Effects of CO_2 Laser Treatment on Fibroblast Attachment to Root Surfaces. A Scanning Electron Microscopy Analysis

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Background: The aim of this study was to analyze the CO_2 laser effects on root surfaces affected by periodontal disease in comparison to scaling and root planing for fibroblast attachment.

Methods: Thirty single-rooted human teeth extracted because of advanced periodontal disease were included in this study. A total of 60 specimens, obtained from all selected teeth, were randomly assigned to 3 groups: 1) control (untreated); 2) hand scaling and root planing (SRP); or 3) laser (CO_2 defocused pulsed) and ultrasonic scaling. All the specimens were incubated in Petri dishes with fibroblast suspension, and then observed by scanning electron microscopy (SEM).

Results: The control group showed the lowest number of attached cells, with no tightly attached fibroblasts. The laser plus scaling group showed the highest number of attached fibroblasts, with the tightly attached fibroblast prevailing. The laser-treated and scaled root specimens did not show any damage or morphologic alteration of the root surfaces.

Conclusion: CO_2 laser treatment in defocused, pulsed mode with a low power of 2W combined with mechanical instrumentation constitutes a useful tool to condition the root surface and increase fibroblast attachment to root surfaces. *J Periodontol 2002;73:1308-1312.*

KEY WORDS

Lasers/therapeutic use; planing; scaling; comparison studies; tooth root; ultrasonic therapy.

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Connective tissue reattachment to periodontally damaged root surfaces is one of the most important goals of periodontal therapy. Periodontal microbiota and bacterial endotoxins contaminate root surfaces in periodontal pockets, inhibiting migration and attachment of fibroblasts.[1,2] Mechanical instrumentation leaves the root surfaces covered with a smear layer that obliterates the orifices of the dentinal tubules, which contain microbiota, bacterial endotoxins, and residual, contaminated root cementum.[3] This could compromise periodontal healing and regeneration of connective tissue attachment. Laser therapy has been recently considered an important tool in improving treatment of periodontal disease, because of its ability to condition dental hard tissues.

The Nd:YAG laser, with an energy ranging from 150 to 87.5 mJ/pulse has shown a bactericidal effect suppressing and eradicating putative periodontal pathogens from periodontal pockets.[4] Other authors[5,6] have studied laser application in vivo and have shown that mechanical instrumentation associated with laser treatment of periodontally involved root surfaces could suppress periodontal pathogens such as *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis,* and *Prevotella intermedia*. Laser treatment has also been reported to eradicate bacteria as well as bacterial endotoxins from dental hard tissue. Yamaguchi et al.[7] showed that Er:YAG laser treatment at an energy density of 300mJ/cm^2 could effectively remove lipopolysaccharides (LPS) from root surfaces. These findings support the hypothe-

sis that laser irradiation with adequate power setting is a useful adjunctive tool in significantly reducing bacterial endotoxins.

The Nd:YAG laser has been reported to remove the smear layer, uncovering dentinal tubules and exposing collagen fibers on root surfaces. The Nd:YAG laser, with non-contact delivery mode and short irradiation time, has shown no damages and/or alterations to dental hard tissues.[8] More recently, CO_2 laser treatment at 3W for 1 second completely removed the smear layer on periodontally involved root surfaces after root planing. This low-energy density and short exposure time protocol for CO_2 laser treatment was associated with no damage to the root cementum and with minimal changes in the diameter of the dentinal tubules.[9]

Despite the above-reported studies on laser application as an adjunctive tool in the treatment of periodontal disease, several questions still remain unresolved, such as maximum energy level in order to avoid damaging the treated tissues, exposure time required to yield the best therapeutic effects with minimal damage, and the ability of fibroblasts to migrate and attach to root surfaces after laser irradiation. The purpose of this study was to analyze the effects of a CO_2 laser with a wavelength of 10.6 µm on periodontally involved root surfaces and to compare this treatment with scaling and root planing in terms of fibroblast attachment and growth.

MATERIALS AND METHODS

Specimen Preparation and Treatment

Thirty single-rooted human teeth extracted because of extensive loss of periodontal supporting tissues were used in this study. Teeth from patients with a history of systemic disease, under antibiotic treatment in the last 4 months, or who underwent periodontal debridement in the last 6 months were excluded. All teeth were extracted under local anesthesia in a non-traumatic fashion; immediately after extraction, blood, saliva, and soft tissue debris were removed by light scrubbing with a sterile scrub brush and by rinsing with sterile saline solution. Two specimens cut with a sterile diamond disk running at low speed with sterile water coolant were obtained from each tooth. The first section was 2 mm apical to the cemento-enamel junction; the second section was 2 to 3 mm coronal from the root apex. A longitudinal bucco-lingual section was cut to expose the pulpal wall and to obtain 2 specimens from each root. To avoid contamination from the pulp, the pulpal wall was separated from the remaining outer portion of root dentin by a bur at low speed kept parallel to the longitudinal axis of the root. Sixty specimens were obtained and randomly assigned to 1 of 3 groups: 1) control group: 10 specimens rinsed with sterile saline solution; 2) scaling and root planing group: 25 specimens scaled with ultrasonic instruments and root planed with hand curets until all visible calculus was removed; or 3) CO_2 laser group: 25 specimens treated with a CO_2 laser[§] with a wavelength of 10.6 µm in defocused, pulsed mode. Laser beam was emitted with a spot size of 2.5 mm in a pulse mode with a frequency of 1 Hz, a power of 2 W, and a duty cycle of 6% for 15 seconds. The duty cycle is defined as laser pulse duration divided by the duration of a single repetition period, and varies between 2% and 40%.[10] The laser beam was positioned perpendicularly 4 cm from the surface of the specimen. After laser treatment, the root specimens were scaled using an ultrasonic instrument.

Fibroblast Culture and Incubation

The continuous mouse fibroblast cell line L-929, which readily adheres and spreads onto most substrates, was chosen for this study. The cell culture media was Dulbecco's modified Eagle's medium[‖] with 10% fetal bovine serum,[‖] 100 µg/ml streptomycin,[‖] 100 U/ml penicillin,[‖] 2.5 µg/ml amphotericin B,[‖] and 2 mmol/l glutamine.[‖] The cells were cultured at 37°C in a humidified incubator with 5% carbon dioxide in air. Fibroblasts were harvested prior to confluence by means of a sterile trypsin-EDTA solution, resuspended in the experimental cell culture medium, and diluted to 1×10^5 cells/ml. Five ml of the cell suspension was seeded into the tissue culture polystyrene containing root samples and incubated for 3 days. At the end of this period, cells on samples were rinsed with Dulbecco's phosphate buffered saline (DPBS) and fixed by DPBS solution containing 4% glutaraldehyde.[‖]

SEM Preparation and Statistical Analysis

Fixed samples were dehydrated by several passages in an ethanol/water solution. After the last passage in absolute ethanol, dehydration was completed by a 30-minute immersion in hexamethyldisilazene. The dehydrated cells were sputter coated by gold (Agar sputter coater) and observed by SEM.[¶] SEM observation was performed as described by Trylovich et al.[11] Briefly, 3 photomicrographs were taken of each root specimen and were considered to be representative of the total surface area. Photomicrographs were taken with a positive angle of 15° at 3 non-overlapping points along an imaginary diagonal line. Medians, minimum, and maximum were calculated from the cell count data (Table 1). Statistical significance of differences between the 3 groups was determined using the Kruskal-Wallis 1-way analysis of variance.

RESULTS

Fibroblasts observed on experimental root specimens were either flat or round in morphology. The fibroblasts

§ Elan, Florence, Italy.
‖ Sigma-Aldrich, Milan, Italy.
¶ Leo 420, Electron Microscopy, Frankfurt, Germany.

tightly attached to specimens by numerous lamellopodiae were flat in appearance; in contrast, the fibroblasts poorly attached to specimens exhibited few attachment processes and were round in appearance (Table 1). The control (untreated) group showed the lowest number of attached cells, with no flat fibroblasts and a median of 6 round fibroblasts per each examined sample. The control group specimens frequently exhibited bacterial cells (Fig. 1). The SRP group had a median of 17 flat and 9 round fibroblasts per each examined sample (Figs. 2 and 3). The number of flat fibroblasts was statistically significantly higher (P <0.001) in the SRP group than in the control group. SEM analysis revealed some residual bacterial cells along the root surface in the SRP group.

The laser and scaling-treated group contained the highest number of cells, with a median of 36 flat and 12 round fibroblasts per each examined sample (Figs. 4 and 5). The number of well-attached (flat) fibroblasts was significantly higher compared to the control (P<0.001) and SRP (P<0.001) groups. SEM analysis of the laser-treated and scaled root specimens revealed no damage to the root surface; moreover, no residual bacterial cells were recovered from the root specimens.

Table 1.

Number of Round and Flat Fibroblasts Attached to Root Surfaces

	Control		SRP		Laser/Scaled	
	Flat	Round	Flat	Round	Flat	Round
Median	0*	6	17*	9	36*	12
Minimum	0	2	0	2	11	4
Maximum	0	12	24	15	46	33

*Statistically significant (P <0.001) between the 3 groups.

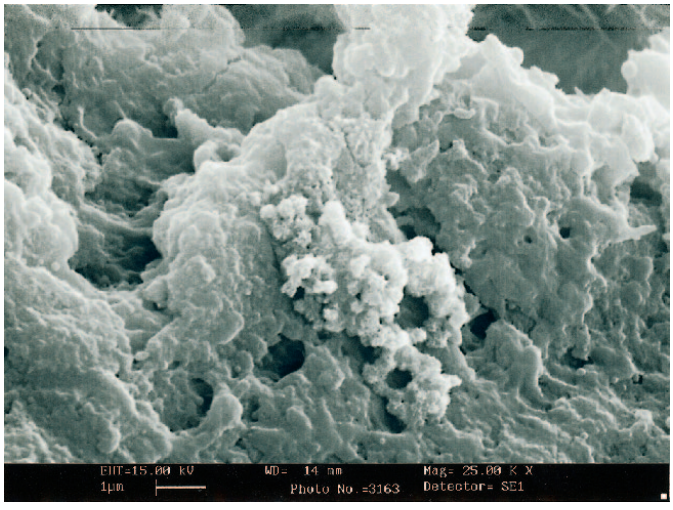

Figure 1.
Control specimen in which round bacterial cells were observed (original magnification ×25,000).

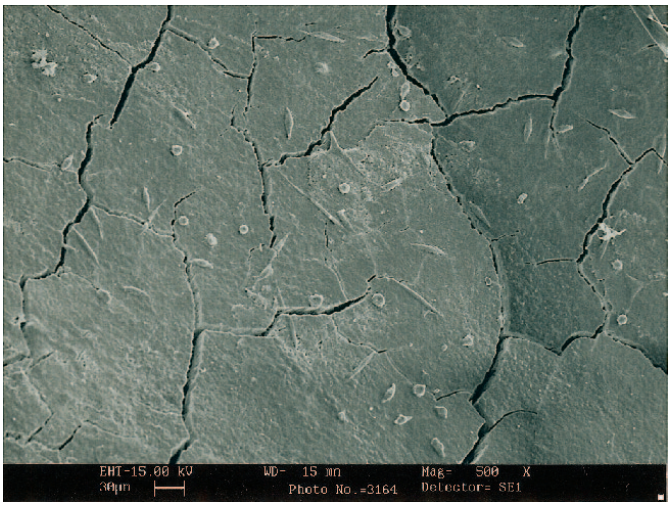

Figure 2.
Specimen treated by scaling and root planing shows round fibroblasts (original magnification ×500).

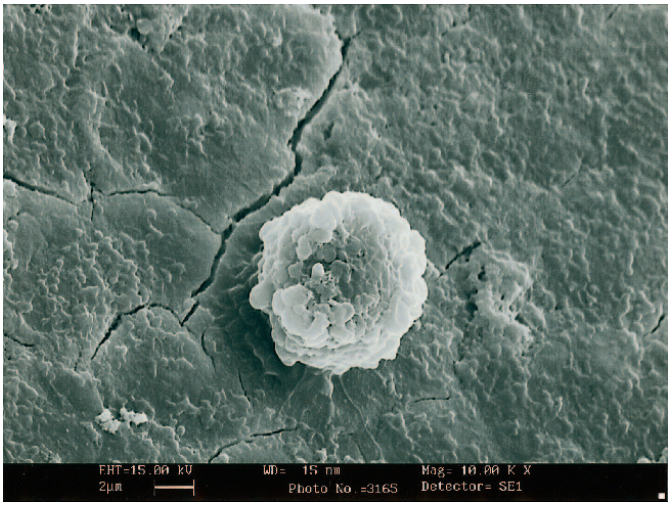

Figure 3.
Same specimen as Figure 2 at higher magnification (×10,000), showing a round fibroblast cell attached to the root surface.

DISCUSSION

The eradication of subgingival microbiota and bacterial endotoxins from root surfaces is an important factor in the outcome of periodontal therapy and often impossible to obtain by mechanical debridement alone.[12] Adriaens et al.[13] observed deep bacterial invasion of the radicular dentin and of dentinal tubules in periodontally involved teeth. This location could rep-

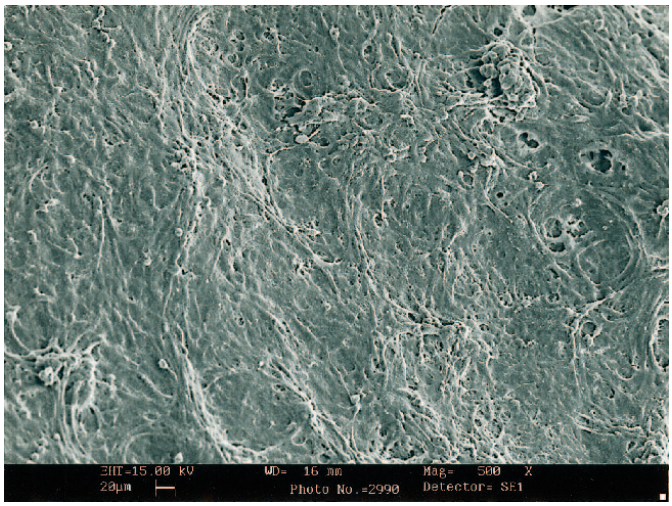

Figure 4.
Lased and scaled specimen which exhibits a high number of well-attached flat fibroblasts (original magnification ×500).

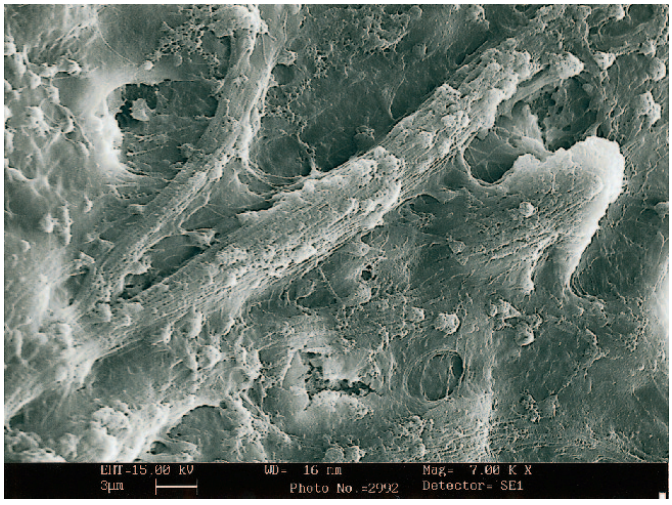

Figure 5.
Lased and scaled specimen shows a flat fibroblast considered healthy and firmly attached due to the presence of well-developed lamellopodia (original magnification ×7,000).

resent a bacterial reservoir for recolonization of instrumented root surfaces, explaining why mechanical treatment alone may be unsuccessful.

Laser treatment has been shown to reduce the amount of subgingival microbiota[14] and to significantly decrease the levels of suspected periodontal pathogens.[5] Other authors have also shown that laser treatment removes LPS from root surfaces.[7] This bactericidal effect could support the hypothesis that laser therapy represents a useful tool in periodontal treatment.

In contrast to the favorable effects of laser treatment shown in the above studies, questions still remain unresolved, such as the ability of fibroblasts to attach to and grow on laser-treated root surfaces, resulting in periodontal healing. Fibroblast attachment has been evaluated to test the biocompatibility of the dental hard tissue after laser treatment.[11,15] Our study was designed to evaluate fibroblast attachment to root surfaces treated by CO_2 laser without any damage to root surfaces.[16] Well-attached fibroblasts appear flat at SEM observation and could be a measure of biocompatibility of laser-treated surfaces.

Findings from the present study showed that the lowest number of fibroblasts were observed on control specimens. This could be explained by the presence of contaminating bacterial endotoxins on root specimens. The SRP group exhibited significantly more flat fibroblasts than the control samples. This was probably due to a reduction in the amount of bacteria and bacterial endotoxins on root surfaces obtained with mechanical debridement alone. A significantly higher number of flat fibroblasts were observed on CO_2 laser/scaled treated root samples than on the other 2 experimental groups. This could suggest that CO_2 laser treatment combined with ultrasonic scaling could enhance fibroblast attachment on the root surfaces better than SRP alone.

Many authors have pointed out the bactericidal effect of the CO_2 laser and its application to detoxify root canal walls as well as cementum root surfaces.[17,18] Talebzadeh et al.[19] examined CO_2 laser effects on different Gram-negative bacteria, at energy power levels lower than those used to cut and ablate dental hard tissues. These authors showed that the CO_2 laser demonstrated bactericidal action on all bacterial strains tested. These reports suggest that CO_2 laser treatment, at an adequate energy power setting, could condition root surfaces without inducing damages or morphologic alterations.

Trylovich et al.[11] found that Nd:YAG laser treatment modifies biocompatibility of root surfaces and reduces the number of attached fibroblasts in comparison to untreated controls and a laser-untreated/endotoxin-treated group. The differences between the findings of Trylovich et al.[11] and our study could be attributed to the different experimental protocols employed. Trylovich and coworkers analyzed Nd:YAG laser treatment on unerupted wisdom teeth, the root segments of which did not show any of the root surface modifications associated with naturally occurring periodontal disease. This could reduce the absorption of *Escherichia coli* endotoxin to the cemental surfaces used by the authors[11] to cause bacterial contamination of root surfaces. Moreover, the endotoxin employed by Trylovich and coworkers did not significantly alter fibroblast attachment.[11] In our study, we used a CO_2 laser in defocused pulsed mode, and the experimental specimens were collected from periodontally

involved teeth naturally contaminated by microbiota and bacterial endotoxins. The Nd:YAG laser treatment reported by Trylovich et al.[11] exhibited under SEM analysis surface alterations such as charring, crater formation, and cementum meltdown. Experimental root segments were not exposed to any mechanical instrumentation after laser treatment, leaving a root surface with altered biocompatibility, in contrast to our study protocol, where the root specimens were scaled after laser treatment to remove any possible contaminants. Moreover, our SEM analysis showed no damage to the root surfaces. These differences could explain why, in the study of Trylovich et al.,[11] the laser-irradiated/endotoxin-treated group showed the lowest number of attached fibroblasts compared to our study, where the laser-treated and scaled root surfaces showed the highest number of attached fibroblasts. The results of our study indicate that laser treatment combined with traditional mechanical instrumentation could be considered a useful tool to condition the root surfaces and enhance fibroblast attachment to root surfaces. More extensive, well-controlled in vivo studies are necessary to confirm and validate these in vitro observations.

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