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# Comparative Study of Dentine Permeability after Apicectomy and Surface Treatment with 9.6 μm TEA $CO_2$ and Er:YAG Laser Irradiation

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## ABSTRACT

**Failure of apicectomies is generally attributed to dentine surface permeability as well as to the lack of an adequate marginal sealing of the retrofilling material, which allows the percolation of microorganisms and their products from the root canal system to the periodontal region, thus compromising periapical healing. The purpose of this study was to evaluate dentine and the marginal permeability after apicectomy and surface treatment with 9.6 μm TEA $CO_2$ or Er:YAG 2.94 μm laser irradiation. Sixty-five single rooted human endodontically treated teeth were divided into five experimental groups: group I (control), apicectomy with high speed bur; group II, similar procedure to that of group I, followed by dentinal surface treatment with 9.6 μm $CO_2$ laser; group III, similar procedure to group I followed by dentinal surface treatment with Er:YAG laser 2.94 μm; group IV, apicectomy and surface treatment with $CO_2$ 9.6 μm laser; and group V, apicectomy and surface treatment with Er:YAG laser 2.94 μm. The analysis of methylene blue dye infiltration through the dentinal surface and the retrofilling material demonstrated that the samples from the groups that were irradiated with the lasers showed significantly lower infiltration indexes than the ones from the control group. These results were compatible with the structural morphological changes evidenced through SEM analysis. Samples from groups II and IV (9.6 μm $CO_2$) showed clean smooth surfaces, fusion, and recrystallized dentine distributed homogeneously throughout the irradiated area sealing the dentinal tubules. Samples from groups III and V (Er:YAG 2.94 μm) also presented clean surfaces, without smear layer, but roughly compatible to the ablationed dentine and without evidence of dentinal tubules. Through the conditions of this study, the Er:YAG 2.94 μm and the 9.6 μm $CO_2$ laser used for root canal resection and dentine surface treatment showed a reduction of permeability to methylene blue dye.**

## INTRODUCTION

THE SUCCESS of endodontic treatment is based on very carefully conducted series of steps, from the opening of the pulpar chamber to the root canal filling. Correct access surgery offers intimate contact conditions of the instruments with the root canal wall together with the irrigation solutions which clean, and promote the adequate desinfection of the root canal during the chemical preparation so that the root canal filling can be done properly, sealing the canal and also avoiding the fluid changes among the root canal systems and periapical tissues.

On the other hand, failures of the treatment may occur because of several factors even with the correct execution of these steps; thus, a new endodontic intervention might be considered. A periapical surgery becomes the indicated therapeutic alternative[1] when all the resources of a retreatment are exhausted, in cases with presence of clinical signs and symptoms and the difficulty of achieving the ideal limit measure for the obturation because of

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anatomical processes or for previous iatrogenical procedures, or for calcified teeth, perforations and curved roots, intra-root pins which are very hard to be removed,[2] or even for contaminated root canals persistent to the conventional endodontic therapy with the maintenance of the harmful apical process.[3]

Generally, failures of the apical surgeries have been related to the remaining microorganisms, their products and the organic fluids percolation of the root canal systems to the periapical region, being possible to penetrate the obturation interface due to the reduced sealing capacity of the retrofilling material,[4,5] or through the exposed dentinal tubules on the resection surface.[1,6] Such microorganisms are capable of being present in extra-root canal infections,[3,7] and to invade the periapical tissues.[8] For these reasons, several techniques have arisen with the purpose of creating a better apical surgical treatment in order to enhance the marginal sealing in the retrograde filling material as well as the dentinal surface sealing enabling biological conditions for tissue healing and cement repositioning.

Some high-intensity lasers have the capacity of removing hard dental tissues by the ablation process because they are highly absorbed by the target tissue components, water and hydroxiapatite, and have been considered efficient for cutting hard dental tissue and bone tissues with extremely small thermal damage.[9,10] *In vitro* studies using the laser for apicectomy have been conducted in order to obtain a smoother and less permeable dentinal cut surface producing the melting and recrystallization of the dentinal structure with the dentinal tubules obliteration.[11–17] Dederich et al.[61] were the first to report the melting and recrystallization of root canal dentine followed by Nd:YAG laser irradiation with the potential decrease of permeability to fluids on the basis of the nonporous aspect of the dentine under scanning electron microscopy examination.

Furthermore, lasers have been used clinically for periapical surgeries[18–20] and evaluated long term with clinical and radiographic follow-up.[21] The laser light acts also with a biomodulation effect stimulating the cellular activity of the tissue healing processes, promotes the microbiological reduction besides the ability to cut the apical portion of the root through the ablation process.

The aim of this study was to analyze the 9.6 μm $CO_2$ and the 2.94 μm Er:YAG laser effects on the dentinal surface after apicectomy and surface treatment, focusing on marginal retrofilling and dentine cut surface permeability.

## MATERIALS AND METHODS

### *Features of the lasers used in this study*

The pulsed 9.6 μm carbon dioxide laser (Opus 96, Opus Dent), known as TEA $CO_2$ laser (transversely excited at atmospheric pressure) possesses carbon dioxide gas as the active material and the He, $N_2$, and $CO_2$ as facilitators gases. This laser is localized in the far infrared range of the electromagnetic spectrum. For this reason a Helium-Neon guide laser beam (He-Ne laser) with a 5 mW power which emission is parallel adjusted to the $CO_2$ laser is necessary. Delivery system of the beam is through an articulated arm and the handpiece has a lens for the laser beam emission. The edge of this piece is an apparatus in order to keep a constant 10 mm focal distance between the lens and the target tissue. Refrigeration with air/water spray is made through a hole on the handpiece towards the laser irradiation focus. Pulse length is 60 μsec, with an average power which can vary from 1 to 7 W, peak power of 700 W and repetition rate which can be adjusted from 20 to 250 Hz. Laser beam emission follows a circular, rectangular or linear pattern configuration. The circular pattern diameter can vary from 1 to 2.5 mm with a diameter beam of 300 μm.

The Opus 20 equipment (Opus Dent) can emit at two wavelengths: the continuous wave 10.6 μm $CO_2$ laser and the 2.94 μm Er:YAG laser. In this study, only the erbium laser system was used; therefore the wavelength characteristics described are only for the Er:YAG laser. The pulsed 2.94 μm Er:YAG laser has as the active medium a YAG (yttrium-aluminum-garnet) solid crystal, highly doped with erbium ions. However, this system does not present a guide beam and for this reason the irradiation is always made with a straight or angled handpiece with a sapphire fiber optic or an inserted hollow metallic tip to be used in the contact mode with the target tissue. Beam delivery is made through a hollow wave-guide and the straight or angled handpiece presents with a lens where the sapphire or metallic probe is inserted. Both the fiber and the metallic probe measured 17 mm but when inserted in the handpiece focal distance is 10 mm. Refrigeration occurs through a hole on the handpiece where the air/water spray flows parallel to the laser beam emission. Pulse length is 250–500 msec; average power is 1–12 W, repetition rate can be adjusted between 7 and 12 Hz, and the diameter beam is 1 mm.

### *Samples preparation*

Sixty-five recently extracted single-rooted human incisors, stored in physiologic saline solution were used in this study. The periapical radiographs of the teeth were taken on the mesial-distal and buccal-lingual orientations in order to verify the absence of deep curves, calcifications, double root canals or even evidences of previous endodontic interventions. All the teeth with the presence of these characteristics were not used in this study. Crowns were separated from the root canals using a low speed carborundum disk.

The root canals were instrumented with a no. 10K file and the instrumentation was performed until the no. 40K file throughout until the apical limit. Endo PTC and the 1% sodium hypochlorite solution were used and irrigated at each instrument change with a total of 50 mL for each root canal prepared. These root canals were then filled with EDTA-T and agitated inside the root canal with a no. 15 Kerr file for 3 min. In order to neutralize the EDTA-T, the root canals were irrigated with 10 mL of physiologic saline.

After preparation, the root canals were dried with absorbent paper points and filled with a number 40 Gutta Percha point, secondary points and AH-26 endodontic cement, overflowing the apical limit and following the active lateral condensation technique with a manual spacer. After filling, the excess cervical and apical cuts of the Gutta Percha cones were made with Paiva rammers and cervical accesses were sealed with zinc phosphate cement. Then, new radiographs were taken to check the root canal filling quality.

### *Experimental groups*

Filled root canals were divided in five experimental groups (Table 1) with 13 samples in each group following these steps:

TABLE 1. EXPERIMENTAL GROUPS

| Group | Apicectomy | Dentinal surface and retrocavity |
|---|---|---|
| I | High-speed | No treatment |
| II | High-speed | 9.6 μm $CO_2$ laser |
| III | High-speed Er:YAG laser | |
| IV | 9.6 μm $CO_2$ laser | 9.6 μm $CO_2$ laser |
| V | Er:YAG laser | Er:YAG laser |

1. The apices were resected with laser (Er:YAG or 9.6 μm $CO_2$ laser) or with a high-speed cylindrical bur (1090).
2. All the retro cavities were prepared in a standardized way with 3 mm depth with a low-speed number 1 straight stainless steel spherical bur (with an adhesive cursor to delimitate the depth).
3. The dentinal cut surfaces and the retro cavities were treated or not with the laser (Er:YAG or 9.6 μm laser) according to Table 1.
4. All the retro cavities were filled with IRM cement.
5. Roots were coated with two nail-polish layers.
6. Samples were submersed in a methylene blue solution.
7. They were worn on their long axis for the infiltration qualitative analysis.
8. Three apices from each group were analyzed using SEM.

In Group I, control group, the apices were cut with a high-speed cylindrical bur and the dentinal surface had no treatment.

In Group II, apices were similar to the control group, however the dentinal surface and the retro cavity walls were irradiated with the 9.6 μm pulsed $CO_2$ laser with constant air/water spray refrigeration. Irradiation was made following the circular configuration of the laser beam first on all the dentinal cut surface on the buccal-lingual and mesial-distal orientation and then on the retro cavity walls with total exposition time of 10 sec (Fig. 1).

In Group III, apices were cut similar to the control group, however the dentinal surface was irradiated with the Er:YAG laser using constant air/water spray refrigeration. All the dentinal cut surface and the retro cavity walls were irradiated the same way as the previous group (Fig. 2).

**FIG. 2.** Treatment of the dentinal cut surface with the Er:YAG laser.

In Group IV, apices were cut with the 9.6 μm pulsed $CO_2$ laser, and the dentinal cut surface and the retro cavity walls were also treated with the same 9.6 μm pulsed $CO_2$ laser (Fig. 3).

In Group V, apices were cut with the Er:YAG laser with constant air/water spray refrigeration. All the dentinal cut surface and the retro-cavity walls were irradiated the same way as in the other groups.

## *Laser irradiation*

*The parameters used with the 9.6 μm $CO_2$ laser.* For apicectomy, average power was 5 W; energy 250 mJ; frequency 20 Hz; beam diameter 300 μm; focal distance 10 mm; energy density 352.73 J/cm²; beam scanning pattern circular; and beam scanning diameter 1.6 mm. For cut surface treatment, average power was 3 W; energy 150 mJ; frequency 20 Hz; beam diameter 300 μm; energy density 211.64 J/cm²; beam scanning pattern circular; and beam scanning diameter 2.0 mm.

*Parameters used with the Er:YAG laser.* For apicectomy, average power was 10 W; energy 1000 mJ; frequency 10 Hz; beam diameter 1 mm; and energy density 126.98 J/cm² For cut surface treatment, average power was 5 W; energy 500 mJ; frequency 10 Hz; beam diameter 1 mm; and energy density 63.49 J/cm².

**FIG. 1.** Treatment of the dentinal cut surface with the $CO_2$ laser.

**FIG. 3.** Apical resection with the $CO_2$ laser.

### *Sample preparation for infiltration with the dye*

After laser irradiation for the apicectomy, for the dentinal surface treatment and for the cavity preparation, the retro-cavities were filled with IRM zinc oxide with an increased proportion of powder/liquid. From each group, three samples had their apical portion sectioned 1 mm thick for morphological change analysis through SEM.

Following this step, 10 roots from each group were coated with two layers of nail-polish on all their external root surface extension, with the exception of the dentinal cut surface. Then, the coated layer drying the roots were submersed in a 0.5% methylene blue solution, pH 7.2 during 48 h, and rinsed in running water for 12 h for the removal of dye excess. Then, the roots were abraded on the long axis until exposure of root filling material and the retrofilling. (Fig. 4).

### *Qualitative analysis of the infiltration*

The images of the root canal hemi-sections were captured by the Microteck Scanmaker and then digitalized and evaluated on the ImageLab 2.3 software for determination in each sample, of the relation between the total root dentine and the dye infiltrated area, in pixel units (Fig. 5).

Initially, for the standardization of the root canal area to be measured, a height of 291.45 pixels from the vertex cut surface, both mesial and distal, was defined. This measure was correspondent to the smaller dentin surface sample. From the straight line between these two points the total dentin area was delimitated excluding the Gutta Percha filling area and the IRM cement. Following this, the infiltrated area by the dye in each sample was also determined (Figs. 5 and 6).

The data were then arranged in tables and statistically analyzed using the biological research software GMC version 8.0 for the Kruskal-Wallis test.

### *Scanning electron microscope analysis*

After apicectomy and treatment of the dentinal surface and retro-cavity, three samples from each group were prepared for SEM analysis in order to verify the morphological changes on the surface after the $CO_2$ and Er:YAG laser irradiation.

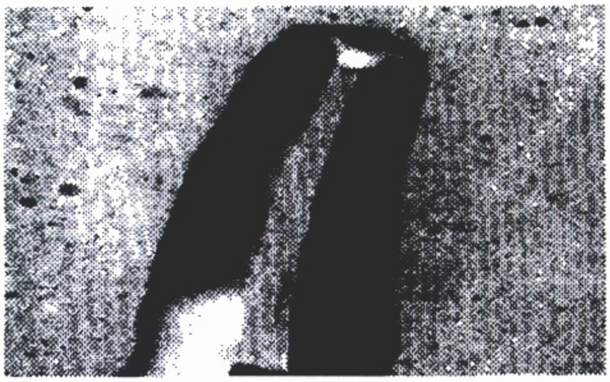

**FIG. 4.** Embedded root in the gypsum for the long axis abrasion.

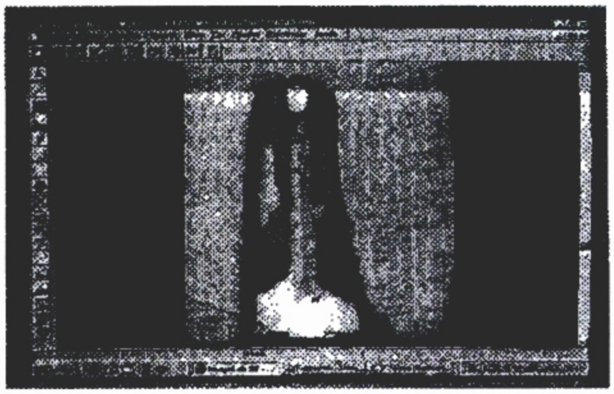

**FIG. 5.** ImageLab 2.3 software used for the determination of the dye infiltrated area and for the total dentine area.

## RESULTS

### *Infiltration indexes*

Measurements of the total dentine area and from the infiltrated area by the methylene blue dye were made from the digitalized images and inserted in the ImageLab software. The measurement of the areas were standardized in pixels, and the average values of the infiltration indexes from each group were calculated (Table 2, Fig. 7).

These original data of the samples from all the experimental groups submitted to the adherence test to the normal curve (Biological Research Software—GMC version 8.0) demonstrated that the 50 specimens did not present normally in the samples distribution (Table 3). This result was expected since the samples from the control group had higher infiltration indices when compared with the indexes from the samples of the laser irradiated groups.

The data were submitted to the Kruskal-Wallis test for the comparison among the experimental groups (Table 4).

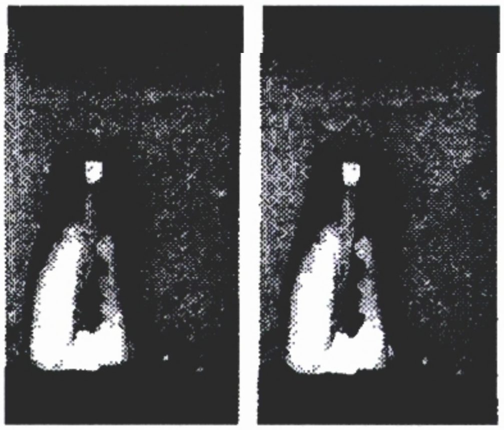

**FIG. 6.** Digitalized images before the delimitation of the infiltration area (left) and after the delimitation (right) made with the ImageLab 2.3 software.

TABLE 2. INFILTRATION INDEXES (%)

| *Samples* | *Group I* | *Group II* | *Group III* | *Group IV* | *Group V* |
|---|---|---|---|---|---|
| 1 | 16.22 | 11.81 | 6.29 | 5.78 | 6.05 |
| 2 | 17.17 | 17.86 | 8.73 | 7.68 | 0.62 |
| 3 | 24.71 | 7.37 | 15.87 | 5.48 | 9.79 |
| 4 | 30.96 | 0.95 | 6.63 | 7.41 | 1.55 |
| 5 | 20.55 | 4.56 | 6.54 | 6.22 | 4.70 |
| 6 | 22.08 | 1.29 | 14.16 | 5.56 | 12.13 |
| 7 | 10.70 | 3.11 | 10.54 | 5.69 | 1.84 |
| 8 | 21.36 | 1.27 | 9.94 | 2.72 | 6.94 |
| 9 | 46.24 | 2.14 | 8.58 | 7.65 | 11.28 |
| 10 | 24.97 | 2.81 | 2.95 | 1.34 | 5.14 |
| Averages infiltration | 23.50 | 5.32 | 9.02 | 5.55 | 6.00 |

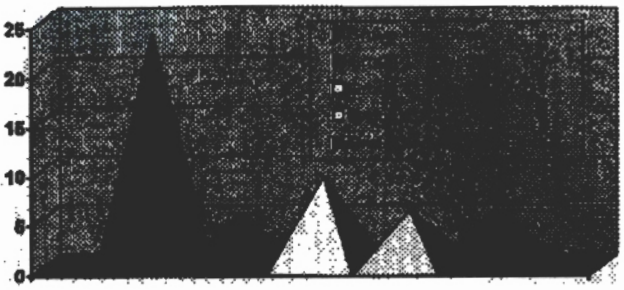

**FIG. 7.** Average of the infiltration indexes from each group.

The results demonstrated that all the samples presented dye infiltration, in different degrees. The samples irradiated with $CO_2$ and Er:YAG laser presented the lowest infiltration indexes when compared with the samples from the control group (Table 5).

The lowest infiltration indexes were from groups II, IV, and V. Group II had the lowest infiltration index (5.32%); however, there was no statistical significant difference between indices from groups IV and V (5.55% and 6.0%, respectively). The infiltration indexes from the group III and V did not present statistical difference between them (9.02 and 6.0, respectively).

### *Scanning electron microscopy*

The photomicrographs obtained with scanning electronic microscopy allowed to differentiate the structural changes that each wavelength caused on the dentinal cut surface and also to relate the changes with the marginal and superficial infiltration index.

It was possible to observe on the microradiographs of the sample surfaces from the control group (group I—high-speed) the smear layer without the dentinal tubules exposition, undulations produced by the high speed bur (Figs. 8 and 9).

The surfaces of the samples from group II (high-speed + $CO_2$ laser) presented similar aspect to the group IV ($CO_2$ laser + $CO_2$ laser) with images showing a high degree of melting and recrystallization of the dentine on all the irradiated surface distributed homogeneously creating a vitrified layer alternated with small craters (Figs. 10, 11, 14, and 15).

The surfaces of the samples from group III (high-speed + Er:YAG laser) presented similar aspects to group V (Er:YAG laser + Er:YAG laser) with rough irregular surface, with characteristics of ablationed dentine, and with the same pattern on all the irradiated area and no dentinal tubules exposition (Figs. 12, 13, 16, and 17).

## DISCUSSION

The results of the conventional periapical surgeries have been evaluated by several authors,[22–25] but the failure indices vary a great deal.[26] Previous authors mention that lack of clarity of the evaluation criteria and the obtained results in short-term clinical and radiographic follow-ups are responsible for the great variation of these indexes, reaching 33.8% on the posterior teeth. For this reason, most of the studies suggest clinical and radiographic follow-up at 5–10 years, since most of the failures could be evidenced only after long postoperative periods.[27]

On the other hand, only a few studies have been made with the objective to analyze if the straight or rough dentinal cut surface has any influence over the speed of the wound healing process. But the acquisition of a smooth surface is commonly indicated, because it would be favorable for the obturation material to be inside the preparation only without the retention on the dentin surface, and thereby increasing the reparation area of the periodontal ligament. Furthermore, the rough surface would make the visualization and probing more difficult for

TABLE 3. ADHERENCE TEST: NON-NORMAL SAMPLE DISTRIBUTION

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A. Frequencies per class intervals | | | | | | | |
| Class intervals | M-3s | M-2s | M-1s | Med. | M+1s | M+2s | M+3s |
| Normal curve | 0.44 | 5.40 | 24.20 | 39.89 | 24.20 | 5.40 | 0.44 |
| Experimental curve | 0.00 | 2.00 | 28.00 | 52.00 | 14.00 | 2.00 | 2.00 |
| B. Chi-square calculus | | | | Results | | | |
| Freedom liberty | 4 | | | Non-normal sample distribution | | | |
| Qui square value | 12.85 | | | | | | |
| Probability of Ho | 1.2000% | | | — | | | |

TABLE 4. KRUSKAL-WALLIS TEST

| | |
|---|---|
| Kruskal-Wallis calculated value (H) | 26.0584 |
| $X^2$ value for four freedom degrees | 26.06 |
| Ho probability for this value | 0.00% |
| Significant to the 1% level | $\sigma = 0.01$ |

TABLE 5. COMPARISONS AMONG THE AVERAGE VALUES OF THE SAMPLES

| | *Group 2 (5.32)* | *Group 3 (9.02)* | *Group 4 (5.55)* | *Group 5 (6.00)* |
|---|---|---|---|---|
| Group 1 (23.50) | 0.1% | 1% | 0.1% | 0.1% |
| Group 2 (5.32) | | 1% | ns | ns |
| Group 3 (9.02) | | | 5% | ns |
| Group 4 (5.55) | | | | ns |

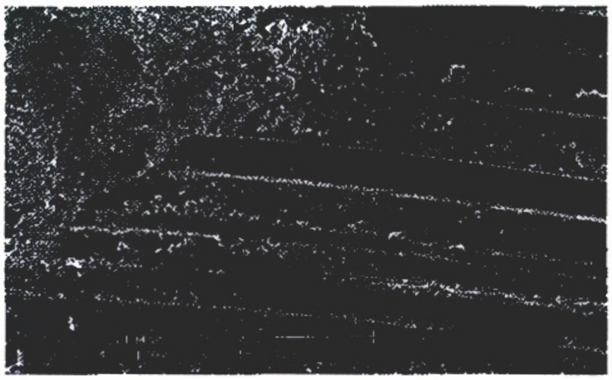

**FIG. 8.** Photomicrograph of the sample surface from the control group. Observe the smear layer and waving aspect of the dentine surface due to abrasion produced by the high-speed bur.

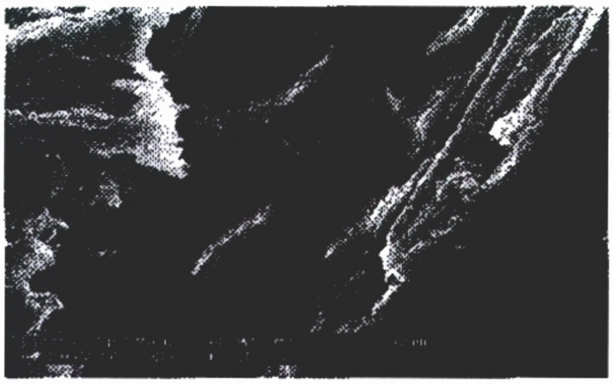

**FIG. 9.** Photomicrograph of the sample surface from the control group. Observe the smear layer on the dentine surface.

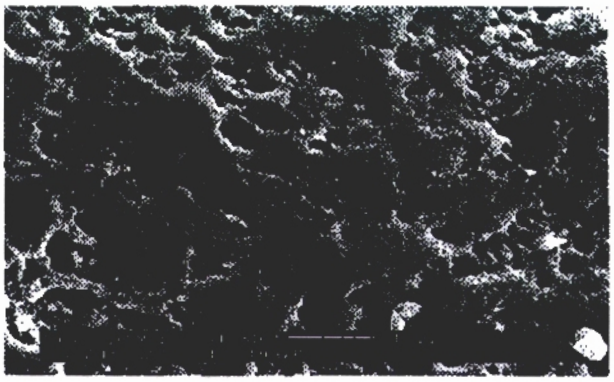

**FIG. 10.** Photomicrograph of the sample surface from group II. Observe the melting and recristalization area with sealing of most part of the dentinal tubules.

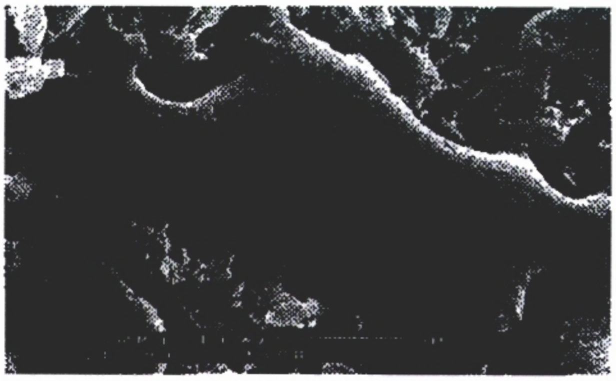

**FIG. 11.** Photomicrograph of the sample surface from group II. In a higher magnification, observe the elevated dentine fusion and melting degree.

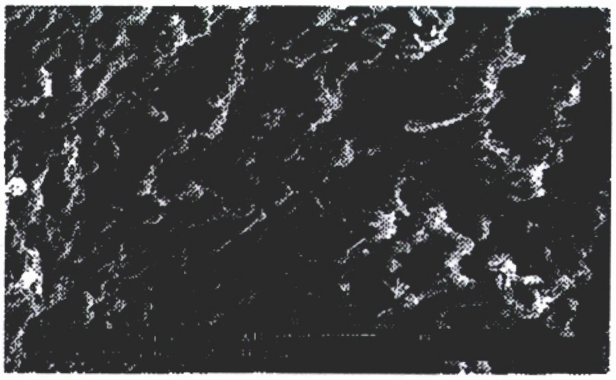

**FIG. 12.** Photomicrograph of a sample from group III. Observe the rough surface, without the smear layer and without the exposition of the dentinal tubules.

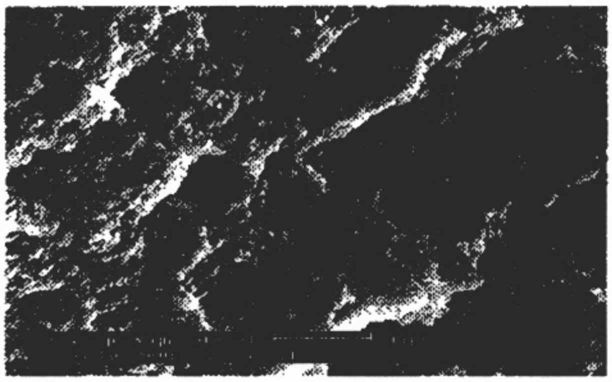

FIG. 13. Photomicrograph of a sample from group III. Observe the rough surface, clean and without the dentinal tubules exposition.

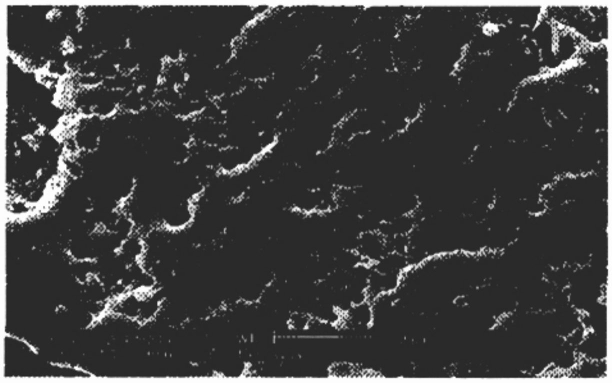

FIG. 14. Photomicrograph of a sample from group IV. Observe the surface with a melting of the dentine and also the resolidification homogeneously distributed, clean and without the dentinal tubules exposition.

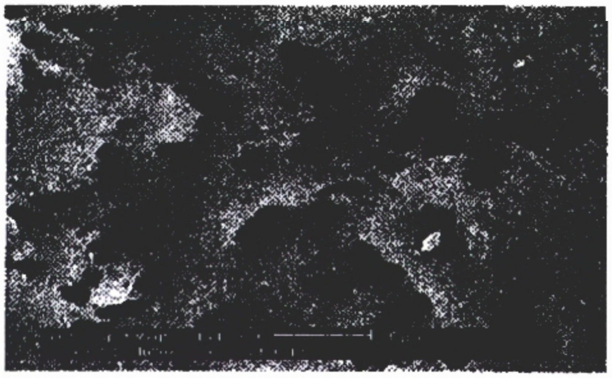

FIG. 15. Photomicrograph of a sample from group IV. Observe the surface with an elevated dentin melting and recrystallization degree homogeneously distributed, clean and without the dentinal tubules exposition.

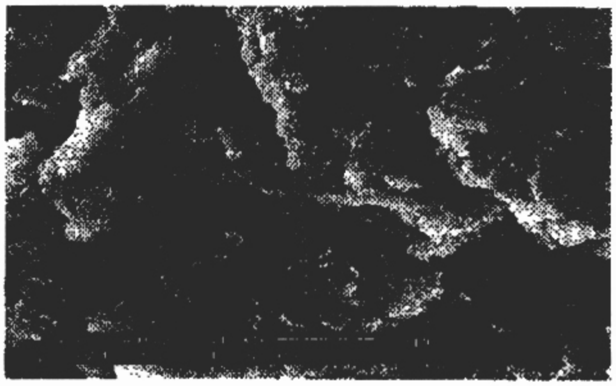

FIG. 16. Photomicrograph of a sample from group V. Observe the rough, clean, no smear layer and no dentinal tubules exposition on the dentine surface.

the detection of eventual fractures and could also favor microorganisms' growth.

A literature review reveals that, among the failures that occur in the apical surgeries, the presence of microorganisms associated with the exposed dentin permeability on the cut surface has been pointed out as one of the main causes for the recurrence of the pathological process. For this reason, it is paramount not only to achieve the maximum microorganisms reduction from the periapical region as well as the acquisition of smooth, regular, and impermeable apical resection surfaces.[28–30]

With the advent of the laser and the diffusion of its use in dentistry, research demonstrates the capacity of some wavelengths to remove the dental hard tissue by the ablation process being for this reason possible its use for cavity preparation and also for apicectomy. The Er:YAG and the 9.6 μm $CO_2$ laser are highly absorbed by the water and minerals content of the enamel and dentine. Electromagnetic energy absorbed by components of the tooth are transformed in thermal energy with a local degradation in heat. Thermal action heats up the water content of the minerals causing micro explosions and disrupts the hard tissue. However, emission of the irradiation must be pulsed, with a short thermal relaxation time and a short pulse duration

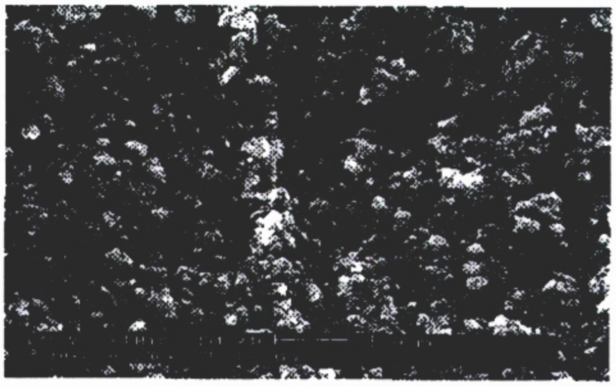

FIG. 17. Photomicrograph of a sample from group V. Observe the rough, clean, with ablation characteristics and no dentinal tubules exposition of the dentine surface.

so that thermal harmful effects do not occur. Thermal relaxation time is the time that the remaining energy, which is not absorbed for the hard tissue removal on the ablation, takes to spread in the layer beneath the ablationed tissue. As to the micro-explosion ablation processes mediated by water, if the pulse width and the thermal relaxation time are low, the deposited heat will only depend on the energy density.[31]

This study evaluated if the dentin morphological changes caused by the 2.94 μm Er:YAG laser and also with the 9.6 μm $CO_2$ laser on the apicectomies and treatment of the cut surface promote the reduction of the marginal and superficial permeability once the bactericidal action of the high intensity lasers has also been exhaustively explored in several studies using different wavelengths,[15,32–36] and even showed their potential for the bacterial reduction in depth.[37,38] It also presents an anti-inflammatory potential action, analgesia, and biomodulation, which stimulates the cell activity optimizing the wound healing process.[39]

Due to the deep thermal damages caused to the hard tissues irradiated with the continuous 10.6 μm $CO_2$ laser,[12,20] its use has been indicated for soft tissues.

The main $CO_2$ laser transition is the wavelength of 10.6 μm. However, it has the capability to emit at several wavelengths alternating some factors in the active medium like the pressure inside the tube and the concentration of the facilitator gases like the helium, nitrogen, and carbon dioxide.[40]

Recent studies demonstrated that the $CO_2$ laser irradiation with a pulse beam mode and with wavelengths of 9.6–1.06 μm, more specifically the pulsed 9.6 μm $CO_2$ laser, have the capacity to remove the dental hard tissue by the ablation process in general followed by fusion, since this wavelength is highly absorbed by the water components and hydroxyapatite of dental tissues but with predominant resonant absorption for the phosphate radical of the hydroxiapatite crystals. On the infrared transmission spectra of the hydroxiapatite crystals (Fig. 18) the low energy transmission percentage on the wavelengths near the 10 μm band shows that the lowest percentage occurs for the 9.6 μm wavelength meaning that this one is highly absorbed by the inorganic content of the hard dental tissues. For this reason, the use of these wavelengths, mainly the 9.6 μm, presents a great potential for cavity preparations,[10,31] as well as for apicectomy.[41]

Previous authors [10,41–48] demonstrated the use of wavelengths between 9.3 and 10.6 μm with pulsed emission, irradiating dentin and enamel samples in cavity preparations and apicectomies, without causing the carbonization of the hard tissues.

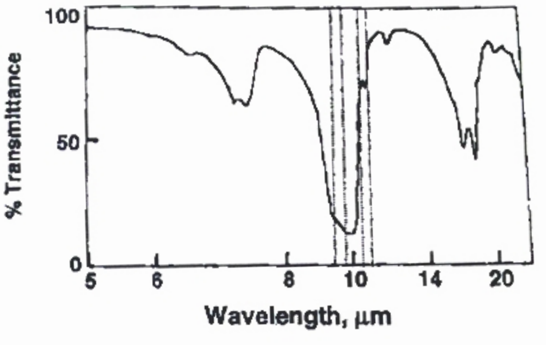

**FIG. 18.** Transmission spectrum for the carbonated hydroxiapatite as a function of wavelength.

The ablation process was also evaluated with the Er:YAG laser. Several in vitro studies demonstrated the ability to remove hard dental tissue with this wavelength. Hibst and Keller, and Keller and Hibst were the pioneers that mentioned the irradiation with the pulsed Er:YAG laser on the enamel and dentin for cavity preparation without causing thermal damage to the adjacent tissues under constant refrigeration and with the use of adequate energy parameters since this wavelength is highly absorbed by the components of the dental tissues, mainly by water and hydroxiapatite. Several studies[16,18,20] have demonstrated that the resected root dentine with Er:YAG laser presented a clean, no smear layer, with dentinal tubules exposed and slightly irregular surface.

In the attempt to obtain a vitrified, homogeneous, and continuous irradiated resection surface area, the pulsed 9.6 μm $CO_2$ laser was used to evaluate the dentinal permeability. This pulsed laser, in addition to presenting the capacity to cause the ablation, can cause the melting and resolidification of the dentin throughout the irradiated area,[19,48] and a less permeable dentinal surface. In fact in the conditions of this study, it was possible to demonstrate that with the 9.6 μm $CO_2$ and 2.94 μm Er:YAG laser irradiation for the apical resection and superficial treatment of the cut dentine decreased the dentinal superficial and marginal permeability leading to less infiltration of the dye when compared with the apexes cut with high-speed drill. For this reason, the use of these two wavelengths (Er:YAG and 9.6 μm $CO_2$ laser) in apical surgeries could promote less permeable surfaces and with this the occurrence of a lower liquid percolation degree between the root canal system and the apical periodontum. However, it is worth noting the importance of the development of future studies that evaluate if the resection surface changed by the laser irradiation would promote better conditions for the cement apposition and if the apical reparation would be faster.

In this present study, the original data of the infiltration indexes in percentage from each group, demonstrate dye infiltration in all the samples but in different degrees. On the other hand, the samples from the control group presented significantly higher infiltrations than the samples from the other experimental groups. The group II, which samples had their apexes cut with a high-speed drill and the surfaces treated with the 9.6 μm $CO_2$ laser, and the group IV, with the apexes cut and treated with the 9.6 μm $CO_2$ laser presented the lowest dye infiltration indexes, 5.32% and 5.55%, respectively, with no statistical difference between the two groups demonstrating lower marginal and superficial permeability among the experimental groups. Those result was expected since previous studies with the 9.6 μm $CO_2$ laser demonstrated its capacity to promote the ablation changing morphologically the enamel and dentine structure as well as change the chemical composition of the enamel inhibiting the carious lesion progression.[50,51] Recently, other authors[41,48] demonstrated that the dentine irradiation with the 9.6 μm $CO_2$ laser produces morphological changes of the structure with formation of elevated fusion and resolidification degrees of the dentine. In our experiment, the lowest dye infiltration degree is due to the decrease of the number and diameter of the dentinal tubules obliterated by the fusion. Marginal infiltration of the dye was lower since the laser irradiation caused the smear layer removal allowing a better adaptation of the retro filling material with the cavity walls. Previous studies demonstrated that the removal of the smear layer and the fusion areas on the root canal dentinal surface from the Nd:YAG laser irradiation, caused better adaptation of the filling material to the root canal walls because they presented better infiltration degrees than the samples from the control group on which the smear layer was not removed.[52,53]

Evaluation of the photomicrograph of the samples from groups II and IV allowed to observed that all the irradiated surface by the 9.6 µm $CO_2$ laser presented evaluated characteristics of fusion degrees of the dentine distributed homogeneously and continuously, with a vitrified aspect and with the presence of small craters. This fact leads us to believe that the irradiated dentine surface with the 9.6 µm $CO_2$ laser presents less permeability than the one produced by the Nd:YAG laser irradiation since the fusion and resolidification due to the Nd:YAG laser irradiation occur in discontinued areas and so only partial sealing of dentinal tubules was visible on the surface.[13,14,54–57]

Regarding group III, apicectomized with the high-speed drill and the surfaces treated with the Er:YAG laser, and group V, with the apexes removed and the surfaces treated with the Er:YAG laser, it was observed that the infiltration degrees with the dye were lower in group V, but there was no statistically significant differences between these two groups. The results of the infiltration indexes of cut dentine permeability irradiated with the Er:YAG laser were surprising since a higher dye infiltration degree was expected because previous studies demonstrate infiltration degrees very close to the control group (apicectomized with high-speed drill) and to promote the exposition of the dentinal tubules allowing thus the infiltration of the dye.[16,57] However, in this study, it was not possible to prove through the photomicroradiographs from both samples from the group III and also from the group V, the opened dentinal tubules contradicting previous studies that demonstrated this characteristic on the Er:YAG irradiated dentine.[9,44,56,57] Perhaps the slightly rough surface aspect also without the dentinal tubules exposure might be the reason for the low infiltration degree of the dye. Other studies must be made to reinforce this, irradiating the dentin with other energy densities to verify the influence of the irradiation conditions on the dentinal tubules exposure.

On the other hand, low marginal infiltration index may be explained due to the removal of the smear layer form the retro preparation walls, enhancing the material adaptation and with an adequate sealing between the filling material and the walls reducing thus the marginal permeability.[52–53]

Studies evaluating the temperature increase due to 9.6 µm $CO_2$ laser irradiation using the Opus 96 equipment (Opus Dent) have not yet been done. In the present study the parameters of 5 W (average power), 20 Hz (frequency), and 353.73 J/cm$^2$ (energy density), following the circular irradiation profile with 1.6 mm diameter for the apicectomies, and 3 W (average power), 20 Hz (frequency), and 211.64 J/cm$^2$ (energy density) with the circular irradiation profile with 2.0 mm diameter for the dentin surface treatment under intense air/water refrigeration, it was not possible to observe the heating of the external root canal surface not even the sample carbonization.

Authors like Fried et al.[43] irradiating the bovine teeth enamel with the 9.6 µm pulsed $CO_2$ prototype laser with energy densities ranging from 5 to 25 J/cm$^2$ and pulse duration of 100 µsec, had a temperature measurement of 600°C on the enamel surface, however at the 125 µm depth the temperature did not exceed 5°C since all the energy is absorbed by a very thin enamel thickness, less than 10 µm. In another study by Fried et al.[44] in 1997, irradiating the dentine surface with the $CO_2$ laser (Pulse System, Los Alamos, NM), pulse duration of 100 µsec, frequencies ranging from 1 to 10 Hz, and energy densities from 0.5 to 8 J/cm$^2$, with an irradiation profile following the rectangle configuration, they had temperature increase from 1 to 2°C.

The Er:YAG laser (Opus 20, Opus Dent), demonstrated in an *in vitro* study a temperature increase from 1 to 2°C, with the maximum increase of 3°C during class V cavity preparations with parameters from 500 to 850 mJ and 10 Hz.[58,59,60] The same way as the $CO_2$ laser, the Er:YAG laser irradiation in this study conditions with energy density of 352.73 J/cm$^2$ for the apicectomies and of 63.49 J/cm$^2$ for the dentine surface treatment, did not cause external root canal surface heating not even carbonization.

The literature review shows that the use of Er:YAG and Nd:YAG lasers have been reported frequently in studies to evaluate the dentinal permeability allied with their confirmed microbial reduction capacity. On the other hand, regarding the 9.6 µm $CO_2$ laser, which is still a prototype with the commercial release in the near future, most research is in the caries prevention area. For this reason, this study was considered relevant, because it evaluates its capacity to promote the dentinal permeability. Although in this study all the samples had shown some dye infiltration, the results were favorable because the dentine morphological change by to the 9.6 µm $CO_2$ laser irradiation and the energy parameters used were able to promote the sealing in most parts of the dentinal tubules, resulting in lower superficial and marginal dye infiltration degrees. However, other dentine irradiating conditions with this new laser system must be evaluated in the attempt to obtain total dentinal tubules sealing without causing the tissue carbonization.

## CONCLUSION

Under the conditions of this study, the 9.6 µm TEA $CO_2$ and the Er:YAG laser irradiation reduced the permeability of dye penetration on dentine cut surface after apicectomy and treatment of surface.

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