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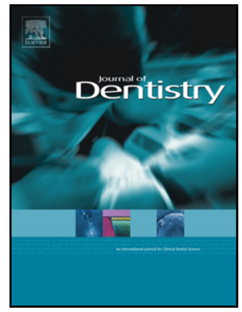

Title: Effect of the Nd:YAG laser on sealer penetration into root canal surfaces: a confocal microscope analysis

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Title: 'Effect of the Nd:YAG laser on sealer penetration into root canal surfaces: a confocal microscope analysis'.

Short title: Effect of the Nd:YAG laser on sealer penetration.

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Key words: Confocal microscope; Nd:YAG laser; endodontic instrumentation; dentinal permeability; endodontic sealer; sealer penetration.

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Abstract

Objectives: The objective of this *in vitro* study was to evaluate the use of the Neodymium:Yttrium-Aluminium-Garnet (Nd:YAG) laser as part of the root canal treatment on the penetration of sealer into dentinal tubules. **Methods:** Eighty extracted lower premolars were randomly assigned to two groups (*n* = 40 each): Control group (CG), subjected to a conventional protocol of endodontic instrumentation and obturation; and Laser group (LG), in which Nd:YAG laser irradiations were combined with conventional preparation and obturation. Endodonted samples were sectioned at 3 and 5 mm from the apex and observed under a confocal scanning microscope (CLSM). The penetration depth into the dentinal tubules and the extension of the intracanal perimeter infiltrated by sealer were measured. The Student-Newman-Keuls test was run for between-group comparisons (α=.05). **Results:** The depth of sealer penetration into dentinal tubules did not differ among groups. LG samples showed the significantly highest percentage of penetrated perimeter at 3 mm from the root apex. Within each group, the greatest depth of penetration (*P*=.0001), and the major percentage of penetrated perimeter (*P* <.001), were recorded at 5 mm.

Conclusions: The application of the Nd:YAG laser after instrumentation did not improve the depth of sealer penetration into the dentinal tubules. The laser enlarged the total penetrable perimeter near the apex.

Clinical significance: The Nd:YAG laser may be an appropriate complement in root canal treatment, as it enhances the sealer adaptation to the dentinal walls in the proximity of the apex.

Introduction

The smear layer (*i.e.,* a biofilm of detritus that covers the walls of prepared and unprepared root canals) obstructs the dentinal tubules, reduces the infiltration of irrigant solutions, and interferes with the adhesion of sealer agents.[1-5] For ensuring a suitable endodontic obturation, the smear layer, soft-tissue debris, inflammatory irritants, and microorganisms must be totally eradicated.[6-9] Although these resist mechanical instrumentation and irrigation, they seem to be more amenable to disruption by pressure waves generated by pulsed lasers.[10]

Moreover, the filling materials must reach a high level of adaptability to the cleaned root canal walls, which may be achieved thanks to the penetration of sealers into the dentinal tubules.[11-13] Conventional protocols of chemomechanical root canal preparation (even in conjunction with chelants, *i.e.,* EDTA), do not completely eliminate the inorganic material at the tubules' entrance.[14]

However, laser technology has widened the spectrum of endodontic indications in the last few years by achieving an effective intracanal ablation of the smear layer[7,15,16] and melting of dentinal surfaces, so that the underlying tubules would be more impermeable.[15,17-20] The Neodymium:Yttrium-Aluminium-Garnet (Nd:YAG) laser system has been the most analyzed because of its important effect on dentinal permeability.[8,12,20-26] Goodis *et al*[20] stated that Nd:YAG laser irradiations in combination with manual instrumentation of the root canals could eliminate the smear layer leaving no remaining tissues on them. Dederich *et al*[27] confirmed that the dentinal walls could recrystallize in a glazed non-porous surface devoid of organic tissues with this type of laser, which was supported by Michiels *et al.*[28]

Accordingly, several authors[12,15,17,21,25] attributed to this laser both an improved cleanliness and a reduced dentinal permeability of the intracanal surfaces caused by dentine fusion that contributed to close and seal the dentinal tubules. Nonetheless, these results may depend upon the different factors that define the energy level, such as power of irradiation, duration of exposure, and color of the dentine.[8,12,18,22,27,29] Compared with the scanning electron microscope and other methods used in these studies,[8,12,18,21,22,27,29] the analysis with confocal scanning microscope (CLSM) has the advantage of providing detailed information about the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at low magnifications (50×-100×) through the use of fluorescent rhodamine-marked sealers.[30] Although this technology would seem to be ideal to determine the degree of penetration and adaptation of the filling materials, clinical achievements with a wide variety of sealers, canal preparation, and obturation techniques have not been forthcoming.[13] Actually, this is the first investigation in which the effect of the Nd:YAG laser on sealer penetration has been evaluated at different root levels from the foraminal apex using confocal scanning microscopy and related specific software.

The aim of this *in vitro* study was to evaluate the effect of Nd:YAG laser irradiations on the penetration of sealer into dentinal tubules under confocal microscopy. The null hypothesis tested was that neither the use of the Nd:YAG laser after endodontic instrumentation, nor the root level assessed from the apical foramen influenced the depth of sealer penetration into the dentinal tubules and the extension of root canal surfaces infiltrated by sealer.

Materials and Methods

Preparation of samples

Eighty single-rooted human lower premolars with a closed apex that had been extracted because of orthodontic reasons were used in the study. The exclusion criteria were: presence of more than one canal, caries, calculus, open apex, root resorption, and/or root fractures.

The teeth were conserved in an injection saline solution of 900 mg NaCl/ 100 ml water (Fresenius Kabi, Barcelona, Spain), and randomly assigned to two groups: Control group (CG) (n = 40), which followed a conventional endodontic instrumentation and obturation protocol; and Laser group (LG) (n = 40), in which Nd:YAG laser irradiations were applied after conventional instrumentation, prior to obturation.

The pulp chamber was opened with 330 tungsten-carbide and EndoZ burs (Dentsply-Maillefer, Tulsa, USA) under water cooling. The coronal flaring was prepared using Gates Glidden drills No. #1, #2, and #3 (Dentsply-Maillefer). A K10 file (Dentsply-Maillefer) was introduced through the access cavity to determine the working length, which was established 1 mm short of the apical foramen. The manual glide path was performed with flexofile K15-20 files (Dentsply-Maillefer) and the instrumentation was completed with a rotatory nickel-titanium MTwo system (VDW, Munich, Germany) using 10.04, 15.05, 20.06, 25.06, 30.05, 35.04, and 40.04 files. During the procedure, 15% EDTA gel (Dentaflux, Madrid, Spain) and irrigations with 5 ml of 5.25% sodium hypochlorite (NaOCl) were applied. Root canals were dried by introducing appropriately sized (Ø 40) absorbent paper points (Dentsply-Maillefer) to the working length. The classic 17% EDTA solution was not used for final flush in order to preserve the smear layer.[31]

An Nd:YAG laser (Deka, Florence, Italy) with a 1064 nm wavelength and a 300 μm optic fiber with a rubber stopper was selected for the study.[32,33]

The laser parameters programmed were: 1.5 W, 15 Hz and 100 mJ. The laser power emitted at the fiber tip was measured by a wattmeter (Field Master, Coherent Inc., Auburn, CA, USA) before each irradiation to ensure stable and standardized power outputs.[34]

After the canal was dried, the laser tip was introduced 1 mm short of the working length and then moved in circles inside the canal making contact with the entire walls. Five 5-s cycles with a 20-s break between them were performed.

Root canals were filled inserting a size 40, 0.2 tapered gutta-percha master cone (Dentsply-Maillefer) impregnated with resin-based root canal sealer (AH-Plus Jet, Dentsply-Maillefer) mixed with 1% rhodamine B isothiocyanate (Sigma-Aldrich, St. Louis, MO, USA). Lateral condensation was performed using a size A finger spreader (Dentsply-Maillefer) with two size 25, 0.2 tapered gutta-percha points (Dentsply-Maillefer), and size C accessory points (Dentsply-Maillefer).

Excess gutta-percha was removed from the coronal cavity up to the level of the cementoenamel junction using a hot instrument (A.S.A. Dental, Massarosa, Italy). Warm vertical compaction of the remaining coronal gutta-percha was performed using a prefitted hand plugger (Machtou, Dentsply-Maillefer).

The study was conducted following the ethical principles of medical investigation under the Helsinki Declaration of the World Medical Association (http://www.wma.net), and the Spanish Law 14/2007 of July 3rd for Biomedical Research (http://www.boe.es). The Ethics Committee Approval (Court of Ethics at the University of Seville, US, Spain) was obtained.

Confocal microscope examination

The root-treated teeth were prepared for confocal microscope analysis. Premolars were cut perpendicularly to the occlusal/apical axis both at the 3 and 5 mm levels from the apex using a 102 × 0.3 mm diamond blade mounted in an IsoMet Low Speed Saw precision cutter (Buehler, Lake Bluff, IL) with running water. The same root levels have been evaluated in related studies on sealer penetration.[13,35]

Samples were polished with an 8” sanding disk for 5 s to reduce cut irregularities and provide smooth surfaces, allowing for better visibility during confocal microscopic imaging.

The specimens were prepared for microscope analysis based on the protocol proposed by Janda[36] for natural teeth at room temperature (RT: 23.0±1.0 ºC). This method requires an effective dehydration and drying of the samples for minimizing the appearance of artefacts that may result from preparation. The specimens were embedded in 70%, 80%, 96%, and 100% ethyl alcohol for 24 h each, submerged in 3 consecutive baths of 100% acetone for 24 h, and dried in a vacuum desiccator (vacuum achieved by a glass filter pump)[36] at 60 °C for 2 h.

Dehydrated samples were examined on an inverted Leica TCS-SPE confocal microscope (Leica, Mannheim, Germany) along the Z-axis with a 60-μm range. The parameters analyzed were: depth of sealer penetration into dentinal tubules (in microns), and penetrated perimeter (both in millimeters and percentages) at 3 and 5 mm from the foraminal apex.

The respective absorption and emission wavelengths for rhodamine B were 540 nm and 590 nm. The specimens were first observed using the HC PL Fluotar 10×/0.30 lens (Leica). Visualized layers were selected 10 μm below the sampled surface.[13,37] For the analysis of the penetration depth and superficial extension of sealer at each root level (*i.e,* 3 and 5 mm from the apex), 40 images equally distributed on 5 allocations along the intracanal perimeter of every sample were captured with the HCX PL APO 40×/1.25-0.75 oil lens (Leica), and acquired with the IM50 Image Manager Software, v1.20 (Leica Microsystems, Buffalo Grove, IL, US) (Fig. 1). These 40× pictures were taken by using 40 sections of 1 μm step size each in a format of 1,024 × 1,024 pixels along the Z-axis. Among such images (numbered from 1 to 40), 5 were analyzed per specimen and section from the apex (No.: 1, 8, 16, 32, and 40).

On each selected micrograph, the depth of sealer penetration was measured at 0° (N), 45° (NO), 90° (O), 135° (SO), 180° (S), 225° (SE), 270° (E), and 315° (NE). When penetration was observed, the length of the sealer tag from the canal wall along the entire tubule was recorded in microns. The canal wall served as the starting point, and sealer penetration into dentinal tubules (sealer tags) was calculated to a maximum depth of 1,000 μm with the ruler tool of the Leica Application Suite Advanced Fluorescence Lite software (Leica Microsystems) (Fig. 1).

The intracanal perimeter of each sample was measured at 3 and at 5 mm from the root apex using the Leica software (Leyca Microsystems). The extension of the intracanal perimeter infiltrated by sealer was registered using the described method and measuring points as for the penetration depth at each root level. Percentages of sealer penetration were also calculated.

For statistical analysis, the abovementioned measured points of each variable were averaged per specimen and canal section from the apex.

Statistical analysis

Descriptive statistics were obtained for each variable. The depth of sealer penetration into dentinal tubules and the penetrated perimeter were reported for each group by both a measure of centrality (mean) and a measure of variability (standard deviation: SD).[19,38]

The Kolmogorov-Smirnov test confirmed that the data was normally distributed. The Student-Newman-Keuls test was run for between-group comparisons.[39,40]

Data were processed using the Statistical Package for the Social Sciences (software v.20) (SPSS/PC+, Inc.; Chicago, IL, USA) taking the cut-off level for statistical significance at

α = 0.05.[5,31,41] The statistical probes utilized in this study adhere to the requirements of Hannigan and Lynch for oral and dental research.[38]

Results

No significant differences were found among CG and LG samples concerning the depth reached by the sealer into the dentinal tubules at 3 and 5 mm from the root apex (Table 1).

The extension of the intracanal perimeter penetrated by the sealer (in mm) was significantly higher in LG than in CG specimens regardless of the root level assessed (Table 1). However, given that CG and LG showed significant differences in the total perimeter at 5 mm from the apex (P =.0001), the 'percentage of penetrated perimeter' may be a more accurate measurement than the 'penetrated perimeter in millimetres'. In this regard, while LG showed higher percentages of penetrated perimeter than CG at both root-levels evaluated, significant differences were only recorded at 3 mm from the apex (P = .034) (Table 1).

When comparing the sections made at 3 and 5 mm from the apex within each study group (CG and LG), the depth of penetration (P =.0001), the penetrated perimeter (in mm) (P =.0001), and the percentage of penetrated perimeter (P <.001) were significantly greater at 5 mm. Significant differences in the total perimeter were found between both root levels assessed within each study group (P =.0001) (Table 2). Therefore, the percentage of penetrated perimeter may be more representative than the penetrated perimeter in millimetres for these comparisons.

Discussion

The smear layer acts as a physical barrier that prevents the adhesion and reduces the sealer penetration into dentinal tubules, thus affecting the efficacy of sealers.[1-3,5] Its removal may improve the sealer penetration, enhancing the interface between filling material and root canal walls for achieving a proper obturation.[6,9] It has been shown that hand preparation with irrigation does not result in complete cleaning of the root canal, mainly when fins and other anatomical irregularities are present, as it usually occurs in the apical third of the root.[8]

Although the use of lasers in Dentistry have been studied for a number of years,[25,39,42] very little research exists with respect to their efficacy in endodontics.[29] Hence, this is the first study aimed to evaluate the effect of Nd:YAG laser irradiations on the penetration of sealer into dentinal tubules at different root levels from the apex using confocal laser microscopy, which makes comparisons difficult. The smear layer removal together with the melting of the dentin surfaces and the consequent partial closing of dentinal tubules caused by the laser were implicitly analyzed in terms of sealer penetration. The null hypothesis was rejected, as the depth of sealer penetration into the dentinal tubules and the extension of the intracanal perimeter infiltrated by sealer depended upon both the use of Nd:YAG laser after endodontic instrumentation and the root level assessed from the apical foramen.

Confocal laser scanning microscopy offers advantages compared with scanning electron microscopy and other methodologies previously used to evaluate penetration and interfacial adaptation of root sealers.[7,12,19,27,43] Visualization of the penetration of sealers in horizontal sections was evident at low magnifications by the presence of rhodamine B fluorescence in dentinal tubules, which was confirmed at higher magnifications. Hence, a higher fluorescence revealed a complete obturation of dentinal tubules, whereas a lower fluorescence corresponded to a partial or incomplete obturation.[13]

All reasonable attempts were made to minimize variables or operator bias in this research. Despite the fact that the procedure of placement does not seem to affect the distribution of sealer in root-canal walls,[44] the sealer was introduced in standard fashion (impregnating the gutta-percha points) by a single, skilled operator, who was experienced in this technique.[13]

The protocol for irradiation used was that proposed by Gutknecht and Behrens,[32] who stated that laser fibers should be in continuous movement and close contact with the canal walls to produce fusion and recrystallization. Eriksson and Albrektsson[45] stated that root surfaces should not increase their temperature more than 10 ºC over the corporal temperature. In this regard, Gutkecht and Behrens,[32] and Camargo *et al*[26] recommended making helicoidal movements with the fiber tip into the root canals and having a break every 20 s to avoid overheating. All of these recommendations were followed in this study.

The application of the Nd:YAG laser at 3 W in the apical part of the root canals of dogs has shown detrimental effects on the periapical tissues, causing cell necrosis in the periodontal membrane 1 day after treatment and ankylosis and cementallysis 30 days afterwards.[46] Even though most types of interactions are strongly wavelength-dependent due to the inherent optical absorption properties of various materials and tissues, there are other energy-dependent interactions that rely on the control of variables such as energy density, pulse duration, and frequency.[12] These variables were precisely set and regulated in the present experiment. In a previous investigation,[27] the Nd-YAG laser did not create a glazed surface by melting; rather craters and perforations were caused as a result of dentine vaporization. This may be explained by the fact that the teeth were split prior to lasing and therefore the impact of the laser beam was perpendicular to the root canal wall, making the energy dose quantifiable. Conversely, in our study, the clinical application of the laser beam was represented. Hence, the root canal was intact and the fiber-optic cable was kept parallel to the root canal with the sides of the fiber in contact with the walls. Although it is difficult to quantify how much energy reaches the dentin surface, such clinical application reduces the energy density the dentine receives,[8] and so may produce a different effect from the craters observed by Dederich *et al.*[27]

Theoretically, the more extensive the sealers penetrate along the circumference of the root canal wall, the better the three-dimensional seal.[41] Several studies[13,43,47] have demonstrated that the maximum penetration depth and superficial extension infiltrated by sealer occurs at the coronal third. Our findings recorded at 5 mm from the apical foramen compared with those obtained closer to the apex (at 3 mm) point in the same direction; there were significant differences in depth and penetrated perimeter regardless of the type of endodontic preparation (*i.e.,* conventional instrumentation or conventional plus the Nd:YAG laser) (Table 2).

This might be attributed to the better access for removing intracanal debris and to the presence of more and bigger tubules in the coronal third of the root canal.[10] For this reason, the use of a laser may be more interesting in the apical third to facilitate the smear layer removal in this area. Laser irradiated samples showed a larger percentage of perimeter penetrated by the sealer than did the controls (Table 1). Such difference was statistically significant at 3 mm from the root apex (Table 1). Thus, under the tested conditions, laser enhances the sealer adhesion and adaptation to the dentin surfaces of the root canals in the proximity of the apex, which is the most inaccessible location for manual instrumentation and irrigation. As the fiber tip is more adjusted to the root canal in the apical third, higher energy would be transmitted to the dentin. In this regard, it is also interesting to remark the importance of a tapered preparation (which was carried out in the present research), using rotary files of greater taper to allow an optimal fiber access and an easier way for irrigants and sealers.[48]

Based on the study findings (Tables 1 and 2), the use of the Nd:YAG laser in combination with conventional instrumentation may be recommended for improving the adaptation of root sealers to the dentinal walls near the root apex. We are limited in our ability to ascertain the exact mechanisms responsible for the interactions observed in this study.[12] However, the possible cleaning and melting effect caused by the laser at the entrance of the dentinal tubules,[15,17-20] might have resulted in a rough surface that favors the adhesion of sealers.[24]

In attempt to exclusively analyze the effect of laser irradiations on sealer penetration and, implicitly, on smear layer removal, the classic 17% EDTA solution was not used for final flush.[31] Otherwise, results might have been partly attributed to the action of this solution. However, it seems prudent to address the fact that different outcomes in the variables tested could have been achieved with routine smear layer removal using EDTA prior to laser therapy.

The Nd:YAG laser could be especially useful in cases of dental necrosis or apical periodontitis, as it would allow a hermetic isolation of the root canal system by sealing the dentinal tubules reducing apical leakage and bacterial recolonization,[23,33,49,50] which would be essential for posterior reconstruction with posts.[51,52] Nevertheless, more *in vitro* and *in vivo* studies are necessary before widespread use is recommended.

Conclusions

The use of the Nd:YAG laser as part of the endodontic instrumentation did not improve the depth of penetration of root canal sealers into the dentinal tubules.

The sealer tested failed to show a consistent adaptation of sealer tags to the total circumference of the root canal walls. However, the Nd:YAG laser may be an appropriate complement in root canal treatment, as it enlarges the superficial extension infiltrated by sealer in the proximity of the apex.

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Figure legends

Figure 1: Confocal image of the inner perimeter of a laser-irradiated sample sectioned at 5 mm from the root apex (HC PL FLUOTAR 10×/ 0.30 oil lens). The depth of sealer penetration into the dentinal tubules and the extension of the intracanal perimeter infiltrated by sealer were recorded using LAS AF Lite software (Leica Microsystems) focussing on the dentinal tubules. (**ROI:* region of interest).

Figure 2: Confocal image of the sealer penetrating the dentinal tubules of a laser-irradiated sample sectioned at 3 mm from the root apex (HCX PL APO 40×/ 1.25-0.75 oil lens).

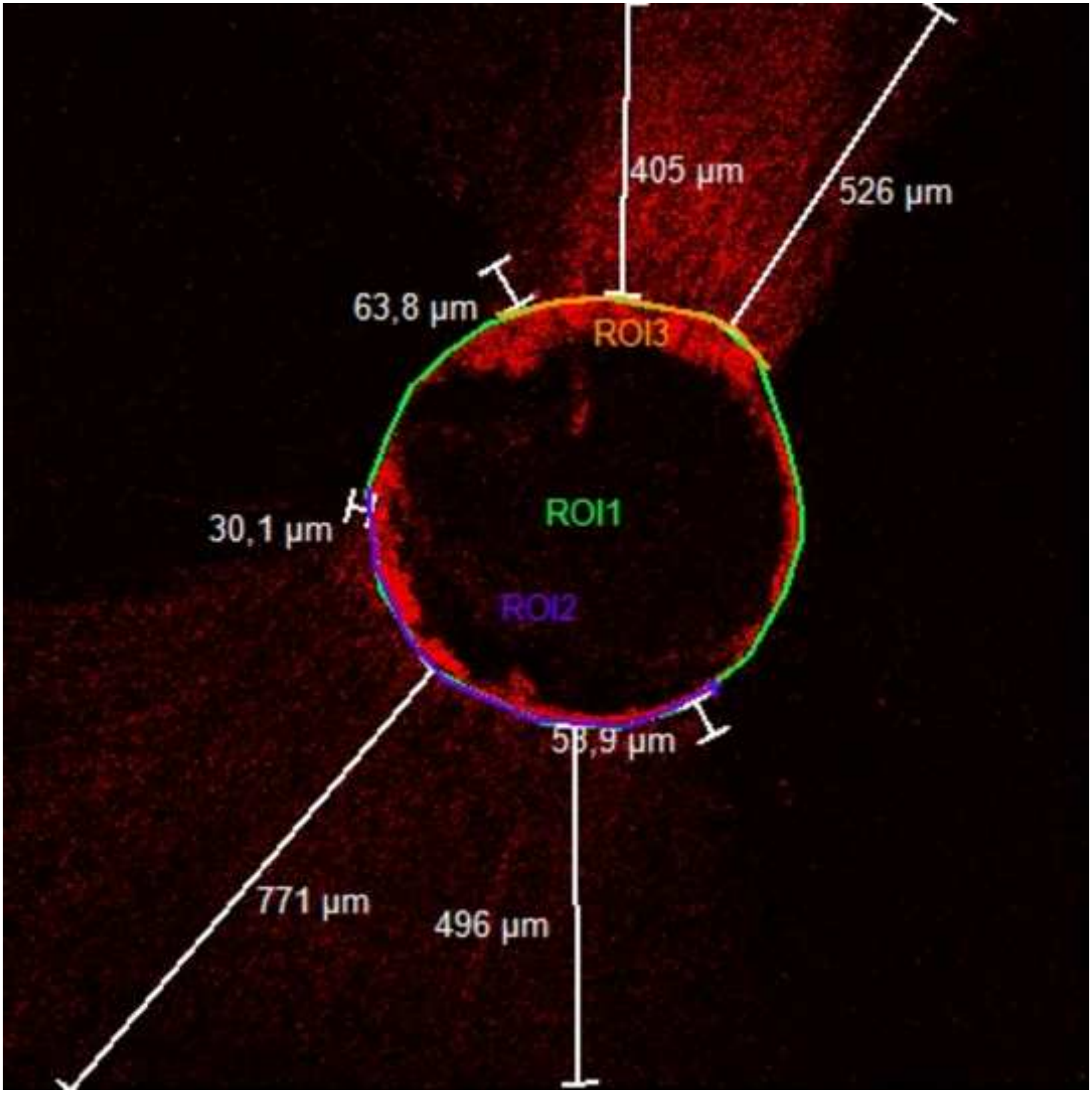
Tables

Table 1: Comparison of the results obtained between control and laser groups (SD: standard deviation; *: $P < 0.05$).

Group	Depth of penetration (μm)	Total perimeter (mm)	Penetrated perimeter (mm)	Penetrated perimeter (%)
Control (*n* = 80)	106.81 ± 87.4	1.95 ± 0.2	0.84 ± 0.5	42.57 ± 23.6
Laser (*n* = 80)	105.13 ± 78.6	2.15 ± 0.4	1.14 ± 0.7	50.90 ± 23.4
P value	0.895	0.0001*	0.001*	0.022*
3 mm from the apex				
Control (*n* = 40)	63.11 ± 53.8	1.82 ± 0.2	0.61 ± 0.3	33.29 ± 18.8
Laser (*n* = 40)	66.73 ± 59.2	1.88 ± 0.2	0.81 ± 0.4	42.86 ± 21.4
P value	0.770	0.121	0.019*	0.034*
5 mm from the apex				
Control (*n* = 40)	150.52 ± 93.1	2.01 ± 0.2	1.07 ± 0.5	51.75 ± 24.4
Laser (*n* = 40)	143.53 ± 77.3	2.42 ± 0.5	1.47 ± 0.7	58.94 ± 22.8
P value	0.71	0.0001*	0. 004*	0.164

Table 2: Comparison of the results obtained at 3 mm and at 5 mm from the root apex (SD: standard deviation; *: $P < 0.05$).

Distance from the apex	Depth of penetration (μm)	Total perimeter (mm)	Penetrated perimeter (mm)	Penetrated perimeter (%)
3 mm (*n* = 80)	65.03 ± 56.4	1.85 ± 0.2	0.71 ± 0.4	38.41 ± 20.6
5 mm (*n* = 80)	146.82 ± 84.6	2.26 ± 0.4	1.28 ± 0.6	55.55 ± 23.7
P value	0.0001*	0.0001*	0.0001*	0.0001*
Control group (CG)				
3 mm (*n* = 40)	63.11 ± 53.8	1.82 ± 0.2	0.61 ± 0.3	33.39 ± 18.8
5 mm (*n* = 40)	150.52 ± 93.1	2.01 ± 0.2	1.07 ± 0.5	51.75 ± 24.4
P value	0.0001*	0.0001*	0.0001*	0.0001*
Laser group (LG)				
3 mm (*n* = 40)	66.73 ± 59.2	1.88 ± 0.2	0.81 ± 0.4	42.86 ± 21.4
5 mm (*n* = 40)	143.53 ± 77.3	2.42 ± 0.5	1.47 ± 0.7	58.94 ± 22.8
P value	0.0001*	0.0001*	0.0001*	0.001*


405 µm
526 µm
63,8 µm
ROI3
ROI1
30,1 µm
ROI2
53,9 µm
771 µm
496 µm