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Adaptation and penetration of resin-based root canal sealers in root canals irradiated with high-intensity lasers

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Abstract. This research analyzed the quality of resin-based sealer adaptation after intracanal laser irradiation. Extracted teeth ($n = 168$) were root canal treated and divided into four groups, according to dentin surface treatment: no laser; Nd:YAG laser (1.5 W, 100 mJ, 15 Hz); diode laser (2.5 W in CW), and Er:YAG laser (1 W, 100 mJ, 10 Hz). The teeth were divided into four subgroups according to the sealer used: AH Plus, EndoREZ, Epiphany, and EpiphanySE. For testing the sealing after root canal obturation, the penetration of silver nitrate solution was measured, whereas to evaluate the adaptation and penetration of the sealer into the dentin, environmental scanning electron microscopy (ESEM) was used. The ESEM images were analyzed using a four-grade criteria score by three evaluators. The inter-examiner agreement was confirmed by Kappa test and the scores statistically compared by the Kruskal-Wallis' test ($p < 0.05$). Both adaptation and sealer penetration in root canals were not affected by the laser irradiation. Nd:YAG and diode laser decreased the tracer penetration for AH Plus, whereas EndoREZ and EpiphanySE performances were affected by Nd:YAG irradiation ($p < 0.05$). It can be concluded that intracanal laser irradiation can be used as an adjunct in endodontic treatment; however, the use of hydrophilic resin sealers should be avoided when root canals were irradiated with Nd:YAG laser.
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Keywords: adaptation; tag formation; resin sealers; high-intensity lasers; apical sealing; root canal; endodontics.
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1 Introduction

Complete seal of canal systems is considered paramount to achieve the successful outcomes in root canal treatment. Many methods have been tried to accomplish complete disinfection and sealing of the root canal system.[1,2] Regarding the filling materials, the sealer must have a perfect adaptation to the dentin walls as well as the largest possible area of contact with dentin surfaces and penetrate into the dentin tubules. Studies using methacrylate resin-based sealers have shown promising results when compared with conventional sealers. They possess hydrophilic characteristics that improve the penetration of the sealer into dentinal tubules, thus improving the sealing capacity.[2–7]

As an adjunct in endodontic treatment, the use of high intensity laser devices has demonstrated significant intracanal disinfection and morphological changes of the dentin surface that interfere with dentin permeability and root canal sealing.[5,6,8–16] The interaction between laser wavelengths and irradiated tissues will assume the level of energy absorbed by the latter and therefore the intensity of tissue alteration.[3,9,11–,15–18] Wavelengths between 800 and 1100 nm (i.e., diode and Nd:YAG lasers) have poor interaction with dental hard tissues like enamel and dentin. These lasers can melt their surfaces, creating a new pattern and occluding dentinal tubules.[9,11,13,15–19] On the other hand, wavelengths in the range of 2700 and 3000 nm (Er, Cr:YSGG and Er:YAG lasers) have an optimal interaction with water and hydroxyapatite, and therefore the laser energy is highly absorbed by dental structures causing surface ablation.[3,10,12,13,18]

Despite the favorable properties of the methacrylate resin-based sealers and high intensity laser irradiation, it is not well understood if the association of these two resources improves the root canal seal, and therefore might enhance the success of root canal treatment. Thus, the aim of the present study is to evaluate the quality of the apical seal of root canals irradiated with high-intensity lasers and filled with resin-based sealers. The hypothesis is that intracanal irradiation with high-intensity lasers interferes with the sealer adaptation and penetration.

2 Material and Methods

This study was approved by the Ethics Committee of the School of Dentistry of the University of São Paulo, Brazil. (Approval 182507) 168 freshly extracted single-rooted teeth with fully developed roots and without calcifications, internal root resorption or previous endodontic treatment were selected for the

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experiment (radiographs were taken to investigate inclusion criteria). The teeth were stored in 1% formalin solution at 4°C.

2.1 *Sample Preparation*

All crowns were removed using a diamond wheel under water cooling and the length of the roots was standardized between 13 and 15 mm. A size 15 K file (Dentsply/Maillefer, Baillaigues, Switzerland) was inserted into the canal until visible at the apical foramen. The working length of each root canal was then established 1 mm short of the apical foramen. All samples were prepared using the ProTaper Universal rotary system (Dentsply/Maillefer) up to a F4 file together with 5 ml 2.5% sodium hypochlorite with each change of file. Next, the root canals were rinsed with 10 ml of EDTA-T (17% ethylenediaminetetraacetic acid in combination with 1.25% sodium lauryl ether sulfate solution) to remove the smear layer and debris, followed by a final rinse with 10 ml sterile water. All irrigating solutions were delivered with 30-gauge NaviTip needles (Ultradent Products Inc., South Jordan, Utah) and 3 ultrasonic activations of 20 s. The roots were kept moistened with sterile water in gauge during instrumentation.

2.2 *Experimental Groups*

The samples were randomly divided into four groups of 42 teeth each ($n = 42$), according to the treatment employed before obturation. The specimens in Group GØ received no laser treatment and served as control teeth. Teeth in Group Neodymium had the root canals irradiated with a 1064-nm Nd:YAG laser (Pulse Master 1000; American Dental Technologies, Southfield, Michigan) operated at 1.5 W, 100 mJ, 15 Hz, and 124,34 $\mathrm{J/cm^2}$, in pulsed mode.[9,11,16] Group diode was irradiated with an 808-nm diode laser (Zap Softlase; Zap Lasers Ltd., Pleasant Hill, California) with a setting of 2.5 W in continuous mode.[5,6,19,20] In the fourth group (Erbium), the root canals were irradiated with a 2940 nm Er:YAG laser (KavoKey laser 2; KaVo Co., Biberach, Germany) operated at 1 W, 100 mJ, 10 Hz, and 38,03 $\mathrm{J/cm^2}$ in pulsed mode.[12,13,18] All laser treatments were carried out according to the manufacturer's instructions. The roots treated with a laser contained sterile water and were irradiated four times with circular movements, from apex to crown at a speed of 2 mm/s with a 20 s interval between applications. For that, a 300-μm fiber optic tip was inserted into the root canal until it reached the working length. At this moment, the laser was activated, and the irradiation was performed as described. All power settings were checked using a power meter (model 841P, Newport Corp., Irvine, California) before and after each irradiation.

2.3 *Scanning Electron Microscope Observation of Surface Morphology*

Two samples of each group were randomly selected to analyze the surface micromorphology after the previous procedures. The roots were split in a bucco-lingual direction and the sections flushed with 20 ml of sterile water. They were then fixed in 2.5% glutaraldehyde, dehydrated in a series of graded ethanol solutions (30%, 50%, 70%, 80%, 90%, and 100%), and then coated with a gold 15 nm layer using a sputter coater (MED 020; Bal-Tec AG, Liechtenstein). The dentin surface of the apical third, 4 mm from the apex and in a central region was examined with a scanning electron microscope (SEM) at 2500× magnification operating at 15 kV (Quanta 600 FEG; FEI Company, Eindhoven, The Netherlands).

2.4 *Root Canal Obturation*

The remaining teeth ($n = 40$/group) were randomly divided into four subgroups ($n = 10$), according to the root canal sealer used for obturation. Prior to obturation, all teeth were externally coated with two layers of nail varnish, except for the last 1 mm at the apical third. One operator (CMN) filled the root canals of all groups using a single cone technique. A standard-size taper. 04 cone was fitted to the working length. In the AH Plus (Dentsply/deTrey) subgroups, a gutta-percha cone (Dentsply/deTrey) was used. The EndoREZ (Ultradent Products Inc.) subgroups used EndoREZ points (Ultradent Products Inc.), a gutta-percha point covered with a thin layer (approximately 15 μm) of UDMA resin. The Epiphany and Epiphany SE (SybronEndo Corp., Orange, California) subgroups used Resilon cones (SybronEndo Corp.), a synthetic polymer-based resin (polycaprolactone). The sealers were mixed according to the manufacturer's instructions and placed in a Skini syringe (Ultradent Products Inc.) attached to a 27G needle to fill the canal. Because Epiphany is not a self etch sealer, it was necessary to apply a layer of Epiphany primer in the root canal walls before the resin filling. For that, a ProTaper F4 sterile paper point moistened with the primer was used, brushing all root canal surfaces.

Before obturation, the teeth from the AH Plus subgroups were completely dried with suction using 30G NaviTip needle (attached to a air-vacuum suction device) and ProTaper F4 sterile paper points, while the roots from the other groups were lightly dried using suction with 30 G NaviTip needle only, to maintain a moist dentin condition. The canals were filled with the sealers and the master cone gently seated to the working length. Excess material was removed using a hot plugger followed by cold vertical condensation. After obturation, the apical foramen was covered with a thin plastic film to avoid oxygen contact and all the samples were stored in an incubator for 72 h at 37°C and 100% humidity.

2.5 *Silver Nitrate Penetration Assay*

Upon completion of the root canal filling procedures, the teeth were immersed into 50% ammoniacal silver nitrate solution (pH 9.5) for 24 h, according to a previously published methodology.[6,21,22] This assay aims to indirectly measure the quality of the dentin tubule sealing after obturation of the root canal. The samples were washed in sterile water and embedded individually in acrylic blocks. The roots were cut in a longitudinal direction using a 0.3-mm-thick diamond blade (Isomet 1000 Precision Saw, Buehler Ltd., Illinois), operated at 200 rpm and 400 g of load, under running water. The cut root sections were then polished with 600 and 1200 grit abrasive papers (3 M St. Paul, Minnesota) using an Ecomet 3 polisher (Buehler) and ultrasonicated with sterile water for 1 min. The blocks were immersed in photo developing solution (Kodak Bras. Ind. Com., São Paulo, Brazil) for 8 h under fluorescent light to reduce silver ions into metallic grains within voids along the interface root surface/filling material. The samples were then scanned with a high-optical resolution scanner (24,000 dpi–CX7300, Epson, São Paulo, Brazil), and the penetration on each side of the sample was blindly measured (mean value) by one operator, with Image J Software (NIH, Bethesda).

Table 1 Adaptation to dentin walls and sealer penetration criteria.

Score	Criteria for adaptation	Criteria sealer penetration
1	<25% adaptation	<25% of penetration/short tags, few or absent
2	25% to 50% adaptation	25% to 50% of penetration/short but uniform tag formation
3	51% to 75% adaptation	51% to 75% of penetration/long but sparse tags
4	>75% adaptation	>75% of penetration/long tags with dense formation

2.6 *Environmental Scanning Electron Microscopy Analysis*

The root sections were prepared for environmental scanning electron microscopy (ESEM) analysis in order to evaluate the adaptation of the filling material to the dentin walls as well as sealer penetration into the dentinal tubules. In order to visualize the morphology, the sectioned surfaces were conditioned with 37% phosphoric acid for 30 s and then rinsed with 10 ml of distilled water. Subsequently, the roots were immersed in 2.5% NaOCl solution for 10 min followed by a 10 ml rinse of distilled water according to Tay et al.[22] The sample was kept hydrated and attached to a specific platform for ESEM analysis using a Quanta 600 FEG microscope (FEI Company, Eindhoven, The Netherlands). To standardize the analysis of the surface, the ESEM images (1500× magnification) were acquired in the same region as previously mentioned, 4 mm coronal to the apex examining the interface between filling material and dentin on both mesial and distal sides. Twenty ESEM images were acquired for each group. Three calibrated examiners did a blind evaluation of the adaptation of the filling material to the dentin wall and sealer penetration into the dentinal tubules (tag formation) using a four-grade score table for each parameter (Table 1). The criteria have been reported in previous studies.[23,24]

2.7 *Statistical Analysis*

For adaptation and sealer penetration, all data of each examiner were tabulated and a Kappa test was used to confirm inter-examiner agreement. Then, the scores were compared using the Kruskal-Wallis nonparametric test complemented by the Dunn's test ($p < 0.05$). For the silver nitrate penetration assay, the normality of the data was verified using the Kolmogorov-Smirnov test. After that, the groups were compared using an analysis of variance (ANOVA), complemented by the Tukey's test ($p < 0.05$).

3 Results

3.1 *Scanning Electron Microscope Observation of Surface Morphology*

SEM analysis of the control group [Fig. 1(a)] showed a dentin surface, free of smear layer with open dentin tubules. In

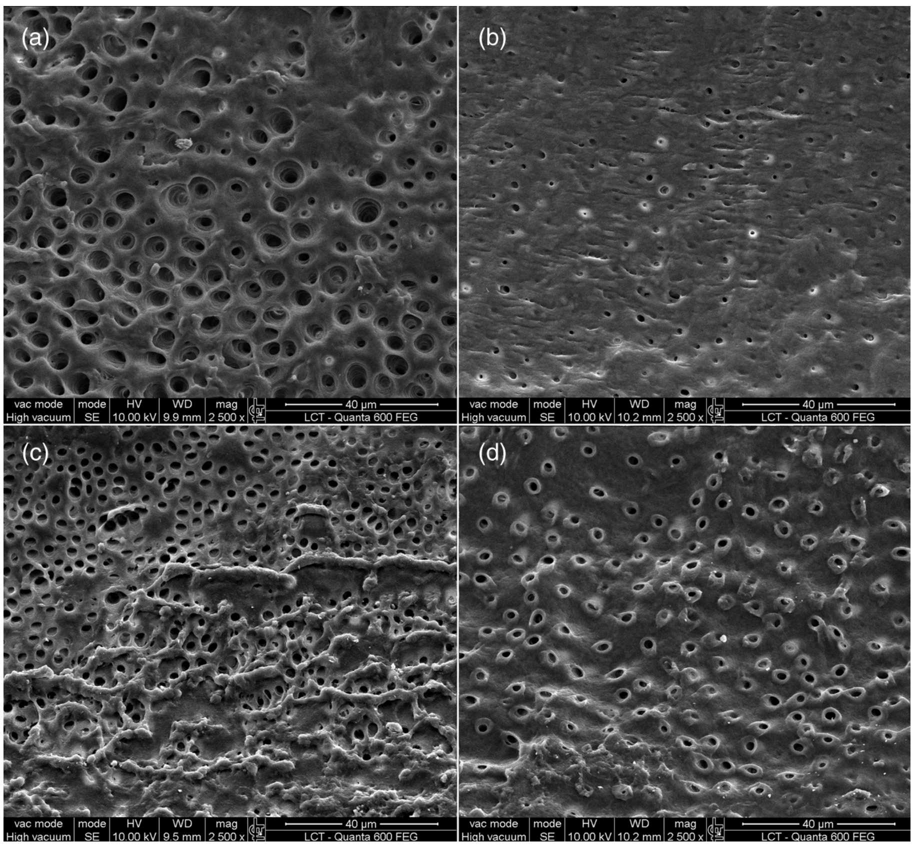

Fig. 1 Composite scanning electron microscope (SEM) images of dentin. Control group with no surface changes (a); neodymium group (Nd:YAG laser) showing melting and resolidification of dentin surface (b); diode group (diode laser) also showing melting and resolidification but in a different pattern (c); erbium group (Er:YAG) showing ablation of the dentin surface (d).

Neodymium [Fig. 1(b)], an irregular surface with fusion of dentin and resolidification was observed and the absence of a smear layer and debris. There were few open dentin tubules while others were partially or completely occluded. The surfaces irradiated with the diode laser [diode group-Fig. 1(c)] also showed aspects of fusion and resolidification of the dentin wall with more open and partially occluded dentin tubules than in neodymium group.

In group erbium [Fig. 1(d)], the dentin surface appeared clean with some localized fusion of dentin and mostly opened dentinal tubules that demonstrated ablated intertubular dentin.

3.2 *Environmental Scanning Electron Microscopy Analysis*

The scores for sealer adaptation to the dentin walls were mostly 3 and 4 (more than 50% of adaptation) irrespective of the laser irradiation or sealer used. The Kappa analysis showed a strong agreement between examiners ($K > 0.7$). The Kruskal-Wallis' test did not reveal statistically significant differences between groups ($p > 0.05$).

The results of the sealer penetration into dentin tubules are presented in Table 2. Despite the surface changes caused by laser irradiation, all sealers tested penetrated into the dentinal tubules. The Kappa analysis showed a strong inter-examiner agreement ($K > 0.75$). The Kruskal-Wallis' test revealed significant differences between groups ($p < 0.05$). The Dunn post-hoc test showed differences with regard to the sealers used. Table 2 shows the comparison between sealers in the same group and between groups in the same sealer. ESEM images in Fig. 2 illustrate the pattern of adaptation and tag formation of each sealer.

3.3 *Silver Nitrate Penetration Assay*

The penetration of tracer solution was observed in all groups. The means and standard deviations of penetration measurements are presented in Table 3. The ANOVA test demonstrated that the factors laser irradiation ($p = 0.003$), sealer ($p < 0.001$), and the interaction between them ($p < 0.001$) significantly affected the silver nitrate penetration.

Independently of the dentin treatment, the silver nitrate penetration results were similar in the teeth filled with Epiphany sealer. For all other sealers, the laser irradiation affected the outcome of the silver nitrate penetration. The use of the Nd:YAG and diode lasers had a positive effect on the AH Plus sealer penetration leading to a significant lower index of tracer penetration, when compared with the nonlased group. However, the irradiation with the Nd:YAG laser significantly increased the tracer penetration index in the roots filled with EndoREZ and Epiphany SE. The tracer penetration in the Er:YAG laser group in the teeth filled with Epiphany SE was significantly higher when compared with those filled with the other sealers. The surface irradiation with Er:YAG laser did not interfere in the

Table 2 Scores of sealer tag formation into dentinal tubules.

		Scores					
Groups	Subgroups	1	2	3	4	Within groups	Versus GØ
Control (no irradiation)	AH Plus	8	2	0	0	b,c	–
	EndoREZ®	2	0	4	4	a	–
	Epiphany®	5	0	3	2	a,b,c	–
	Epiphany SE®	8	0	2	0	b,c	–
Neodymium (Nd:YAG)	AH Plus	2	4	2	2	a,b	n.s.
	EndoREZ®	0	0	3	7	a	n.s.
	Epiphany®	8	0	2	0	b	n.s.
	Epiphany SE®	9	0	1	0	b	n.s.
Diode (diode)	AH Plus	1	2	6	1	a,c	n.s.
	EndoREZ®	0	0	2	8	a	n.s.
	Epiphany®	1	0	2	7	a	n.s.
	Epiphany SE®	8	1	1	0	b,c	n.s.
Erbium (Er:YAG)	AH Plus	3	2	5	0	n.s.	n.s.
	EndoREZ®	1	5	4	0		n.s.
	Epiphany®	5	2	2	1		n.s.
	Epiphany SE®	2	8	0	0		n.s.

Note: n.s.: not significant. Different letters denote statistically significant differences between groups.

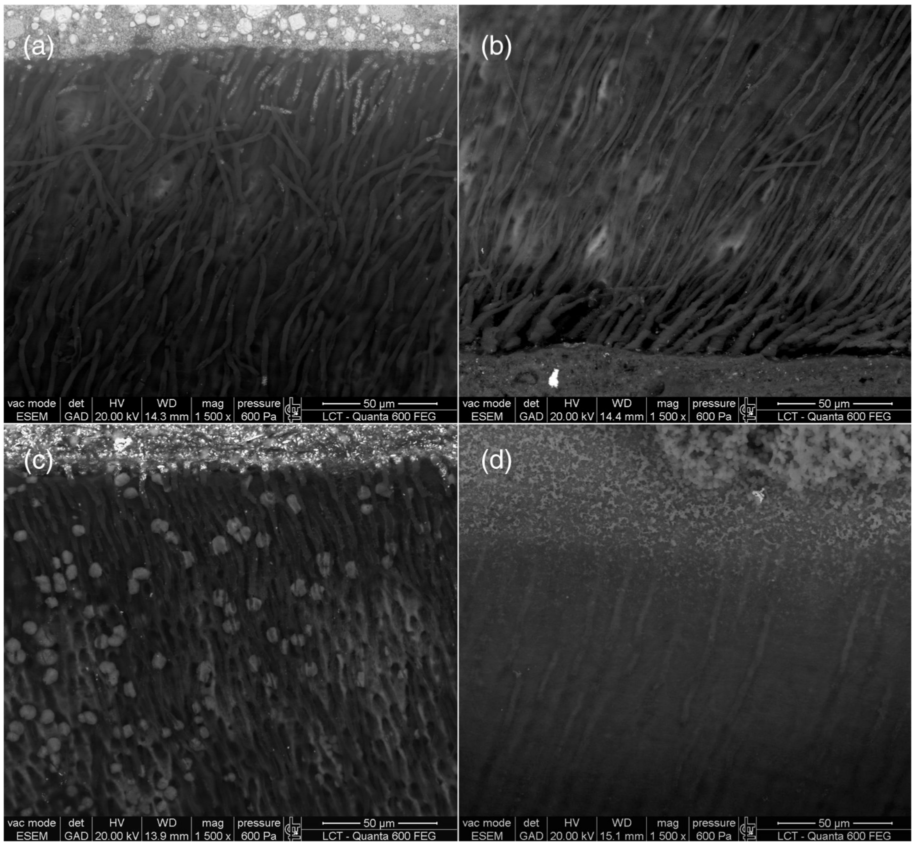

Fig. 2 Environmental scanning electron microscope (ESEM) images of resin tags from sealers. AH Plus (a) and EndoREZ (b) showing considerable tag formation; Epiphany (c) with more discrete tags; Epiphany SE (d) showing few tags.

outcome of the root canal fillings, independently of the sealer used when compared with the control group ($p > 0.05$).

4 Discussion

The pursuit of a perfect apical marginal seal of the root canal system represents a fundamental ideal for successful endodontic therapy. Treatment of the wall of the canal prior to obturation may affect the apical sealing ability of root canal sealers, as demonstrated in previous studies using different approaches and materials from EDTA to laser irradiation.[3–5,10,12,13,16,17] It is well-known that high intensity laser intracanal irradiation has other advantages. Several studies demonstrated its efficacy in cleanliness and disinfection.[8,9,11–15] Few studies have compared the resin-based sealers that were investigated in the present study and the influence of high-intensity laser irradiation on the adaptation and penetration into dentin tubules of these sealers.[3,5,6] Therefore, the hypothesis of this study is that the treatment of dentin prior to obturation using high intensity laser irradiation interferes with the quality of the apical seal when using resin-based sealers was accepted.

In order to better understand the effect of laser irradiation of dentin and the subsequent interaction with sealers, the surface morphology after laser treatment was investigated. Two specimens of each group serving as control were split longitudinally and processed for conventional SEM analysis. The SEM images

Table 3 Means and standard deviations (mm) of silver nitrate penetration.

	Sealer			
Surface treatment	AH Plus	EndoREZ	Epiphany	Epiphany SE
Control-no laser	$1.28 \pm 0.24^{A,a}$	$0.91 \pm 0.17^{B,a}$	$1.15 \pm 0.37^{A,B,a}$	$1.36 \pm 0.21^{A,a}$
Neodymium-Nd:YAG	$0.83 \pm 0.13^{A,b,c}$	$1.22 \pm 0.32^{B,b}$	$1.31 \pm 0.24^{B,a}$	$1.84 \pm 0.34^{C,b}$
Diode-diode	$0.78 \pm 0.26^{A,b}$	$1.05 \pm 0.22^{A,B,a,b}$	$1.19 \pm 0.31^{B,a}$	$1.33 \pm 0.24^{B,a}$
Erbium-Er:YAG	$1.09 \pm 0.32^{A,a,c}$	$0.99 \pm 0.12^{A,a,b}$	$1.19 \pm 0.13^{A,a}$	$1.49 \pm 0.22^{B,a}$

Note: For each line, different capital letters denote significant statistical differences between sealers ($p < 0.05$) for the same surface treatment. For each column, lowercase letters denote significant statistical differences between the surface treatments using the same sealer ($p < 0.05$).

demonstrated that the laser tested produced different morphological features. The nonirradiated group presented a clean dentin surface with open dentinal tubules. Both Nd:YAG and diode laser promoted fusion and resolidification of dentin with occlusion of some dentinal tubules. These effects were more evident with the Nd:YAG laser. These results concur with other reports in the literature.[9,15–19] On the other hand, Er:YAG laser irradiation caused ablation of the dentin surface, which was more evident in the intertubular than the peritubular dentin. This resulted in more exposed dentinal tubules as previously reported.[3,10,14,18]

When evaluating the sealer adaptation as well its penetration into the dentinal tubules, it was observed that the laser treatment did not have a negative effect. With conventional SEM, specimens must be dehydrated and coated with a conductive gold layer under vacuum. This process can result in artifacts in the form of resin contraction and formation of gaps and cracks. Tay et al.[22] cautioned using this technique when analyzing resin-based materials using SEM. Therefore, the use of an ESEM working with a low vacuum is more suitable, since the specimens are kept hydrated during all analysis, and thus avoiding processing artifacts. In our study, a field emission SEM was used under environmental conditions to analyze the adaptation of sealer to the dentin wall and its penetration into dentinal tubules. Using this technique and rating the adaptation of filling material according to the percentage of contact to the dentin wall was possible and led to the conclusion that none of the laser treatments interfered with the adaptation. Furthermore, a comparison of the filling materials showed similar results regardless of prior dentin treatment. This finding suggests that, besides adaptation, other factors are important in establishing a seal. Therefore, the analysis of sealer penetration into the dentinal tubules becomes a relevant factor in achieving a better seal. This evaluation was also made using a four-grade scoring system and was based on the morphology and length of the resin tags at the sealer/dentin interface (Table 2).

When scored for sealer penetration into dentinal tubules, the depth of penetration of EndoREZ = Epiphany > Epiphany SE and AH Plus > control group. Although the similarity between Epiphany and Epiphany SE sealers, the latter demonstrated worst results. Epiphany SE sealer is the self-etch version of Epiphany that needs a primer application on the root canal walls before its insertion. This additional step became a disadvantage for the dentists who prefer simpler materials. However, based on the findings of this study, it seems that the use of the primer on the root canal walls before sealer insertion was mandatory for better results. Studies have been reported that evaluated resin-based sealer penetration, most of them in the past 8 years. Mamootil and Messer[25] analyzed AH26, EndoREZ, and Pulp Canal Sealer EWT. They found sealer tags up to 1337 μm for AH26, 863 μm for EndoREZ, and just 71 μm for Pulp Canal Sealer EWT, the latter a zinc oxide-based sealer. The above authors dried the root canal prior to the use of EndoREZ, which is contraindicated as it affects the penetration of the hydrophilic resin. Indeed, penetration can exceed what the authors reported as demonstrated by Bergmans et al.[26]

These results showed the superiority of resin-based sealers with respect to tag formation. Patel et al.[27] also reported high values of tag formation for RealSeal, on average higher than 900 μm. In the present study, the laser irradiation did not interfere with sealer adaptation or the penetration into dentinal tubules. As described by other authors, it is not possible to deliver the same amount of laser energy to all root canal walls, mostly because it is a manual procedure.[3,8,9,12,13,15,18–20]

The analysis of silver nitrate penetration showed that laser irradiation interfered with sealer interaction of the apical seal. This method was used as it was reported in previous studies that this tracer solution produced good results.[5,21,22] The 50% ammoniacal silver nitrate solution has a pH = 9.5, which prevents the dissolution of calcium phosphate salts at the root canal interface, unlike other acidic tracer solutions.[21,22]

Because there are few studies in the literature, a comparison of our data is difficult to make. It was verified, however, that the Nd:YAG and diode laser had a positive influence on AH Plus sealing capacity. Being a hydrophobic epoxy resin sealer, this result was to be expected. The wavelengths of these lasers cause melting and resolidification of dentin, with occlusion of dentinal tubules, a condition that decreases the permeability of canal walls. Furthermore, these lasers probably promoted the loss of dentin hydration, and thus improving the sealing capacity of this sealer. Similar results have been previously reported.[5,6,16] On the other hand, the Nd:YAG laser treatment increased the penetration index of root canals filled with EndoREZ and Epiphany SE sealers. Because both are methacrylate resin-based sealers with hydrophilic properties, the loss of hydration associated with a presumed degradation of collagen fibers caused by the laser treatment had a negative effect on the sealing capability of these sealers. On the other hand, the Er:YAG laser irradiation had no effect on the penetration index. This can be explained as this wavelength causes ablation of dentin and thus exposing the dentinal tubules. Some studies have reported that this phenomenon causes an increase in dentin permeability.[13,18] However, others have reported that Er:YAG laser irradiation also causes dehydration and degradation of collagen fibers, factors that compromise the hybridization of resin sealers to the dentin.[10]

Therefore, within the limitations of this study, it can be concluded that the interaction of the resin-based sealers and laser irradiation does not neither negatively influence the adaptation of filling material to the dentin walls, nor the sealer's penetration into dentinal tubules. However, the use of hydrophilic resin-based sealers should be avoided when root canals are irradiated with a Nd:YAG laser.

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