

# Effect of Combined Use of Photon-Initiated Photoacoustic Streaming and Chitosan on Smear Layer Removal

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## ABSTRACT

**Objective:** Ethylene diamine tetraacetic acid (EDTA) is an appropriate irrigant for smear removal. This study aims to compare the smear-removing capacity of chitosan in combination with photon-initiated photoacoustic streaming (PIPS) to that of EDTA.

**Methods:** Forty-five human mandibular premolar teeth were included. Root canals were prepared with OneShape files. The samples were randomly divided into three equal groups. The final rinsing was done as follows. Group 1: 0.2% chitosan irrigation with PIPS irradiation; Group 2: 0.2% chitosan alone; and Group 3: 17% EDTA. All the roots were longitudinally split into two halves and examined under SEM to assess the remaining smear. A statistical analysis was performed using the Kruskal-Wallis test.

**Results:** In the overall evaluation, the remaining smear was significantly less for group 1 as compared to the other groups ( $p < 0.05$ ). In groups 2 and 3, the remaining smear significantly increased in the apical one-thirds, while in Group 1, the remaining smear was significantly less than the apical one-thirds of the other groups ( $p < 0.05$ ).

**Conclusion:** Photon-initiated photoacoustic streaming combined with 0.2% chitosan improved the removal of smear layers.

**Keywords:** Photon-initiated photoacoustic streaming, chitosan, ethylene diamine tetraacetic acid, scanning electron microscopy, smear layer

## INTRODUCTION

The removal of smear layers during root canals is strongly recommended due to the presence of bacteria and tissue remnants in these layers (1, 2). The smear layer also reduces the penetration of irrigants, medicaments, and sealers into dentinal tubules (3, 4). There are various chemicals, including ethylene diamine tetraacetic acid (EDTA), citric acid, acetic acid, and, more recently, chitosan, that have been used to remove smear layers (5, 6). Furthermore, laser systems such as erbium-doped yttrium aluminum garnet (Er:YAG) and erbium, chromium-doped yttrium, scandium, gallium, and garnet laser (Er,Cr:YSGG) lasers are other contemporary options for the removal of smear layers.

Studies involving this subject have revealed that cleaning of the apical third is more challenging as compared to the other portions of root canals, and residual smear scores following treatment protocols increase from the coronal part toward the apical region (2, 7, 8). In order to increase the effect of irrigation solutions, particularly in the apical region and isthmuses, different systems including ultrasonic activation (2, 5), erbium-family lasers (8, 9), and photon-initiated photoacoustic streaming (PIPS)

(10) have been employed. A PIPS system comprises an Er:YAG laser equipped with a specially designed tip. In this technique, the chelators and irrigants are agitated in the root canals, which enhances the antibacterial and/or chelation potential of these solutions (11).

Chitosan is obtained from the shells of shellfish (12). It is biocompatible and it has low cost and no toxicity (6, 13). Its superiority in terms of smear removal (6) and its antibacterial effects (14, 15) have been proven in earlier studies. Furthermore, chitosan improves the effects of different medicaments, pastes, and chemicals (15-17). However, in literature, information regarding the combined use of chitosan and PIPS is insufficient. Therefore, the present study aims to compare the smear removal potential of chitosan in the presence and absence of PIPS and to compare these results with EDTA. In addition, we also aimed to determine whether the PIPS technique can improve the smear removing potential of chitosan, particularly in the apical one-third of the root canals. The null hypothesis reveals that there is no difference among EDTA, chitosan, and chitosan + PIPS in both general and partial (coronal, middle apical) evaluations.

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## METHODS

### Sample Preparation

A total of 45 sound human mandibular premolars chosen from a collection of teeth that had been extracted for periodontal or orthodontic reasons were included in the present study with the approval of the Ethics Committee Commission of Gaziantep University dated/numbered 12.01.2015/2015-3. This study is an ex vivo study and does not include human participants. Thus, no consent form was required. The teeth were randomly collected following the tooth extraction procedure of a patient who attended the clinic at the Gaziantep University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery. The study samples were radiographically controlled as they had single straight canals. The teeth with any abnormalities, such as immature apexes, calcification, cracks, or resorption, were not included in this study. The teeth were stored in 5.25% NaOCl solution for disinfection. Following the cleaning of the root surfaces of any soft tissue remnants with ultrasonic scalers, the teeth were stored in distilled water at 4°C until used. The crowns of the teeth were separated with a water-cooled diamond fissure bur, and a 13-mm root was obtained.

### Root Canal Instrumentation Procedures

Working length assignment was attained by inserting a size 15 K-file until it was visible at the apex and subtracting 1 mm from this working length. Then, the root canals of the teeth were prepared with 0.06-tapered, size-25 OneShape files (Micro-Mega, Besançon, France) mounted onto an endodontic motor (VDW Reciproc, Munich, Germany) using the settings for OneShape recommended by the manufacturer (400 rpm; 2.5 N-cm). During the instrumentation process, the root canals were irrigated with 1% NaOCl: when pressure was detected, the progress of the file was stopped. The instrumentation process continued until the working length was achieved. After the completion of the canal preparation procedures, the canals were irrigated again with 1% NaOCl and dried using absorbent paper points.

### Final Rinsing Protocols

The samples were randomly divided into 3 study groups, each including 15 teeth according to the final irrigation protocol.

Group 1: Chitosan solution was prepared as follows: 0.2 mg chitosan powder (Aldrich Chemistry, St. Louis, USA) was added into 100 mL 1% acetic acid and mixed under magnetic stirring at room temperature for 2 h. An Er:YAG laser with a wavelength of 2940 nm (AT Fidelis, Fotona, Ljubljana, Slovenia) was used with a special quartz tip used for PIPS (14 mm long, 300 µm diameter) (Preciso

300/14, Fotona, Ljubljana, Slovenia). The parameters of the Er:YAG laser were set as follows: 1 W, 20 Hz, and 50 mj per pulse. The water and air systems were turned off. The laser tip was placed into the canal orifice and an irrigation needle was inserted into the root canal, positioned superior to the laser tip. In the course of the irrigation process, the laser was activated for 5 s. Thus, simultaneous irrigation with chitosan solution and irradiation with PIPS was performed. This application was repeated 5 times following a 5-s break for each cycle. Therefore, simultaneous irradiation and irrigation was applied for a total of 25 s. The canals were rinsed with distilled water and dried with a paper point.

Group 2: Each root canal was slightly rinsed with 5 mL chitosan solution for 3 min. The canals were irrigated with deionized water and dried with a paper point.

Group 3: Each root canal was rinsed with 5 mL 17% EDTA (Imicryl, Konya, Turkey) solution for 3 min. The canals were irrigated with deionized water and dried with a paper point.

### Sectioning and SEM Analyses

For the SEM analysis, all the roots were longitudinally separated into 2 halves (30 halves were obtained for each group). A diamond disk was used to prepare longitudinal grooves at the buccal and lingual sides of the roots: care was taken to not penetrate into the canals. The roots were split into two halves with a chisel. All the root halves were overlaid with gold and examined under SEM (magnification: 2000×). The images were taken from the 2<sup>nd</sup>, 7<sup>th</sup>, and 11<sup>th</sup> millimeter distances to the apices for the smear evaluations of the apical, middle, and coronal one-thirds, respectively (Figure 1). The smear amount was scored according to the criteria described by Hulsmann (18):

- 1: free of smear: all the dentin tubules are open.
- 2: small amount of smear: most of the dentin tubules are open.
- 3: nearly half the surface is coated with smear: half of the tubules are open.
- 4: large amount of smear: only a few tubules are open.
- 5: surface is totally coated with smear: no visible open tubule is present.

### Statistical Analysis

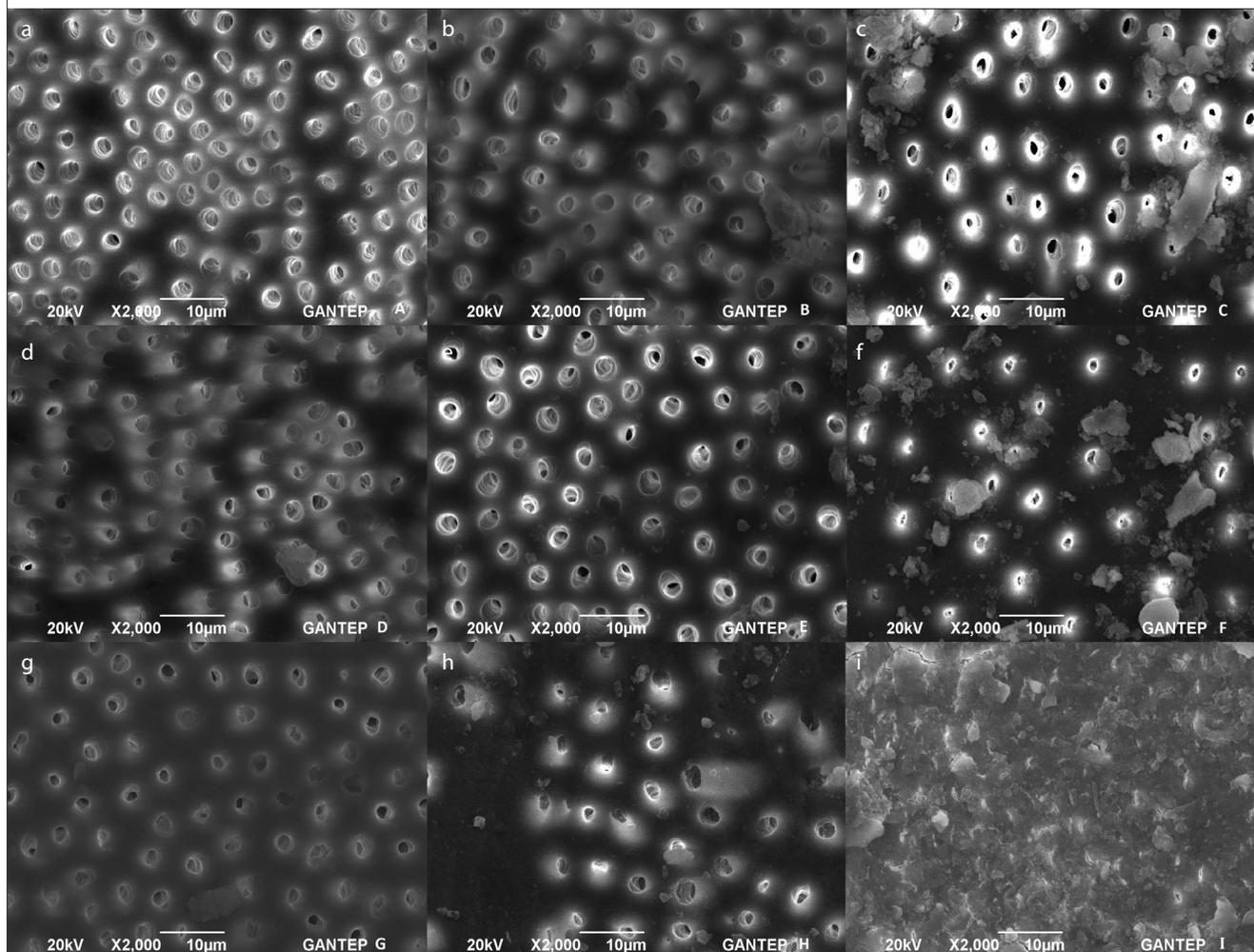
The smear scores for each group were recorded and statistically analyzed both in general and for the apical, middle, and coronal

**Table 1.** Mean smear score values for the groups and their standard deviations

|         | Coronal                  | Middle                   | Apical                   | Total                  |
|---------|--------------------------|--------------------------|--------------------------|------------------------|
| Group 1 | 1.80±0.67 <sup>A,a</sup> | 2.13±0.63 <sup>A,a</sup> | 2.47±0.74 <sup>A,a</sup> | 2.13±0.72 <sup>A</sup> |
| Group 2 | 1.93±0.70 <sup>A,a</sup> | 2.20±0.41 <sup>A,a</sup> | 4.33±0.48 <sup>B,b</sup> | 2.82±1.21 <sup>B</sup> |
| Group 3 | 2.00±0.75 <sup>A,a</sup> | 2.33±0.48 <sup>A,a</sup> | 4.40±0.50 <sup>B,b</sup> | 2.91±1.22 <sup>B</sup> |

<sup>A, B</sup>: Different uppercase letters (column) represent the statistically different groups. <sup>a, b</sup>: Different lowercase letters (row) represent the statistically different groups

Figure 1. a-i. SEM images of the specimens (2000×). Surfaces: (a-c) Group 1: coronal, middle, and apical, respectively. (d-f) Group 2: coronal, middle, and apical, respectively. (g-i) Group 3: coronal, middle, and apical, respectively



one-thirds. The Kruskal-Wallis test was used to perform statistical analysis and the significance was set to 0.05.

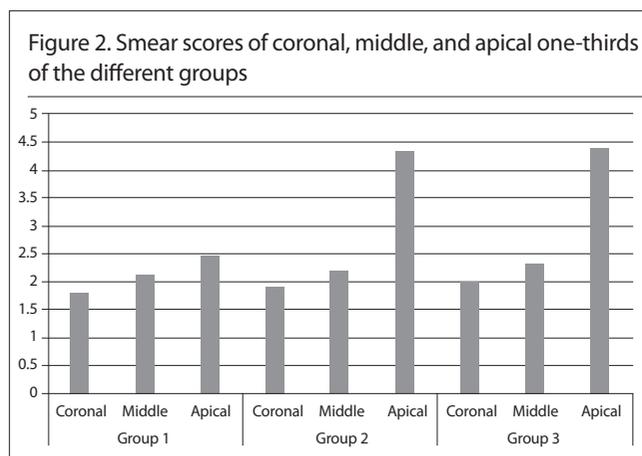
## RESULTS

The mean smear scores and standard deviations (SD) are listed in Table 1 and shown in Figure 2. In the overall evaluation, group 1 (chitosan + PIPS) revealed significantly less smear scores as compared to the other groups ( $p < 0.05$ ). Groups 2 and 3 were statistically similar ( $p > 0.05$ ). In the partial evaluations, the smear scores significantly increased in the apical one-thirds as compared to the middle and coronal one-thirds ( $p < 0.05$ ), except for group 1. The apical one-third of group 1 exhibited a similar amount of smear as compared to the middle and coronal parts ( $p > 0.05$ ) and lesser amount of smear as compared to the apical one-thirds of groups 2 and 3 ( $p < 0.05$ ).

## DISCUSSION

The findings did not conform to the first null hypothesis in which the smear removal efficiencies were similar among the different irrigation procedures. The presence of a smear layer in root canals may be considered as a cause of failure because it harbors

bacteria and tissue remnants (2) and also reduces the penetration of irrigants, medicaments, and sealers into the dentinal tubules (3, 4). Furthermore, the penetration of resin cements used with fiber posts is interrupted by the presence of smear (5). EDTA is a widely used chemical to remove smear layers by chelating the inorganic component of the root dentin (19), while chitosan is a contemporary material used for this purpose. The optimum concentration and duration for chitosan was reported as 0.2% and 3 min, respectively, by Silva et al. (20). Furthermore, its microhardness-reducing effect—a disadvantage of chelators—is found to be not more than that of EDTA, as discussed by Pimenta et al. (21). For these reasons, 0.2% chitosan was used for 3 min in group 2. Our results are in good agreement with those of Silva et al. (6). They found that EDTA and chitosan have similar smear-removing capacities. Different from EDTA (which is not natural and is considered as a pollutant (22)), chitosan is a natural product. It is biocompatible and has no toxicity (23). It has a low cost and has chelating capacity toward various metallic ions (12). In the study of Silva et al. (6), 0.2% chitosan prepared by the mixing of chitosan powder with 1% acetic acid, as in the present study, was found to be superior to using only 1% acetic acid. Hence, they



concluded that the chelating properties of chitosan should be attributed to chitosan rather than acetic acid. These advantageous properties make chitosan a suitable substitute for EDTA. However, it should be noted that the amount of smear increases from the coronal one-third toward the apical one-third, regardless of the chelator, as stated in the studies of Schmidt et al. (2) and Srirekha et al. (5). This is in accordance with the results of the present study for groups 2 and 3. In these groups, the amount of smear in the apical one-third was significantly higher than the coronal and middle one-thirds. These results are possibly related to the inability of EDTA and chitosan solutions to perfectly reach and affect the apical root dentin.

In order to increase the effects of irrigants and chelators, particularly in the apical portion, laser systems have been used in recent years (7). Guidotti et al. (8) revealed that Er:YAG laser irradiation with EDTA is effective in terms of smear removal even in the apical one-third. This is also verified by the study of Murugesan et al. (9), where it was found that an Er,Cr:YSGG laser increased the smear-removing capacity of EDTA in curved canals. One of these systems is PIPS, which constitutes an Er:YAG laser equipped with a special, radial tip. In the study of DiVito et al. (24), the smear-removing capacity of EDTA increased when used in combination with PIPS. The results of the present study revealed that a chitosan solution could remove smear similar to EDTA in 3 min. However, both these solutions remained inadequate for reaching the apical one-third. When used in combination with PIPS, chitosan effectively removed smear, particularly in the apical one-third. Thus, the null hypothesis was rejected. Olivi and DiVito (11) reported that PIPS strongly agitates the intra-canal irrigants and generates faster streaming of these fluids distant to the source. This mechanism explains why the apical one-third of group 1 yielded significantly less smear scores as compared to the apical parts of the other groups.

In this study, the root canals were prepared to an apical size of 25 with a 0.06 tapered instrument. It is noteworthy that preparing root canals to larger diameters may overcome the limitation of solutions by facilitating solutions to reach more areas. The disparity in the results of the present study and the study of Silva et al. (6) who found chitosan effective in both the middle and apical one-thirds may be related to the larger apical preparation size in

that study. However, in the PIPS group of the present study, the smear layer in the apical portion was also effectively removed. Hence, it can be concluded that the streaming effect of PIPS mentioned above does not depend on the size of the preparation.

Teixeira et al. (25) and Lui et al. (26) found that EDTA is more capable of removing the smear layer in cases of its use in combination with 1% NaOCl. For this reason, 1% NaOCl was used during canal preparation before final rinsing in the present study.

In the study of Akcay et al. (27), an EDTA solution was refreshed when the coronal reservoir decreased during irradiation with PIPS, while in the study of DiVito et al. (24), continuous irrigation was simultaneously performed with PIPS irradiation. However, during PIPS irradiation, an accurate observation of the level of intra-canal fluids may not be possible. For this reason, we preferred to continuously irrigate the root canals during PIPS irradiation in order to maintain the irrigants at a constant level.

The present study is aimed to investigate the effectiveness of smear removal after different final rinsing protocols. Further studies are needed to examine the effect of different laser parameters and bond strength of resin cements.

## CONCLUSION

Within the limitations of the present study, the following can be advised:

- 1) Chitosan can be used as the final irrigant, instead of EDTA, to effectively remove the smear layer at a concentration of 0.2% for 3 min.
- 2) Using PIPS with chitosan enhances the effect of chitosan in terms of smear removal, particularly in the apical one-third and considerably reduces the time required to obtain the expected results related to chitosan.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: 12.01.2015, Number: 2015-3).

**Informed Consent:** Informed consent was not obtained from patients due to this study is an ex vivo study and does not include human participants.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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