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Original Research

# Effect of Agitation of Ethylenediaminetetraacetic Acid with Sonic and Photon-Initiated Photoacoustic Streaming Tecniques on Dentin Microhardness

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**ABSTRACT**
**Objective:** The aim of this study was to evaluate and compare the effect of the ethylenediaminetetraacetic acid (EDTA) agitation with a sonic system and the photon-initiated photoacoustic streaming (PIPS) technique on the microhardness of the root canal dentine.
**Methods:** A total of 24 extracted single-rooted human mandibular incisor teeth were collected. The root canals were instrumented according to the manufacturer's instructions using the reciprocating single-file system Reciproc. The teeth were decoronated, and the roots were longitudinally split into two halves. Following initial microhardness measurements of root canal dentine, the halves were connected, and the samples were divided into four groups, according the final irrigation protocol (Group 1, only distilled water; Group 2, EDTA; Group 3, EDTA+sonic agitation, Group 4: EDTA+PIPS agitation). Final microhardness values were calculated after the irrigation procedures. The Shapiro–Wilk test was used to evaluate the normal, or abnormal distribution of the values. For multi-comparison of the groups, one-way analysis of variance and post-hoc Tukey tests were used.
**Results:** The EDTA significantly reduced microhardness compared to the distilled water group ($p<0.001$), while the results of the sonic and PIPS activation groups were statistically similar to the EDTA alone group ($p=0.053$ and 0.266, respectively). No significant difference was found between the agitation groups ($p=0.853$).
**Conclusion:** The results of the present study revealed that neither sonic nor PIPS agitation resulted in further microhardness reduction.
**Keywords:** Dentin, ethylenediaminetetraacetic acid, laser, microhardness

## INTRODUCTION

The success of a root canal treatment depends on the removal of microorganisms and organic and inorganic tissue remnants from the root canal system. Particularly, the removal of a smear layer formed during canal preparation is important because of its microbial and necrotic tissue content (1). Different irrigation solutions have been employed for this purpose.

Ethylenediaminetetraacetic acid (EDTA) is a widely used chelating solution to remove the inorganic content of the smear layer, and it acts by decalcifying calcium ions from the root dentin and smear layer (2). However, this mechanism of action may cause alterations in the physical properties of dental hard tissues (3). Previous studies revealed that EDTA resulted in a significant reduction of root dentin micro hardness (3-5). Furthermore, the effectiveness of EDTA in the apical region during irrigation with conventional syringes is limited due to a decreased flow of the solution to this zone (1). To overcome this problem, sonic and ultrasonic devices and lasers have been used to increase the efficiency of intra-canal solutions by agitation of the solutions. In a previous study, it was demonstrated that the EDTA agitation with a diode laser may result in a further decrease of root dentin micro hardness (6). While sonic activation agitates the intra-canal fluids by using vibrating devices with a frequency of 2-3 khz (7), photon-initiated photoacoustic streaming (PIPS) is a novel technique that constitutes of an Er:YAG laser equipped with a specially designed tip. It agitates the intra-canal solutions by producing photoacoustic shock waves (8). Although it was stated that PIPS enhances the smear layer by removing the efficiency of intra-canal EDTA, to the best of our knowledge, no data are available regarding whether this technique may lead to further decrease in the micro hardness of root dentin.

Thus, the present study aimed to evaluate micro hardness changes in the root canal dentin after the agitation of EDTA with PIPS and sonic activation and to compare the results when EDTA is used alone without agitation.

## METHODS

The present study was approved by the Ethical Committee of İzmir Katip Çelebi University School of Medicine numbered/

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dated 157/08.22.2017. 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 due to periodontal, orthodontic, or prosthetic problem at the İzmir Katip Çelebi University School of Dentistry, Department of Oral and Maxillofacial Surgery. Twenty-four freshly extracted, human mandibular incisor teeth from patients of both sexes aged 40-50 years with single canals free of any cracks, fractures, hypoplastic defects, and resorption were included. After the removal of soft tissue remnants with a scaler, the teeth were kept in +4°C distilled water until they were used. Coronal access cavities were achieved conventionally with a high-speed round carbide bur (Diatech; Coltene Whaledent, Altstatten, Switzerland) under water cooling. The canal patency was established with a #15 K-file (Dentsply Maillefer, Ballaigues, France), and working length was determined by subtracting 1 mm from the point it was viewed at the apex. The root canal preparation was performed with Reciproc R25 (25.08) and R40 (40.06) (Reciproc; VDW, Munich, Germany) instruments according to the manufacturer's recommendation using VDW Silver Reciproc endodontic motor (VDW, Munich, Germany) with settings "Reciproc ALL" program, special to Reciproc files.

The teeth were decoronated from the cemento-enamel junction, and split longitudinally into two halves. In this manner, 48 samples were obtained. Each sample was embedded into acrylic block as the root canal surface is visible at the top. Initial microhardness measurements were performed throughout the mid-root region at a constant mark by using a Vickers indenter (HMV-G20 Series; Shimadzu Corp., Shiga, Japan) using a 50 gr load for 15 seconds following surface polishing with 500, 800, 1000, and 1200 grit abrasive papers with a grinding machine (Microtest grinder-polisher; Microtest Ltd., İstanbul, Turkey). The average of three measurements was accepted as the microhardness value and recorded.

After initial microhardness measurements, the two halves of each tooth were reconnected with boxing wax to perform the intra-canal procedures. All teeth were then divided into four groups according to the irrigation protocol:

Group 1 (negative control; n=3 teeth; 6 halves): Each canal was rinsed with 5 ml distilled water.

Group 2 (n=7 teeth; 14 halves): Each canal was rinsed with 5 mL 17% EDTA for 60 seconds, 5 mL 5% NaOCl for 60 seconds, and 5 ml distilled water, respectively.

Group 3 (n=7 teeth; 14 halves): Each canal was rinsed in 2.5 mL 17% EDTA for 15 seconds followed by 15 seconds of sonic activation with activator (EndoActivator; Dentsply Tulsa, OK, USA) equipped with the blue tip (30/0.6) at a distance 2 mm short of the working length. Its frequency was 10,000 cycles (in-and-out motion) per minute. This was repeated 2 times, and by this way, an irrigation with 5 mL 17% EDTA for 60 seconds (30 seconds irrigation + 30 seconds sonic activation) was achieved. Following 17% EDTA, 5 mL of 5% NaOCl irrigation for 60 seconds and irrigation with 5 mL distilled water was also performed like in other groups.

Group 4 (n=7 teeth; 14 halves): Each canal was rinsed with 1 mL 17% EDTA for 6 seconds, and intra-canal EDTA was agitated with a 12-mm-long, 300 nm quartz-stripped tip attached to the Er:YAG laser (Fidelis AT; Fotona, Ljubljana, Slovenia) (0.3 W, 15 Hz, and 20 mj) for a further 6 seconds. Following a 10-second break, the same procedure was repeated 5 times. In this way, the irrigation with 5 mL 17% EDTA for 60 seconds (30 seconds irrigation+30 seconds PIPS activation) was achieved. Again, 5 mL 5% NaOCl irrigation for 60 seconds, and irrigation with 5 mL of distilled water was performed as the final irrigation.

Irrigations was performed by using a 30-gage double-side vented, close-ended irrigation needle (C-K Endo Needles; C-K Dental, Korea) for all groups. Following irrigation, the teeth were re-split, and the last indentations were performed at the same previous marks. The differences between the initial and the last indentations were recorded as the change in the microhardness value. The mean changes in microhardness as percentage were calculated and recorded for each specimen.

### Statistical Analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 20.0 (SPSS IBM Corp.; Armonk, NY, USA). The standard descriptive methods such as the mean, standard deviation, median, frequency, and minimum and maximum were applied to determine the characteristics of the sample. The Shapiro-Wilk test was used to evaluate the normal or abnormal distribution of the values. For multi-comparison of the groups, one-way analysis of variance and post-hoc Tukey tests were used. The significance was set to <0.05.

## RESULTS

The mean difference in microhardness and standard deviations are presented in Table 1. The negative control group (distilled water) revealed a significantly smaller microhardness change compared to the other groups (p<0.001). Group 2 (EDTA), Group 3 (EDTA with sonic activation), and Group 4 (EDTA with PIPS activation) had statistically similar results (p>0.05).

**Table 1.** Mean values of microhardness change and their standard deviations (SD) shown as percentage

| Groups | N | Change in Microhardness (%)±SD |
|---|---|---|
| Group 1 | 6 | 4.04±3.31[a] |
| Group 2 | 14 | 16.70±4.60[b] |
| Group 3 | 14 | 22.88±7.90[b] |
| Group 4 | 14 | 21.01±6.41[b] |

Different lowercase letters represent the statistically different groups

## DISCUSSION

According to the results of the present study, EDTA resulted in a significant decrease in the microhardness of root dentin in accordance with the results of previous studies (4, 5). However, neither sonic nor PIPS agitation resulted in a further decrease in microhardness compared to the not agitated EDTA irrigation. The rationale for the use of these irrigation agitation protocols is to increase their effectiveness particularly in the apical region. The EndoActivator is a sonic activation device. It acts by agitating intra-canal fluids with an in-and-out motion, and in this way, it generates a hydrodynamic activation (7, 9). This synergistic effect provides more penetration of irrigation solution in the root canal system. PIPS is another irrigating agitation technique that uses laser energy to generate photoacoustic shock waves, and in this way, it increases the efficiency of intra-canal irrigants. Its most prominent advantage compared to other agitation techniques is its ability to act by placing the tip only in the coronal part of root canals (10), while the sonic activation requires the advancement of the tip closer to the apex. However, according to our results, the effect of both agitation techniques in terms of a further decrease in microhardness compared to EDTA alone is slight and insignificant. We assume that the agitation of EDTA only enhances the penetration capacity and flow features of solution, and it cannot alter the demineralization mechanism or capacity of the solution. However, slightly more decreased microhardness values of dentin in the agitation groups than EDTA alone may have been due to an increased surface contact of the EDTA solution with dentin in agitation applications. This is in accordance with the study by Arslan et al. (6) who found that the ultrasonic activation of EDTA did not cause an additional decrease in microhardness. We assume that similarity among the groups (except the control group) of the present study is related to the same application interval of EDTA (60 seconds for each group), regardless of the activation protocol. In a previous study, it was stated that the EDTA exposure time had a significant effect on dentin microhardness and in turn, fracture resistance (11). This is related with the organic-inorganic composition of dentin. Particularly, the calcium-to-phosphorus ratio is a critical determinant for the psychical properties of dentin, which is affected from the contact time of EDTA with dentin (12). In the study by Gurbuz et al. (13), it was demonstrated that laser irridation reduces this ratio in dentine. However, in the present study, similar durations of EDTA application may have resulted in similar calcium absorption and thus similar microhardness changes. In other words, it can be stated that the effect of the sonic activation and PIPS is to increase the penetration of EDTA in the root canal system, not to aggravate its mechanism of action. On the other hand, the study by Arslan et al. (6) revealed that the agitation of EDTA with a 2 watt diode laser resulted in a significant decrease when the duration of laser application reached 40 seconds, while shorter durations (10, 20, and 30 seconds) did not resulted in a significant decrease compared to EDTA alone. They correlated their results to the morphological and chemical changes of dentin, as stated by Al-Omari and Palamara (14), who reported that the laser irridation vaporized the organic matrix of dentin, and such a disintegration causes more reduction in microhardness because collagen constitutes nearly one-fifth of dentin and provides resistance to dentine by reducing stress (15). This may explain why PIPS technique did not resulted in further decrease in the present study because the effect of laser in the PIPS technique is obtained by its effect on the intra-canal irrigation solution rather than directly on dentin. Thus, its effect on dentin may have remained limited. To the best of our knowledge, no previous studies evaluated the effects of the PIPS technique on the microhardness reduction. Our results showed that the PIPS technique did not result in a further microhardness decrease. Furthermore, those previous studies showed that microhardness was significantly reduced following laser irridation with an output power ranging between 2 W and 4.5 W during a time interval ranging from 40 seconds to 2 minutes, which may indicate that both the output power and duration may affect microhardness (6, 14). Al-Omari and Palamara (14) stated that the reduction in microhardness is directly proportional to the output power. This may be another explanation why our PIPS groups did not significantly reduce microhardness because it was used with a lesser output power (0.3 W) for lesser duration (30 seconds).

As in the PIPS group, the sonic activation of EDTA did not result in a further microhardness decrease. This is presumably related to its mechanism of action, which is only mechanical. It agitates intra-canal fluids and thus provides more penetration of irrigation solutions. Although it enhances the efficiency of EDTA (16), it does not cause any alteration in the chemical composition of root dentine due to its limited mechanical effect. That is why the sonic activation group did not show a significant microhardness reduction compared to the EDTA alone group.

## CONCLUSION

In the present study, EDTA decreased the microhardness of root dentine in accordance with the previous studies. Sonic and PIPS agitation techniques did not result in further reduction in microhardness. Further studies may be beneficial to better clarify this issue.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of İzmir Katip Çelebi University School of Medicine (numbered/dated 157/08.22.2017).

**Informed Consent:** This study is an ex vivo study and does not include human participants. Thus, no consent form was required.

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