Carbon Dioxide Laser for De-Epithelialization of Periodontal Flaps

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Regeneration of mineralized and soft connective tissue components of the attachment apparatus is the main goal in the treatment of periodontal diseases. Often, apical migration of epithelium (long junctional epithelium) effectively prevents the formation of bone and connective tissue attachment after periodontal surgery. The purpose of the present study was to compare conventional periodontal surgery combined with carbon dioxide laser and conventional periodontal surgery alone with respect to epithelial elimination and degree of necrosis of mucoperiosteal flaps. After signing a consent form, five patients with at least two comparable bilateral periodontal defects needing pocket elimination surgery participated in this study. The investigators randomly divided each side into test and control sites. Each patient received oral hygiene instruction and initial therapy prior to surgery. At surgery, the test site received a sulcular incision and carbon dioxide laser de-epithelialization of the outer and inner aspects of the flap. The control group received reverse bevel incision only. The surgeon performed open flap debridement on all teeth. At the time of surgery, the surgeon did a biopsy of each site and submitted specimens for histologic evaluation. A matched pairs t-test was used to analyze the data. The results show significant differences between the carbon dioxide laser and reverse bevel incision with respect to sulcular ($P \leq 0.025$) and gingival (external) ($P \leq 0.01$) flap surface epithelial elimination and tissue necrosis ($P \leq 0.005$). These results should be replicated with a larger number of subjects. The carbon dioxide laser eliminated sulcular and gingival (external) epithelium without disturbing underlying connective tissue. This finding supports the concept that the carbon dioxide wavelength has little or no effect on tissues beyond the target. However, neither laser nor blade eliminated all the epithelium. Researchers observed chronic inflammation in the control and test sites, with a predominance of plasma cells. Lining the sulcular and gingival (external) lased areas, investigators found coagulation necrosis covered by fibrin and coagulated blood. The laser appears to effectively remove epithelium at the time of surgery; however, future long-term, well-controlled quantitative histologic studies are needed to evaluate the effect of repeated carbon dioxide laser de-epithelialization of the gingival (external) surface of mucoperiosteal flaps at intervals during the healing period. J Periodontol 1997;68:763–769.

Key Words: Connective tissue/radiation effects; epithelium/radiation effects; laser surgery; periodontal diseases/surgery; periodontal diseases/therapy; radiation effects; surgical flaps.

In periodontal surgery, tissue healing may be accompanied by apical migration of epithelial cells, thus preventing the regeneration and restoration of periodontal attachment lost to disease. New connective tissue attachment and cementum regeneration can be achieved by cells originating from the periodontal ligament. Many attempts to prevent apical migration of epithelial cells include: subgingival curettage, cryotherapy, chemical substance application (e.g., phenol camphor, and antiflammatory), free palatal grafts, different types of incision, biological barrier membranes, and carbon dioxide laser.

The carbon dioxide (CO$_2$) laser emits a continuous beam with a (10,600 nm) wavelength in the invisible infrared part
of the electromagnetic spectrum.\textsuperscript{23-24} Causing only thermal
damage when its energy contacts tissue, there is no change
in atomic structure of cells within the tissue and therefore
no chance for genetic mutations.\textsuperscript{22} Biologic tissue, regardless
of pigmentation or vascularity, absorbs CO\textsubscript{2} laser energy
because the target of interaction is water.\textsuperscript{1,22,26-29} Thus, the
laser destroys tissue by rapidly heating and vaporizing in-
tracellular water, resulting in minimal lateral energy spread.
This feature virtually assures no heat conduction to deeper
soft tissue layers. Thus, the CO\textsubscript{2} laser has the potential to
de-epithelialize tissue.\textsuperscript{19}

Recent research suggests that gingiva can be totally
de-epithelialized using CO\textsubscript{2} laser while leaving the con-
nective tissue basically undisturbed in monkeys\textsuperscript{19,20} and in
humans.\textsuperscript{21} CO\textsubscript{2} laser treatment of flaps at the time of sur-
gery delayed epithelial downgrowth along the root sur-
face for up to 14 days longer than conventional tech-
niques.\textsuperscript{19} However, no research yet demonstrates that the
CO\textsubscript{2} laser removes all epithelium from a flap during the
initial surgical procedure. By blocking epithelial down-
growth, the necrotic layer formed on the wound area after
laser delivery may give time for cells of the periodontal
ligament to repopulate the root surface and form a new
attachment. The purpose of this study was to histologi-
cally compare conventional periodontal surgery combined
with carbon dioxide laser de-epithelialization with con-
tventional periodontal surgery alone with respect to ep-
ithelial elimination and degree of necrosis of mucoperios-
steal flaps.

MATERIALS AND METHODS

Sample
Five volunteer patients from the University of Maryland
at Baltimore Dental Clinic, 4 males and 1 female, in need
of resective periodontal surgery, comprised the patient
population. All patients signed an informed consent doc-
ument approved by the University of Maryland at Balti-
more Institutional Review Board.

Inclusion criteria were: 1) no medical conditions that
precluded periodontal or restorative treatment; 2) at least
two comparable bilateral periodontal defects needing re-
sective pocket elimination surgery; and 3) periodontal de-
fects that did not extend beyond the keratinized gingiva.

Exclusion criteria were: 1) any medical condition or
medication that delayed normal wound healing; 2) any
condition that posed a risk to the dental team; 3) any
condition that precluded effective oral hygiene; and 4)
any type of antibiotic therapy, including subacute bac-
terial endocarditis (SBE) prophylaxis.

Research Design
The research used a split-mouth design with similar de-
fects on randomly selected test and control sites. Six
weeks prior to the surgical procedure, each patient re-
ceived, as initial therapy, full-mouth scaling, root planing,
and oral hygiene instruction (modified Bass intrasulcular
technique and interdental brushing and flossing). To allow
for healing, 4 weeks following initial therapy, patients
received a standard baseline examination consisting of: 1)
Löe and Silness gingival index\textsuperscript{30} (GI); 2) Silness and Löe
plaque index\textsuperscript{31} (PLI); 3) sulcus depth measured with a
pressure-sensitive periodontal probe; and 4) gingival
crevicular fluid (GCF) flow.

The control and test sites received block anesthesia using
2% lidocaine\textsuperscript{1} with 1:100,000 epinephrine and, to control
hemorrhage, local infiltration with 1:50,000 epinephrine 2%
xylcocaine. At the control site, the surgeon made a su-
cular and reverse bevel incision with a Bard Parker #15 scalpel
blade at least ½ to 1 mm away from the free gingival mar-
gin, then directed the incision toward the alveolar crest
parallel to the long axis of the tooth and extended it one tooth
laterally in either direction from the surgical site. The op-
erator then removed and discarded the sulcular wall tissue
thus resected, reflected facial and lingual full-thickness mu-
coperiosteal flaps, and performed soft tissue degranulation
and root planing using hand instruments.

The surgeon made a sulcular incision and managed the
test site in the same manner as the control, except for the
following: 1) prior to reflection, the surgeon lased the outer
aspects of the mucoperiosteal flap from the free gingival
margin to the mucogingival junction labially and 5 mm api-
tal to the free gingival margin on the palatal/lingual surface;
the operator used a sweeping motion with the carbon di-
oxide laser set at 8 watts, pulsed mode 7 (repetition rate 20
times per second with a 20 msec exposure-40% duty cycle)
using a 0.8 mm ceramic tip in a focused beam (1 to 3 mm
away from tissue)\textsuperscript{31} (Fig. 1); 2) the surgeon then wiped
down the lased area with a cotton swab embedded in chilled
saline solution to confirm removal of the epithelial layer and
cool the tissue;\textsuperscript{9,20} 3) noted connective tissue exposure by
either red coloration (relative to the epithelium) or minor
bleeding upon wipe down; 4) reflected tissue and then lased
the inner aspect of the facial and lingual flaps in the same
manner as above to remove any remaining epithelium (Fig.
2); and 5) avoided any laser contact with root surface or
alveolar bone by placing a periosteal retractor between hard
and soft tissue and aiming the laser beam at a 90\degree angle
to the soft tissue flap.\textsuperscript{20} The operator resected control and test
sites and placed the tissue in 10% neutral buffered formalin,
used interrupted sutures to close the flaps, placed periodontal
dressing when necessary, and gave patients oral and written
postoperative instructions. Ten days after the procedure, the
surgeon removed the sutures and periodontal dressing (if
present). To ensure consistency, one operator performed all
surgeries.

Each patient yielded four specimens: two from the con-
trol site (facial and lingual) and two from the test site

\textsuperscript{1}Astra USA, Inc., Westborough, MA.
(facial and lingual). The operator divided each specimen buccolingually in approximately three equal sections (mesial, distal, and middle) (Fig. 3).

**Histological Processing**
All specimens were fixed in 10% neutral buffered formalin for 24 hours and embedded in paraffin. Eight µ histologic slides were cut and stained with hematoxylin and eosin. Between 5 and 16 slides were obtained per section. Two calibrated examiners (the operator and an oral pathologist), blind as to which section was test or control, evaluated all readable samples. The examiners reached consensus on all slides. For calibration, the examiners observed and scored histologic sections (other than those studied in this project) until they reached consistency. The examiners looked for: 1) gingival (external) epithelium; 2) sulcular (internal) epithelium; 3) gingival necrosis; and 4) sulcular necrosis.

The investigators scored the data for test and control sites as follows:
Epithelium (gingival/sulcular): 1 = complete absence of epithelium; 2 = partial presence of epithelium (the investigators considered partial presence of epithelium from as little as one cell to groups of epithelial cells attached to or detached from the rest of the tissue); and 3 = intact epithelium.
Necrosis (gingival/sulcular): 1 = no necrosis; and 2 = necrosis (if investigators noted any area of necrosis, they scored that section as necrotic).

**Statistical Tests**
The matched pairs t-test was used to analyze epithelial elimination and degree of necrosis of gingival (external) and sulcular (internal) aspects of the mucoperiosteal flap on control and test sites. For analysis, the mean of the six sections on each patient (facial/mesial, facial/middle, facial/distal, lingual/mesial, lingual/middle, lingual/distal) generated a single score for each tooth (control and test). Therefore, for each t-test, n = 5 with a significance of P ≤ 0.05.

**RESULTS**

**Histologic and Statistical Observations**
Investigators employed two methods for examining samples. Although 639 slides were available in the first method, 587 were read (295 test and 292 control); the other
52 samples were at the edge of the laminate or disrupted, making evaluation impossible. In the second method, called the “representative sample” procedure, examiners chose the best histological slide in each of the six sites on each patient, read them, and obtained the mean and standard deviation of all six sites per patient. Both methods gave exactly the same results, showing the accuracy of the representative sample procedure as stated in the literature.32-33 For completeness, the investigators chose to use the entire sample for the statistical analysis.

**Test Specimens**

The test specimens showed almost complete elimination and carbonization of gingival (external) and sulcular (internal) epithelium (Figs. 4 and 5). Areas of coagulation necrosis, manifested by basophilic staining and increased tissue opacity, covered by fibrin and coagulated blood, appeared adjacent to the area of carbonization. Also, detachment and shredding of keratin and separation from the lamina propria were noticed (Fig. 6).

The connective tissue displayed three zones: a zone of tissue necrosis next to the laser wound that displayed coagulated collagen with almost no cells present; a zone of thermal effect; and a zone of undisturbed connective tissue (Fig. 6). The zone of thermal effect displayed collagen fibers with a glassy appearance; the intercellular stroma is composed of a mixture of normal collagen fibers and homogenized bundles. In this zone and immediately adjacent to it, investigators noticed small arteries, arterioles, and veins coagulated and occluded by clot, as well as moderate inflammation with a predominance of plasma cells. In de-epithelialized areas, the basement membrane was detached from the supporting tissue and the free border of the connective tissue traceable in its entire morphology (Fig. 6).

Investigators observed partial elimination of sulcular epithelium at 8 sites, total elimination at 21 sites, and intact epithelium at 1 site, for a total of 30 sites. On the gingival epithelial site, investigators observed partial elimination of gingival epithelium at 27 sites, total elimination at 2, and intact epithelium at 1 site, for a total of 30 sites. Every patient had gingival epithelium at one or more sites along the wound (incision) surface and presented gingival and sulcular necrosis along the wound surface.

**Control Specimens**

The control specimens displayed intermittent islands of epithelium on the sulcular surface. Investigators observed keratinized stratified squamous epithelium on the gingival surface as well as a mild to moderate inflammatory infiltrate with minimal connective tissue necrosis (Fig. 7).

Investigators observed partial removal of sulcular epithelium at 9 sites, total elimination at 18 sites, and completely intact epithelium at 3 sites, for a total of 30 sites, and gingival epithelium partially removed at 4 sites and
Significant differences were found between test and control sites for sulcular and gingival de-epithelialization and degree of necrosis. Differences at the sulcular sites are important because, on the test site, a laser was used and, on the control site, a blade was used. The significant differences between test and control for the gingival sites were expected because a laser was used on the test site, while no manipulation was employed on the control site. Results indicate that even though the CO₂ laser eliminated more sulcular epithelium than conventional periodontal surgery alone, neither laser nor blade eliminated all the epithelium. In the control and test sites, remnants of sulcular epithelium appeared more frequently in the interproximal regions and less frequently at the mid-facial and mid-lingual surfaces. This finding was probably due to the greater thickness of epithelium in interproximal areas and problems of access. In the test group sulcular wall, 2 of 5 patients had no areas of interproximal epithelium in contrast with the control group, where every patient had one or more sites of interproximal epithelium. In the test group gingival (external) wall, investigators observed remnants of epithelium in every patient. Reasons for this finding could be the deep epithelial ridges surrounding finger-like connective tissue papillae on this surface and difficulty in clinically determining complete flap de-epithelialization. The literature shows that dependence on visual feedback to gauge depth of ablation may be distorted by a zone of thermal damage or char advancing ahead of and lateral to the ablation front.36 This inability to control ablation volume may contribute to failure in removing all the epithelium.

Control site results concur with previous studies showing the blade’s inability to predictably remove all epithelium from the wound edge in humans.14,15,37 This study’s findings differ from those in rhesus monkeys, where researchers found complete epithelial removal following reverse bevel incision.46 The different results may be related to the animal model system. In monkeys, periodontal pockets are artificially created and generally of short duration. However, periodontal pockets in humans are usually the result of longstanding chronic inflammation, often associated with pseudo epitheliomatous hyperplasia and elongation of rete ridges. The plasma cell infiltrate observed in this study indicates a chronic inflammatory state. Deep penetration of rete ridges could account for the inability to remove all epithelium in humans.14

The fate of residual epithelium is uncertain. Some investigators consider residual epithelium from whatever source as “seed areas.”19 Others state that residual nests of junctional epithelium do not participate in regeneration of new junctional epithelium; rather, adjacent oral epithelium is the source.38,39 The viability of epithelial remnants could not be determined by this study. More study is necessary to determine the viability of epithelial remnants.

In the areas in which laser removed epithelium, the
basement membrane detached from the connective tissue, and the investigators could clearly trace the connective tissue border, free of epithelial cells (Fig. 6). This finding indicates that under the correct conditions, the laser can totally remove epithelium while leaving connective tissue essentially intact, coinciding with other studies. Possible explanations for this specificity include: 1) the higher water content of the epithelium in comparison to connective tissue; 2) the hemidesmosome junction weakness between epithelium and connective tissue; and 3) the thermal stability of connective tissue structural proteins: elastin and type I collagen.

Researchers observed moderate plasma cell infiltration on test and control sites (Fig. 7), indicating presence of chronic inflammation within connective tissue. This observation may be due to the patient's periodontal disease, not the laser or scalpel surgery. This periodontal condition may explain the great sinuosity of rete pegs and may be a contributing factor to epithelium remaining after lasing. Some thermal diffusion occurred during laser delivery. This study observed distinct areas of CO₂ laser-tissue interaction: a zone of vaporization and tissue necrosis; a zone of reversible thermal damage; and a zone of undisturbed connective tissue (Fig. 6). These results coincide with previous studies. This study found areas of coagulative necrosis that may delay the apical downgrowth of epithelium during flap surgery, as shown in monkeys and in humans. Therefore, this covering of necrotic tissue may give time for cells of the periodontal ligament to repopulate the root surface and form a new attachment.

The surgeon found the carbon dioxide laser technique very time-consuming because one must make numerous passes and wipe away charred debris, as observed by other researchers. For future studies, the authors suggest vertical releasing incisions for better access of the CO₂ laser handpiece into the surgical area and reverse bevel incision instead of single incision to help achieve epithelial elimination as suggested by previous studies. The articulated arm and handpiece of the CO₂ laser could be improved for better access in the oral cavity. The authors also suggest lasing the gingival (external) and sulcular surface of the flap next to the lased surgical area to eliminate interproximal epithelial remnants and control hemorrhage. The effect of this procedure on healing is unknown and more study is necessary.

Conclusions

Within the limits of this study, the following conclusions can be made. Results indicated that:

1) The carbon dioxide laser will eliminate significantly more sulcular epithelium when compared to conventional periodontal surgery, but neither laser nor scalpel will completely remove all the epithelial lining of the sulcus.

2) The laser will not remove all the epithelium on the gingival (external) surface.

3) The carbon dioxide laser can completely de-epithelialize inner and outer aspects of mucoperiosteal flaps with adequate: a) access to surgical areas; b) fluid control; c) visual feedback; and d) tissue health (reasonable control of chronic inflammation and hyperplasia).

4) The carbon dioxide laser will eliminate epithelium while leaving connective tissue basically undisturbed.

5) The carbon dioxide laser technique will produce significantly more necrotic tissue adjacent to the wound area than conventional periodontal surgery.

6) Future long-term, well-controlled quantitative histologic studies are needed to evaluate the effect of repeated carbon dioxide laser de-epithelialization of the gingival (external) surface of a lased mucoperiosteal flap to retard epithelial downgrowth.

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REFERENCES


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