-c), 4 (d-f), and 7 (g-i)

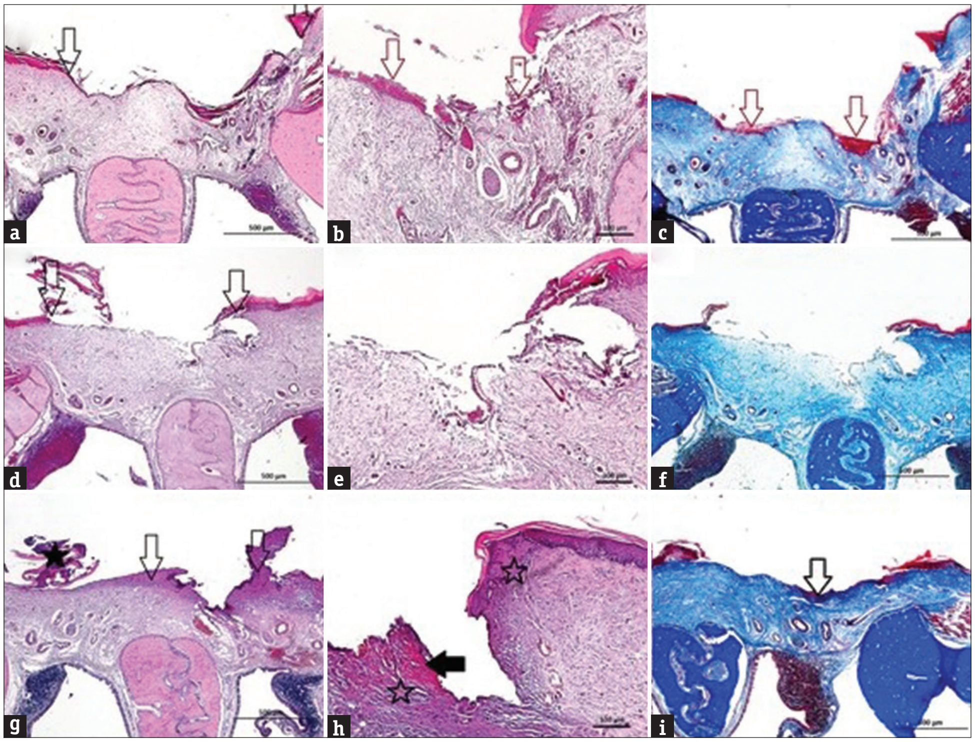

Figure 7: Results at Day 0. (a-c) At the scalpel-induced wound area (lips were marked), the epithelium was lost, the connective tissue was partially lost, otherwise damaged. Hemorrhage to the surface (red arrows) and into the damaged tissue was evident. (d-f) At the laser-induced wound area (lips are marked), the epithelium was absent. Connective tissue loss and damage were less than those of the scalpel-induced wound. Hemorrhage to the surface and into the damaged tissue was nearly absent. (g,i) Radiofrequency-induced wound area seemed larger than those of the scalpel and laser-induced wound area. The epithelium was lost; the connective tissue was highly damaged. Large, damaged tissue pieces were observed at the surface (black asterisk). The surface was highly uneven. Hemorrhage into the tissue was observed (black arrow). Connective tissue next to the surface was condensed, as in the burns (arrows). There were many pyknotic nuclei at that area (asterisks. *H-E; ×4, H-E; ×10, Massond area seemed larger than those, for each line*

In all groups, the epithelial gap was present on days 2 and 4, except in the laser group on day 4, and was almost closed. However, the presence of keratinized stratified squamous epithelium confirmed the closure of the epithelial gap in all three groups at day 7.

Discussion

Although previous studies have investigated the healing process of palatal wounds, data comparing secondary healing created by different instruments remain incomplete. This study evaluated the effects of excision using a scalpel, diode laser, and radiofrequency device on secondary wound healing, which is characterized by hemostasis, inflammation, proliferation, and remodeling.[13] The animals were observed for seven days as the seventh day typically corresponds with the end of the inflammatory phase.[12] Chaushu *et al*.[4] studied palatal wounds in rats with primary intention and observed healing for 14 days. They made a single incision to expose the periosteum. Weinberg *et al*.[14] created 4.2-mm excisional palatal wounds in rats and examined the healing for three weeks. In our study, part of the connective tissue was left to mimic the periodontal soft tissue procedures in humans. Thus, healing was observed for seven days, keeping the number of animals at a minimum. Closure of the epithelial gap in all groups on day 7 confirmed our study protocol.

Chaushu *et al*.[4] created an experimental rat model to investigate palatal wound healing. A single incision was made using a full-thickness flap and subsequently repositioned with sutures. A year later, the same group assessed excisional palatal mucoperiosteal wound closure and compared the difference between young and old rats.[3] Excisional palatal wound healing has also been observed by Weinberg *et al*.[14] in a rat model. Parallel to the findings of a recent clinical study,[15] they highlighted the importance of the amount of soft tissue remaining at the wound area and suggested comparing the macroscopic and microscopic results of different harvesting techniques. In this study, palatal wounds were created by secondary intention using a scalpel, diode laser, and radiofrequency device and subsequently analyzed clinically and histologically. Despite the use of different techniques, similar to other animal and human studies,[4,14,16] the periphery of the mucosal wounds healed faster than the center of the wound in our study. Thus, macroscopic soft tissue wound healing by secondary intention during periodontal procedures may be similar when different instruments are used.

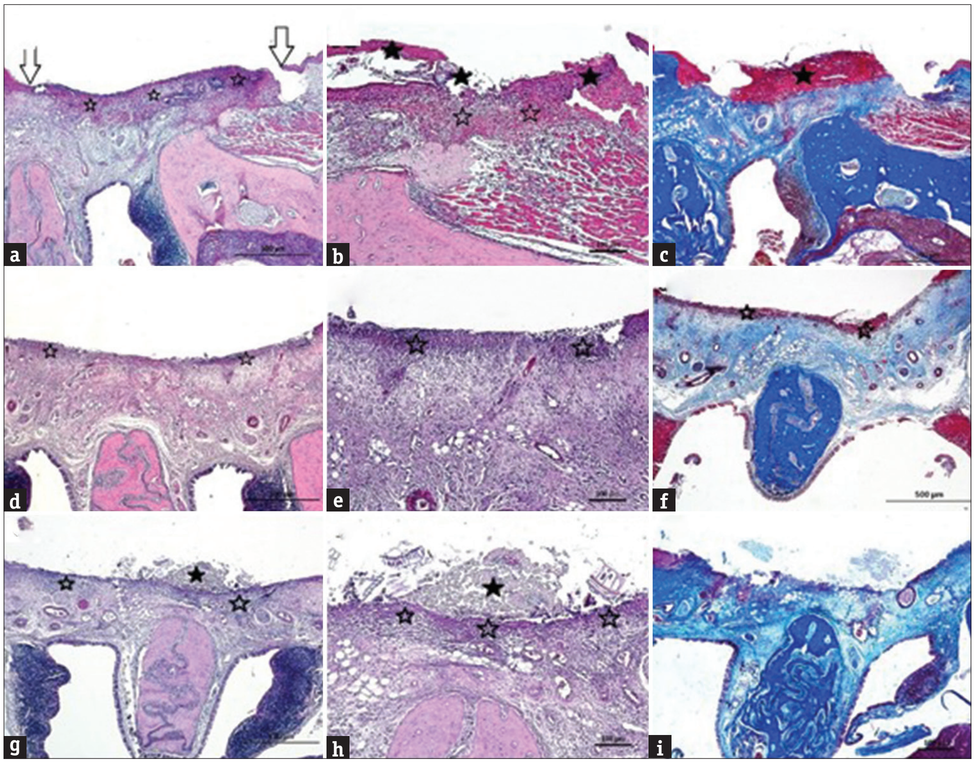

Figure 8: Results at Day 2. (a-c) At the scalpel-induced wound area, a prominent inflammatory reaction and hemorrhage were observed (asterisks). Lots of debris were seen at the wound surface (black asterisks). (d-f) The laser-induced wound area was highly even and clean. Hemorrhage was absent. Mild inflammatory reaction next to the surface was observed (asterisks). (g,i) Prominent tissue debris was detected at the surface of the radiofrequency-induced wound (black asterisks). Mild-to-moderate inflammation (asterisks) and occasionally small area of hemorrhage were observed. Tissue condensation was not obvious anymore. *H-E; ×4, H-E; ×10, Massonrisks) and occasionally small ar, for each line*

Scalpel wounds cause the extravasation of blood and lymph, inducing a more pronounced inflammatory response, including swelling.[9] In our clinical examination, hemostasis was significantly higher in the scalpel group than in the other groups, which can be explained by the damage in capillary. A significant increase was also observed in the L-L distance in the laser group compared to that in the other groups on day 2. A delay in capillary proliferation might have caused an increase in the wound area. In addition, thermal injury, varying from transient heating to protein denaturation and burning, may also have caused impaired healing in laser-induced wounds.[9] However, the histological findings of this study did not confirm this hypothesis. The application of lasers at lower outputs is known to be less effective, although it minimizes thermal injury. In this study, the diode laser was used in continuous mode at an output power of 1.5 W, which is considerably low and thus explaining the absence of thermal injury. In scalpel wounds, thermal injury does not occur owing to better clinical outcomes in the total wound area on days 2 and 4, as observed in this study. However, at the end of day 7, no clinically significant difference was observed between the scalpel and laser groups. Conversely, healing was slower in the radiofrequency group. Thus, for periodontal soft tissue procedures, a clinician can consider alternative techniques to scalpel with special caution regarding the amount of energy applied to the tissues to prevent LTI.

In histological examination, hemostasis and inflammatory reaction were maximum at the scalpel-induced wound area at day 2. Conversely, no surface debris was present in the laser group, and a minimal inflammatory reaction was observed. Irradiation with a diode laser conventionally eliminates bacteria.[17] This may have positively influenced the improved outcomes of laser-induced wound healing observed histologically on day 2. The remodeled epithelium almost covered the scalpel-induced and laser-induced wound area, but not the radiofrequency-induced area on day 4. Despite the slow healing in radiofrequency-induced wounds, as observed by incomplete papillae remodeling, separate epithelium and lamina propria, and dense connective tissue, all wound types recovered on day 7. Arcangelo *et al*.[9] compared diode laser and scalpel incision in a split-mouth study in rats. They found better outcomes in favor of the scalpel procedure owing to the LTI created by the diode laser. However, they applied power outputs of 4 and 6 W, which were almost four times higher than those used in this study. The type of electrode affects the precision

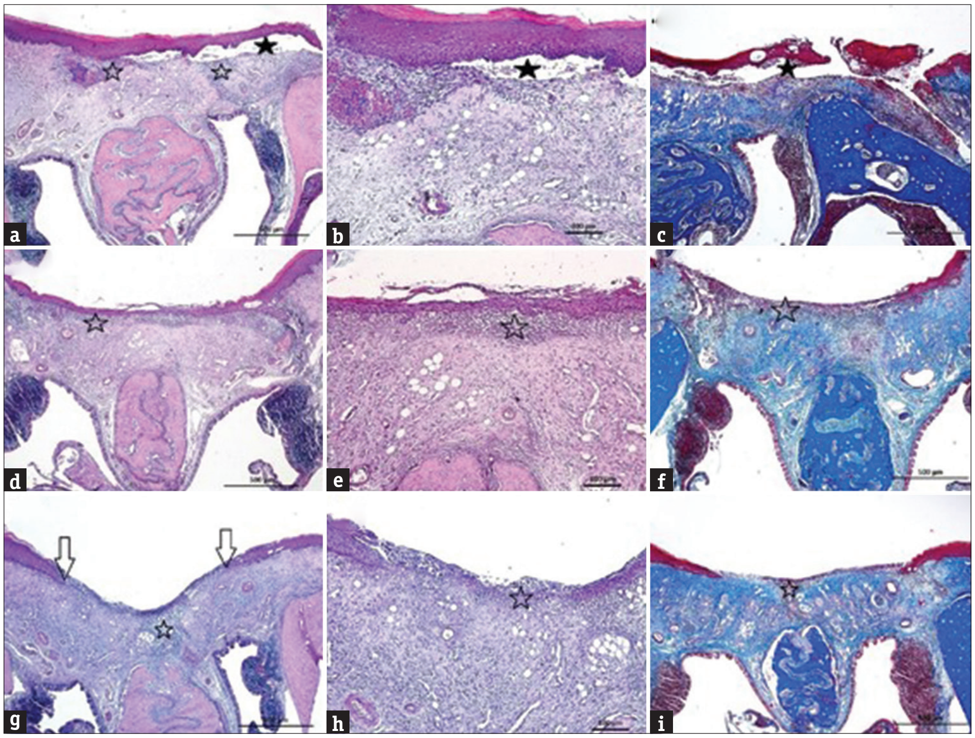

Figure 9: Results at Day 4. (a-c) At the scalpel-induced wound area, tissue was not completely healed. Connection between the epithelium and lamina propria was not intact (black asterisks). Inflammatory reaction was obvious (asterisks). Hemorrhage into the connective tissue was seen (blue asterisks). (d-f) Laser-induced wound area was clean. The keratinized stratified squamous epithelium was remodeled although thin and not fully keratinized. Epithelium-connective tissue connection was intact. Mild-to-moderate inflammatory reaction was observed. (g,i) Radiofrequency-induced wound area was not completely healed. Wound lips were still separated (arrows). Mild-to-moderate inflammatory reaction was observed (asterisks). *H-E; ×4, H-E; ×10, Massonion was observed (asterisks). le, for each line*

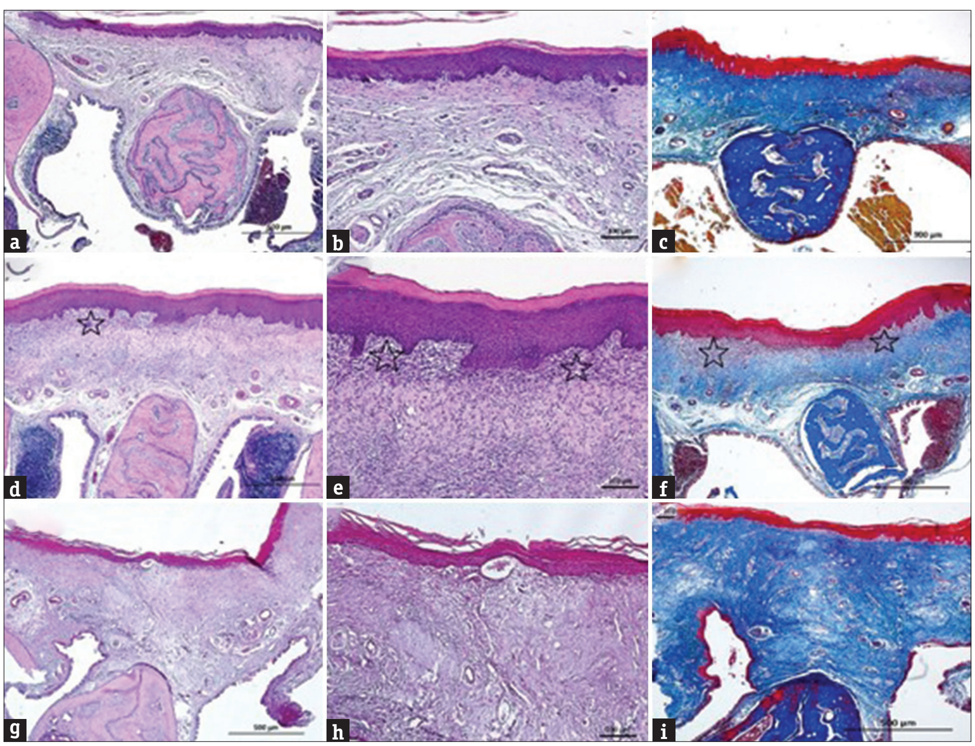

Figure 10: Results at day 7. (a-c) Scalpel-induced wound area was regenerated. Epithelium was intact although its width was not always uniform. Connective tissue was highly dense. (d-f) Laser-induced wound area was regenerated. The keratinized stratified squamous epithelium was highly thick and highly keratinized. (g,i) Radiofrequency-induced wound area was regenerated. The keratinized stratified squamous epithelium was remodeled although it was thin. Connective tissue was highly dense. Inflammatory reaction and hemorrhage were not detected. *H-E; ×4, H-E; ×10, Massonnd area was regenerated. The ker, for each line*

of the tissue cut and the LTI.[18] Unlike electrocautery, resistance develops within the tissue, and the cutting tip does not heat, even in a fully rectified waveform.[6] In our study, the slower healing in the radiofrequency group may be explained by the type of electrode tip used, which was considerably large, and the relatively low power output of 15 W. Studies with radiofrequency devices at different output levels and electrodes of different sizes are needed to define the optimum soft tissue healing.

One limitation of this study was the use of an animal model. Human studies evaluating soft tissue wound healing using different techniques would have provided more appropriate results for application in clinical practice. Another limitation is that, owing to the limited number of animals, a diode laser and radiofrequency device were used at one specific output in this study. Studies comparing varying energy levels in the same instruments, with particular attention to LTI, should be conducted. To improve wound healing, stimulation by low-level laser therapy and the use of some anti-inflammatory agents is a new area of research[19,20] and should be investigated in more studies.

In conclusion, in palatal wounds created by secondary intention in a rat model, hemostasis was significantly better in the laser and radiofrequency groups than in the scalpel group. Generally, healing was slower in the laser group on day 2 and in the radiofrequency group on days 4 and 7 than in the other groups. However, the epithelial gap was closed, and all wound types healed in all groups by the end of day 7. Clinicians should consider patient comfort, cost-effectiveness, and the need for experience when choosing the optimum technique for periodontal soft tissue procedures.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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