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RESEARCH ARTICLE

EVALUATION OF SMEAR LAYER AFTER ER: YAG LASER IRRADIATION IN MIDDLE AND APICAL THIRD OF MESIAL ROOT CANALS: A COMPARATIVE SEM INVESTIGATION

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ABSTRACT

Background: Laser-assisted endodontic treatment is a suitable instrument for smear layer removal in root canals. Objective: This ex-vivo study evaluated the efficiency of an Er: YAG laser (2940nm) at different pulse energy levels to remove smear layer with or without chelators from the middle and apical third of mesial roots. **Methods:** This study evaluated the efficiency of an Er: YAG laser (2940nm) at different pulse energy levels to remove smear layer with or without chelators from the middle and apical third of mesial roots. 48 mesial root canals of first mandibular molars were divided into 4 groups of 12 teeth each. Each group consisted of 2 subgroups each (A and B), regarding the irrigation protocol. In subgroup A, teeth were rinsed only by distilled water whereas in subgroup B, teeth were rinsed by 5ml 17% EDTA for 60sec, 5ml 5% NaOCL and 5ml distilled water. Four different pulse energy values were tested, namely 30mJ, 50mJ, 70mJ, 80mJ in order to irradiate the roots in Group 1, Group 2, Group 3 and Group 4 respectively. Control groups (n=4) was instrumented and rinsed as experimental groups (subgroup A & B) but not irradiated. Teeth were sectioned longitudinally and observed under SEM. **Results:** Results were statistically analyzed with the Kruskal-Wallis Test. There is statistically significant difference between groups irrigated with chelators and groups with distilled water before laser irradiation in the apical third. Group 1B (0.75W) showed statistically significant outcome in apical part. The results showed no statistically difference between subgroup B and control group. **Conclusion:** The presence of chelating factor may play an important role in the laser mechanism of smear layer removal from apical part of narrow and curved root canals.

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INTRODUCTION

The interaction between laser wavelength and specific tissues is strongly dependent on the optical properties of the tissue. Mid-infrared wavelengths between 2.7– 3μm exhibit a high absorption in water and hydroxyapatite thus making them highly effective in ablating hard dental tissues (Fried, 1998; Zuerlein, 1999). Currently, the most common lasers of mid-infrared spectrum used in dentistry, are the Er: YAG laser (2940nm) and the Er, Cr: YSGG laser (2790nm) (Hibst, 2002). Specifically Er: YAG lasers with a wavelength of 2940nm, have the highest absorption in water with an absorption coefficient μA of 800cm^{-1}. Considering that hard dental tissues consist of water in different percentages (Hibst, 1989), Er: YAG laser is a suitable instrument for ablating them (Lukac, 2004; Baraba, 2009). Previous studies have tested the ability and the effects of this laser in laser-assisted endodontic treatment and have indicated that the Er:YAG laser is a suitable instrument for smear layer removal in root canals (Takeda, 1998a; Takeda, 1998b; Takeda, 1999; Pecora, 2000). Dental hard tissue removal can be achieved by two mechanisms either thermo-mechanical ablation or explosive vaporization (Fried, 2000; Seka, 1996). In Erbium lasers, the explosive thermo-mechanical ablation is the process that occurs (Seka, 1996). Understanding the morphology of the root canal is of primary importance in achieving long-term success of the treatment (Vertucci, 1974). The available literature related to root anatomy and root canal configuration of the permanent mandibular first molar varies significantly. Anatomically permanent mandibular first molar typically displays a mesial and a distal root with two mesial and one distal canal (Al-Nazhan, 1999; Al-Qudah, 2009; Wang, 2010). The most common canal configuration in mesial roots of first is type IV and type II (31.5%-28%) (de Pablo, 2010). The purpose of the study was to evaluate smear layer removal in the middle and apical third of mesial root canals irradiated by an Er: YAG laser and determining the optimal values of pulse energy and average power. Therefore, the null hypothesis of this study was that Er: YAG laser using a conical designed fiber tip can effectively remove smear layer in the apical root third, without the aid of any chemical irrigants.

MATERIALS AND METHODS

The protocol of this study was accepted by the Research Ethic Committee of Dental School of Aristotle University of Thessaloniki (Greece) (no 363/16.01.18). A total number of fifty two (52) freshly extracted mandibular first molars with two roots were collected. The mesial root was separated from distal and was placed in coronal-apical direction inside the acrylic holder. Cone-beam computed tomography (CBCT) (Planmeca PROMAX Mid, 120KV, 16mA) was used for scanning and separation of mesial roots according to the criteria following: configuration anatomy (type II Vertucci), no visible root caries, no fractures, cracks, internal and external resorption, calcification, completely formed apex and no former root canal therapy (Figure 1).

Each tooth was sealed with wax and placed apical-coronally inside the acrylic holder. Coronal access was achieved and subsequently, with the help of a K-type #10 and #15 files, the working length was set at a distance of 1 mm shorter than the apical foramen. Root canals were instrumented using Protaper Gold (Dentsply-Maillefer, Ballaigues, Switzerland) up to size F3 (30/0.09) and irrigated with 1ml distilled water between instrumentations. At the end of the process, teeth were randomly assigned into 4 groups with 2 subgroups each namely A and B, depending on the protocol used to remove the smear layer. In subgroup A, teeth were rinsed only by 5% distilled water whereas in subgroup B, teeth were rinsed by 5ml 17% EDTA for 60sec, 5ml 5% NaOCL for 60sec and 5ml distilled water. Teeth were incubated in a thermostatic incubator at 37 ^{0}C temperature and 100% humidity (Memmert, Schwabach Germany) until use. An Er: YAG laser system (2940nm) (Morita AdvErl Evo, Kyoto, Japan) was used. The tip utilized was a 300μm diameter radial tip (R300T), applied in a suitable handpiece (Morita, N8001736) (Figure 2). The laser tip was inserted into the root canal 1mm short of the apex. The movement was longitudinal circular movement from the apical to the coronal part of the root. The speed of the movement was 2mm/s. Each root canal was irradiated for 4 times.

The samples were irradiated according to the following protocol (Table 1):

- In Group 1, the laser parameters set were: pulse energy 30mJ, average output power of 0.75W, pulse repetition rate of 25Hz, pulse duration of 300μs and 7:7 water/ air ratio.
- In Group 2, the laser parameters set were: pulse energy 50 mJ, average output power of 1.25W, pulse repetition rate of 25Hz, pulse duration of 300μs and 7:7 water / air ratio.
- In Group 3, the laser parameters set were: pulse energy 70mJ, average output power of 1.75W, pulse repetition rate of 25Hz, pulse duration of 300μs and 7:7 water / air ratio.
- In Group 4, the laser parameters set were: pulse energy 80mJ, average output power of 2W, pulse repetition rate of 25Hz, pulse duration of 300μs and 7:7 water / air ratio.
- In control group A (n=2), teeth were instrumented and rinsed by distilled water. No laser irradiation was applied.
- In control group B (n=2), teeth were rinsed by 5ml 17% EDTA for 60sec, 5ml 5% NaOCL for 60sec and 5ml distilled water. No laser irradiation was applied.

All sample irradiations were performed by a single operator. After irradiation, two longitudinal grooves were made in the outer root surface - on the buccal and lingual surfaces- with the help of a thin long diamond bur. Teeth were split along their long axis in a buccolingual direction using a hammer and chapel. Paper points were put in the root canals so as obstruct the extrusion of debris into them. From the two halves, only the mesial half of mesial root was selected and the distal part was discarded. Mesial parts were set suitably in round bases and were carbon coated, in order to be observed under the Scanning Electronic Microscope (Cam scan MV 2300, Oxford Instrument, UK) at 2000x magnification. Digital images were taken from common apical third of buccal and lingual root canal.

Statistical analysis

To estimate the percentage of smear layer residues, a specific rating system was followed:

- **Score 0:** No smear layer; dentinal tubules open
- **Score 1:** Small amount of smear layer; many dentinal tubules open
- **Score 2:** Homogeneous smear layer covering the root canal walls; only a few dentinal tubules open
- **Score 3:** Complete root canal wall covered by a homogeneous smear layer; no dentinal tubules open

Evaluation was performed by two independent calibrated examiners and in a blind manner. The Cohen's kappa coefficient was calculated to analyze the agreement between the two evaluators. The data were analyzed using the Kruskal–Wallis nonparametric analysis of variance. Statistical analysis was performed with SPSS Statistics Software and significance level was set at 5% ($P<0.05$).

RESULTS

The Cohen k analysis showed excellent reliability and reproducibility between the evaluators with values ≥1 for both. Different results were observed in experimental groups when comparing different laser parameters and irrigation protocol applied. The results for the smear layer scores in the middle and apical third of the mesial root canals in each group are shown in Table 2.

Table 1. Experimental groups

	GROUPS	Average power	Pulse energy	Repetition rate	Pulse duration	Water/ air ratio	Irrigation
Group 1	1A (n=4)	0.75W	30mJ	25Hz	300µs	7:7	dw
	1B (n=4)	0.75W	30mJ	25Hz	300µs	7:7	EDTA-NaOCL-dw
Group 2	2A (n=4)	1.25W	50mJ	25Hz	300µs	7:7	dw
	2B (n=4)	1.25W	50mJ	25Hz	300µs	7:7	EDTA-NaOCL-dw
Group 3	3A (n=4)	1,75W	70mJ	25Hz	300µs	7:7	dw
	3B (n=4)	1,75W	70mJ	25Hz	300µs	7:7	EDTA-NaOCL-dw
Group 4	4A (n=4)	2W	80mJ	25Hz	300µs	7:7	dw
	4B (n=4)	2W	80mJ	25Hz	300µs	7:7	EDTA-NaOCL-dw

dw: distilled water

Table 2. Kruskal–Wallis non parametric test between Groups

Root Thirds	Groups	Rank	Groups	Rank
Middle third 1st root canal	1A	13,50	1B	9,92
	2A	17,25	2B	13,75
	3A	4,50	3B	14,50
	4A	14,75	4B	11,83
Apical third 1st root canal	1A	13,25	1B	7,50
	2A	13,25	2B	13,17
	3A	13,25	3B	11,83
	4A	10,25	4B	17,50
Middle third 2nd root canal	1A	14,25	1B	9,92
	2A	15,50	2B	11,83
	3A	4,75	3B	14,50
	4A	15,50	4B	13,75
Apical third 2nd root canal	1A	12,83	1B	7,67
	2A	14,75	2B	14,83
	3A	14,75	3B	11,00
	4A	7,67	4B	16,50

		Middle 3rd 1st rc	Apical 3rd 1st rc	Middle 3rd 2nd rc	Apical 3rd 2nd rc
GROUPS	Kruskal-Wallis	11,358	1,038	11,024	5,600
1A,2A, 3A, 4A	Df	3	3	3	3
	Asymp. Sig.	0,010	0,792	0,012	0,133
GROUPS	Kruskal-Wallis	2,113	6,967	2,113	6,660
1B, 2B, 3B, 3B	Df	3	3	3	3
	Asymp. Sig.	0,549	0,073	0,549	0,084

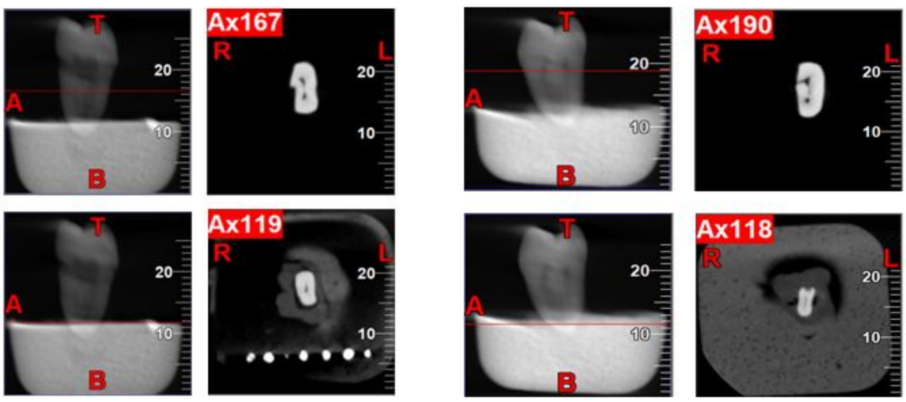


Figure 1. CBCT images- type II Vertucci

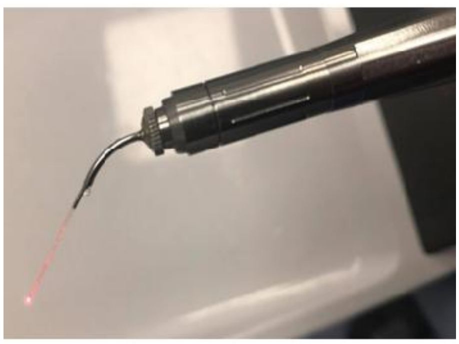


Figure 2. Tip laser Er: YAG Laser R300T (Morita Corporation)

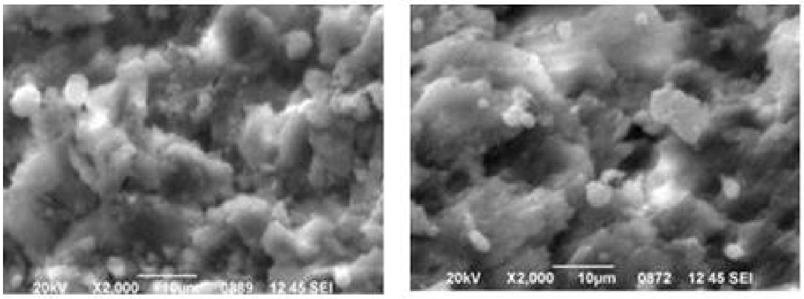


Figure 3. Groups 1A 2A, 3A, 4A: Representative SEM images from apical third

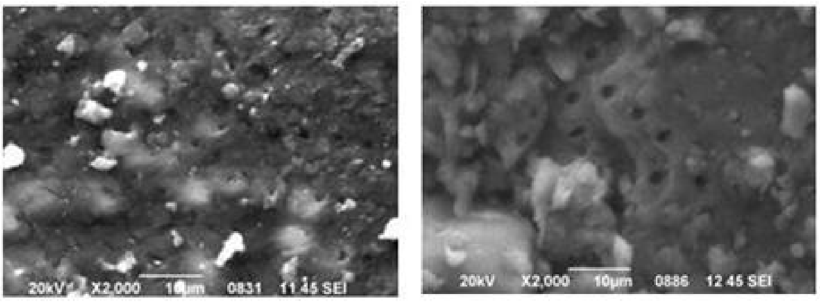


Figure 4. Groups 1A 2A, 3A, 4A: Representative SEM images from middle third

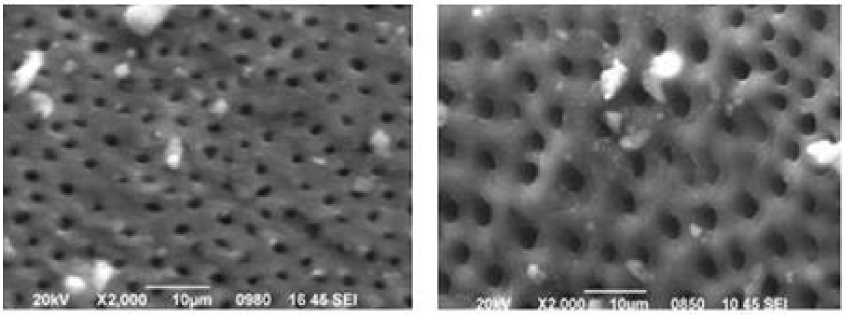


Figure 5. Groups 1B, 2B, 3B, 4B: Representative SEM images from apical third

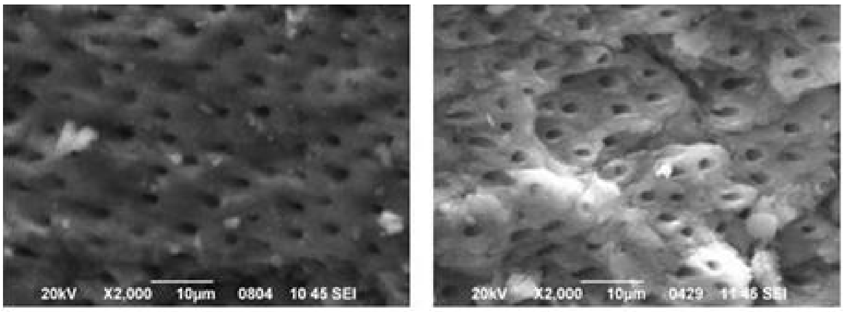
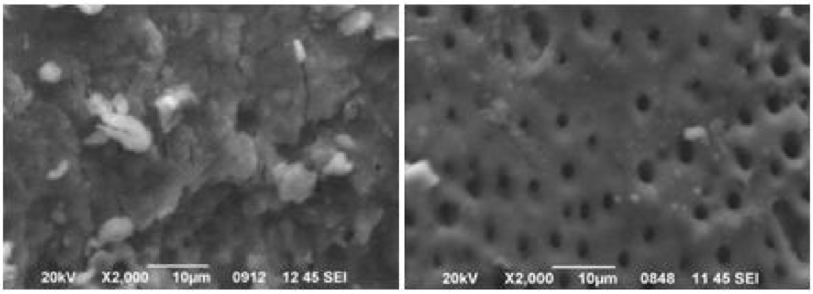


Figure 6. Groups 1B, 2B, 3B, 4B: Representative SEM images from middle third



A **B**

Figure 7. Apical third in control group A and B respectively

The results for the present study showed considerable amounts of smear layer on the canal walls in apical third, regardless of the pulse energy applied. On the other hand, in groups 1B, 2B, 3B, 4B, samples were irrigated following an irrigation protocol (5ml 17% EDTA for 60sec, 5ml 5% NaOCL for 60sec and 5ml distilled water) before irradiation. SEM pictures reveal that there were samples where smear layer was totally removed in apical third (Figure 5). In these groups, there were samples free of smear layer (score 0), some with small amount of smear layer (score 1) and a small percentage with homogeneous smear layer covering the root canal walls (score 2). In middle part, in all the samples of subgroup B, smear layer was completely removed or it is left a small amount of debris (Figure 6). The Kruskal- Wallis test showed that there was no significant difference in the middle and apical third. The results for the present study showed that the increase of the pulse energy applied does not influence the smear layer removal from the canal walls. In control group A, samples were totally covered by smear layer whereas in control group B, samples were free of smear layer in middle and apical part, (Figure 7).

More specifically, samples of subgroups 1A, 2A, 3A, 4A, were irrigated only by distilled water before irradiation. In apical part of root canal, SEM pictures showed the presence of heavy and intact smear layer regardless the energy pulse and average power applied. There were no areas where smear layer was slightly removed (Figure 3). In the middle part of these samples, magnification images showed much lower percentages of smear layer residues. In half of the samples, small amount of smear layer with many dentinal tubules open was observed (Figure 4). The Kruskal- Wallis test showed a significant difference among laser subgroups 1A, 2A, 3A, 4A, regarding the smear layer scores in the middle third of both two root canals. In the opposite, there was no significant difference in the apical third.

DISCUSSION

This study evaluated the cleanliness of dentinal walls in middle and apical third of mesial root canals after Er: YAG laser irradiation. Laser parameters -average power and pulse energy- were compared concerning the efficiency in smear layer removal. The null hypothesis was there is no difference between groups irrigated with chelators and the other groups irrigated with distilled water before laser irradiation.

Smear layer is an amorphous, irregular layer which is formed after mechanical preparation of root canal (Mader, 1984). There are several methods for removing the smear layer such as chemical solutions, use of ultrasonic dental devices and dental lasers.

For accurate dental tissue procedures, Erbium lasers offer the safest and most efficient solution. Of the available Erbium laser technologies -Er: YAG and Er, Cr: YSGG laser-, the Er: YAG has the optimum absorption characteristics in water which explains its increased efficiency (Diaci, 2012). The water content of the tissue plays a major role in the ablation process (Armengol, 1999). Er: YAG laser irradiation is strongly absorbed by water and the energy causes evaporation. The vapor bubble starts to expand and form a void in front of the laser – tissue interaction point. These bubbles, the formation of an empty space in a liquid, are the basis of cavitation and smear layer removal (Brugnera, 2003; Kivanc, 2012). DiVito and al (Di Vito, 2011) studied the effectiveness of Er: YAG laser in removing smear layer (25mJ, 15 Hz, 50 μs) with the radial and stripped tip in different irradiation times with and without the use of 17% EDTA. All irradiated groups had good results in removing the smear layer. The combination of Er: YAG laser with 17% EDTA was the most effective. In present study, different pulse energy settings were tested (30-80mJ) in the same different irradiation times with and without the use of irrigation solutions (NaOCL and EDTA).

Another study confirmed the successful removal of smear layer using Er: YAG laser with lower power settings (20mJ, 10Hz, 50μs) for 20 or 40s with 17% EDTA irrigation (DiVito, 2012). In both previous studies, the SEM analysis of the mentioned groups, showed open tubules, exposed intact collagen fibers and no thermal damage to the root canal wall. The smear layer was successfully removed.

The sample of this study consisted of mesial roots of 1st mandibular molars. It is now well accepted that mandibular first molar exhibits a number of anatomical variations not only in number of roots but also in canal morphology (Prabhu, 1995; Schumann, 2008). The new lanced endodontic tip R300T tip is used for first time for smear layer removal from molars. Herman (Herrman, 2017) supported that after root canal preparation with mechanical nickel- titanium instrumentation, R300T (50mJ, 25pps) can be used. A recent vitro study (Henninger, 2019) aimed at evaluating the antimicrobial activity of Er: YAG laser (70mJ, 20pps) and R300T on several microorganisms associated with persistent endodontic infection.

The traditional laser applications necessitate conventional preparation for at least up to size 30 and the laser tip need to reach apical third of the root. However, R300T tip of 300μm necessitates larger root canal diameter than ISO 30. In our studies, in subgroups A, it was very difficult the instrumentation of such narrow and curved root canals without chelator. Many samples were rejected due to broken file.

Ramalho *et al.* (2005) showed that the Er: YAG laser did not remove the smear layer from dentinal wall surfaces, because they were not reachable by its optical fiber. Better results have been reported when comparing Er: YAG laser with other methods of smear layer removal. Er: YAG laser is reported to have the most effective removal of smear layer from the dentine wall compared to 17% EDTA, 6% citric acid, 6% phosphoric acid and CO_2 laser (Takeda, 1999).

In another study Er: YAG laser had also shown to have better smear layer removal when compared to other wavelengths such as Argon and Nd: YAG lasers (Takeda, 1998a). Irrigation with EDTA was used in this study. Acid solutions have been recommended for removing the smear layer, including EDTA, citric acid and orthophosphoric acid (Perez, 1996). These irrigants dramatically improve the cleaning ability of root canals.

On the other hand, studies have shown that a combination of NaOCL and EDTA removed the smear layer only partially (Ciucchi, 1989) and unable to clean in the apical portion of the root canals, and hence, to improve that in apical portion laser-activated systems were used in the recent study of Dhawan *et al*. (2020).

Comparing the two major groups from a clinical point of view, it can be concluded that Er: YAG laser is more effective in combination with EDTA solution. Er: YAG laser adequately cleaned dentinal walls at middle third. The absence of chelating factor played an important role in the laser mechanism of smear layer removal in apical third. Pulse duration has been found to influence ablation (Hibst, 2002; Meister, 2004; Apel, 2002).

At shorter pulse durations like 100µs then 150µs, enamel ablation begins already with 7J/cm^2, which is 3J/cm^2 lower than the ablation threshold (Apel, 2002). Shorter pulse durations leads to less thermal transfer to surrounding tissue and results also in more efficient ablation (Meister, 2006). Unfortunately the device used had a constant pulse duration of 300µs which leads to smaller peak power values.

Er: YAG laser due to its high water absorption and hydroxyapatite has low penetration into the dentinal walls of the root canal system (Torabinejad, 2003). Its antimicrobial action is limited in removing smear layer and superficially cleaning the dentinal walls better than conventional methods, but has significantly less transmission in dentine than a near-infrared diode laser (Gutnecht, 2008).

Conclusion

The outcome of the present study showed that laser-assisted smear layer removal with an Erbium laser with the tested parameters is not predicable for the apical third of mesial root canals. The presence of chelating factor may play an important role in the laser mechanism of smear layer removal from apical part, mainly, of narrow and curved root canals.

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Glossary of Abbreviations

- **CBCT:** Cone beam Computated Tomography
- **DW:** distilled water
- **EDTA:** Ethylene- Diamine- Tetra-Acetic acid
- **Er,Cr:YSGG laser:** Erbium Chronium doped: Yttrium Scadium Gallium Garnet
- **Er: YAG:** Erbium doped: Yttrium Aluminium Garnet
- **LASER:** Light Amplification by Stimulated Emission of Radiation
- **MAF:** Master Apical File
- **NaOCL:** Sodium hypochlorite solution
- **SEM:** Scanning Electron Microscope

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