Comparative Evaluation of Effect of Irrigation Solutions with Various Exposure Time on Microhardness of Root Canal Dentin (In Vitro Study)

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ABSTRACT

Background: It is important to test the effect of the irrigating solutions on dentin, as they may come in contact during irrigation procedures. These irrigants cause alterations on dentin and enamel surfaces and affect their interactions with materials used for obturation and coronal restorations. The aim was to study the microhardness of root canal dentin after irrigation with different irrigant solutions for different periods.

Materials and Methods: Twenty five newly extracted non carious human permanent incisors were sectioned at cementoenamel junction and splitted longitudinally then divided into five groups; Gr1 (control) distilled water, G2: 5.25% sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (1 min), G3: 5.25% sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (5 min), G4: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (1 min) and G5: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (5 min). Vickers microhardness was evaluated.

Results: Data were analyzed using one-way ANOVA and paired t-test. The results indicated that all treatment time with 5.25% NaOCl and 17% EDTA decreased dentin microhardness significantly compared to distilled water (control). There were significant differences (P<0.001) between the tested groups with increasing time of exposure of irrigation solutions. Treatment with distilled water (control) showed significantly the highest microhardness value, while 5.25% sodium hypochlorite for 20 minute followed by 5 minutes (G5) with 17% EDTA showed significantly the least microhardness value followed by G4, G3 and G2.

Conclusions: Increasing irrigation time with both 5.25% sodium hypochlorite and 17% EDTA decreased dentin microhardness.

Key words: Sodium hypochlorite, EDTA, microhardness, exposure time.

تقييم مقارن لتاثير محاليل الغسل في فترات مختلفة على الصلادة الدقيقة لعاج قناة الجذر (دراسة مختبرية)

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المستخلص

من المهم اختبار تأثير محاليل الغسل على العاج، لأنها تأتي في تماس مع العاج أثناء عمليات الغسل. هذه المحاليل تسبب تغيرات في سطح العاج والمينا وتؤثر على تفاعلها مع المواد التي تستخدم في حشوات الجذور وحشوات التيجان. الهدف من هذا البحث هو دراسة الصلادة الدقيقة لعاج قناة الجذر بعد غمرها بمحاليل غسل مختلفة لفترات مختلفة.

٢٥ من القواطع الدائمية بدون تسوسات مقلوعة لاسباب التهابات في انسجة اللثة استخدمت في الدراسة الحالية. تم قطع التيجان وفصل الجذور الى جزئين بصورة عمودية وتقسيمها الى خمس مجاميع:

المجموعة الاولى (الضبط):ماء مقطر,

المجموعة الثانية:٥,٢٥٪ صوديوم هيبوكلوريت (١٠دقائق) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (دقيقة).

المجموعة الثالثة: ٥,٢٥٪ صوديوم هيبوكلوريت (١٠دقائق) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (٥ دقائق).

المجموعة الرابعة:٥,٢٥٪ صوديوم هيبوكلوريت (٢٠دقيقة) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (دقيقة).

المجموعة الخامسة:٥,٢٥٪ صوديوم هيبوكلوريت (٢٠دقيقة) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (٥ دقائق).عينت معدلات الصلادة الدقيقة لسطح العينات بواسطة جهاز قياس الصلادة .

بعد تحليل النتائج احصائيا ,اظهرت الدراسة ان جميع المحاليل المستخدمة وفي جميع الفترات سببت انخفاض كبير في صلادة العاج مقارنة مع الماء المقطر. المجموعة الاولى التي تم معاملتها مع الماء المقطر سجلت اعلى قيمة للصلادة بينما المجموعة الخامسة سجلت اقل قيمة تليها المجموعة الرابعة, المجموعة الثالثة والمجموعة الثانية. الاستنتاجات المستنبطة لهذه الدراسة تشير الى ان الانخفاض في الصلادة يزداد بزيادة وقت المعالجة مع هذه المحاليل.

INTRODUCTION

Mechanical instrumentation of the root canal produces a smear layer that covers the dentinal tubules. The smear layer is an amorphous irregular layer containing inorganic dentin debris as well as organic materials like pulp tissue, odontoblastic process, necrotic debris, microorganisms and their metabolic products [1].

There is a controversy over whether to remove or maintain the smear layer, but a recent systematic review and meta-analysis of leakage studies concluded that the removal of the smear layer improves the fluid tight seal of the root canal system. It also hinders the penetration of intracanal medications and sealers into dentinal tubules [2]. Effective cleaning of the canal system requires the use of irrigation solutions during instrumentation and irrigation, which serve variety of purposes including antibacterial action, tissue dissolution, cleaning and chelating [3].

The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl), because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue [4,5]. However, NaOCl solutions exert no effects on inorganic components of smear layer. Chelant and acid solutions have been recommended for removing the smear layer from instrumented root canals, including ethylene diaminetetraacetic acid (EDTA), citric acid and phosphoric acid [6,7]. Ethylene diaminetetraacetic acid (EDTA) is generally accepted as the most effective chelating agent in endodontic therapy. It is used to enlarge root canals, remove the smear layer and prepare the dentinal walls for a better adhesion of filling materials. The disodium salt of EDTA at 17% concentration and neutral pH is widely preferred for root canal treatment [8]. Root canal irrigation with the previously described solutions can lead to structural changes, as evidenced by the reduction of dentin strength, microhardness and changes in surface roughness [9,10]. **Baumgartner and Mader** [11] reported that when EDTA and NaOCl solutions were alternately applied to an uninstrumented root canal wall dentin showed an eroded appearance and tubular orifice diameters were enlarged. **Oliveira et al.** [12] reported that 1% NaOCl for 15 min decreased root dentin microhardness.The decalcifying effect of chelating agents depends largely on application time, solution pH and concentrations [13].

Although a reduction in microhardness facilitates the instrumentation throughout the root canal, it may also weaken the root structure [14]. Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues [15].The time of exposure to the irrigants is a factor that has gained little attention in endodontic studies. Even fast-acting biocides such as sodium hypochlorite require an adequate working time to reach their potential [16].Therefore, the purpose of this study was to examine the effect of irrigant solutions that applied for different time on microhardness of root canal dentin.

MATERIALS AND METHODS

Samples selection and preparation

Twenty five newly extracted non carious human permanent incisors extracted primarily for periodontal reasons were selected for this study. The teeth were stored in distilled water after the root surface was cleaned with curettes. Teeth are examined under 20x magnification in a microscope (Langenfeld, Germany), those having cracks were eliminated to prevent false results.The pulp of all selected teeth were removed using barbed broaches [17]. Then crowns were sectioned at the cementoenamel junction by using diamond disk under water cooling.The roots then sectioned longitudinally in the buccolingual direction to obtain root halves (n=50) using diamond disk under water coolant. Root specimens were horizontally embedded in autopolymerizing acrylic resin leaving the dentin surface exposed. The surface of each root half was polished with silicon carbide paper (500, 800 and 1000 grit) (Leco, St. Joseph, USA) under constant water coolant [17].The prepared samples divided into five experimental groups(n=10):

Group 1: Distilled water (control).

Group 2: 5.25% sodium hypochlorite (NaOCl) for (10 minutes) then 17% EDTA for (1 minute)

Group 3: 5.25% sodium hypochlorite (NaOCl) for (10 minutes) then 17% EDTA for (5 minutes)

Group 4: 5.25% sodium hypochlorite (NaOCl) for (20 minute) then 17% EDTA for (1 minute)

Group 5: 5.25% sodium hypochlorite (NaOCl) for (20 minute) then 17% EDTA for (5 minutes)

All specimens received a final flush with 10 ml distilled water immediately after treatment for the determined time to avoid the prolonged effect of chelating solution and dried with sterile paper point. The same procedure was carried out after treatment with NaOCl [17].

The specimens were mounted on stage of Vickers microhardness tester. The midroot portion is halfway from the outer surfaces was focused for testing. Indentations were made with Vickers diamond indenter using 300 gm load with a dwell time of 20 second. These indentations were measured and converted into Vickers hardness number (VHN) values by the monitor [18].

RESULTS

Vickers microhardness values (means ± SD) for the irrigating regimens are summarized in (Table 1). Group1 (control) showed significantly the highest microhardness value and group5 demonstrated the least microhardness value.

Table (1): Descriptive statistics of microhardness values for all groups

Groups	*N*	*Mean*	*±S.D*	*Min*	*Max*
Group1 (control)	10	62.56	±1.03	61.10	64.25
Group2	10	54.74	±.97	53.42	56.08
Group3	10	53.62	±.99	52.33	55.15
Group4	10	51.54	±.94	49.65	53.28
Group 5	10	50.05	±.65	48.87	50.74

Data were analyzed using one-way ANOVA and paired t-test. In these tests, P>0.05 (Non significant), P<0.05 (Significant), P<0.001 (Highly significant). One-way ANOVA test demonstrated that the time of treatment with 5.25% sodium hypochlorite (NaOCl) and 17% EDTA had a significant influence on microhardness of root dentin (P<0.001).

Table (2): One-way ANOVA test

	Sum of Squares	*DF*	*Mean Square*	*F*	*P-value.*
Between Groups	947.13	4	236.78	91.92	.000 (HS)
Within Groups	115.92	45	2.58		
Total	1063.04	49			

Further analysis was done by using paired t-test to compare between each two groups with different treatment time. Comparison between group1 (control) and group2 showed significant difference (P<0.05), while the differences were highly significant between group1 and all other tested groups with increasing exposure time to both 5.25% sodium hypochlorite (NaOCl) and 17% EDTA (P<0.001).

Table(3):Paired t.test for all groups

Paired	*t-value*	*DF*	*p-value*	*Sig.*
G1 - G2	3.03	9	.014	S
G1 - G3	7.54	9	.000	HS
G1 - G4	12.86	9	.000	HS
G2 - G3	6.04	9	.000	HS
G2 - G4	8.60	9	.000	HS
G3 - G4	3.65	9	.005	HS

DISCUSSION

Irrigants used in endodontic treatment caused alterations in the chemical and structural composition of dentin during removal of smear layer (19,20). Recently, **Uzunoğlu et al.** (21) stated that fracture resistances of root canal treated teeth were affected by irrigation procedures. Ideally, mechanical properties like strength, composition and hardness of dentin should not be affected in any negative aspect after irrigation procedures or this effect should be minimized. However, the sequential use of EDTA (or any acid) and NaOCl causes a progressive dissolution of dentin at the expense of peritubular and intertubular areas (22). The efficacy of chemical agents used to remove smear layer and demineralize and soften root dentine during root canal treatment has been examined by various means, including microhardness measurements, micro-radiographic assessments, spectrometry studies (**Verdelis et al.**(23), **Dogan & Calt** (24), **Scelza et al.**(25), **Machado-Silveiro et al.** (26), **Ari & Erdemir 2005** (27), **Gonzalez-Lopez et al. 2006** (28)) and especially electron microscopy studies (**Calt & Serper** (29), **Di Lenarda et al.**(30), **Ayad** (31), **Haznedaroglu** (32), **Perez-Heredia et al.**(33)). The assessment of the microhardness of a material is one of the simplest nondestructive mechanical characterization methods. Hardness is measured as the resistance to the penetration of an indenter that is harder than the sample to be analyzed (34). In our study Vickers microhardness test was used because previous studies have shown the suitability and practicability of Vickers microhardness test for evaluation of surface changes of dental tissues treated with chemical agents (15,31). Sodium hypochlorite (NaOCl) at a higher concentration (5.25%) was more effective in disinfection of the dentinal tubules (35) so in this study we used this concentration to study its effect on root canal dentin when used as root canal irrigant.

The present study showed that all irrigation periods with 5.25% sodium hypochlorite and 17% EDTA decreased dentin microhardness significantly. Treatment with 5.25% sodium hypochlorite for 10 minutes followed by 5 minutes 17% EDTA (group3) showed a significant decrease in microhardness from control group that treated with distilled water and also significantly lower than irrigation for same treatment time with 5.25% NaOCl but for 1 minute with 17%EDTA (group2). This in accordance with other studies which reported that EDTA when used for more than 1 minute causes erosion of dentinal tubules, thus reducing the dentin microhardness and consequently causing root fragility (36,37).

A previous study showed that 17% EDTA either alone or in combination with a tensoactive cationic detergent (Cetavlon) caused a more significant reduction of root dentin microhardness than 10% citric acid (34). **Cruz-Filho et al.** (38) reported that the action of 17% EDTA in decreasing dentin microhardness can be observed within the first minute after application of this chelator and that dentin microhardness decreases as the contact time with the solution increases.

The use of NaOCl as an initial irrigant creates an apatite-rich collagen-sparse dentin subsurface (39,40) that is more brittle than untreated mineralized dentin (41).This collagen-sparse subsurface zone has the potential to create non uniform deproteinization channels (42) that facilitate subsequent EDTA penetration and apatite dissolution, removal of the "superficial subsurface" organic phase from the mineralized dentin by NaOCl is both concentration and time-dependent. The combined action of NaOCl and EDTA causing changes on collagen matrix and demineralization of root dentin with consequent exposure of collagen respectively results in a decrease of dentin microhardness as observed in the presented study [43].

Calt and Serper [44] studied the time-dependent effect of EDTA followed by NaOCl which can be the evidence for the dentin microhardness decrease. EDTA as a time-dependent solution after 5 min decreased dentin microhardness more than its 1 min application at a depth of 100 mm from the pulp-dentin interface. Many studies have shown that different concentrations of chelating agents and citric acid can reduce dentin hardness [17] and this effect increases with increase exposure time [22].

The result of this study demonstrated that the treatment with 5.25% sodium hypochlorite for 20 minute followed by 5 minutes 17% EDTA (group5) significantly decrease the microhardness from control group and also significantly lower than all other groups including the group treated for same time with NaOCl but for 1minute with 17%EDTA (group4). This result in accordance with **Slutzky-Goldberg et al.**[45], they used irrigation with 2.5% or 6% NaOCl for 5, 10, or 20 min without subsequent EDTA they concluded that exposure of dentin to sodium hypochlorite solution for more than 10 min decreased dentin microhardness significantly. The decrease in microhardness was more marked after irrigation with 6% NaOCl than with 2.5%. **Zhang et al.** [46] showed different concentrations of NaOCl cause a time-dependent linear increase in removal of the organic phase from mineralized dentin and the extent and rate of removal were more severe with the use of higher concentrations (5.25%) when NaOCl was used as the initial irrigant. **Saleh and Ettman** [36] studied the effect of endodontic irrigation solutions (3% H2O2 and 5% NaOCl or 17%EDTA for 60 second) on the microhardness of root canal dentin, the results showed that irrigation with H2O2/NaOCl or EDTA significantly reduced the microhardness of root dentin.

Unlike what is commonly accepted, the treatment of dentin with NaOCl may not only remove the organic matrix but also some of the inorganic content that ultimately renders dentin much weaker than normal [47]. Although NaOCl is not a chelating agent, it can significantly decrease the C (calcium)/P (phosphorus) ratio of superficial root dentin [24] and its microhardness [15] depending on the concentration of the solution.

CONCLUSION

Within the limitations of this study, irrigation of root canal with 5.25% sodium hypochlorite (NaOCl) for (20 min) followed by 17% EDTA for (5 min) resulted in decreasing of root canal dentin microhardness. So the time of irrigation is a factor that should gained special attention during endodontic treatment.

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