Evaluation of lateral thermal damage and reepithelialization of incisional wounds created by CO_2-laser, monopolar electrosurgery, and radiosurgery: a pilot study on porcine oral mucosa

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Objective. This study aims to compare lateral thermal damage (LTD) produced by CO_2-laser, monopolar electrosurgery (MES), and radiosurgery (MRS) and its effects on the reepithelialization of oral mucosa incisional wounds.
Study design. Five adult swine were submitted to standardized incisions at the tongue and gingiva by MES, MRS, CO_2-laser and scalpel. Full-thickness specimens were harvested sequentially on days 1 and 3. All specimens were formalin fixed, paraffin embedded, cut, and stained with hematoxylin-eosin to quantify LTD extent. Sections of day 3 were stained for Ki-67 to assess epithelial proliferation at the incision margins. A multiple linear regression model and an analysis of variance were used to determine the correlation of each instrument with LTD extent and Ki-67 expression respectively.
Results. LTD was most extensive in the CO_2-laser but did not differ between the MES and MRS groups. No statistically significant differences regarding reepithelialization were noted among the investigated instruments.
Conclusions. CO_2-laser produced more extensive LTD, without evident impact on reepithelialization. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;113:741-747)

Although steel scalpel is considered to be the instrument of choice for surgical incisions, CO_2-laser and monopolar electrosurgery have been well documented as alternative modalities in oral and maxillofacial surgery. Despite the easy use, wide availability, incision accuracy, and minimal damage to adjacent tissues, the use of steel scalpel is "limited" in highly vascular areas, such as the head and neck region, owing to the imperative need for hemostasis.[1]

To combine tissue incision and/or ablation with adequate hemostasis alternative instruments, such as monopolar electrosurgery (MES) and CO_2-laser, have been developed. Monopolar radiosurgery (MRS), delivering to the tissues an electrosurgical current of higher frequency (3.8-4 MHz) than conventional monopolar electrosurgery (0.5-1 MHz), has been introduced lately in the clinical practice. On use of all the above instruments, various forms of electromagnetic radiation (i.e., high-frequency alternating electrical current and infrared light, respectively) are converted to heat which is diffused through the surgical field, inducing both beneficial and unintended tissue effects.[2,3]

Heat diffusion through the surgical field, apart from causing vessel sealing and providing excellent hemostasis, also considerably reduces the bacterial population, thus minimizing the risk of intraoperative contamination. It also prevents the occurrence of micrometastasis due to intraoperative dissemination of cancer cells into blood microcirculation upon use of these instruments for incisional biopsy or surgical treatment of malignant lesions.[4-6]

However, heat diffusion also causes denaturation of intracellular proteins and collagen to unintended tissues, producing a characteristic pattern of thermal injury compatible with coagulative necrosis in a varied distance from the incision, described as "lateral thermal damage" (LTD). This has been associated with intraoperative (discomfort or pain) and/or postoperative (disturbed function of adjoining vital structures, such as the maxillary/mandibular bone or the dental pulp, delayed and/or disrupted wound healing, and conspicuous scarring) morbidity and has substantially limited the use of MES and CO_2-laser for skin or oral mucosa biopsy harvesting.[2,7-11]

The objective of the present pilot study was to evaluate the interaction of alternative surgical instruments on the porcine oral mucosa. Comparisons were made regarding lateral thermal damage and its impact on the initial stages of wound healing in oral mucosa incisions performed by CO_2-laser, MES, and MRS. The LTD produced by each investigated instrument was measured and compared with that of the others. Moreover, its effect on the primary reepithelialization process was

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determined and compared among the investigated instruments.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Experimental Surgery and Surgical Research at "Laiko" General Hospital of Athens. The protocol had been approved by the Animal Care Committee of the Prefecture of Athens. The experimental procedure was performed under the guidance of a veterinarian in compliance with international guidelines regarding care and treatment of experimental animals.

Animals

Five adult healthy swine (3 male and 2 female) of similar body weight (22.8-25 kg) were used in this pilot study.

Surgical devices

The following devices were used: 1) an MES unit of the MD62 type provided by Martin, operating at 1 MHz; 2) a continuous wave CO_2-laser unit of the Smart US-20D type provided by Deka; and 3) an MES unit of the Surgitron FFPF EMC-Vet Surg type provided by Elleman International, operating at 3.8 MHz. A steel scalpel (no. 15 blade) was used to create the incisions of the control group. MES and MRS incisions were performed by active electrodes of commensurate dimensions and geometry (needle-like electrodes), using the "cut(ting)" mode of each device.

Surgical procedure

General anesthesia was induced with intravenous injection of propophol and phentanyl and maintained with halothane after intubation of the animals. Chemoprophylaxis and corticosteroids were also administered intravenously. The operative site was cleansed and draped in a sterile fashion.

Standardized full-thickness longitudinal incisions, ~1.5-2 cm long, were made by the 4 investigated instruments (steel scalpel, CO_2-laser, MES, and MRS) at the dorsal surface of the tongue and the maxillary free and attached gingiva, in a randomly assigned sequence (day 1).

Full-thickness rectangular specimens, symmetrically extending on either side of one-half of the incision were harvested by scalpel immediately afterward (day 1); the wounds were closed with 3.0 silk sutures in a simple interrupted fashion. Similar specimens extending on either side of the other half of the incision were harvested by scalpel 48 hours later (day 3).

The animals were killed by intravenous administration of propophol and pentobarbital.

Histologic and immunohistochemical evaluation

All specimens (40 taken on day 1 and 40 taken on day 3) were fixed in 10% neutral-buffered formalin solution and embedded in paraffin. Histologic evaluation was performed in 3–5-μm-thick tissue sections stained with hematoxylin-eosin (HE). The incision margins of the specimens were examined under low- and high-power light microscopy by 2 independent histopathologists, who were completely unaware of the surgical instrument applied in each specimen, using a calibrated eyepiece grid.

Twenty sections derived from specimens taken from the tongue on day 3 were stained immunohistochemically to detect Ki-67 nuclear antigen as a marker of epithelial cell proliferation. For the immunohistochemical staining, the sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 3% v/v H_2O_2 in water for 5 minutes. Sections were incubated overnight with the primary antibody (MIB-1 monoclonal antibody, dilution 1:100; Dako) and then incubated in the biotin-conjugated secondary antibody for 10 minutes. The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibody with the use of an LSAB System Universal Kit (Dako) for 10 minutes; DAB solution was used as chromogen for 5 minutes; all sections were counterstained with Mayer hematoxylin for 1 minute and mounted. For negative control slides, the antibody was omitted.

In the HE-stained sections of day 1, the LTD extent was assessed by measuring the maximum distance (in μm) from the incision margins where coagulative necrosis was detected as a distinct zone of hyalinization with altered staining (at an ×10 magnification). In each immunohistochemically stained section of the 20 specimens harvested from the tongue on day 3, the expression of the Ki-67 nuclear antigen adjacent to the incision was assessed by measuring the number of moderately or intensely stained nuclei in the basal and parabasal layers of the epithelium within a distance of 500 μm from the incision margins (at an ×20 magnification). In HE-stained sections of day 3, inflammatory infiltration (extent and intensity) was assessed adjacent to the incisions.

Statistical analysis

The LTD extent and epithelial proliferation adjacent to the incision margins were compared among the 3 experimental (CO_2-laser, MES, MRS) and control (steel scalpel) groups.

To determine the correlation between LTD extent and applied instrument, a multiple linear regression model was used, after adjustment for the operative site (tongue and gingiva). To investigate the effect of LTD

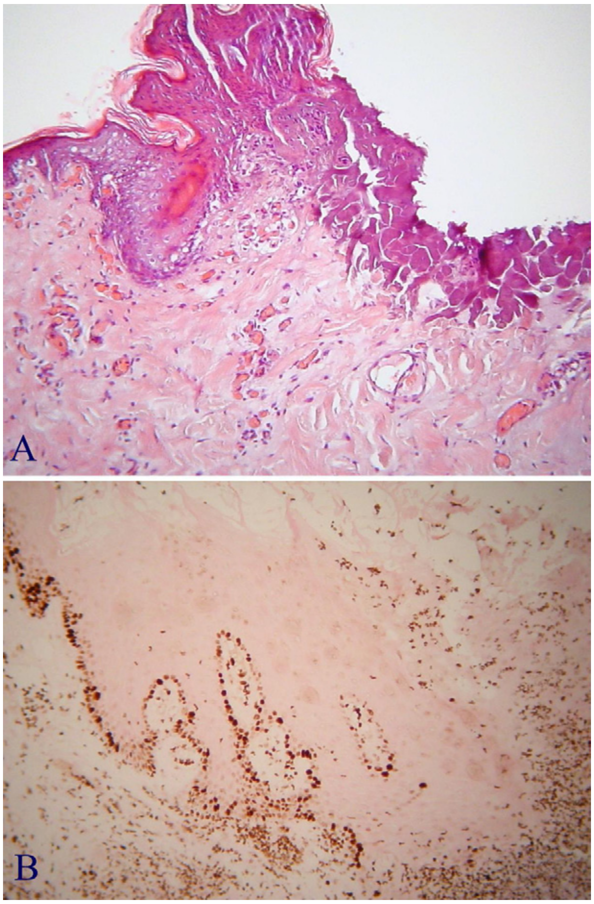

Fig. 1. **A,** Coagulative necrosis adjacently to an incision performed by monopolar radiosurgery on the maxillary free and attached gingiva (hematoxylin-eosin, original magnification ×10). **B,** Ki-67 expression in the basal and parabasal epithelial layers and in scattered inflammatory cells of the subepithelial connective tissue (immunohistochemical staining with MIB-1, original magnification ×20).

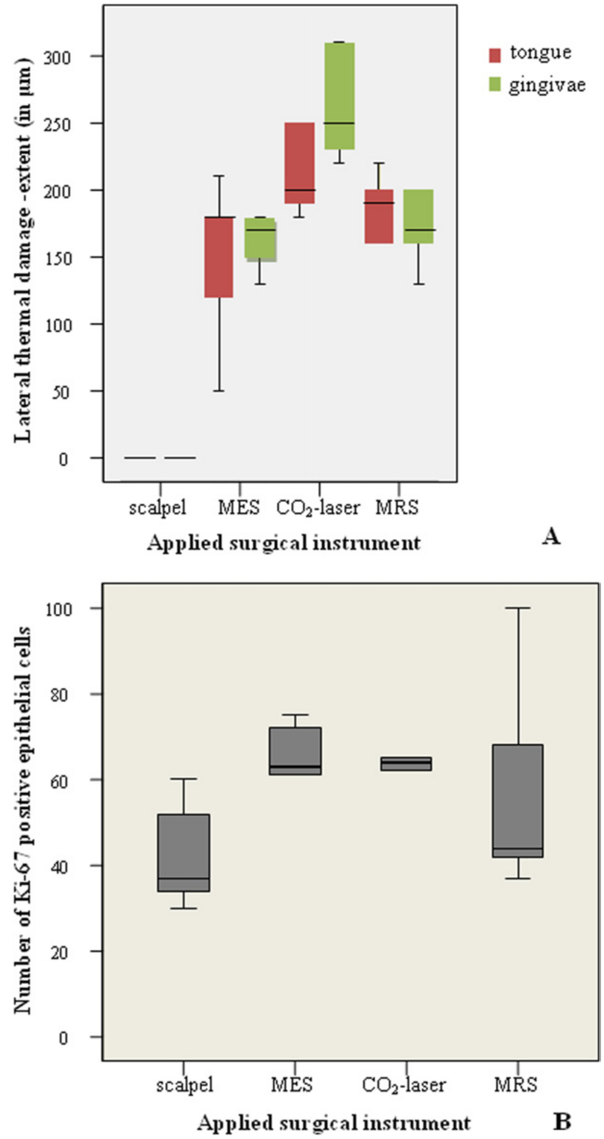

Fig. 2. **A,** Comparison of the representative values of lateral thermal damage extent among the applied instruments, depending on the operative site (tongue and gingiva). **B,** Comparison of the representative values of Ki-67-expression among the applied instruments for specimens taken from the tongue. *MES*, monopolar electrosurgery; *MRS*, monopolar radiosurgery.

on the reepithelialization process, the correlation between Ki-67 expression (as a marker of epithelial cell proliferation) and applied instrument was determined, by using an analysis of variance (ANOVA). For all analyses, performed with the SAS statistical package (version 9.1, SAS Institute, Cary, NC), significance was determined at the 95% level ($P < .05$).

RESULTS

Evaluation of lateral thermal damage

Histologic evaluation of the HE-stained sections from specimens harvested on day 1 revealed lesions compatible with thermal damage adjacent to incisions performed by CO_2-laser, MES, and MRS. Coagulative necrosis was evident as a distinct zone of basophilic hyalinization, exhibiting a characteristic loss of the collagen fibrillar texture (Fig. 1, *A*). A comparison of the representative LTD extent values among the applied instruments, depending on the operative site (tongue and gingiva) for specimens harvested on day 1 is depicted in Fig. 2, *A*.

Results of multiple linear regression determining the correlation between LTD extent and applied instrument, after adjustment for the operative site (tongue and gingiva), are presented in Table I. LTD extent was found to differ significantly among the applied instruments: Considering scalpel as the baseline, mean LTD extent increased by 162 μm, 258 μm, and 179 μm on

Table I. Multiple linear regression results regarding the correlation between lateral thermal damage extent and applied instrument in tongue and gingiva

Variable	*Category*	*Coefficient* β	*SE*(β)	*P value*
Applied instrument	Scalpel	Baseline		
	MES	162.00	24.44	<.0001
	CO_2-laser	258.00	24.44	<.0001
	MRS	179.00	24.44	<.0001
Operative site	Tongue	Baseline		
	Gingiva	25.50	17.28	.149

MES, Monopolar electrosurgery; *MRS,* monopolar radiosurgery.

use of MES, CO_2-laser, and MRS, respectively. LTD extent was not found to differ significantly (P = .149) depending on the surgical site when tongue and gingiva were compared.

Evaluation of reepithelialization

Evaluation of immunohistochemically stained sections from specimens harvested on day 3 revealed considerable Ki-67 expression in the basal and parabasal epithelial layers, and Ki-67 stained cells (mostly inflammatory and endothelial cells, fibroblasts, and skeletal muscle fibers) were also detected in the subepithelial connective tissue (Fig. 1, *B*). A comparison of the representative Ki-67 expression values among the applied instruments for specimens harvested from the tongue on day 3 is depicted in Fig. 2, *B*.

ANOVA results determining the correlation between Ki-67 expression and applied surgical instrument for specimens harvested from the tongue on day 3 are presented in Table II. Mean Ki-67 expression did not differ significantly (P = .267) among the applied surgical instruments, based on the findings from the tongue specimens.

Evaluation of inflammatory infiltration

Histologic evaluation of HE-stained sections from specimens, harvested on day 3, revealed a diffuse inflammatory infiltration of varied intensity, with a mixed-type inflammatory cell infiltrate, featuring predominantly polymorphonuclear cells and mononuclear-macrophages, followed by lymphocytes and plasma cells. The inflammation extended mostly into the subepithelial connective tissue and subjacent muscular substrate, but inflammatory cells were also evident in the epithelium (Fig. 3, *A*).

A tendency toward more extensive inflammatory infiltration was evident adjacent to incisions performed by CO_2-laser, but the relevant findings were not compared among the experimental groups, owing to the presence of infectious and other local confusing factors at the operative sites. Microabscesses and accumulations of microorganisms (mostly cocci) were observed in the upper epithelial layers in almost all specimens (Fig. 3, *B*), taken from the tongue and gingivae on day 3.

Table II. Analysis of variance results regarding the correlation between Ki-67 expression and applied instrument for specimens taken from the tongue

Source of variation	*SS*	*df*	*MS*	*F*	*P value*
Among groups	1,342.80	3	447.60	1.44	.267
Within groups	4,958.00	16	309.88		
Total	6,300.80	19			

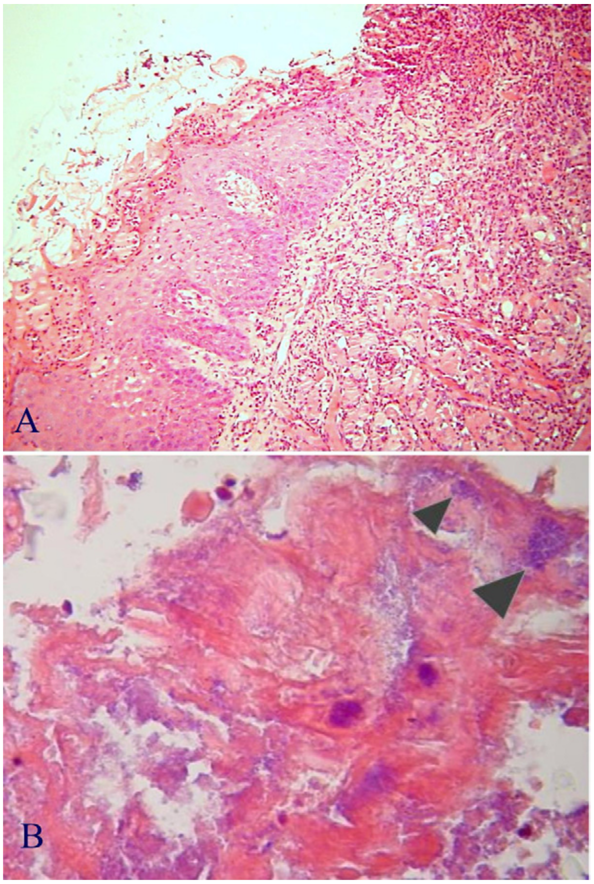

Fig. 3. Specimen taken from the tongue on day 3. **A,** Intraepithelial microabscesses with inflammatory cells as well as diffuse inflammatory infiltration in the muscular substrate of the tongue are evident. **B,** Microorganisms (cocci; *arrowheads*) are noted in the upper epithelial layers. Hematoxylin-eosin, original magnification ×20 (A) and ×40 (B).

DISCUSSION

Much research has been devoted to comparing LTD and healing of incisional wounds created by various heat-generating instruments, such as CO_2-laser and MES, with the healing of incisions performed by steel scalpel.[10,12-18] A relatively limited number of studies have been designed to compare MRS (i.e., MES operating at frequencies >2.5-3 MHz) with other surgical and/or ablative modalities, based mostly on animal models[2,7,19-22] and rarely on clinical trials.[23-25]

The data regarding performance and reliability of these instruments in the pertinent literature remain conflicting. The objective of the present pilot study was to address this issue by determining and comparing the extent of LTD produced by the above instruments and its effect on the early stages of wound healing, appraising both histologic and immunohistochemical findings in an oral mucosa animal model. A study of similar design, comparing the same instruments but based solely on histologic findings, has been conducted by Silverman et al. (2007).[2]

Because of the impossibility of absolute standardization and comparability of the incision depth among all experimental groups (due mainly to the noncontact CO_2-laser surgery) in the present study, only the epithelium and subepithelial connective tissue were taken into consideration in histologic evaluation of the investigated parameters, intentionally omitting assessment of the subjacent muscular substrate. Although the aim of the study was to quantify LTD at the incision margins, regardless of the incision width per se, CO_2-laser–induced lateral photothermal changes may depend on the spot size, thus limiting the comparability of incisions performed by this specific instrument with those performed by the other investigated modalities.[11]

The experimental design of this pilot study was warranted by both ethical and practical reasons. The investigated animal model is common in studies, addressing the same issues, because of the similarity of structural and healing characteristics in porcine and human tissues. However, the results obtained from animal studies should be regarded only as indicators rather than directly transferable predictors of the potential effects in humans.[13,26-28]

Histologic evaluation of specimens harvested on day 1 revealed a significantly more extensive LTD in the CO_2-laser group, compared with the same variable in the MES and MRS groups. This observation has been acknowledged by numerous studies,[7,20-22,29,30] but also contradicted by others.[1,2,4,19,31,32]

The originality of the present pilot study lies mostly in the use of the Ki-67 nuclear antigen as a marker of epithelial cell proliferation adjacent to the incisions performed by the investigated instruments. However, no statistically significant differences regarding the epithelial expression of this marker at the incision margins were detected among the investigated instruments.

The extent of LTD should correlate with wound healing, including the constituent of reepithelialization[3]; in accordance with this statement, Ki-67 expression at the incision margins should be proportional to the thermal injury, instigating the reepithelialization process. However, this hypothesis was not confirmed by statistical analysis of the findings of the present pilot study, possibly because of the relatively small number of observations. This number was further limited by the fact that gingival specimens were not taken into account in evaluation of the Ki-67 expression.

This was warranted to avoid confusion related to the following factors: 1) Because the animals were provided with chow ad libitum, the precise duration of mastication could not be fully designated and standardized to permit delineation of its effect on the reepithelialization process; different masticatory mechanofunctional forces prevailed at the 2 operative sites (considerable at the free and attached gingiva but minimal or negligible at the dorsal surface of the tongue), impinging on both reepithelialization and postoperative inflammation taking place at the incision margins; therefore, it was impossible to reliably compare gingival to lingual incisional wounds; and 2) extension of the gingival incisions to the perioral skin “compelled” focally the closure by secondary intention, thus differentiating the healing process (reepithelialization and postoperative inflammation) between gingival wounds and those created at the dorsal surface of the tongue.

The contamination with oral flora microorganisms combined with masticatory movements constituted severe limitations for the comparison of the postoperative inflammatory infiltration among the examined experimental groups during the early stages of wound healing. However, a tendency toward more extensive inflammatory infiltration at the CO_2-laser incision margins was observed, in proportion to the more extensive LTD produced by this instrument.

Accumulated microorganisms and microabscesses at the upper epithelial layers were detected in most specimens. Bacterial contamination, attributed partly to the inadequacy of antiseptic measures in the oral cavity and partly to the extended duration of mastication, acted as a potential confusing factor on the extent and intensity of inflammatory infiltration at the incision margins. Given the impossibility to designate the causative factor (mechanical and/or thermal injury, induced by incision or infection) of inflammatory infiltration at the incision margins for each specimen and the objective of the study to determine and compare the inflammatory constituent of the healing proces, activated solely by the mechanic and/or thermal injury produced by each of the applied instruments, the registered inflammatory characteristics were intentionally disregarded and no statistical analysis was performed.

The discrepancy between the findings of this pilot study and those of methodologically comparable double-blind randomized controlled experimental studies regarding the evaluated parameters may be ascribed to differences among: 1) the instruments (different parameter configurations of the operating devices) investigated in each study; 2) the examined tissue types (determining various factors, such as water and pigment content, electric conductivity, cellularity, vascularity, and most importantly thermal conductivity); and 3) the animal models used in each study.[1,28] Other to-date-unidentified parameters/factor could also be responsible for contradicting data regarding the addressed issues.

A double-blind randomized controlled experimental study of similar design including a sufficient number of observations is mandatory to fully elucidate differences among the investigated tools regarding lateral thermal damage and its effect on reepithelialization of incisional wounds. To reduce the risk of mechanofunctional (masticatory) stress acting as a confusing factor upon postoperative inflammation and reepithelialization, incisions should preferably be performed at 1 operative site (e.g., the dorsal surface of the tongue) or at least at more or less comparable sites. Finally, the risk of postoperative bacterial contamination of the incisional wounds should be minimized through rigorously controlled provisioning of the experimental animals and, ideally, irrigation of the oral cavity with an antiseptic mouthwash after feeding.

CONCLUSION

The histologic and immunohistochemical findings of this pilot study suggest that CO_2-laser produced significantly greater LTD than MES or MRS. No statistically significant differences regarding the evaluated parameters (LTD and epithelial cell proliferation) were observed between MES and MRS. Further investigation including a greater number of observations is mandatory to determine the effect of lateral thermal damage on early reepithelialization.

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