Retardation of Epithelial Migration in Monkeys Using a Carbon Dioxide Laser: An Animal Study

Jeffrey A. Rossman,* Michael J. McQuade,* and Denise E. Turunen*†

Obtaining a connective tissue attachment to the root surface following mucoperiosteal flap surgery has been a goal of periodontal therapy for a long time. The objective of this study was to examine whether controlled de-epithelialization with the CO₂ laser would retard the apical migration of the epithelium and thereby increase the amount of connective tissue attachment. Elastics were placed on the maxillary premolars and incisors of 7 cynomolgous monkeys to create periodontal defects. Open flap debridement was performed on each side. On the experimental side, the oral epithelium was removed by CO₂ laser irradiation. This report describes the 3 specimens at 7, 14, and 28 days respectively. On the control side sulcular epithelium was seen at 14 days. Sulcular epithelium was first seen on the test side at 28 days. For all specimens over 7 days, there was a trend to less epithelium and more connective tissue attachment on the experimental side than on the control side. However, no statistical analysis was possible on this histologic study. The CO₂ laser may be a useful tool to retard epithelium and thereby enhance new connective tissue attachment. J Periodontol 1992; 63:902–907.

Key Words: Surgical flaps; connective tissue attachment; epithelium.

Successful treatment of intrabony pockets to obtain new attachment continues to represent a serious therapeutic challenge in periodontics. Epithelial proliferation apically, along the root surface, has been shown to interfere with the establishment of a new connective tissue attachment.¹ The presence of inflammation has also been shown to stimulate epithelial migration in an apical direction.² Both the degree of inflammation and the “race” between connective tissue maturation (coronally) and downgrowth of epithelium appear to be important factors influencing development of a new attachment of the dentogingival unit following surgical intervention.

Historically, a number of techniques have been tried to retard epithelial downgrowth. Goldman’s technique eliminated the coronal portion of the periodontal pocket by ginvectomy in conjunction with subgingival curettage.³ Prichard employed a technique in treating intrabony defects (3-walled osseous defects) that left the interproximal areas exposed to heal by secondary intention in an attempt to keep the epithelium away from the defect.⁴ Shapiro’s technique for obtaining new attachment stressed the importance of removing marginal epithelium in the initial incision (inverse bevel) such that the epithelial margin was kept away from the healing defect.⁵

Other techniques for retarding epithelial migration have since been described. Elleegard and co-workers used free gingival grafts as barriers.⁶ ⁷ Their results confirmed observations by Caffesse⁸ that the traditional full thickness flap results in epithelium that is well-preserved, and placement of this flap adjacent to the treated area facilitates the migration of epithelium into the intrabony defect.⁹ Nyman et al. used millipore filters to exclude epithelium and gingival connective tissue from healing surgical sites in monkeys⁹ and man.¹⁰ Bone and connective tissue attachment were regenerated, and it is postulated that the filter provides a selective advantage to periodontal ligament cells. New attachment was demonstrated on previously diseased surfaces when the millipore filters were used in conjunction with complete soft tissue coverage of the entire root.¹¹ More recently, the use of a expanded polytetrafluore (eEPTF) membrane for epithelial exclusion has become common practice.

The aim of the present study was to see if the use of a carbon dioxide (CO₂) laser to de-epithelialize a flap would or could act as an epithelial exclusion technique.

The most fundamental property of laser light which distinguishes it from the other sources is that it is far more coherent (individual waves are “in phase” with one another). Lasers in current use fall between infrared and ul-

*Postdoctoral Periodontics, University of Texas Health Science Center, Houston, TX.
†Postdoctoral Periodontics, Baylor College of Dentistry, Dallas, TX.
*Department of Periodontics.

⁸Gore-Tex, W.L. Gore and Associates, Flagstaff, AZ.
traviolet on the electromagnetic spectrum and the specific
type is defined by the wavelength of radiation emitted. The
most commonly utilized types in medicine and dentistry are
the carbon dioxide (CO₂) laser (wavelength 10,600 nm); the
neodymium-yttrium-aluminum-garnet (Nd:YAG) laser
(wavelength 1,060 nm); the various ultraviolet wave-
lengths such as the excimer (excited dimer); and the argon
laser with a wavelength of 488 to 516 nm.

The CO₂ laser is very versatile due to its ability to excise
and coagulate soft tissues. This is because the beam is rap-
idy absorbed by water in the most superficial tissues with
virtually no heat conductivity to lower layers. The Nd:YAG
and the argon, on the other hand, are more penetrating
lasers with the energy absorbed by deeper and more pig-
mented tissues. This means that the CO₂ laser has the
potential for de-epithelialization while the Nd:YAG laser is
generally not considered useful for this purpose. The depth
of the wound produced by any laser is directly proportional
to the power output, the duration of exposure, and the spot
diameter of the beam.

The laser was developed by Maimon in 196012 and was
first applied in vivo to human teeth in 1965.13 The effects
of the CO₂ laser on dental hard tissues have been studied
quite extensively,14-17 however, histologic reports of its use
as an adjunct to soft tissue dental surgery are quite limited.
Goultchin et al.18 compared hard and soft tissue healing
in the beagle dog following a gingivectomy per-
formed with a CO₂ laser to that performed with a con-
tentional knife. Crater-like defects were observed in the
cementum on the laser-treated specimens where the beam
had touched the tooth. Because of the excisional type of
surgical procedure, the histologic results did not demons-
strate any substantial advantage of the laser over the con-
tentional gingivectomy. Also since the intraoral handpiece
had not yet been invented, the investigators experienced
difficulty in controlling the laser handpiece.

Because of the very precise depths to which the laser can
"cut," the purpose of this study was to determine if it is
possible that the CO₂ laser could de-epithelialize the peri-
odental flap and delay epithelial downgrowth and hence
facilitate regenerative efforts.

MATERIALS AND METHODS

In conducting the research described in this paper, the in-
vestigators adhered to the "Guide for Laboratory Animal
Facilities and Care," as promulgated by the National Re-
search Council.

For this preliminary report, 3 cynomolgus monkeys (Mac-
caca irus) were used. The model followed for experimen-
tally induced periodontitis was that established by Caton
and co-workers.19,20 Orthodontic elastics were doubled
and placed at the gingival margins around the maxillary central
incisors and maxillary premolars bilaterally. The elastics
were changed every 2 weeks and the monkeys were fed a
standard diet of Purina Monkey Chow supplemented with
fresh fruit. The monkeys were maintained on this regimen

until vertical bony defects were obvious by radiographic
evaluation. All animals then underwent open flap curettage
procedures on both sides of the maxillary arch. The experi-
mental side was randomly determined by a coin toss.

Both sides were treated simultaneously using an inverse
bevel incision 1.0 mm below the free gingival margin in
an effort to remove the crevicular epithelium. Full thickness
mucoperiosteal flaps were then reflected on the facial and
palatal aspects of the intrabony defects. The sites were de-
granulated with curets and the root surfaces were lightly
planned (Fig. 1A). Notches were made in the root surface
at the bottom of the defects with a small Wedelstadt chisel
with cemento-enamel junction (CEJ) serving as a fixed ref-
ence point. The areas were irrigated with sterile saline.

On the control side, the tissue flaps were carefully adapted
to the teeth and sutured with 4-0 silk suture to obtain
primary coverage of the intrabony defects. On the experi-
mental side, the outer surface of the flaps was irradiated with
the CO₂ laser.21 The incident laser beam was held perpen-
dicular to the tissue surface and was focused to a 2 mm
circular spot using a 400 mm focal length lens. The tissue
surfaces were exposed to impacts of 0.5 seconds duration
at 10 watts.22 (Fig. 1B)

The impacts were adjoining and slightly overlapping to
ensure complete irradiation of the flap overlying the intra-
bony defects. The carbonized layer was then removed by
wiping with a moist gauze. The de-epithelialized tissue flaps
were then carefully adapted to the teeth and sutured over
the intrabony defects, identical to the control side (Fig. 1C).
Following surgery, each monkey received a rubber cup pro-
phylaxis 3 times a week.

*Model 400, Coherent Medical Products, Palo Alto, CA.
Table 1. Histometric Measurements of Healing Defects

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEJ-AN</td>
<td>CEJ-JE</td>
</tr>
<tr>
<td>7</td>
<td>3.16</td>
<td>*</td>
</tr>
<tr>
<td>14</td>
<td>3.00</td>
<td>2.08</td>
</tr>
<tr>
<td>28</td>
<td>2.61</td>
<td>1.68</td>
</tr>
</tbody>
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Measurements are in mm and represent averages of multiple histologic sections on the same specimen. These are mean values.

AN = apical notch.
JE = junctional epithelium.
CT = connective tissue.
*No epithelial downgrowth was present.

2. The distance in mm from the CEJ to the apical extent of the healed junctional epithelium.

3. The distance in mm from the CEJ to the crest of the alveolar bone. The extent of the epithelial migration along the tooth surface from the CEJ was then computed and the two sides were compared (Table 1).

RESULTS

7-Day Specimens
The control sides showed minimal inflammation; no epithelium was seen and the collagen appeared to be viable. Fibroblastic activity was observed apical to the level of the notch delineating the base of the defect. A fibrinous clot was present at the base of the defects and below this the tissues were edematous. Small fragments of necrotic bone were present in the connective tissue adjacent to the notches.

On the experimental sides, the inflammation was mild to moderate and no epithelium was present. The tissue was edematous and an eosinophilic coagulum was present. Superficial necrosis was observed where the inflammation was most intense. Marked fibroblastic activity was present. Localized areas of osteoclastic activity approximated small bone spicules.

14-Day Specimens
The control sides were slightly inflamed. Keratinized epithelium extended apically into the sulcus. The amount of epithelial downgrowth was less than 25%, was heavily keratinized and had a well-established spinous layer. Few fibroblasts were present but there was early osteoblastic activity (Fig. 2A). Histometrically there was a distance of 2.08 mm from the CEJ to the base of the junctional epithelium and 0.92 mm of connective tissue attachment.

The experimental sides were also slightly inflamed with with no epithelium lining the sulcular aspect of the flap nor any indication of junctional epithelium. In the underlying connective tissue there was intense fibroblastic activity. A fibrin clot was observed at the base of the defects. Fragments of necrotic bone were seen throughout the stroma at the level of the notch and apical to the defects (Fig. 2B). Nearly twice as much connective tissue attachment was

The animals reported in this paper were sacrificed at 7, 14, and 28 days respectively following surgery and the tissues were prepared for histologic examination. Ten mesial to distal representative sections were taken from each animal and the average of these 10 measurements was taken (Table 1). One animal and its 10 sections represented each time period. The sections were measured to the nearest 0.10 mm with a calibrated stereometric microscopic system with the CEJ serving as a fixed reference point. Distances between the CEJ and various landmarks of the periodontal tissues were measured using a calibrated disc in the eyepiece of a light microscope. These measurements included:

1. The distance in mm from the CEJ to the notch in the tooth surface depicting the most apical point of the intrabony defect at surgery.
28-Day Specimens

The control sides were slightly inflamed. The epithelium was several layers thick and extended from 55% to 65% of the defect. Small amounts of connective tissue and cementum were noted but there was only minimal new bone formation in the deeper portion of the defects (Fig. 3A). The average distance from the CEJ to the junctional epithelium base was less than that seen at 14 days (2.08 mm vs. 1.68 mm).

The experimental sides were also inflamed. Epithelial downgrowth was seen for the first time as a thin layer extending halfway to the base of the defect. New connective tissue attachment was observed. New bone formation extended from the notch to the level of epithelial downgrowth (Fig 3B). Much more connective tissue attachment was seen than in the control (1.62 mm vs. 1.10 mm).

DISCUSSION

The purpose of this pilot research was to determine whether or not a CO$_2$ laser could be used to effectively retard the downgrowth of epithelium into a treated periodontal defect. Based on the data collected, our observations were that the CO$_2$ laser did, in fact, alter the course of healing.

In all 3 early cases, irradiation of the experimental side resulted in delayed epithelial downgrowth along the root surface. Comparison of the results at 7 and 14 days suggests
that epithelialization was retarded by at least 7 days when compared to the control, but epithelial migration was roughly equal by 28 days. The healing of the connective tissue on the irradiated side was not seamed to be retarded. In this preliminary report, no hypothesis testing of the data was done. The thrust of this paper is therefore to present histologic observations in the de-epithelialization using a carbon dioxide laser and quantity differences in terms of millimeters.

In light of the significant interest in lasers today, one might speculate on the effect that an Nd:YAG or the argon laser would have versus the carbon dioxide laser. The carbon dioxide laser has a very shallow depth of penetration and a shallower depth of thermal coagulation than the Nd:YAG or the argon. The latter two mentioned laser beams tend to pass through epithelium and be absorbed by the deeper pigmented tissues. Therefore the carbon dioxide laser is more apt to de-epithelialize only and not harm underlying connective tissue. The carbon dioxide laser was thus chosen for this experiment.

The CO₂ laser has been used in otolaryngology and gynecology for almost two decades and has become a valuable tool for controlled tissue devitalization, vascular coagulation, excisional surgery, and endoscopic and microsurgery. There has been much less experimentation to histologically evaluate the laser as a surgical adjunct in dentistry.

Within the limitations of this study, it appears that de-epithelialization by the CO₂ surgical laser can retard the apical downgrowth of epithelium during flap surgery in monkeys. Although in some instances root resorption has been reported as a complication of treatment with other epithelial exclusion techniques, no root resorption was observed in this study. Additionally, the CO₂ laser is less technically demanding and more time efficient than other currently known methods of epithelial retardation, possibly making it a desirable adjunct to periodontal regenerative procedures. Future quantitative histologic studies are needed to evaluate the effect of combined laser/membrane exclusion regenerative techniques.

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Send reprint requests to Dr. Michael J. McQuade, Director, Postgraduate Periodontics, Baylor College of Dentistry, 3302 Gaston Ave., Dallas TX 75246.

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