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The Effect of Four Commonly Used Root Conditioner Agents in Different Time Periods Applied on Periodontally Diseased and Healthy Teeth

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Abstract

***Statement of Problem*:** Root surface contamination or infection can potentially change the consequences of regenerative periodontal therapies and therefore the modification and disinfection of the contaminated root surfaces are necessary.

***Objectives*:** This study aimed to compare the surface characteristics of the extracted human teeth after exposure to four root conditioners in different time periods.

***Materials and Methods*:** The study samples were prepared from 40 freshly extracted teeth including 20 affected teeth with periodontal diseases and 20 healthy teeth. After performing root planning, 240 dentinal block samples were prepared and each affected and healthy sample was randomly allocated to receive one of the following root conditioners; Ethylenediaminetetraaceti acid (EDTA), citric acid, doxycycline, and tetracycline or rinsed with normal saline as the control agent. The prepared specimens were evaluated using scanning electron microscope and the inter-group differences and changes in study indices; dentin (%), tubular spaces (%), and diameter of dentinal tubules (μm²) were compared using one-way ANOVA test.

***Results*:** In the control group receiving normal saline, the changes in the indicators of dentin, tubular spaces, and diameter of dentinal tubules remained insignificant in all time periods. EDTA, citric acid, and tetracycline had chelating effects on the study indices; however, doxycycline led to gradual decrease of the tubular space and diameter as well as increase in dentin percentage.

***Conclusions*:** In different time intervals and when considering healthy or affected tooth surfaces, the effect of conditioning agents could be different. Amongst the four agents used, EDTA and tetracycline consistently increased the diameter of tubules and percentage of patent tubules in both healthy and diseased teeth.

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Introduction

One of the goals of periodontal therapy is predictable regeneration of the periodontium in areas previously affected by periodontal disease [1]. Root surfaces are potentially at risk for hypermineralization as well as contamination by different strains of bacteria and their endotoxins [2]. Root surface contamination or infection can potentially change the consequences of regenerative periodontal therapies and therefore for achieving

optimal appropriate outcome the modification and disinfection of the contaminated root surfaces are necessary [3]. The most minimally invasive procedures for removing the bacterial deposits, accretions and endotoxins from the exposed root surface are scaling and root planning that removes the calculus below the gum line and cleans the root surface which allows the healing process to begin [4]. However, the potential limitations of scaling and root planning have been also described. First, by using this treatment, complete decontamination of the root surfaces affected by periodontitis seems to be impossible. In fact, scaling and root planning provides only a temporary solution to the periodontal disease. Moreover, it has been shown that these methods are less effective in deeper pockets in which removing the calculus is more difficult [1].

This is also true for the posterior teeth which are difficult to reach for mechanical root surface debridement. In order to overcome this difficulty, root conditioning could be considered as an adjunct to mechanical debridement [5]. Different materials and agents have been introduced for removing the smear layer and bacterial endotoxins from the root surface; many application times have been tested ranging from 0.5 to 10 min, and most of the studies have found the best results during 3 to 4 min of application [6].

A systematic review related to the subject was published by Mariotti [7] who concluded that the use of citric acid, tetracycline or EDTA to modify the root surface provides no benefit of clinical significance to regeneration in patients with chronic periodontitis. On the other hand, the author also states that since most of the included study designs are not fully developed, a definite conclusion should be taken into account [7]. There is still a remarkable controversy concerning the need to use chemical agents; this justifies the search for parameters that can support the selection for this procedure in periodontal treatments. In addition, knowing the effect of these agents in different time periods could be helpful for selecting the best application time.

Therefore, the present study aimed to compare the surface characteristics of both healthy and periodontally diseased extracted human teeth at different time periods after treatment with different root conditioning agents.

Materials and Methods

The study samples were 40 freshly extracted molars including 20 affected teeth with periodontal diseases and 20 healthy teeth that were extracted because of other reasons. None of the affected teeth had a history of periodontal treatment such as scaling or root planning within the last 6 months. Also, those with root surface caries and/or restoration, root surface abnormalities, or cervical abrasion and/or erosion were excluded.

Following the extraction, the teeth were thoroughly washed and then root planning was performed on the root surfaces using a curette to obtain smooth, shiny, and hard surface. For facilitating dehydration and coating, the teeth were divided longitudinally by a water cooled high speed fissure bur (Teeskavan, Tehran, Iran) and the pulp and canal spaces were removed. Then, the teeth were washed by soft brush and distilled water and then dehydrated. The crowns were separated and a total of 240 dentinal block sample sizes of 2x3x1 mm were obtained by sectioning the root on the planed area with a flexible double-faced diamond disc.

The samples were randomly allocated to one of the following treatment groups. The chelating agents were provided by the Department of Pharmacology in Hamedan University of Medical Sciences:

A. Control group – rinsed with normal saline

B. Group 1 – received EthyleneDiamineTetraAcetic Acid (EDTA) 24% (pH = 7.3)

C. Group 2 – received saturated citric acid (pH = 1.0)

D. Group 3 – received doxycycline 10% (pH = 2.2)

E. Group 4 – received tetracycline 10% (pH = 1.8)

In each group, 48 samples were included. The groups were divided into subgroups according to the tooth type. Two types of teeth (affected and healthy) were used (n = 24). Each of these two subgroups was further divided into the four application time intervals of 1, 2, 3 or 4 min (n = 6).

Application of the agents on the root was done by rubbing method using cotton pellets for 10 to 15 seconds and set on for 1,2,3 and 4 minutes after which the tooth specimens were flushed with distilled water to stop the reactions. Then, the specimens were dehydrated in an ascending concentration of alcohol solutions (40 min in alcohol 50%, 40 min in alcohol 75%, and finally 40 min in alcohol 100%); and air dried. Dried specimens were mounted on Scanning Electron Microscope (SEM) stubs and sputter coated with gold in a gold sputtering unit Balt-Tec SCD-050 (Balt-Tec, Cheshire, UK). With the gold coating procedure, a very thin layer of gold (about 200 Angstrom) covers the specimens by which the

contrast increases in the SEM view.

Finally, the mounted specimens were evaluated using the SEM JSM8A (JEOL, Tokyo, Japan) by grouping them in 10 groups each consisting of 24 specimens and evaluating one group at each time. The SEM micrographs were transferred to a computer and analyzed by Image J software. The field shown at magnification of 1,000× was taken as a reference for the total area in which the percentage of the areas occupied by the enlarged dentinal tubules and by the dentin, and also the mean diameter of the patent dentinal tubules according to the scale bar 10 µm, were calculated (Figure 1). For better assessment of the changes in study parameters, the following ratio was defined indicating the efficacy of the applied agents (zero indicating no effectiveness of the agent):

$$\text{k index} = \frac{\sum \Delta y}{\sum \Delta x}$$

y: The difference of each study parameter (tubular space percentage, percentage of area occupied by dentin or tubular diameter measured on the 1,000× magnification SEM view) at different application time intervals

x: Time intervals (1-2 min, 2-3 min, and 3-4 min)

Inter-group differences and changes in study indices after interventions were compared using one-way ANOVA test.

Results

In the control group receiving normal saline, the changes in the indicators of the dentin (%) ($p = 0.730$), tubular spaces (%) ($p = 0.751$), and diameter of the dentinal tubules (µm²) ($p = 0.867$) were insignificant in all time periods. Those who received EDTA experienced a significant linear and partial persistent increase in the tubular spaces ($p = 0.001$) and the mean diameter of the tubules ($p = 0.041$), as well as a decrease in the percentage of the dentins ($p = 0.001$) at 3-4 min time periods in both affected and healthy teeth. The use of citric acid led to a non-linear and inconsistent but increasing trend in root conditioning in the affected group ($p = 0.001$).

Doxycycline groups experienced non-linear and non- persistent decreasing trend in root conditioning which led to a gradual decrease in tubular space and diameter as well as an increase in the percentage of area occupied by dentin ($p = 0.001$). In tetracycline groups, although the observed trend in root conditioning was non-linear, it was persistent and increasing ($p = 0.001$) (Table 1,2). As shown in Table 3 and according to the measurement of K index, the use of EDTA, citric acid, and tetracycline had positive chelating effects on the study indices (increasing the tubular space or tubular diameter); however,

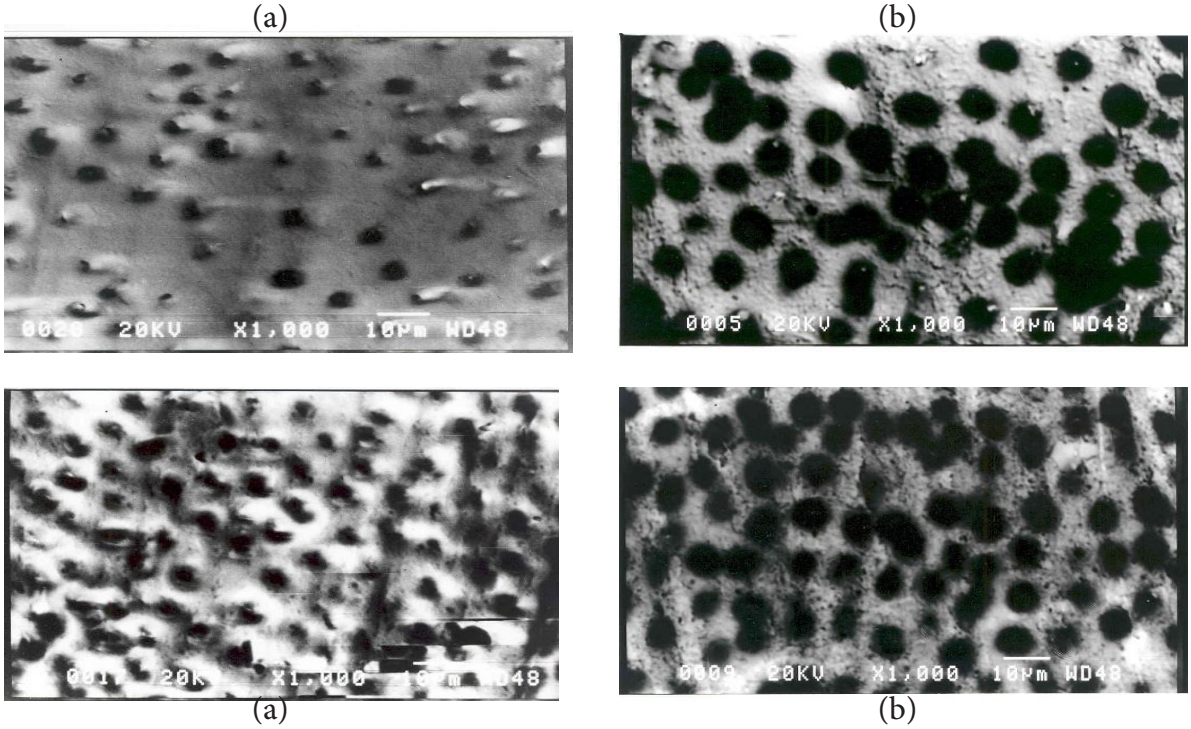

Figure 1: Micrographs of the representative specimens showing the mean diameter of patent tubules and percentage of areas occupied by dentinal tubules and dentin before (a) and after (b) applying conditioning agent viewed by SEM (1,000X).ntin before (a) and after (b) applying conditioning agent viewed by SEM (1,000X).

Table 1: Changes in tubular space, dentin and tubular diameter indices in healthy groups at different time periods following the use of each conditioner

Group	Time (min)	Tubular space (%)		Dentin (%)		Tubular diameter (μm²)	
		Before	After	Before	After	Before	After
EDTA	4	42.7±0.6	52.0±0.3	57.3±0.6	47.9±0.6	4.4±0.5	6.2±0.5
	3	43.0±0.8	46.5±0.5	57.0±0.8	73.4±0.8	4.1±0.5	3.9±0.5
	2	43.2±0.7	43.0±5	56.8±0.7	76.9±0.7	3.8±0.5	4.0±0.6
	1	44.0±1	39.4±0.7	56.0±1.2	80.5±1.2	3.6±0.5	3.7±0.4
Citric acid	4	42.0±0.5	25.1±0.5	58.0±0.5	74.8±0.5	4.3±0.5	7.5±0.7
	3	42.4±0.8	30.2±0.5	57.6±0.8	69.7±0.5	4.1±0.6	6.2±0.5
	2	42.6±0.7	18.0±0.7	57.4±0.7	81.9±0.7	3.6±0.7	6.1±0.6
	1	43.0±0.6	21.3±0.5	57.0±0.6	78.6±0.5	3.7±0.5	4.4±0.7
Doxycy-cline	4	42.9±0.6	34.1±0.6	57.1±0.6	65.8±0.6	5.5±0.5	4.3±0.6
	3	43.0±0.7	35.4±0.5	56.9±0.7	64.5±0.5	5.1±0.4	4.2±0.7
	2	43.0±0.6	37.2±0.5	57.0±0.6	62.7±0.5	6.8±0.4	3.8±0.5
	1	43.0±0.6	35.8±0.8	57.0±0.6	64.1±0.8	7.8±0.6	3.5±0.5
Tetracy-cline	4	41.0±0.8	42.1±0.5	59.0±0.8	57.8±0.8	4.3±0.5	7.6±0.5
	3	43.2±0.7	34.8±0.6	56.8±0.7	65.1±0.7	4.2±0.5	4.9±0.7
	2	42.7±0.6	26.9±0.5	57.3±0.6	73.0±0.5	4.0±0.6	8.0±0.6
	1	41.2±0.4	43.3±0.6	58.8±0.4	56.6±0.6	4.1±0.4	7.2±0.5
Normal saline	4	42.2±0.5	43.3±0.5	57.7±0.5	56.6±0.5	4.4±0.6	4.4±0.4
	3	42.8±0.8	43.9±0.8	57.1±0.8	56.0±0.8	4.1±0.6	4.2±05
	2	43.0±0.8	44.0±0.6	57.0±0.8	56.0±0.6	3.7±0.4	3.9±0.6
	1	44.1±0.7	45.0±0.7	55.8±0.4	55.0±0.7	3.6±0.5	3.7±0.5

Table 2: Changes in tubular space, dentin and tubular diameter indices in affected groups at different time periods following the use of each conditioner

Group	Time (min)	Tubular space (%)		Dentin (%)		Tubular diameter (μm²)	
		Before	After	Before	After	Before	After
EDTA	4	42.7±0.6	49.9±1.2	57.3±0.6	50.0±1.2	4.4±0.5	7.6±0.4
	3	43.0±0.8	47.2±0.3	57.0±0.8	52.7±0.3	4.1±0.5	8.0±0.3
	2	43.2±•,7	42.1±0.7	56.8±0.7	57.9±0.7	3.8±0.5	8.0±0.4
	1	44.0±1.2	41.7±0.7	56.0±1.2	58.2±0.7	3.6±0.5	7.8±0.4
Citric acid	4	42.0±0.5	48.3±0.6	58.0±0.5	51.6±0.6	4.3±0.5	8.1±0.5
	3	42.4±0.8	39.9±0.8	57.6±0.8	60.0±0.8	4.1±0.6	6.2±0.6
	2	42.6±0.7	21.3±0.8	57.4±0.7	78.6±0.8	3.6±0.7	6.1±0.6
	1	43.0±0.6	18.0±0.6	57.0±0.6	81.1±0.6	3.7±0.5	4.5±0.7
Doxycy-cline	4	42.9±0.6	34.1±0.3	57.1±0.6	65.8±0.3	5.6±0.5	4.3±0.6
	3	43.0±0.7	29.5±0.6	56.9±0.7	70.4±0.6	5.1±0.4	4.2±0.5
	2	43.0±0.6	36.4±0.7	57.0±0.6	63.5±0.7	5.7±0.4	3.8±0.3
	1	43.0±0.6	55.6±0.6	57.0±0.6	44.3±0.6	3.5±0.6	7.9±0.3
Tetracy-cline	4	41.0±0.8	53.3±0.4	59.0±0.8	46.7±0.4	4.3±0.5	7.8±0.8
	3	43.2±0.7	27.8±0.7	56.8±.7	72.1±0.7	4.2±0.5	5.5±0.6
	2	42.7±0.6	23.8±0.7	57.3±0.6	76.1±0.7	4.0±0.6	8.2±0.4
	1	41.2±0.4	31.9±0.7	58.8±0.4	68.0±0.7	4.1±0.4	7.3±0.6
Normal saline	4	42.2±0.5	43.3±0.5	57.7±0.5	56.6±0.5	4.4±0.6	4.4±0.4
	3	42.8±0.8	43.9±0.8	57.1±0.8	56.0±0.8	4.1±0.6	4.2±0.5
	2	43.0±0.8	44.0±0.6	57.0±0.8	56.0±0.6	3.7±0.4	3.9±0.6
	1	44.1±0.7	45.0±0.7	55.8±0.4	55.0±0.7	3.6±0.5	3.7±0.5

doxycycline had adverse effects as a root conditioner in the affected teeth. The changes in the study indices across the four treatment regimens in different time periods are presented in Table 4. In each time interval the differences in study indices between treatment regimens were significant ($p = 0.001$).

Table 3: Changes of study indices according to the measurement of K

$\frac{\sum \Delta y}{\sum \Delta x}$	Tubular space (%)	Dentin (%)	Tubular diameter (μm²)
EDTA			
Affected teeth	3.16	-3.17	0.33
Healthy teeth	4.63	-4.63	0.69
Citric acid			
Affected teeth	10.46	-10.60	0.94
Healthy teeth	-1.54	1.60	0.85
Doxycycline			
Affected teeth	-7.14	7.39	-1.06
Healthy teeth	0.55	-0.55	1.03
Tetracycline			
Affected teeth	7.23	-7.17	0.10
Healthy teeth	0.33	-0.33	0.09
Normal saline			
Affected teeth	0.11	0.65	-0.02
Healthy teeth	0.00	0.01	0.01

Discussion

To select the best therapeutic conditioning material for contaminated root surfaces, we compared the effectiveness of different materials applied in different time periods. Regarding the linear and consistent trend of the changes in the parameters, the use of EDTA, followed by tetracycline was preferable. In addition, EDTA and tetracycline samples had a greater percentage of patent tubules when compared with other treated samples.

The proper effectiveness of EDTA has been previously shown. In the study done by Gamal *et al*. [8] applying EDTA gel for 4 minutes after scaling and root planning removed the root surface smear layer and had the best result in adherence and growth of the PDL cells. In addition to the contact time, it has been shown that the curative effects of EDTA quickly become apparent so that it can remove the smear layer in less than 1 minute [9]. Moreover, EDTA can reduce the micro-hardness of the dentin by 17.33% to 29.48%, and this effect is considerably greater than other active and control solutions [10,11]. Thus, on the basis of our result and in comparison with the previous findings, the application of EDTA, even alone, had the maximum results in root conditioning. In fact, the natural pH along with the capability to remove the root surface smear layer makes the use of EDTA highly beneficial in clinical practice.

According to our results, the use of tetracycline can be the second choice for root conditioning. Different studies have evaluated and compared the

Table 4: Effects of treatment regimens on the study indices in different time periods in affected teeth

Material		EDTA	Citric acid	Doxycycline	Tetracycline	Normal saline	*p* value
min 1	TS	-2.2±1.1	-24.1±0.3	12.6±0.2	-9.2±0.3	0.8±0.1	*0.001
	D	2.2±1.1	24.1±0.3	-12.6±0.2	9.2±0.3	-0.8±0.1	*0.001
	TD	4.2±0.4	0.9±0.3	4.4±0.5	3.2±0.2	0.7±0.1	*0.001
min 2	TS	-1.1±0.9	-21.3±0.5	-6.5±0.3	-18.9±0.0	1.0±0.5	*0.001
	D	1.1±0.9	21.3±0.5	6.5±0.3	18.9±0.0	-1.0±0.5	*0.001
	TD	4.1±0.8	2.5±0.3	-1.9±0.4	4.2±0.7	0.1±0.6	*0.001
min 3	TS	4.2±0.7	-2.4±0.3	-13.4±0.2	-15.4±0.1	1.1±0.2	*0.001
	D	-4.2±0.7	2.4±0.3	13.4±0.2	15.4±0.1	-1.1±0.2	*0.001
	TD	3.9±0.8	2.0±0.7	-0.1±0.5	1.3±0.3	0.0±0.2	*0.001
min 4	TS	7.2±1.7	6.4±0.2	-8.8±0.2	12.3±0.7	1.1±0.3	*0.001
	D	-7.2±1.7	-6.4±0.2	8.8±0.2	-12.2±0.7	-1.1±0.3	*0.001
	TD	3.2±0.6	3.7±0.1	-1.2±1.0	3.5±0.3	0.0±0.6	*0.001

TS: Tubular Space, D: Dentin, TD: Tubular Diameter

*$p < 0.01$

efficacy of tetracycline with other materials such as citric acid [12-15]. According to the findings of Grover *et al*., the number of patent tubules present in the citric acid and EDTA test groups was significantly higher than those in the tetracycline hydrochloride test group. However, in their study the average diameter of the patent tubules was greater in the tetracycline hydrochloride group compared with the citric acid and EDTA groups [4].

Shetty *et al*. also showed that the proportion of patent dentinal tubules was significantly higher in the tetracycline HCl group (74%) compared to minocycline (48.3%), doxycycline (42%) and citric acid (52%). The number of patent tubules was also higher in the tetracycline group compared to minocycline and doxycycline and the difference was statistically significant [16]. In fact, it can be concluded that with regard to root conditioning, tetracycline can be a good choice regarding its effects on the dentin smear layer removal and tubule exposure. Because of its efficacy against suspected causative microflora, anti-enzymatic properties, as well as antibiotic impacts, the use of this agent is preferred to other antibiotics [17].

Our results showed that applying tetracycline increased the tubular diameter in less than one minute and the tubular space after 3 minutes. However, a previous study suggested that the application time should be limited to 2-3 minutes [18] since long etching time of 3 minutes and above could impair periodontal healing [19]. Tetracycline is able to enhance fibrin clot adhesion by exposing collagen matrix [20] and it enhances fibroblast chemotaxis and binding leading to a more stable initial clot formation [21].

According to our results despite having the maximum increase in the tubular space and tubular diameter in the first minute, doxycycline had adverse effects as a root conditioner and decreased the tubular space and tubular diameter after 1 minutes of applying. Similarly Chahal *et al.* [22] reported that removal of smear layer by tetracycline and citric acid is better than doxycycline and the percentage of patent tubules and their diameter were lower in the doxycycline group. This finding could be attributed to the lower pH of tetracycline HCl (pH 1.8) and citric acid (pH 1) as compared to doxycycline (pH 2.2).

Citric acid has the ability to remove the smear layer, exposes the dentinal tubules and makes them wider with funnel shaped orifices. It may also act as a potent antibacterial agent [23]. In a study comparing the effect of citric acid applied for 1,2 and 3 minutes removing the smear layer, showed that the efficacy of this root conditioner was best after 3 minutes [24]. In comparison, our results also showed that citric acid increased the tubular diameter and tubular space significantly after 3 minutes in the affected teeth ($p < 0.01$). However some disadvantages have been addressed with applying citric acid such as the formation of extremely acidic environment in the surrounding tissues, which may result in unfavourable wound healing responses [25].

Conclusions

Because of more persistent effectiveness on early root conditioning (chelating effect in less than one minute of applying), EDTA and tetracycline are preferable root conditioner agents which could be used in regenerative periodontal therapies. The use of citric acid agent for surface conditioning showed different outcomes in the healthy and periodontally affected teeth with increasing tubular space significantly in the affected teeth after applying for 3 minutes. Doxycycline has adverse effects as a root conditioner after 1 minutes of application.

Conflict of Interest: None declared.

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