

Original Article

The effect of *Morinda Citrifolia* juice as an endodontic irrigant on smear layer and microhardness of root canal dentin

Mohammad Ali Saghiri^{a,*}, Franklin García-Godoy^b, Kamal Asgar^{c,1}, Mehrdad Lotfi^d

^a Department of Dental Material, Dental Branch, Craniofacial Research Center, Islamic Azad University, Tehran, Iran

^b College of Dentistry, University of Tennessee, Memphis, TN, USA

^c Department of Dental and biological materials, School of dentistry, university of Michigan, MI, USA

^d Research Center for Pharmaceutical Nanotechnology and Department of Endodontics, Dental Faculty, Tabriz University (Medical Sciences), Tabriz, Iran

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ABSTRACT

Purpose: The purpose of this study was to evaluate the effect of *Morinda Citrifolia* Juice (MCJ) on smear layer removal and microhardness value of root canal dentin in compared with various endodontic irrigants.

Material and methods: Eighty-four single-rooted human teeth were prepared to apical size of #35. Since decoronation, samples were divided into seven groups of 12 in each ($n = 12$). Specimens were finally irrigated by either 1: 2.5% NaOCl, 2: 6% MCJ, followed by a final flush of 17% ethylene diaminetetraacetic acid (EDTA), 3: 6% MCJ, 4: 2.5% NaOCl then 17% EDTA, 5: MTAD, 6: 2% chlorhexidine (CHX), and 7: saline. After irrigation, all samples were subjected to Vickers microhardness test at 100 and 500- μm depths and then were examined under scanning electron microscopy (SEM) and ImageJ program was used to calculate open dentinal tubules. One way ANOVA and post hoc Tukey tests were used to reveal any significant differences among and between groups respectively.

Results: The microhardness values at 100 μm and 500 μm for MTAD were significantly lower than for NaOCl + EDTA and MCJ + EDTA groups ($p < 0.05$). MCJ + EDTA, NaOCl + EDTA, and MTAD protocol significantly removed smear layer in compared with control group ($p < 0.05$), with no significant differences among these three groups.

Conclusions: It was concluded that 6% MCJ followed by a final flush of 17% EDTA can be regarded as an effective solution on smear layer removal without any adverse influence on microhardness property of root canal dentin.

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1. Introduction

Successful endodontic therapy needs shaping and cleaning of root canal systems. Smear layer is produced during root canal preparation by the manipulation of the dental canal walls. It is believed that the presence of smear layer contributes to leakage, and it is a source of nutrients for microorganisms [1]. Therefore, elimination of smear layer is an important part of endodontic therapy. Several chemicals and therapeutic agents are used to achieve this goal. The most effective among them are ethylenediamine tetra-acetic acid (EDTA), and mixture of tetracycline, acid, and detergent (MTAD). NaOCl is a broad spectrum antimicrobial agent especially against *Enterococcus faecalis* [2]. NaOCl can dissolve the pulp and the organic phase of smear layer [3]. EDTA is a chelating

agent which has been suggested to remove the inorganic matter of the smear layer [4]. However, the antimicrobial efficacy of EDTA is relatively limited [5]. For effective removal of both organic and inorganic components of the smear layer, it is recommended to use 2.5–6% NaOCl during root canal therapy followed by 17% EDTA [6,7].

An alternative endodontic irrigant containing 3% doxycycline, 4.25% citric acid and 0.5% polysorbate 80 detergent [8] is being commercialized as Biopure[®] MTAD (Dentsply Tulsa Dental, Tulsa, OK). This irrigant is recommended to be used as the final rinse for 5 min after initial rinse with 1.3% NaOCl for 20 min [8]. It has been claimed that MTAD can remove the smear layer efficiently according to the protocol mentioned [9].

CHX is a bis-biguanide with amphiphatic and antiseptic properties [10]. CHX at 2% concentration has been used more recently because it has an affinity to dental hard tissues, which causes its prolonged antimicrobial activity, a phenomenon called substantivity [11]. Moreover, it cannot dissolve the smear layer completely [7,12] and it can discolor teeth [13].

* Corresponding author.

E-mail address: Saghiri@gmail.com (M.A. Saghiri).

¹ Deceased.

A few natural products such as propolis, ArctiumLappa, Triphala, green tea, and Morinda Citrifolia juice (MCJ) have been used as an alternative to help chemomechanical preparation of the root canals [14]. MCJ is commonly known as great morinda, Indian mulberry, nunaakai (Tamil Nadu, India), dog dumpling (Barbados), mengkudu (Indonesia and Malaysia), Kumudu (Balinese), pace (Javanese), beach mulberry, and cheese fruit. The literature has shown that MCJ has antimicrobial and therapeutic effects [14,15] suggesting its potential to be used as an endodontic irrigant. MCJ has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor [16], anthelmintic, analgesic, hypotensive [15] anti-inflammatory, and immune-enhancing effects [17]. MCJ contains the antibacterial compounds L-asperuloside and alizarin [15]. An investigation confirmed some properties of MCJ such as antibacterial effect and removal smear layer allowing the use of MCJ as root canal irrigant [18].

Some investigations have acclaimed that canal irrigants are capable of altering the chemical composition of human dentin and changing the calcium/phosphorus (Ca/P) ratio of the dentin surface [19,20]. Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues [21]. Previous studies have indicated that in all concentrations NaOCl alters the dentin microhardness negatively [22–24]. Previous studies have also confirmed the reduction of microhardness after irrigation with 17% EDTA as a result of its excessive demineralizing effect [22–24]. Murray et al. [18] showed that using 6% MCJ with a flush of 17% EDTA had good antibacterial and smear layer removal properties. Several studies have evaluated the effect of canal irrigants on the dentin microhardness [23–26] and also their capabilities in removing the smear layer [6,7]; a search of the endodontic literature showed the absence of any reports comparing the effect of MCJ on the microhardness of root canal dentin with other canal irrigants. Therefore, the aim of this study was to assess the relationship of smear layer removal and dentin microhardness after irrigating with 6% MCJ with a flush of 17% EDTA in comparison with commonly used canal irrigants.

2. Methods and materials

The protocol of teeth current study was approved by the Research Ethics Committee of Kamal Asgar Research Center (protocol No. KARC/14B2010-74-32).

2.1. Teeth preparation

This study was similar to those carried out by Saghiri et al. [20]. In brief, eighty-four freshly extracted single-canal human mandibular premolar teeth with mature apices and minimum curvature ($<5^\circ$) were selected from patients of both sexes of 20–40 years of age for this study. The degree of canal curvature was determined using the Schneider's method [27]. The selection of teeth was based on their relative dimensions and similarity in morphology and lengths. The teeth were examined to eliminate the roots with any cracks or defects, had not been stored in antibacterial or fixative solutions, and had not received any root canal medicaments. Teeth with root lengths between 12 and 16 mm were included in this study. Debris, calculus, and soft tissue remnants on the root surfaces were cleaned using a Gracey's curettes and a sharp scalpel. All teeth were immediately stored in 0.5% chloramine T solution for 1 week and thereafter stored in distilled water until utilization. After access cavity preparation, the pulp tissues were removed with a barbed broach (Dentsply, Maillefer, Switzerland), and the size of the apical foramen was gauged with a #15 K-file (Dentsply, Maillefer, Switzerland). The working length was determined by measuring the length of the initial file (#15) at the apical foramen minus 1 mm.

The apical part of the roots was put inside green stick compound during instrumentation. The canals of all the teeth were prepared up to file #35. Each instrument was used only in three canals and then was replaced by a new one. Instrumentation was performed by using RaCe rotary instruments (FKG; Dentaire, La-Chaux-de-Fonds, Switzerland). These instruments were set into rotational speed (500 rpm) with an eight:one reduction handpiece powered by a torque limited electric motor (TCM Motor 3000; Novage, Konstanz, Germany). Instrumentation was completed using the crown-down technique according to the manufacturer's instructions. The preparation sequence was as follows: 0.1 tapered #40, 0.08 tapered #35, 0.06 tapered #30, 0.04 tapered #25, 0.04 tapered #30, 0.06 tapered #30, and 0.06 tapered #35 were used to one third, one half, two third, and the rest to the full working length respectively. Saline solution was used as an intracanal irrigant during instrumentation. This procedure followed by a final flush with 5 mL of saline solution. After that, a 4 mm-thick slice was obtained from the mid root region. The slices were sectioned horizontally using a low speed saw (Isomet; Buehler Ltd., Lake Bluff, IL, USA) with a diamond disc, under continuous water irrigation in order to prevent overheating. Vicker's microhardness test requires a flat and smooth surface under examination. Therefore, a standard metallographic procedure was employed, involving grinding and polishing containing ascending grades of abrasive papers (500, 800, 1000, and 1200 grit) under constant water irrigation to reduce adverse effects on the dentin structure and further polished with fine alumina suspension (0.1 μm) to remove any surface scratches. The canal of each section was obstructed with an adhesive wax at the lower surface of the slice to prevent any exposure of irrigants to the lower surface of slices.

2.2. Final irrigations

At this point, all specimens were randomly divided into 7 groups ($n = 12$) according to the irrigants used. The root canal of each group was filled with the following endodontic irrigants and refreshed every 1 min:

- Group 1: 2.5% NaOCl for a total of 10 min
- Group 2: 6% MCJ (Tahitian Noni International Inc, Orem, UT) for 10 min with a final flush of 17% EDTA for 1 min (Pulpdent Corp., Watertown, MA, USA).
- Group 3: 6% MCJ (Tahitian Noni International Inc, Orem, UT) for 10 min
- Group 4: 2.5% NaOCl for 10 min followed by 1 min of 17% EDTA (Pulpdent Corp., Watertown, MA, USA)
- Group 5: MTAD (DENTSPLY Tulsa Dental, Tulsa, OK) according to the clinical protocol, 20 min of 1.3% NaOCl followed by 5 min of MTAD
- Group 6: 2% CHX (Consepsis[®], Ultradent Products, USA) for a total of 5 min
- Group 7: Saline solution for a total of 5 min (Control group)

Distilled water was used between first and second irrigant in group 2:(MCJ+EDTA), 4:(NaOCl+EDTA) to minimize the potential interaction between irrigants. In groups 1–4, the specimens received a final flush of 10-mL distilled water immediately after the treatment, to avoid the prolonged effect of solutions. Group 5 was not rinsed with distilled water according to manufacturer's instructions [28].

2.3. Microhardness measurement

Each specimen was numbered. Prior to application of test solutions, the Vicker's hardness values of the specimens were measured in lower surfaces of slices after irrigation on a MicroMet[®] 5100

microhardness tester (Buehler Ltd., Lake Bluff, IL, USA). In each sample, 3 separate indentations were made at 100 μm and 500 μm away from the root canal spaces. The indentations were carefully observed in an optical microscope, and the average length of their two diagonals was used to calculate the microhardness value (MHV). The representative hardness value for each distance of the samples was obtained as the average of the results for the three indentations. Mean \pm standard deviations of values were evaluated for each group. One-way ANOVA and post hoc Tukey's test were used to evaluate the significant differences among and between groups at 95% level of confidence respectively.

2.4. Smear layer evaluation

After microhardness test, each specimen split into two parts by using custom made Picker/Puncher and prepared into two parts to observe the root canal wall. One half of each sample was randomly chosen, placed in 2% glutaraldehyde for 24 h and then rinsed 3 times with a sodium cacodylate buffered solution (0.1M, pH 7.2). After incubation in osmium tetroxide for 1 h, the samples were dehydrated with ascending concentrations of ethyl alcohol (30–100%), placed in a desiccator for 24 h and mounted on a metallic stub. After coating the samples with 10 nanometer of gold, scanning electron microscopy (SEM) equipped with secondary electron detector (SE) (XL30, Philips, The Netherlands) photomicrographs were taken and analyzed at $\times 2000$ magnifications. All analyses were carried out at 20 kV. Digital images were recorded using a Microsoft picture manager (Redmond, WA) to standardize each picture at 480×666 pixels. Then, open dentinal tubules were calculated with ImageJ program (Rasband WS, ImageJ; US National Institute of Health, Bethesda, MD) (Fig. 2). Each figure was inverted (Fig. 2A) by this program and brightness was adjusted to select the throats of each tubule (Fig. 2B); binary was made for considering the throat of the tubules as a circle and to calculate the total number of circles in each micrograph (Fig. 2E and F). The data were analyzed by one-way analysis of variance and a post hoc Tukey's test at a significance level of $p < 0.05$.

3. Results

3.1. Microhardness

The mean \pm standard deviation of microhardness value at 100 and 500 μm are shown in (Fig. 1B and C). At 100 μm , ANOVA test showed significant differences among the groups ($p < 0.001$). Tukey's test revealed significant differences between the MTAD Protocol and the other groups ($p < 0.05$). However, there was no significant difference between the NaOCl+EDTA and MCJ+EDTA groups. In other words, MTAD significantly reduced microhardness more than the other irrigants tested at the 100 μm level (Fig. 1B). At the 500 μm depth, ANOVA test showed significant differences among the groups ($p < 0.001$). Tukey's test revealed significant differences between the MTAD Protocol and the other groups ($p < 0.05$). However, there was no significant difference between the NaOCl+EDTA and MCJ+EDTA groups. MTAD reduced microhardness of dentin significantly more than the other irrigants tested at 500 μm (Fig. 1C).

3.2. SEM analysis

The mean \pm standard deviations of open dentinal tubules were 64 ± 29 , 132 ± 52 , 31 ± 14 , 99 ± 27 , 118 ± 43 , 50 ± 24 , and 2 ± 3 for 2.5% NaOCl, 6% MCJ + 17% EDTA, 6% MCJ, 2.5% NaOCl + 17% EDTA, MTAD Protocol, 2% CHX, and saline respectively. There were significant differences among the groups ($p < 0.001$). Tukey's test did not

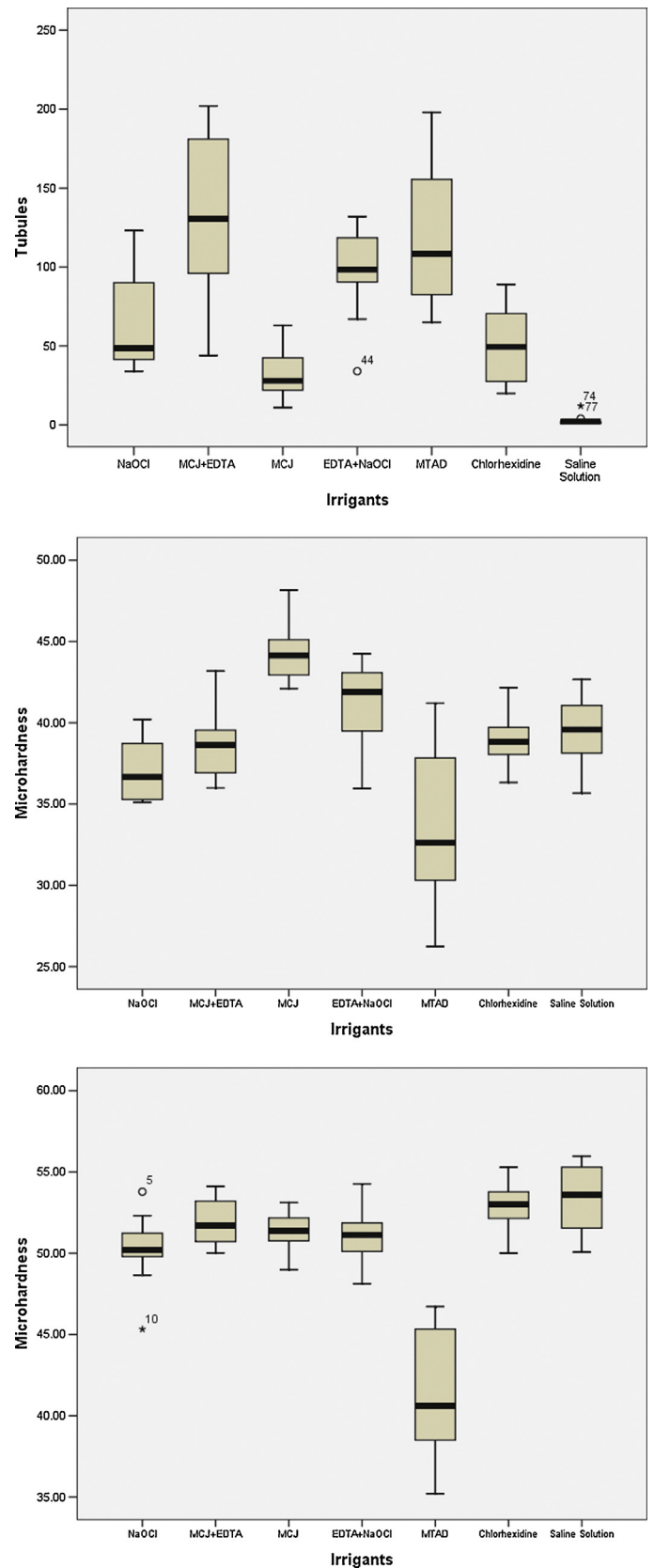


Fig. 1. (A) Box plot of the number of tubules in each group irrigated with 2.5% NaOCl, 6% MCJ + 17% EDTA, 6% MCJ, 2.5% NaOCl + 17% EDTA, MTAD Protocol, 2% CHX, and saline respectively, and Box plots of Vickers value of microhardness at 100 μm and 500 μm depths (B and C) in each group which illustrate the mean \pm standard deviation, minimum and maximum amount of data, as well as the variance in each experimental group.

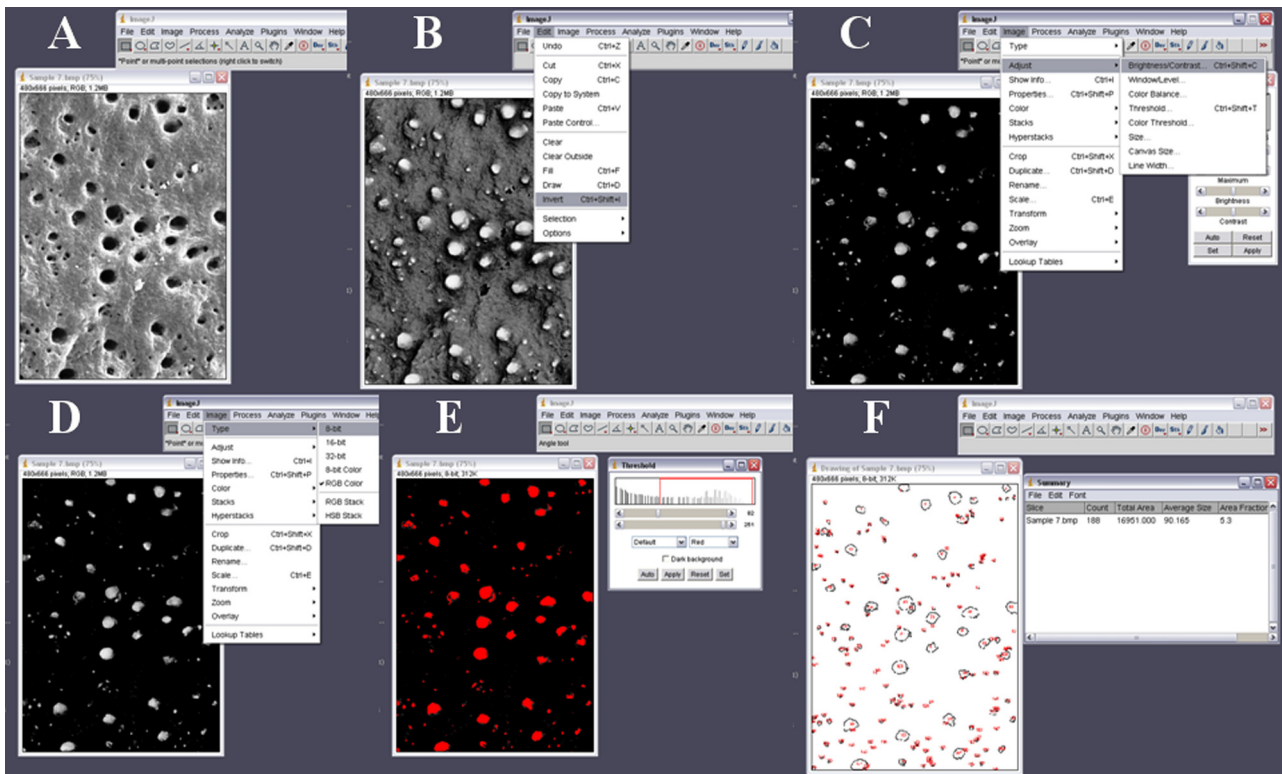


Fig. 2. Sequence of image analysis by ImageJ. (A) Micrograph of root canal dentin wall, (B) invert for feasible calculating. (C) Enhance contrast. (D) Convert to 8-bit type for better calculation. (E) Adjust threshold for better calculation. (F) Calculate the amount of circle or ellipses on the micrograph in regard to the scale.

demonstrate any significant difference among MCJ+ EDTA, MTAD protocol and NaOCl + EDTA groups ($p > 0.05$) (Fig. 1A).

4. Discussion

In this study, the effect of MCJ on microhardness and smear layer removal was demonstrated using Vickers microhardness test and combination of SEM and ImageJ processing software. Vickers microhardness test was used because previous studies have shown the suitability and practicability of the Vickers indenter method for evaluation of dentin microhardness and surface changes of root canal dentin treated with chemical agents [20,23,24,26]. One study reported that root canal irrigants could penetrate up to 130 μm into dentinal tubules [29]. Therefore, the current study measured microhardness in two depths of 100 and 500 μm to evaluate any existing effect of irrigants on microhardness of dentin. According to the suggestion of Ari et al. [22] and Eldeniz et al. [26] 300 g loads and 20-s dwell time were used at each measurement. Mid-root slices were used in this study to eliminate the effect of different numbers of dentinal tubules in coronal, middle, and apical portions of canal (Fig. 2).

EDTA was selected as final irrigation solution in combination of MCJ since a previous investigation [18] confirmed that the use of NaOCl as primary irrigation during instrumentation and final flush with EDTA is the "gold standard" to remove smear layer and because the purpose of this study was to substitute NaOCl with a safer irrigation solution with the same effect on smear layer and the least deteriorating effect on microhardness. Therefore, we selected MCJ for primary irrigant followed by a final flush of EDTA.

Based on the results of this investigation, CHX did not remove the smear layer completely and left most of dentinal tubules occluded after a 5-min application. This is in accordance with other studies showing that CHX did dissolve the pulp remnants and the organic component of the smear layer [7,12,20]. Saghiri et al. [20]

reported that the demineralization kinetic promoted by MTAD was significantly faster than routine irrigants including NaOCl, EDTA, and CHX. These findings are consistent with the current study. The effect of 2.5% NaOCl and 17% EDTA in removing the smear layer was prominent in this study which is consistent with previous studies [6,7]. The reduction of microhardness subsequent to using MTAD in the present study may be related to the fact that NaOCl dissolves the organic portion of the dentin and facilitates decalcification of the inorganic portion of the smear layer by MTAD [9]. Regarding the depths under evaluation, we achieved similar results as demonstrated by a previous study showing a reduction of dentin microhardness after irrigation with 2.6% NaOCl followed by 17% EDTA [30]. This study demonstrated that MTAD significantly decreased the dentin microhardness at both depths in comparison with other irrigants, which is consistent with the findings of Saghiri et al. [20] In the current study, MTAD and MCJ treated samples revealed the cleanest root dentinal walls with almost all dentinal tubules being opened. Moreover despite MCJ, MTAD protocol caused the most reduction of dentin microhardness at both depths among the experimental groups. MTAD can remove the inorganic portion of smear layer and decalcify dentin structure by means of its chelating components [4]. This deleterious effect on dentin microhardness can be due to its capability to decalcify dentin structure by means of its chelating components [8]. The 3% doxycycline hyclate component of MTAD is an isomer of tetracycline [4] which has a low pH and act as a calcium chelator causing root surface demineralization [31]. Moreover, MTAD consists of 4.25% citric acid [8] which is capable of dissolving the mineral contents of dentin [13]. De-Deus et al. [32] reported that the demineralization kinetic promoted by 5% citric acid was significantly faster than 17% EDTA; these findings are in agreement with the results obtained in this study.

According to the present study, MTAD and MCJ both removed smear layer, but MCJ had less effects on microhardness at both

depths evaluated. These findings probably can be due to the viscosity [33] flow [34] and pH [35] of MCJ. As expected, saline was the least effective irrigant for removing smear layer. Similar results were reported in previous studies [7,20].

5. Conclusion

MCJ as a primary irrigant and EDTA as a final flush, showed hopeful results in smear layer removal in compared with other commonly used endodontic irrigants. This regimen can be regarded as an effective solution for this purpose with lower reduction of microhardness value, which is a serious concern in case of other popular endodontic solutions.

Conflict of interest

We affirm that we have no financial affiliations or involvements with any commercial organizations with direct financial interests in the subject or materials discussed in this manuscript and deny any conflict of interests related to this study.

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