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Abstract: Aim: This ex-vivo study evaluated the efficiency of an Er, Cr: YSGG laser (2780 nm) at different power settings to remove smear layer from the apical third of mesial roots of first lower molars by using SEM. Materials & Methods: 42 mesial root canals of first mandibular molars (type II Vertucci) were divided into 4 groups of 10 teeth each. The rest 2 teeth were used as control groups. After coronal access, all teeth were instrumented by Protaper Gold rotary files up to size F2 (25/0.08), followed an irrigation protocol with saline. Then, the experimental groups were irradiated by Er, Cr: YSGG laser. Four different output powers were tested, namely 1.25 W, 1.5 W, 2 W, 2.5 W was used to irradiate the roots in Group 1 (G1), Group 2 (G2), Group 3 (G3) and Group 4 (G4) respectively. Control group (n=2) was instrumented as experimental groups but not irradiated. Teeth were sectioned longitudinally and observed under SEM. Results were statistically analyzed with the Kruskal-Wallis Test and Mann-Whitney Test. Results: None of the tested groups succeeded in removing completely the smear layer from apical dentinal walls. The results showed no statistically significant differences between laser groups in removing smear layer from apical third of lower molars. Group 3 (2 W) showed better outcomes but it was not statistically significant. Conclusion: The outcome of the present study showed that laser-assisted smear layer removal with an Er, Cr: YSGG laser with the tested parameters is not predictable for the apical third of root canals. Clinical significance: The presence of chelating factor may play an important role in the mechanism of laser-assisted removal of smear layer in the apical third of root canals. Further research needs to be performed in order to find the optimal irradiation protocol.

Keywords: Smear Layer Removal, Er, Cr: YSGG Laser, Mesial Root, Molars

1. Introduction

Smear layer is an amorphous, irregular layer which is formed after mechanical preparation of root canal. It is consistsed of organic and inorganic particles of dentin, remnants of pulp tissue, microorganisms, endotoxins and blood cells [1]. Harmful effects may occur in case that the smear layer is not removed during root canal treatment. Smear layer obstructs the penetration of antimicrobial irrigants, medicaments and sealers into dentinal tubules and this compromises the disinfection of root canals [1, 2].

The complexity of root canal anatomy presents clinical challenges concerning cleaning and disinfection of the root canal system. First mandibular molars and in particular, mesial roots present a complicate canal configuration, with two or three root canals, isthmus, apical deltas, and inter-canal communications. It is important for the clinician to take into account this complex anatomy because the outcome of endodontic therapy is directly related to eliminating smear layer and preventing microbial contamination.

Lasers can be considered as an alternative tool for cleaning root canals and removing smear layer [3, 4]. Er, Cr: YSGG laser is a well- investigated wavelength and has proven to have many advantages over traditional methods in root
canal disinfection and cleaning. Er, Cr: SGG emits photons at a wavelength of 2780 nm. This wavelength has high absorption in water and hydroxyapatite, which makes it suitable for smear layer removal and possibly microbe reduction during root canal therapies [5].

Er, Cr: YSGG laser, as a free running pulsed laser is able to ablate by means of explosive thermo-mechanical vaporization. The water molecules of the water spray expand in a very short time frame and explode [6-8]. Therefore, smear layer removal can be attributed to cavitation effects inducing high speed fluid motion into the root canal [9]. Direct laser irradiation combined with cavitation effect created by laser activation of irrigants is considered the most effective protocol in removing smear layer and increasing dentin permeability [10].

Radial firing tips (RFTs) are recently designed to spread energy along the direction of dentinal tubules in a uniform way. This is attributed to the conical shape of the fiber tip, which allows laser light to be emitted in the form of a broad cone with an angle of about 60° [11].

The purpose of the study was to evaluate smear layer removal in the apical third of mesial root canals, irradiated using an Er, Cr: YSGG laser, RFT and determine the optimal OPW (output powers) without the aid of any chemical irrigants such as NaOCl and EDTA.

2. Material and Methods

Forty two freshly extracted mandibular first molars with two roots were collected. Samples were stored in a 5% NaOCl solution for 2 days in order to remove organic residues and in a 0.1% thymol solution until use. Then, teeth were set in custom-made acrylic holder and decrowned. The mesial root was separated from distal with a double-sided diamond disc using a low-speed handpiece.

Cone-beam computed tomography (CBCT; Planmeca Pro-Max3Dmax operated at 96 kV, 10 mA, 12 s) was used for non-invasive CBCT images. Each tooth was placed in coronal-apical direction inside the acrylic holder. After scanned, mesial roots selected 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. Type II anatomy is defined as having 2 separate canals from the coronal third, joining to common apical foramen (Figure 1).

Root canal length was standardized to a length of 14 mm and the teeth were de-crowned with a diamond bur using a high-speed handpiece. Coronal access was achieved and therefore, apical patency was obtained by K-file size #15 (Sybron Endo, Kerr, USA). The files were introduced further into the mesial root canal until just the tip was visible at the apical foramen. Root canals were instrumented using Protaper Gold (Dentsply-Maillefer, Ballaigues, Switzerland) up size F2 (25/0.08) and irrigated with 1 ml saline between the instruments. Canal preparation was performed using an electric engine (X-Smart, Dentsply Maillefer) with contact speed of 300 rpm and rotational force (torque) of 1.6 N. cm, at the working length. At the end of instrumentation, root canals were irrigated by 5 ml saline for smear layer removal.

![Figure 1. Vertucci II configuration (CBCT).](image)

Root canals were dried with absorbent paper points and coronal orifices were sealed with cotton pellets and temporary restorative material Coltosol (Coltene, WholeDent, Switzerland). Teeth were incubated in a thermostatic incubator at 37°C temperature and 100% humidity (Memmert, Schwabach Germany) until use.

An Er, Cr: YSGG laser system (2780 nm) (Waterlase MD, Biolase Technology, Irvine, CA, USA) was used. The tip utilized was a 200 µm diameter radial firing tip (RFT2), applied in a gold handpiece. Four different output powers were tested, namely 1.25 W (79.6 mJ / cm²), 1.5 W (95.5 mJ / cm²), 2 W (127.4 mJ / cm²), 2.5 W (159.2 mJ / cm²) to irradiate the roots in Group 1 (G1), Group 2 (G2), Group 3 (G3) and Group 4 (G4) respectively. The other laser parameters used were: pulse repetition rate of 50 Hz, pulse duration of 140 µs (H-mode) and 80% - 30% water-air spray ratio.

The radial firing tip was inserted into the root canal 1 mm short of the apex. The movement was circular movement from the apical to the coronal part of the root. The speed of the movement was 2 mm/sec. Each root canal was irradiated 7 sec for 4 times. Therefore, the overall irradiation time was 28 sec for every root canal.

Teeth were randomly assigned into 4 groups, according to the protocol used to remove the smear layer (Table 1):

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Average power</th>
<th>Pulse energy</th>
<th>Repetition rate</th>
<th>Pulse duration</th>
<th>Total energy delivered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=10)</td>
<td>1.25W</td>
<td>25mJ</td>
<td>50Hz</td>
<td>140µs</td>
<td>35J</td>
</tr>
<tr>
<td>Group 2 (n=10)</td>
<td>1.5W</td>
<td>30mJ</td>
<td>50Hz</td>
<td>140µs</td>
<td>42J</td>
</tr>
<tr>
<td>Group 3 (n=10)</td>
<td>2W</td>
<td>40mJ</td>
<td>50Hz</td>
<td>140µs</td>
<td>56J</td>
</tr>
<tr>
<td>Group 4 (n=10)</td>
<td>2.5W</td>
<td>50mJ</td>
<td>50Hz</td>
<td>140µs</td>
<td>70J</td>
</tr>
</tbody>
</table>

In control group (n=2), teeth were instrumented as the experimental groups. None of laser protocol nor removal technique was applied.

A single operator performed all irradiations. 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. Randomly, 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 at 1000 X magnification. Digital images were taken from common apical third of buccal and lingual root canal.

The percentage of smear layer residues was estimated according the rating system below:

a. Score 0: Absence of residues
b. Score 1: Small amount of residues (<20%)
c. Score 2: Moderate amount of residues (>50%)
d. Score 3: Large amount of residues

Two calibrated examiners evaluated the amount of residues. A third examiner assisted in the scoring of the sample in case of disagreement.

The Cohen's kappa coefficient was used to analyze inter-rater agreement. Results were statistically analyzed with Kruskal-Wallis and Mann-Whitney test. Kruskal-Wallis test compared the percentage of smear layer remaining among groups, for each third and considering the canal as a whole. Mann-Whitney test was performed as the post hoc multiple comparison method. Statistical analysis was performed with SPSS Statistics Software and significance level was set at 5% (P<0.05).

### 3. Results

The results of the present study were statistically analyzed in order to interpret the significant differences in smear layer scores within each group and between the groups. Cohen's kappa coefficient of inter-rater reliability was 0.887. Control specimens showed dense remnants in all thirds as opposed to the negative controls. Residual smear layer was found in specimens of all experimental groups.

When examining the efficacy of smear layer removal from all thirds, Kruskal-Wallis test revealed that none of laser group was more efficient than other groups. The results for the present study showed considerable amounts of smear layer on the canal walls in apical third, regardless of the output power used (Table 2).

More specifically, in Group 1 (1.25 W), all the magnification images showed the presence of intact smear layer and debris. Most of samples remained covered with smear layer. In Group 2 (1.5 W), smear layer covering the root canal walls was evident in all the samples. The dentine tubules appeared to be closed. There were no areas where the smear layer was removed completely. In Group 3 (2 W), SEM pictures showed the best presence of smear layer removal in most samples. Three samples were evaluated with grade 1 (20% smear layer) and four samples with grade 2 (50% smear layer). It can be observed dentinal tubules completely open and debris free. In Group 4 (2.5 W), seven samples were covered with smear layer. Some cracks could also be seen due to drying procedures (Figure 2). As it was expected, in the Control group, all samples were having a homogeneous smear layer that was obstructing the dentinal tubules.

### Table 2. Results of Smear Layer removal.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Count</th>
<th>20%</th>
<th>50%</th>
<th>100%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>Count</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>% within GROUPS</td>
<td>0.0%</td>
<td>30.0%</td>
<td>70.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>Count</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>% within GROUPS</td>
<td>0.0%</td>
<td>50.0%</td>
<td>50.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>Count</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>% within GROUPS</td>
<td>30.0%</td>
<td>40.0%</td>
<td>30.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>Count</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>% within GROUPS</td>
<td>0.0%</td>
<td>30.0%</td>
<td>70.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>3</td>
<td>15</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>% within GROUPS</td>
<td>7.5%</td>
<td>37.5%</td>
<td>55.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

### Figure 2. Representative SEM images: A. Group 1 (1.25W), B. Group 2 (1.5W), C. Group 3 (2W), D. Group 4 (2.5W).

### 4. Discussion

Smear layer is produced as a result of mechanical root canal instrumentation. Despite controversial views regarding its removal, the evidence based studies have decided on removing and eliminating smear layer. Lasing parameters vary considerably and their influence in smear layer removal...
remains unclear.

This study evaluated the various methods to improve smear layer removal at the apical third of the instrumented mesial root canals. The main goal was to verify the effect that laser produced in apical third without the combination of a chelating agent. We investigated only the influence of power on the cleaning efficacy of laser-activated irrigation according to manufacturer’s parameters. Apical preparation was extended to size 25/0.08 to allow adequate access for the laser firing radial tip No 2 (200 µm) to the apical third of the canals. The protocol was common in all experimental groups. Teeth were irradiated with a wavelength of 2780 nm, with an average output power of 1.25 W, 1.5 W, 2 W and 2.5 W, pulse repetition rate of 50 Hz and pulse duration of 140 µs.

Strict criteria were applied in sample selection to standardize the specimens, and to avoid sample related bias thanks to CBCT imaging. The sample was consisted of mesial roots of first mandibular molars type II (Vertucci configuration anatomy) [12]. Typically, mandibular first mandibular molars are two-rooted with two mesial canals and one distal canal. In a total of 28 studies (n=6,959 teeth) reported that the most common Vertucci type of canal configuration is type II (52.0%) and type IV (28.7%) [13]. 83% of mesial roots have 2 canals with isthmus and there are frequent inter-canal communications or anastomoses [14]. It is speculated that this complex anatomy was responsible for the highest percentage of smear layer residues in all experimental groups. There is not any previous study in molars.

In the present study, the cleanest surfaces among the experimental groups were observed in the third group (2 W). However, this finding was not statistically significant. Likewise, Ali et al (2005) [15] observed that at 2 W, in apical third, there was not much debris and smear layer. In fourth group, higher output power (2.5 W) not only caused thermal damages and carbonization but also failed to remove smear layer when compared with lower. The high power laser itself might become a source of smear layer formation because of the structural damage that it causes in the dentinal structure [16].

In a previous study, Er, Cr: YSGG laser, with an average output power of 1.5 W, removed effectively the smear layer in apical, middle and coronal parts of the root canal walls and had similar results as the conventionally treated group with irrigation of EDTA and NaOCl. This is not consistent with the results of present study. In group 2 (1.5 W), smear layer had covered over the half of apical root surface.

As mentioned, the increased incidence of narrow mesial root canal of molars where laser beam cannot reach may be the reason for low efficiency of smear layer removal. The need for large canal preparation has been proposed so as to enable fiber tip to reach the canal end, resulting in canal transportation or destruction of root anatomy while canal ramifications remain still untouched by any irrigant. In our study, Er, Cr: YSGG laser was equipped with a newly designed tip of 21 mm length and 200 µm diameter, called Radial Firing Tip No 2. The tip was inserted into each root canal, 1 mm less from working length. The movement was circular movement from apical to coronal part, with speed movement 2 mm/sec.

In most studies, experimental groups showed better smear layer removal in coronal and middle third than in apical [10, 17, 18]. Additionally, Al-Karadaghi et al (2015) [19] observed that Er, Cr: YSGG laser had an uneven smear layer removal especially in the apical third of the root canal wall. This is attributed to many reasons. Firstly, the taper and diameter of apical third are much smaller than those of coronal and middle [20] which in turn hinder the circulation and action of irrigating solution [21].

Secondly, dentin at the apical region is sclerotic and transparent, has more irregular structure and reduced permeability comparing to dentin of coronal or middle root third [22]. All above characteristics contribute to the complexity of the apical region [23, 24].

Thirdly, inadequate debridement in apical region may be resulted from apical vapor lock effect. Since root canal behaves as a close-ended channel, it is observed air entrapment and bubble formation [25]. The radial firing tip was inserted into the root canal 1 mm short of the apex, taking into consideration patient’s safety and probably preventing the thorough cleaning of the apical third.

To date, a final irrigation sequence with EDTA and then NaOCl seems to remove effectively the smear layer [26] but not completely. In present study, saline was the only irrigant used as a lubricant between the instruments. Saline could remove neither organic nor inorganic particles of smear layer. This reduced the amount of variables, and allowed the evaluation of the contribution of Er, Cr: YSGG laser.

Mid-infrared erbium lasers (Er: YAG laser and Er, Cr: YSGG laser) demonstrate a high absorption coefficient for both aqueous solutions and hydroxyapatite. Due to their high water absorption in smear layer, they have extremely low transmission into the dentinal walls of the root canal system [20]. Therefore, their antimicrobial action is limited in deeper dental layers. Both wavelengths can have better results in disinfection of the main canal compared with conventional methods [27]. On the other side, near infrared range diode lasers (810, 940, 980 nm) and Nd: YAG laser (1064 nm) are mostly absorbed by hemoglobin and melanin [28] and their disinfection is even deeper (Gutknecht 2008). Therefore, in a recent study of Martins et al (2018) [29], it is suggested the double-wavelength application (erbium and diode laser) for a better outcome of the endodontic therapy as each laser wavelength has a specific absorption coefficient in every tissue [30].

5. Conclusion

Results of the present study showed that root canal disinfection aided by an Er, Cr: YSGG laser at 2 W output power was quite effective in smear layer removal but not complete and satisfactory. In clinical practice, the apical part of the root plays an important role in the success of endodontic therapy. The absence of chelating factor may play an important role in the mechanism of smear layer removal. Further research is required in order to find the optimal irradiation protocol (average power, pulse energy, pulse duration) for the laser systems, which could remove the
smear layer completely.

References


