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RESEARCH ARTICLE

Removal Efficiency of Calcium Hydroxide Intracanal Medicament with Er:YAG Laser: A Scanning Electron Microscopic Study

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ABSTRACT

Aim: This *ex vivo* study compared the efficiency of Er:YAG laser to remove calcium hydroxide from root canal walls, especially from the apical third, with manual and ultrasonic irrigation technique by using a scanning electron microscopic (SEM).

Materials and methods: Sixty-four single-rooted teeth were divided into 3 groups of 20 teeth each. The rest 4 teeth were used as control groups (2 positive and 2 negative control group). After coronal access, all teeth were instrumented by Protaper Next rotary files (Dentsply-Maillefer, Ballaigues, Switzerland) up to size F3, followed an irrigation protocol and filled with pure calcium hydroxide powder mixed with saline. Teeth were stored in an incubator for 7 days and then calcium hydroxide was removed using 3 techniques: Manually (group I), by ultrasonic irrigation (group II), by laser Er:YAG and x-pulse tip (group III). The teeth of control groups were instrumented as the experimental groups; no removal technique was applied in positive group, whereas in negative one, the root canals were left empty. Teeth were sectioned longitudinally and observed under SEM. Results were statistically analyzed with the Kruskal–Wallis test and Mann–Whitney test.

Results: The results showed significant difference between laser and the other two groups in coronal and middle root third, but no statistic difference in apical third.

Conclusion: Laser improved the removal of calcium hydroxide in comparison with conventional techniques.

Key words: Calcium hydroxide, Removal methods, Root canal, Scanning electron microscopy.

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INTRODUCTION

Calcium hydroxide is the most common antimicrobial intracanal dressing used between the sessions of endodontic therapy, because of its antimicrobial property and organic tissue dissolution capacity.[1] The antibacterial efficacy is attributed to its alkaline pH[1] and is directly proportionated to its ability to dissociate into calcium and hydroxyl ions.[2,3] According to some authors, the speed of ions dissociation and diffusion can be affected by the vehicle used for the mixing.[4]

Ex vivo studies have reported that residual calcium hydroxide on the root canal walls prevents sealer from penetrating the dentinal tubules, reduces dentin bond strength of resin sealer,[5,6] increasing the apical leakage of root fillings on the long-term.[7] and finally, reacts with zinc oxide and eugenol-based cements, make them brittle and granular.[6,8] Therefore, despite its unique physicochemical properties, calcium hydroxide should be completely removed before root filling.

To date, there is no consensus on which method was the best for calcium hydroxide removal. Several techniques have been described to remove calcium hydroxide from root canals.[9,10] The most popular technique for removal of calcium hydroxide is instrumentation with the use of master apical file (MAF) at working length, in combination with copious irrigation.[10,11]

Conventional irrigation with syringes has been advocated as an efficient method of irrigant delivery, using syringe with agents, such as sodium hypochlorite (NaOCl), ethyene diamino tetra acetic acid (EDTA) and citric acid in different combinations. This technique is still widely accepted by both general practitioners and endodontists. However, when conventional irrigation is used, stream action is relatively weak because of complex root canal anatomy.[12,13] The effectiveness of irrigation depends importantly on stream action[14] and not on the volume of irrigant.[13] Therefore, current endodontic techniques fall short of the goal to remove all calcium hydroxide dressing consistently. In order to overcome the previous problem, different irrigation techniques have been proposed to improve the efficiency of irrigation, including the use of sonic and ultrasonic agitation,[15] which generates continuous irrigant movement.[16] The recent years, dental Light Amplification by Stimulated Emission of Radiation (laser) has established the acceptance of the scientific community due to its unique

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properties. With the introduction to the field of endodontics last 15 years, laser has proven to have many advantages over conventional methods. Results suggest that laser is an effective tool for the disinfection and removal of debris, smear layer and obturation materials, as long as it seems to overcome the problem of insufficient depth of penetration of commonly used disinfecting agents.[14,15] The ability of Er:YAG laser to work on the dental tissue has been well established in literature. It is used to enhance fluid dynamics, by inducing vapor bubbles which expand within the root canal system[15,17] and remove debris from regions, which remain untouched by endodontic instruments during shaping procedures is enhanced.[18] On the contrary, there is little evidence of the Er:YAG laser concerning its capacity to remove calcium hydroxide which impairs obturation.[19-21]

The aim of this *ex vivo* study is to compare the effectiveness of Er:YAG laserusing photoacoustic streaming in comparison with ultrasound and manual techniques in removing calcium hydroxide from root canals.

MATERIALS AND METHODS

Sixty-four permanent single-rooted human teeth extracted were selected and radiographed in the buccal-lingual and mesial-distal directions. Criteria for tooth selection included a single root canal, no visible root caries, no fractures, cracks, internal and external resorption, calcification, completely formed apex and finally no intense curvature- less than 5^{o} according to Schneider's methodology.[22]

The teeth were stored in a 5% NaOCl solution for 2 days in order to remove organic residues and then, they were placed in a 1% thymol solution until the use. They were set in acrylic blocks and were de-crowned with a double-sided diamond disk using a low-speed handpiece. Root canal length was standardized to a length of 14 mm. Firstly, K-files sizes #10 and #15 (Sybron Endo, Kerr, USA) obtained the apical patency. The files were introduced further into the root canal until just the tip was visible at the apical foramen. The working length was determined by subtracting 1 mm from this length. The teeth were instrumented using Protaper (Dentsply-Maillefer, Ballaigues, Switzerland) up size F3, irrigation with 2 mL 5% NaOCl between the instruments –6 mL NaOCl totally. Canal preparation was performed using an electric engine (X-Smart, Dentsply Maillefer) with contact speed of 300 rpm and rotational force of 1,6 N.cm, at the working length.

At the end of instrumentation, root canals were irrigated by 10 mL 17% EDTA for smear layer removal followed by final irrigation with 10 mL 5% NaOCl and dried with absorbent paper points. The root canals were filled with pure powder calcium hydroxide mixed with saline using the MAF. The coronal orifices were sealed with cotton pellets and temporary restorative material Coltosol (Coltene, WholeDent, Switzerland). The teeth were incubated for 7 days at 37°C temperature and 100% humidity in a thermostatic incubator (Memmert, Schwabach Germany).

Then the teeth were separated into 3 groups, according to the protocol used to remove calcium hydroxide dressing:

- *First group (n = 20)*: Manual. The calcium hydroxide was removed by manual technique, by the use of MAF – a size of #30 K-file. Root canals were rinsed with 10 mL 5% NaOCl and 10 mL 17% EDTA, and finally irrigated with 10 mL 5% NaOCl, using a 10-mL syringe with a 30-gauge needle.
- *Second group (n = 20)*: Ultrasonic. The calcium hydroxide was removed by ultrasonic technique. Root canals were rinsed with 10 mL 5% NaOCl and 10 mL 17% EDTA, using a 10-mL syringe with a 30-gauge needle. The use of ultrasonic (Electro Medical Systems. Switzerland) was followed, using 5% NaOCl. The solution was delivered using master delivery tip which was located at the root canal orifice. It was ensured continuous irrigant supply for 60 seconds. Finally, root canals were irrigated with 10 mL 5% NaOCl.
- *Third group (n = 20)*: Er:YAG Laser (Fotona, Ljubljana, Slovenia). Root canals were rinsed with 10 mL 5% NaOCl and 10 mL 17% EDTA, as 1st and 2nd group. During laser irradiation, the root canals were continuously irrigated with 2 mL of 5% NaOCl to maintain hydration and fluid levels using a 30-gauge needle, positioned above the laser tip in the coronal aspect of the access opening.

Then, root canals were irradiated at a wavelength of 50 µsec, with an output of 0,3 Watt, a pulse energy of 20 mJ, and a pulse frequency of 15 Hz, according to researcher parameters (Table 1). During irradiation, the tip (600 µm, diameter, 14 mm length) was inserted into the canal and held stationary, parallel to the canal wall, at the first 1 mm of coronal third for 60 seconds. Finally, root canals were rinsed with 10 mL 5% NaOCl and 10 mL 17% EDTA.

Table 1: Laser parameters

Laser parameters	
Pulse energy	20 mJ
Irradiation time	60 second
Pulse length	50 µsecond
Fibre tip shape	Conical
Fibre tip diameter	600 µm
Fibre tip position	Coronal part

- *Positive control (n = 2)*: The teeth were instrumented as the experimental groups. The root canals were filled with calcium hydroxide but none of removal technique was applied.
- *Negative control (n = 2)*: The teeth were instrumented as the experimental groups but the root canals were left empty.

All teeth were split longitudinally in half. Therefore, grooves were prepared on the buccal and lingual surfaces with a diamond bur used with a high-speed handpiece. Teeth were split along their long axis in a buccolingual direction using a hammer and chapel. The roots were set suitably in round bases and were carbon coated, in order to be observed under the scanning electronic microscope (SEM) at 3 levels of magnification (2000×, 500×, 100×). Digital images were taken at the center of coronal (9 mm from apex), middle (6 mm from apex), and apical (3 mm from apex) thirds (Fig. 1).

All samples were analyzed using energy dispersive spectrometry (EDS) (Inca software, Oxford, UK) at 2000× magnification within an area (30 µm) at every third. The identification of the calcium was achieved using NORAN System SIX software V.1.8 (Fig. 2).

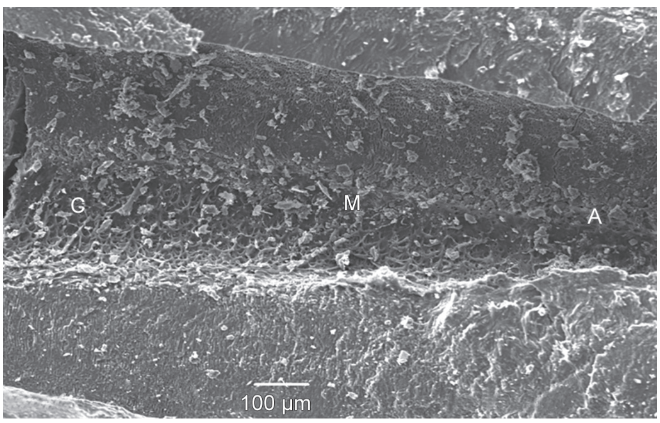


Fig. 1: Scanning electron image of the root canal: C (coronal), M (middle), A (apical)

To estimate the percentage of calcium hydroxide residues, it was followed a specific rating system (Fig. 3):

- Score 0: Absence of residues (Fig. 3A)
- Score 1: Small amount of residues (<20%) (Fig. 3B)
- Score 2: Moderate amount of residues (>50%) (Fig. 3C)
- Score 3: Large amount of residues (Fig. 3D)

Evaluation was performed by two calibrated examiners independently and in a blind manner. If the examiners could not reach an agreement, a third examiner would assist in the scoring of the sample.

The Kruskal-Wallis test was used to compare the percentage of calcium hydroxide remaining among groups, for each third and considering the canal as a whole. The Mann–Whitney test was performed as the *post hoc* multiple comparison method. The significance level was set at 1%. Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) statistics software.

RESULTS

A total of 64 teeth were included in the present study, with 4 being used as control group. A total of 60 teeth were randomly distributed into 3 groups. The Kruskal–Wallis test indicated statistic difference in the percentage of calcium hydroxide residual in the coronal and middle part of group III (Table 2).

More specifically:

- *Group I*: The root canal walls in this group are moderate in coronal part (Fig. 4A). It is possible to see some open tubule orifice in the middle third (Fig. 4B). However, in apical third, all the magnification images show the presence of intact calcium hydroxide and debris (Fig. 4C).
- *Group II*: The root canal walls in this group are not cleaner than in 1st (Fig. 4D) but it is possible to see a less percentage of remnants in the middle third (Fig. 4E)
- *Group III*: In all thirds of root canal, dentin surfaces appeared 50% less calcium hydroxide residues than groups I and II (Figs 4G to I).

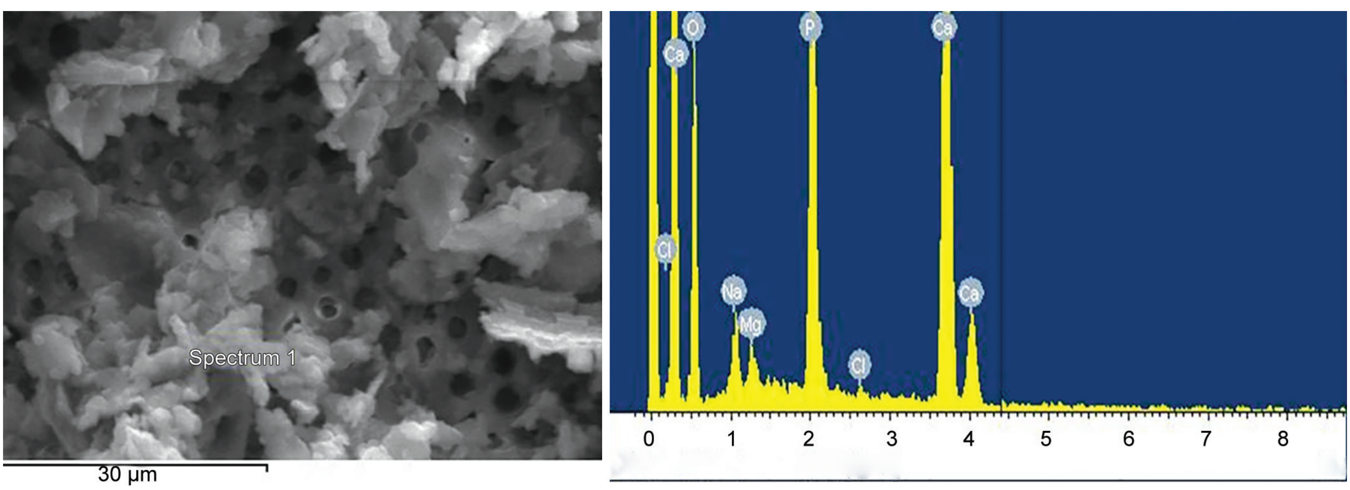
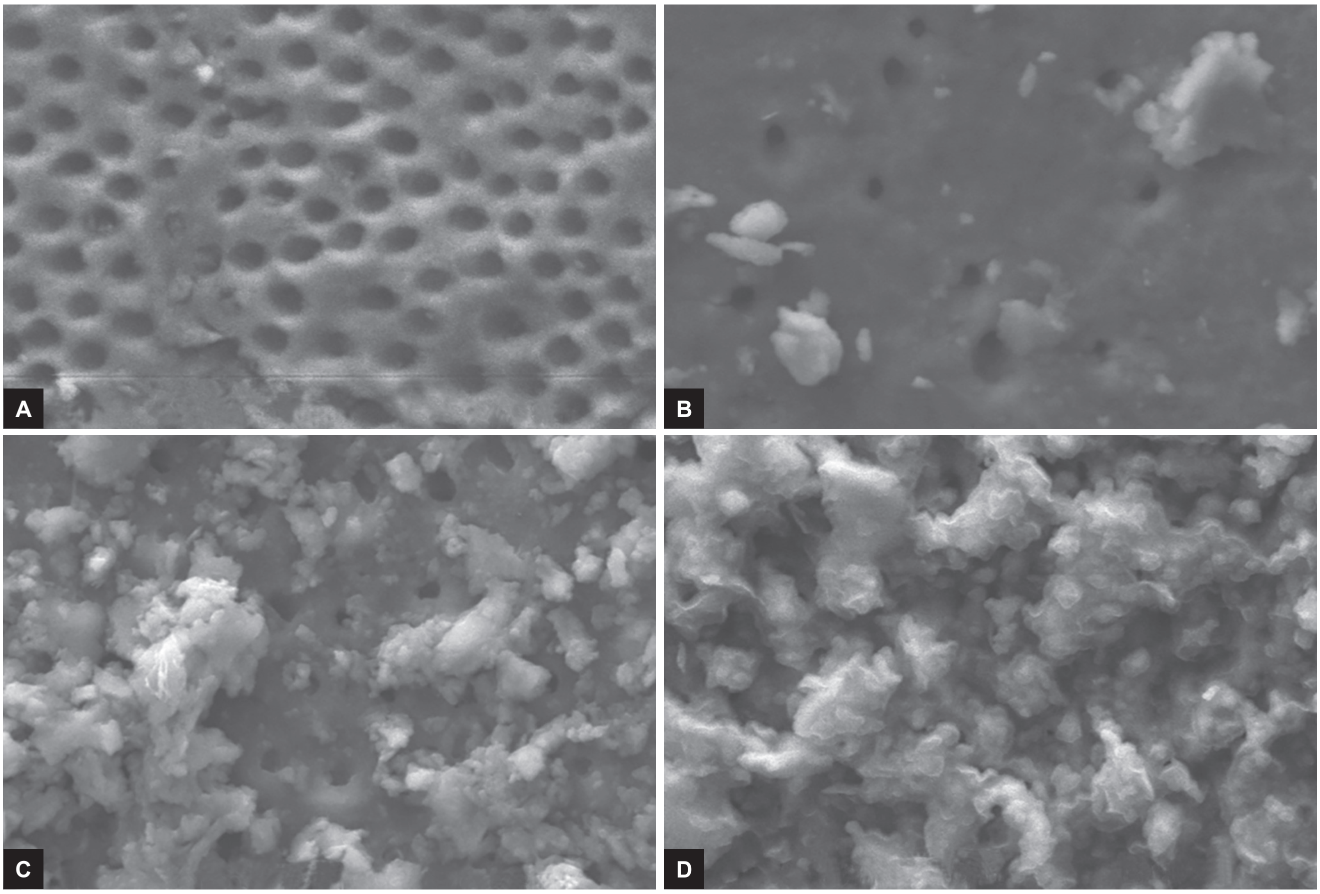


Fig. 2: Scanning electron microscopic/energy dispersive spectrometry analysis (2000× magnification)



Figs 3A to D: Scanning electron microscopic images representative of scores attribution: (A) Score 0: Absence of residues; (B) score 1: Small amount of residues; (C) score 2: Moderate amount of residues; and (D) score 3: Large amount of residues

Table 2: Results of percentage of score of residual $Ca(OH)_2$ residues at every canal third

	Coronal third	*Middle third*	*Apical third*
Third groups	*Mean (%)*	*Mean (%)*	*Mean (%)*
I	40	35,2	31,4
II	32,5	36,9	31,4
III	19	19,4	28,7
Kruskal–Wallis	χ^2 = 17,818 p < 0,01	χ^2 = 13,385 p < 0,01	χ^2 = 0,462 p > 0,01

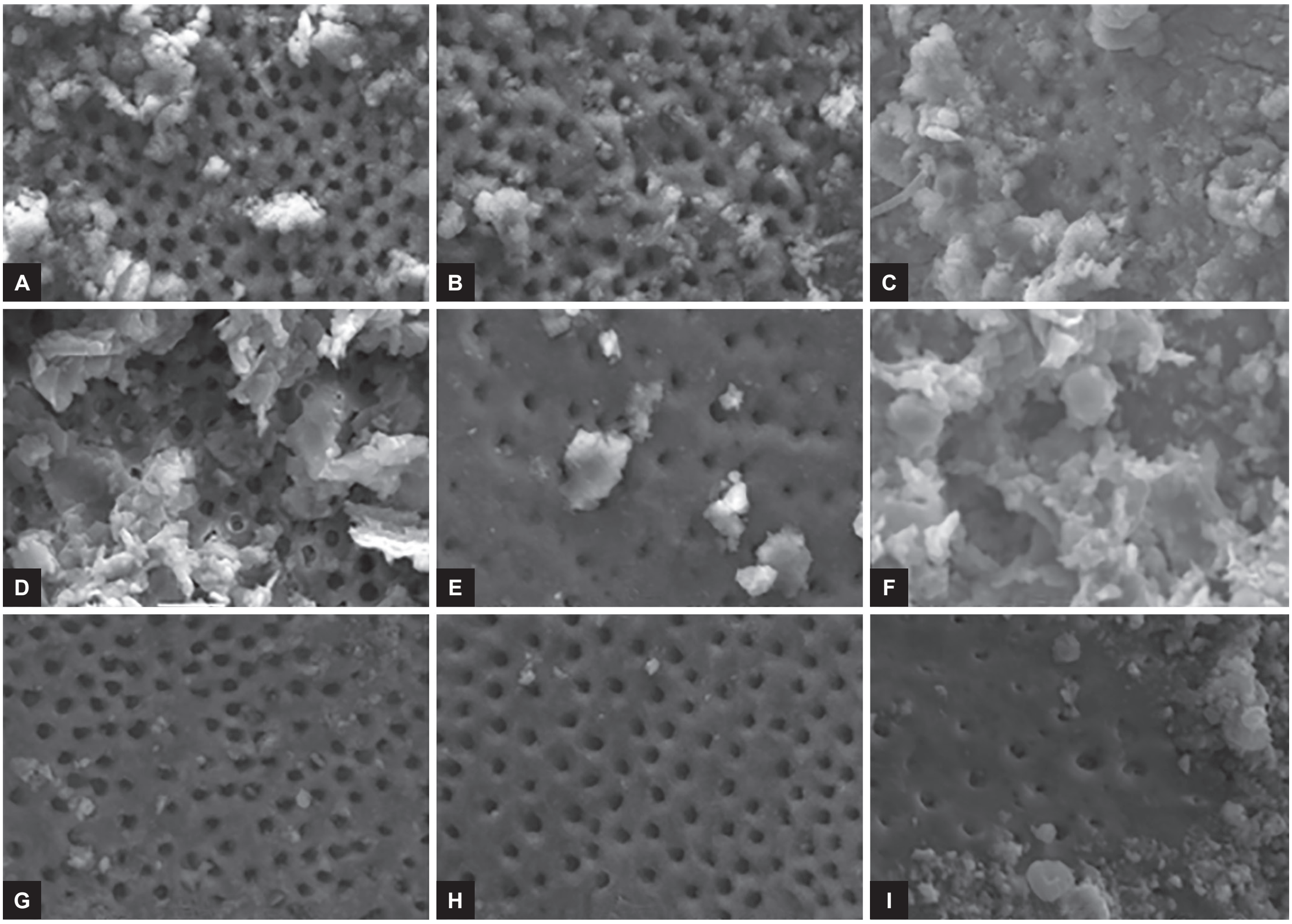
None of the techniques removed the calcium hydroxide debris completely from every third of tooth. Remnants of the intracanal medication were found in all experimental groups regardless of the removal technique. However, in all experimental groups, the percentage of calcium hydroxide debris was higher in comparison with the negative control group ($p < 0.01$). Positive control teeth in all groups showed densely packed remnants in all thirds.

DISCUSSION

Calcium hydroxide is the widely used intracanal medicament between sessions for its efficient antimicrobial activity. Its total removal before obturation is first priority as remnants may have negative impact on the sealing ability.

The Er:YAG (yttrium aluminium garnet) laser is the most investigated wavelength in endodontics and has proven to have many advantages over conventional methods. Firstly, Er:YAG laser is suggested to be an effective technique for the disinfection and removal of debris, smear layer and obturation materials, as long as it seems to overcome the problem of insufficient depth of penetration of commonly used disinfecting agents.[14,15] Laser-activated irrigation using Er:YAG lasers is an irrigant agitation technique with great potential for improved cleaning of the root canal system.[14] This could be attributed to the photoacoustic streaming effect seen, when light energy is pulsed in liquid. Laser emits photons to generate photoacoustic shockwaves into root canals. With the irrigant continuously delivered by means of a syringe into the pulp chamber during the laser process, the resultant shock wave is directed 3-dimensionally in the fluid and effectively removes both vital and necrotic tissue remnants.[19]

Some authors have suggested the need for large canal preparation so as to enable a 27 G needle to reach the canal end and reduce the microbial load,[23] resulting in canal transportation or destruction of root anatomy while canal ramifications remain still untouched by any



Figs 4A to I: Scanning electron microscopic images representative of group I (A = Coronal third; B = middle third; C = apical third), group II (D = coronal third; E = middle third; F = apical third), group III (G = coronal third; H = middle third; I = apical third)

irrigant.[24] Gutarts et al, mentioned that only energized irrigants permit fluid interchange throughout root canal system.[25] In our study, Er:YAG laser was equipped with a newly designed tip of 14 mm long 600-µm diameter, called x-pulse. The tip was placed and held stationary into the coronal access opening of chamber only. The advantage of the tip is that permitted minimal preparation and not over-instrumented root canal in order to achieve sufficient disinfection.[26]

We speculate that this phenomenon was responsible for the lowest percentage of calcium hydroxide residues among the other experimental groups, in coronal and middle root third the results of SEM observations show that the biggest number of teeth with 0 score belongs to group III.

However, our findings were not similar with those of Kaptan et al who compared the calcium hydroxide removal efficiency of Er-YAG and conventional techniques, using EDTA and NaOCl as irrigants. Although they concluded that the calcium hydroxide residues were reduced to 60,58% in comparison to 46,3% by conventional technique, the difference between the two groups was not statistically significant. Furthermore, noticing the results of that study, there was no comparison between the three parts of root canals as in our study, in which each third was evaluated concerning the presence or not of remnants.[27]

More recently, Arslan et al evaluated the effect of various techniques including photon-initiated photoacoustic streaming (PIPS), ultrasonic, sonic and conventional irrigation on the removal of calcium hydroxide from artificial grooves created in root canals. They concluded that PIPS, was superior to other techniques which mentioned, providing complete removal of the intracanal medicament.[28]

Lasing parameters vary considerably and their influence remains unclear. In present study, according to manufacturer's parameters, the teeth were irradiated at a wavelength of 2940, with an output of 0,3 Watt, pulse energy of 20 mJ and pulse frequency of 15 Hz. We did not investigate the influence of different lasing parameters on the cleaning efficacy of laser-activated irrigation.

Meire et al showed that pulse energy, pulse length, pulse frequency, irradiation time and position of the fiber tip all significantly affected the cleaning efficacy of laser. Higher pulse energy, shorter pulse length, longer irradiation time, placement of the fiber tip close to the groove, and, to a lesser extent, higher pulse frequency resulted in better debris scores. On the contrary, the shape and diameter of the fiber tip had no statistically significant influence on the results.[29]

Despite the unique advantages of laser, it may have negative impulse on root canals. Because of large amount of irrigation fluid, there is the danger of irrigant extrusion from apical end and side effects in periapical tissues.[30] Additionally, the formation of gas bubbles may cause the irrigation fluid to vanish from the respective canal section completely, which again may cause thermal damage of dental hard tissue.[30]

The effect of irradiation with the Er:YAG laser is enhanced by the presence of irrigation solutions NaOCl and EDTA. This leads to significantly better removal of the intracanal medicament from root canal walls contributing to an improvement in treatment efficacy.[14,16]

In addition, we should take into consideration that removal efficiency may be also dependent on the composition of calcium hydroxide. For instance, the vehicle which is used for mixing, affects the calcium hydroxide removal. Lambrianidis et al found that Pulpdent demonstrated significantly higher retention capacity than pure calcium hydroxide.[31] Likewise, the mixture of $Ca(OH)_2$/CHX (gel) paste was associated with significantly larger amount of residue, whereas $Ca(OH)_2$/CHX (solution) paste was associated with less.[32]

Concerning 1st and 2nd group-conventional and ultrasonic technique there was not statistically significant difference. That comes in disagreement with some authors who compared the efficacy of conventional technique and passive ultrasonic irrigation for the removal of calcium hydroxide.[33,34] They found that ultrasonic agitation of NaOCl was significantly more effective than irrigant-only techniques. Likewise, Khaleel et al evaluated that Endoactivator and ultrasonic technique removed significantly more residues than conventional technique.[35]

In the study of Tahan et al, there was no statistically significant difference between all experimental groups and the negative group in each canal third.[36] However, there was statistic difference between the experimental groups and the positive control. In the present study, only 3rd group – and only in coronal and middle canal part – showed statistically significant difference from negative control.

Finally, the data for the present study showed that none of group removed totally calcium hydroxide from the apical third. This result was similar to the results of previous study, which showed considerable amounts of calcium hydroxide on the canal walls, regardless of the removal technique used.[33,35,36] This drawback could be overcome by some additional interventions and results could be different if the irradiation duration was increased or the tip was located deeper.

CONCLUSION

It can be concluded that laser improved the removal of calcium hydroxide in comparison with conventional techniques. Laser application seems to be promising concerning calcium hydroxide removal. More studies are required with different laser parameters, different designs and position of tips as well as with other textures of calcium hydroxide paste with various mixture vehicles.

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