A Histopathological Study of Wound Healing in Rabbit Oral Mucosa After Incisions Made with Electrocautery or CO_2 Laser

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Purpose: The aim of this preliminary study involving 6 rabbits was to histologically compare the wound healing of incisions in the oral mucosa made by a CO_2 laser focus probe, a CO_2 laser nonfocus probe, and electrocautery.

Materials and Methods: Incisions 5 mm long and 1 mm deep were made in the buccal mucosa of 6 male rabbits weighing between 3 to 4.5 kg. Punch biopsies, 5 mm in diameter, were obtained immediately postoperatively (day 0) and on the 14th day. The presence or severity of the following histological parameters was evaluated: hyperemia, ulcer, edema, thermal depth, acanthosis, parakeratosis, fibrosis, erosion.

Results: Histological findings demonstrated that the performance of the focus laser was similar to electrocautery. However, the performance of nonfocus laser was generally inferior to both as it was the only technique to produce ulceration on the 14th day.

Conclusion: The results of this preliminary study confirm that further studies must be done on CO_2 lasers for oral mucosa surgery.

Keywords: CO_2 laser, electrocautery, wound healing.

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The CO_2 laser is the most commonly used laser for tissue ablation and incision in oral mucosal diseases.[1-4] The continuous-wave (CW) CO_2 laser provides a highly controllable, hemostatic incision in procedures where the placement of a conventional scalpel incision would be restricted. Much research has been devoted to comparing wound healing of incisions created by tissue ablation with the CO_2 laser and incisions made by scalpel.[2,5,6] Because the CO_2 laser beam is absorbed by water, and typical soft tissue has a high water content, the laser has been clearly demonstrated to act by vaporizing tissue at its focal point while leaving adjacent tissues practically unaffected, thus enabling fine hemostasis of the incision site without precluding primary repair of the resulting wound.[4,7,8]

The CO_2 laser is a useful surgical instrument for excising oral soft tissue lesions and has been reported to possess many advantages for both surgeon and patient over conventional techniques.[1-8] The CO_2 laser has a wavelength in the infrared region (10.6 μm) and is absorbed primarily by water.[2,4,7] The penetration depth in tissue is between 25 and 50 μm, depending on the

water content of the tissue. The water in the target tissue absorbs this energy and is vaporized, resulting in thermal ablation. The tissue lateral to the ablation crater has a characteristic pattern of thermal damage.[3,9]

In the treatment of a soft tissue lesion in the oral cavity, the surgeon has a choice of two techniques: excision or vaporization in situ.[2,10-12] The laser beam is said to cause only localized destruction and the zone of the damaged tissue around the point of impact is narrow, although it has been reported to produce certain inadvertent thermal effects on surrounding tissue. The wound is initially covered by a carbonized layer and after 24 h by a thick fibrous coagulum.[9,13,14] The acute inflammatory reaction is minimal, few myofibroblasts are present during healing, and little collagen is laid down.[8,13,14]

Several studies have reported the histological effects of CO_2 lasers on primary healing in oral mucosa,[3,5,8,10-12,14] but none of them have compared oral mucosa wound healing produced by CO_2 lasers with different probes. The aim of this study therefore was to histologically compare wound healing of incisions made by a CO_2 laser focus probe, a CO_2 laser nonfocus probe, and electrocautery in the oral mucosa of rabbits.

MATERIALS AND METHODS

Six male rabbits weighting from 3 to 4.5 kg were used in this study. On day 0, the rabbits were anesthetized by 0.5 mg/kg intravenous injections of Ketamine HCl (Kethalar flacon, Eczacibasi, Turkey). Each of the following instruments/techniques were used to make one incision 5 mm long and 1 mm deep in the buccal mucosa of two rabbits by two passages over the tissue lasting 1 s: a CO_2 laser (Sharplan 15-F, Tel Aviv, Israel) with continuous focus probe, CO_2 laser with continuous nonfocus probe (Sharplan), and electrocautery (Whaledent Perfect TCJ, Mahwah, NJ, USA) set at 5 W. After the incision, in order to observe the oral epithelial turnover, a punch biopsy (Unipunch, India) 5 mm in diameter was performed immediately postoperatively on day 0 in one of the pair and at day 14 in the other. Animals were killed immediately after biopsies by an overdose of urethane anesthesia (1.2g/kg, ip). Each specimen was fixed in 10% formaldehyde, embedded in paraffin, sectioned, and stained with hemotoxylin-eosin. All specimens were evaluated by a single observer.

RESULTS

Semiquantitative histological findings are presented in Table 1. The most important of these findings and additional descriptions not reflected by the table are discussed below.

CO_2 Laser Focus Probe Incision

On day 0, minimal acanthosis was observed on the surface epithelium, and a diathermal artifact was under the erosion. Moderate hyperemia and edema were detected, and fibrosis was minimal (Figs 1 and 2). By day 14, there was moderate acanthosis and minimal parakeratosis in the epithelium. Minimal fibrosis and chronic inflammation elements were observed in the subepithelial plane (Fig 3).

CO_2 Laser Nonfocus Probe Incision

On day 0, minimal parakeratosis on the border of the erosion, a diathermal artifact under the erosion with focal bleeding beneath it, and moderate edema but no fibrosis were observed (Figs 4 and 5). By day 14, an ulcer in the epithelium, a pseudoepitheliamatotic hyperplasia near the ulcer, acute inflammation in the stroma, neovascularization, minimal fibrosis in the deep layer, and minimal edema were observed (Figs 6 and 7).

Electrocautery Incision

On day 0, there was minimal parakeratosis, a diathermal artifact, and moderate edema in the epithelium (Figs 8 and 9). By day 14, there was minimal acanthosis and parakeratosis in the epithelium, minimal edema, capillary vascular proliferation and minimal hyperemia in the subepithelial plane (Figs 10 and 11).

In terms of edema severity, the wound healing of the incisions made by the CO_2 laser focus probe was superior to that of those made by both the CO_2 laser nonfocus probe and electrocautery. However, electrocautery was superior to both laser techniques in terms of acanthosis severity. The nonfocus laser was the only technique to result in ulceration at day 14 and initially produced deeper thermal depth than the other two methods did.

Table 1 Histological findings (severity: - none; + minimal; ++ moderate)

Histological parameter	Incision Technique: CO_2 laser focus probe, Day 0	CO_2 laser focus probe, Day 14	CO_2 laser nonfocus probe, Day 0	CO_2 laser nonfocus probe, Day 14	Electrocautery, Day 0	Electrocautery, Day 14
Hyperemia	++	+	++	+	+	+
Ulcer	-	-	-	+	-	-
Edema	++	-	++	+	++	+
Thermal depth	1 mm	-	1.5 mm	-	1 mm	-
Acanthosis	+	++	-	++	-	+
Parakeratosis	-	+	+	+	+	+
Fibrosis	-	+	-	+	-	-
Erosion	+	-	+	-	+	-

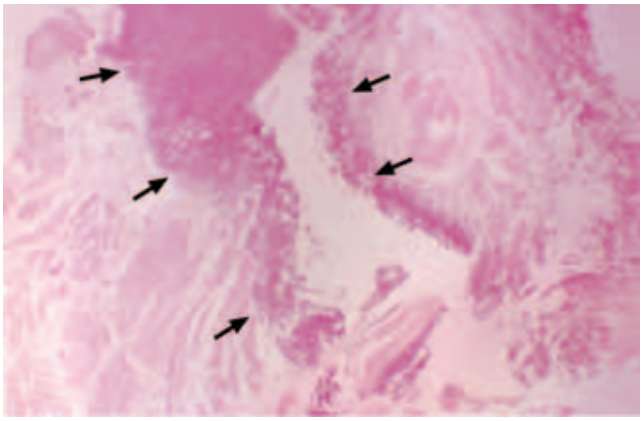

Fig 1 CO_2 laser focus probe incision site at day 0 showing diathermal effect on the mucosa, loss of surface epithelium, diathermal effect in the deep layer of the tissue, and surface epithelium and connective tissue interface (→). HE stain, 40X magnification.

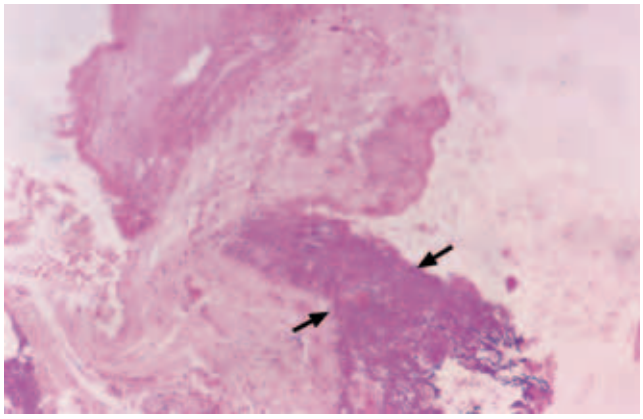

Fig 2 CO_2 laser focus probe incision site at day 0 showing diathermed effect on the mucosa, loss of surface epithelium, diathermal effect in the deep layer of the tissue, and surface epithelium and connective tissue interface (→). HE stain, 100X magnification.

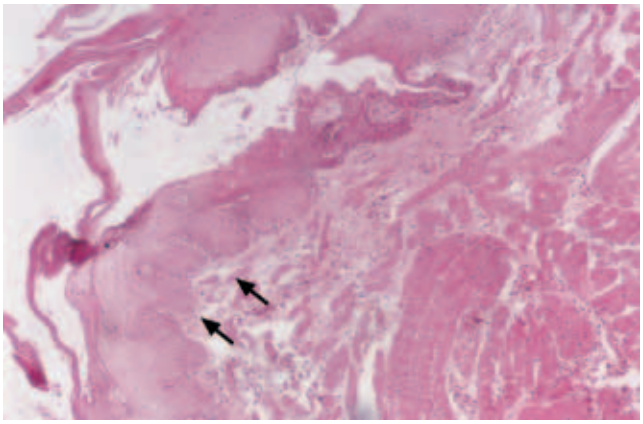

Fig 3 CO_2 laser focus probe incision site at day 14 showing mild acanthosis in the squamous epithelium, focal parakeratosis and superficial separation, reactive and regenerative changes, and minimal edema in the subepithelial layer (→). HE stain, 100X magnification.

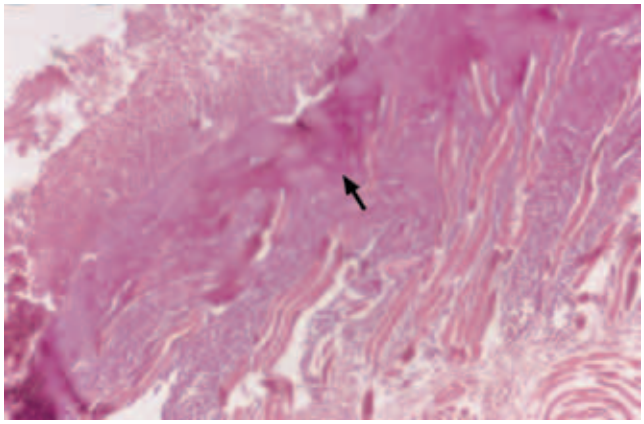

Fig 4 CO_2 laser nonfocus probe incision site at day 0 showing separation and loss of surface epithelium, focal epithelial loss and disintegration, diathermal artifact continuing to the middle layer of the tissue (→). HE stain, 100X magnification.

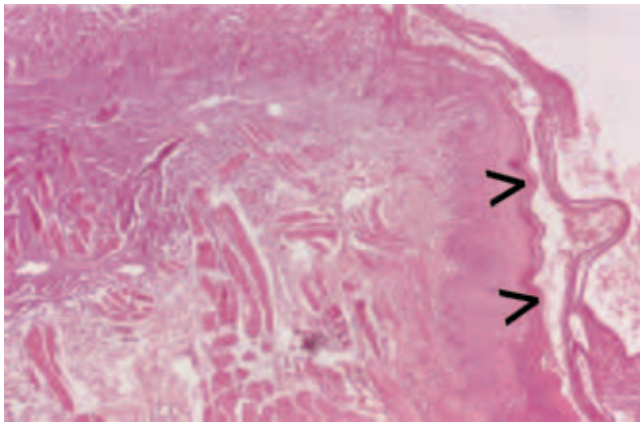

Fig 5 CO_2 laser nonfocus probe incision site at day 0 showing separation and loss of surface epithelium (>). HE stain, 100X magnification.

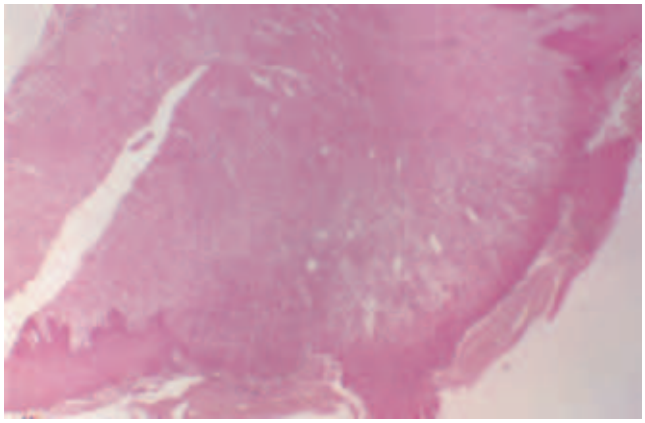

Fig 6 CO_2 laser nonfocus probe incision site at day 14 showing an ulcer on the mucosa, pseudoepithelial hyperplasia on the epithelium near the ulcer. HE stain, 40X magnification.

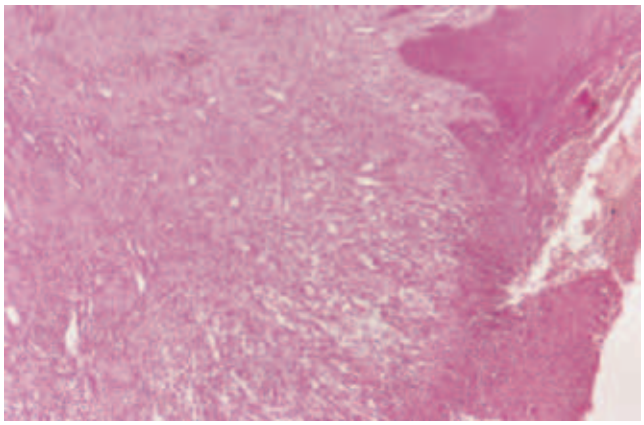

Fig 7 CO_2 laser nonfocus probe incision site at day 14 showing acute inflammatroy reaction of the floor of ulcer, neovascularization, edema, and mild fibrosis in the deep layer. HE stain, 100X magnification.

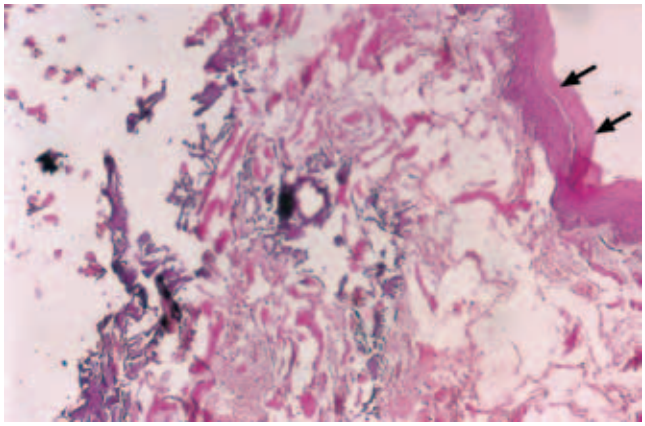

Fig 8 Electrocautery incision site at day 0 showing focal parakeratosis in the squamous epithelium and subepithelial edema, diathermal artifact continuing to the middle layer of the tissue (→). HE stain, 100X magnification.

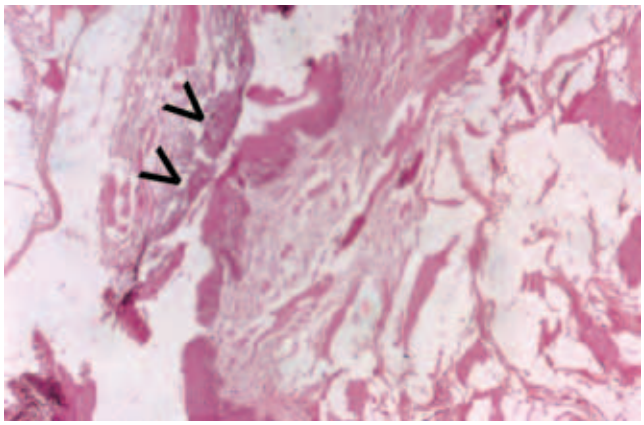

Fig 9 Electrocautery incision site at day 0 showing separation in collagen tissue due to diathermy (>). HE stain, 100X magnification.

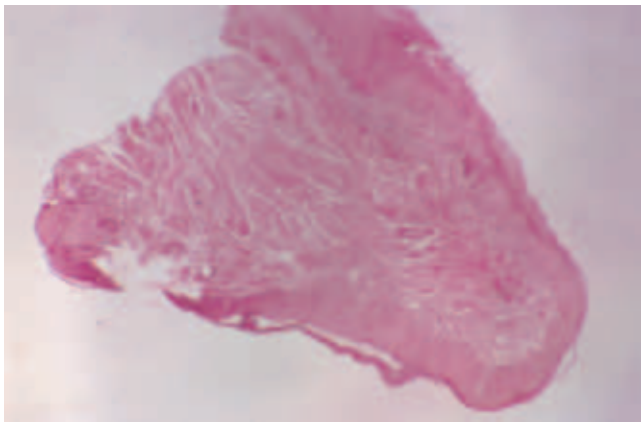

Fig 10 Electrocautery incision site at day 14 showing acanthosis on the surface epithelium. HE stain, 40X magnification.

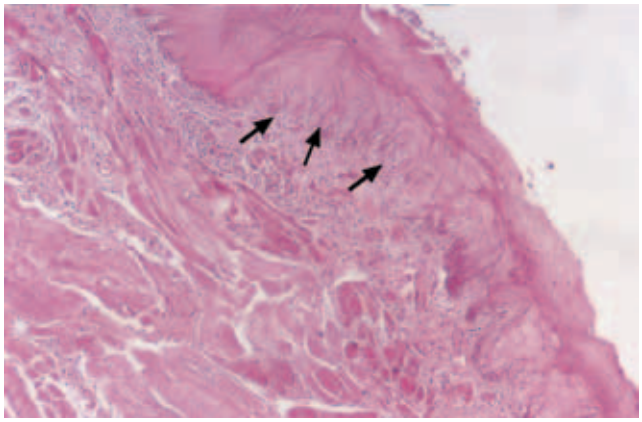

Fig 11 Electrocautery incision site at day 14 showing focal parakeratosis, regenerative variation, and slight fibrosis on the stroma (→). HE stain, 100X magnification.

DISCUSSION AND CONCLUSION

The biological effects of laser beams on tissues have been throughly investigated.[3,5,6,8-12] Our results are generally similar to these studies. However, detailed comparisons are difficult because other researchers compared different devices to ours.

Bryant et al[3] histologically studied healing of a wound in the canine oral mucosa produced by a scalpel, a carbon dioxide laser, and a pulsed laser. They found that wound healing was similar for all techniques, but only the CO_2 CAST system incision exhibited a lack of excessive fibrosis wound healing on day 14.

Carew et al[5] histologically evaluated the wound healing of incisions created by scalpel, electrocautery, and CO_2 and KTP lasers in rat tongues. They reported that the wound surface had re-epithelialized and normal skeletal muscle formation could be seen laterally on either side of the wound. They found the scalpel to produce the least wound depth and the KTP laser the greatest.[5]

Finsterbush et al[6] studied CO_2 laser incision and scalpel wounds in 36 rabbits and observed wound healing after 23 days which was similar to ours at day 14. In our preliminary study, we compared CO_2 laser focus probe, CO_2 laser nonfocus probe, and electrocautery incisions in wound healing, but we did not investigate wound healing in scalpel incisions. A further factor which may make comparisons with other studies difficult was the sample size of the present study, kept low for ethical reasons. The observation time in our study (14 days) is based on the turnover of oral mucosal epithelization, which is approximately two weeks. However, examination at more time points between 0 and 14 days might be useful.

Other authors have reported laser beams to have similar basic effects on tissue to those of electrocautery.[10,11,14] Our preliminary study has also shown that the healing pattern of the wounds produced by the focus probe CO_2 laser to be similar to that produced by electrocautery. However, the healing of the nonfocus probe CO_2 laser wound was found to be inferior to that inflicted by the other 2 methods.

The results of this study therefore suggest that further studies must be done on CO_2 lasers for oral mucosa surgery.

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