

The Effects of Natural Collagen Cross-Linking Agent “Proanthocyanidin” on the Microhardness of The Radicular Dentin: An In Vitro Study

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Abstract

Aim: The aim of this study was to evaluate the effects of Proanthocyanidin as a collagen cross-linking agent on the microhardness of root dentin.

Materials and Methods: twenty-four straight palatal roots of upper first molars were decoronated and instrumented by ProTaper universal hand system till size F4 (40,06). Based on the final irrigating regimen, samples were divided into three groups (n=8): group1; distilled water, group2; sodium hypochlorite (5.25%)/ ethylenediaminetetraacetic acid (17%) and group3; the same protocol used in group2 followed by 7% Proanthocyanidin. All samples were filled with gutta percha and AH Plus sealer then incubated at 37°C for seven days. The roots sectioned longitudinally and horizontally embedded in acrylic disc. Microhardness was measured with Vicker indenter at six different points for each sectioned root (coronal, middle and apical thirds). In each third, the indentation was made on the dentin surface approximately at 100µm and 500µm from the canal-dentin interface.

Results: at 100µm, group1 scored the highest values along the canals with no significant difference with group3, while group2 was significantly the lowest one. At 500µm, group1 was significantly the higher at all levels. Group2 was higher than group3 at coronal and middle thirds, while group3 was higher than group2 at the apical third with no significant different between them.

Conclusions: final irrigation with 7% Proanthocyanidin improve the microhardness of radicular dentin.

Keywords: proanthocyanidin; collagen cross-linking; dentin; hardness; irrigant

Introduction

Hardness can be defined as a measure of material resistance to localized plastic deformation [1]. Microhardness determination provides indirect evidence of change in mineral content hard tissue [2]. Thus, microhardness presents the first step toward predicting the behavior of dentin under stress [3]. Chemical irrigants, used in endodontic treatment, cause alterations in the chemical structure of dentin and change the ratio of calcium to phosphorus. These irrigants affects the microhardness of dentin subsequently; weaken the root structure due to the reduction in modulus of elasticity and flexural strength of dentin [4], hence increase the susceptibility to fracture [5]. Sodium hypochlorite (NaOCl) and ethylenediamine tetra-acetic acid (EDTA) have wide used as efficient irrigation solutions to remove the smear layer. NaOCl acts to dissolve the organic part of the smear layer, while EDTA can eliminate inorganic elements [6].

However, the synergistic effects of NaOCl and EDTA could cause harmful changes of the mechanical properties of the tooth structure consequently render these endodontically treated teeth more susceptible to vertical root fracture [5]. Proanthocyanidin (PA) as a natural cross-linking agent is profoundly presented in grape seed extract. Several studies stated that, PA improved the biomechanical properties and bond strength of dentin [7]. Till date, there are no study reported on the effect of PA on the microhardness of root dentin. Hence, the rational of the current in vitro study was to evaluate the effects of this agent as a final root canal irrigant on the microhardness of the radicular dentin in comparison with the mostly used irrigation protocol (NaOCl/EDTA).

Materials and Methods

Samples Selection: twenty-four human palatal roots of maxillary first molars were selected and stored in 0.2% thymol solution until they were used.

Samples Preparation: stainless steel K-file 10 # was inserted into root canal until the tip was seen just exiting at the apical foramen (observed under magnifying lens). All roots were standardized at 12mm. working length was determined by subtracting 1mm from this length. The apical foramen was sealed with sticky wax (GC, chemical Co, Japan) to prevent extrusion of irrigant out of the apex. All samples were prepared using hand universal ProTaper system (Dentsply, Maillefer, Swiss), till F4 (0.4/06) according to the manufacturer's instructions. During preparation, the canals were irrigated with 2ml of distilled water (DW), this was repeated each time the instrument was removed.

Samples Grouping: the roots were randomly divided into 3 groups (n=8) relative to the final irrigation protocol. The concentrations of NaOCl, EDTA and PA were 5.25%, 17% and 7% respectively.

Group 1: irrigation process was performed with DW only.

Group 2: the sequence of final irrigation was as follows:

1. 1ml of NaOCl (Wohciech, Pawlowski, Poland), Endoactivator (Dentsply, Maillefer, Switzerland) for 30 seconds.
2. 5ml of DW and dried with absorbent paper point (#40).
3. 1ml of EDTA (Siaulial, Lithuania), Endoactivator for 30s.
4. 1ml of EDTA, Endoactivator for 30s.
5. 5ml of DW and dried with absorbent paper point (#40).
6. 1ml of NaOCl, Endoactivator for 30s.
7. Canals were finally flushed with 5ml of DW and dried with absorbent paper points (#40).

Group 3: the same protocol used in group 2 followed by these subsequent steps:

1. 1ml of PA (HerbStore, USA), Endoactivator for 30s.
2. Fresh 1ml of PA, Endoactivator for 30s, and then the canals were dried with absorbent paper point (#40).

Root Canals Obturation: after final irrigation, the experimentally root canals dried with absorbent paper points and filled with ProTaper F4 gutta-percha (Dentsply, Maillefer, Switzerland), and an epoxy-resin sealer (AH Plus; Dentsply, Konstanz, Germany) using single cone technique. Radiographs were taken to confirm the absence of voids in the fillings. The canal access was restored with a temporary restorative material. The roots were stored in an incubator at 37°C and 100% humidity for 1week to allow the sealer to get fully set.

Preparation of Specimens: longitudinal grooves on the buccal and palatal external root surface were prepared using diamond disc (China) in a micromotor straight handpiece (W&H, Austria) at a speed of 1500rpm, under constant copious amount of water coolant. Each root specimen was splitting with a chisel and hammer into two segments and the one with less surface aberrations on dentin was selected and horizontally embedded in autopolymerizing acrylic disc (Figure 1). The dentin surface of the mounted specimen was then grounded flat and smoothed with a series of fine carbide papers (800, 1200 and 2400 grit) (China) to remove any surface scratches and it was finally polished with fine grades of composite polishing kit (PD, Switzerland), using 50µm sized alumina suspension on a rotary felt disk.

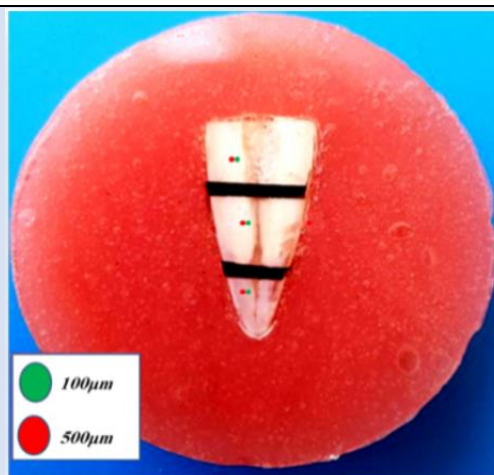


Figure 1: The specimen embedded in autopolymerizing acrylic disc showing the location of the first and second points measurements.

Microhardness Test: the surface microhardness of the root dentin was determined with a digital Vicker's hardness tester (TH-714, Inspec, China), using 100g load for 15seconds (Figure 2). The indentation was made with a diamond indenter, and then the indented area was scanned by microscopic lens (400x). The microhardness measurements were taken at six different points for each sectioned root

(coronal, middle and apical thirds). In each third, the indentation was made on the dentin surface approximately at 100 μ m and 500 μ m from the canal-dentin interface. The representative hardness value at each point was obtained as the average of the three indentations in order to produce a single value. All readings were performed by the same examiner, using the same calibrated machine.

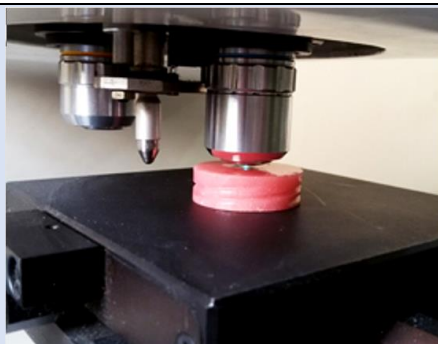


Figure 2: Mounting the specimen on Vicker hardness tester.

The statistical analysis was performed by statistical package for social science (SPSS, version 20.0; IBM, Incorporation, USA).

Results

Table 1: descriptive statistics of VHN of the three groups at 100 μ m.

Groups	Levels	No.	Min.	Max.	Mean	$\pm$ SD
1	Coronal	8	60.15	77.91	69.18	6.08
	Middle	8	57.82	72.79	65.03	5.20
	Apical	8	52.57	73.81	63.20	6.35
2	Coronal	8	39.11	54.15	45.19	5.47
	Middle	8	40.53	56.74	47.39	5.31
	Apical	8	37.41	52.87	43.58	5.49
3	Coronal	8	58.12	73.44	66.31	7.12
	Middle	8	55.41	72.63	63.90	6.87
	Apical	8	53.82	67.23	59.91	4.20

From Table 1, the highest microhardness value at coronal area was recorded in group1 (69.18 $\pm$ 6.08). The second was scored for group3 (66.31 $\pm$ 7.12), while the lowest value was observed in group2 (45.19 $\pm$ 5.47). At the middle third the highest value was scored in group1 (65.03 $\pm$ 5.20), the second was observed in group3 (63.90 $\pm$ 6.87), whereas the lowest value was recorded for group2 (47.39 $\pm$ 5.31). Apically, the sequence of the microhardness values was the same as coronal and middle thirds and as follow: the highest value was scored in group1 (63.64 $\pm$ 7.89), the second

Microhardness at 100 μ m

Microhardness values (mean, standard deviation, minimum and maximum) of the three groups are illustrated in Table 1.

was observed in group3 (54.89 $\pm$ 6.40), while the lowest value was recorded for group2 (51.84 $\pm$ 5.68). For inferential statistics; one way ANOVA with LSD test was applied to find out differences among the groups. ANOVA test (Table 2) revealed significant differences among all groups. Intergroup multiple comparisons were done by LSD test (Table 3). Respective to all levels, there were significant differences of group2 with group1 and group3, whereas group1 did not differ significantly from group3.

Table 2: One-way ANOVA test to compare the microhardness at all levels at 100µm.

Levels	F	P-Value	Sig.
Coronal	34.95	0.000	HS
Middle	22.81	0.000	HS
Apical	30.00	0.000	HS

Table 3: LSD test among all groups along the three levels at 100µm.

Levels	Groups		Mean Difference	SE	P-Value	Sig.
Coronal	1	2	23.98	3.13	0.000	HS
		3	2.87	3.13	0.370	NS
	2	3	21.11	3.13	0.000	HS
Middle	1	2	17.64	2.92	0.000	HS
		3	1.13	2.92	0.702	NS
	2	3	16.51	2.92	0.000	HS
Apical	1	2	19.62	2.71	0.000	HS
		3	3.29	2.71	0.239	NS
	2	3	16.33	2.71	0.000	HS

Microhardness at 500µm: Microhardness values (mean, standard deviation, minimum and maximum) of the three groups are illustrated in Table 4.

Table 4: descriptive statistics of VHN of the three groups at 500µm.

Groups	Levels	No.	Min	Max.	Mean	±SD
1	Coronal	8	60.67	78.07	71.17	7.22
	Middle	8	56.78	76.94	66.86	7.71
	Apical	8	53.06	70.97	63.64	7.89
2	Coronal	8	46.15	67.90	53.96	8.90
	Middle	8	49.23	64.72	54.68	5.98
	Apical	8	45.39	59.78	51.84	5.68
3	Coronal	8	42.08	61.30	51.17	8.50
	Middle	8	48.36	60.32	53.30	4.31
	Apical	8	46.82	61.75	54.89	6.40

Rendering to the Table 4, the maximum mean value of VHN at coronal third was recorded in group1 (71.17 ± 7.22) followed by group2 (53.96 ± 8.90), whereas the minimum microhardness value was observed for group3 (51.17 ± 8.50). At the middle area the highest mean value of microhardness was scored in group1 (66.86 ± 7.71) and the second highest value was observed in group2 (54.68 ± 5.98), while the lowest value was recorded for group3 (53.30 ± 4.31). Apically, the highest mean value was scored in group1 (63.64 ± 7.89), followed by group3 (54.89 ± 6.40), while the lowest value was recorded for group2 (51.84 ± 5.68). For inferential statistics; one way ANOVA with LSD test was applied to find out differences among the groups. ANOVA test (Table 5)

revealed that, there were statistically significant differences among the three groups at all levels.

Table 5: one-way ANOVA test to compare the microhardness at all levels at 500µm.

Levels	F	P-value	Sig.
Coronal	13.81	0.000	HS
Middle	13.73	0.000	HS
Apical	6.62	0.000	HS

Intergroup multiple comparisons were done by LSD test (table 6) which showed significant differences of group1 with group2 and group3 at the three levels, while there was no significant difference between group2 and group3 along the canals.

Table 6: LSD test among the all groups at the three levels at 500 μ m.

Levels	Groups		Mean Difference	SE	P-Value	Sig.
Coronal	1	2	17.20	4.12	0.000	HS
		3	19.99	4.12	0.000	HS
	2	3	2.78	4.12	0.506	NS
Middle	1	2	12.18	3.08	0.001	HS
		3	13.56	3.08	0.000	HS
	2	3	1.38	3.08	0.658	NS
Apical	1	2	11.76	3.36	0.002	HS
		3	8.74	3.36	0.017	S
	2	3	3.05	3.36	0.374	NS

Discussion

Microhardness at 100 μ m

In group1, microhardness test revealed no statistical differences between coronal and middle segments, while there was reginal significant difference between coronal and apical thirds. Obviously, the microhardness was declined apically, and this might be attributed to the histological pattern of the root canal dentin and relative nature of dentin in the apical region. These findings were in accordance with the study of Ballal et al. [8] who reported that, there was an increase in microhardness from apical to coronal thirds. Inoue et al. [9] concluded that the mechanical characteristics are in direct proportion to the mineral percentage of the tissue, and the coronal dentin contains more minerals than those of radicular dentin. Contrarily, the work of Carrigan et al. [10] showed that, tubules density decreased from cervical to apical dentin. Pashley et al. [4] reported that, there was an inverse correlation between dentin microhardness and tubular density. The controversy of these results with other studies might be attributed to different types of root canal preparation, differences in methods of applications of these irrigants and different evaluation techniques for assessment of hardness. In group2, NaOCl/EDTA significantly reduced microhardness at 100 μ m along the three levels in comparison to the other groups. These findings indicated that, these solutions could diffuse into dentinal tubules about 100 μ m, and this was in agreement with previous study [5].

Khoroushi et al. [11] revealed that, NaOCl decreases the stiffness of intratubular dentin matrix as this agent breaks down to sodium chloride and oxygen, causing oxidizing some components in the dentin matrix, and thus decreasing the hardness of dentin which is attributed to the loss of organic substance from the dentin. Furthermore, the effect of the combination of EDTA with NaOCl in this sequence cause decreased

hardness values [3]. This was definitely demonstrated in the current study. Although there were no statistical differences in microhardness among the three levels of group2, the coronal part had the higher reduction of dentin microhardness in comparison with the apical area. These findings might be due to that NaOCl and or EDTA were not effective in the apical region as they were in the coronal and middle thirds. This might be attributed to the less effectiveness of these solutions in reducing the surface tension apically than in the middle and coronal thirds, and/or because of the less penetration in the apical third of the canal with little amount of irrigants in contact with canal walls. The above results also agree with other researches [12]. Moreover, the relatively small canal diameter in the apical third possibly exposes the dentin to a lesser volume of irrigants and hence compromising their efficiency. This outcome was in consensus with the previous studies [13]. According to Mohammed et al. [14], the taper confinement and air bubble entrapment could affect the flow and penetration of an irrigant within the root canal system.

In group3, PA significantly improved the microhardness in comparison with group2. This improvement could be attributed to the ability of PA to act as a scaffold to accelerate the deposition of the minerals onto the collagen matrix, increasing the hardness of dentin. These results were in accordance with the published studies [15,16]. These researches presented that; PA can increase the remineralization of carious enamel lesions. Xie et al., [17], as well, revealed that, after treatment of carious dentin with PA that is combined with the calcium ions, enhanced remineralization may occur. Moreover, Cheng et al. [18] showed that, the gallic acid, a major constituent of PA, facilitates minerals deposition. Fine [19], also supports these findings demonstrated that, PA has a chelating mechanism with calcium ions, which enhance minerals deposition on the surface of dentin.

These results might reveal the ability of PA as an irrigant solution to recover the obligatory deleterious effects of NaOCl/EDTA on the microhardness of superficial radicular dentin, as it restored the microhardness to the first state of non-significant differences with control group.

Microhardness at 500µm

The values of microhardness in group1 declined apically with significant differences between coronal and apical thirds. Hardness values at 500µm were higher than at 100µm respective to all levels. Although this difference was no significant, it could be attributed to the fact that a greater number of widely opened dentinal tubules are found near the pulp, which offer least resistance to the microhardness testing indenter. These evidences were supported by previous study that reported; the microhardness of dentin is increased when tested from dentin near the pulp to outer regions [4]. In group2, NaOCl/EDTA still caused significant reduction of dentin hardness compared to group1 along the root. Thus, these solutions might penetrate deep into the dentin and negatively affect the minerals contents, consequently minimize the microhardness. According to Saghiri et al. [20], the depth of irrigant penetration might be the key factor that causes reduction in dentin hardness. Along the same line, previous investigation by Slutzky-Goldberg et al. [13] showed a reduction in microhardness at 500µm from the lumen of the root canal in samples irrigated with 6% and 2.5% NaOCl. Additionally, Oliveira et al. [21] concluded that, NaOCl solutions significantly minimize the microhardness of root canal dentin at 500 and 1000µm from the pulp-dentin interface. Baldasso et al. [5] as well, determined that, 17% EDTA was significantly reduce dentin microhardness at 500µm depth.

In group3, although PA caused significant improvement of dentin hardness at 100µm; it did not considerably change the hardness at 500µm. These findings might indicate that, PA failed to penetrate to this depth. Molecular weight (Mw) of the irrigant may affect the penetration of disinfected solutions into dentin. However, the penetration depth of NaOCl (Mw:74.45 g/mole) may be higher than that of the acid fuchsin dye (Mw: 585.54 g/mole). Obviously, this might be related to the difference of molecular weight of the two materials [22]. Notably, PA is a heavy material (Mw: 592.553 g/mole) compared with NaOCl (Mw:74.45 g/mole) and EDTA (Mw: 292.244 g/mole). According to the manufacturers, NaOCl

and EDTA, which were used in this study, contained surfactants that enhance their penetration into dentinal tubules; whereas, PA was used without any surface-active agents. The abovementioned explanation might clarify the limited penetration of PA at 500µm. In fact, more investigations needed to improve the diffusion of PA into dentin.

Conclusion

PA showed a tendency to enhance the microhardness at 100µm of radicular dentin. However, at 500µm, microhardness was not improved.

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