Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 7 Issue 2 February 2023

Research Article

Evaluation of the Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate (Cpp-Acp) on Edta Treated Root Dentin Microhardness when used as Final Irrigant in Single Rooted Teeth - An *Invitro* Study

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DOI: 10.31080/ASDS.2023.07.1562

Received: December 28, 2022

Published: January 11, 2023

Abstract

Aim: The aim of this study was to evaluate the effect of irrigation with CPP-ACP solution on EDTA treated root dentin microhardness when used as a final irrigant in single rooted teeth.

Methodology: Twenty one extracted human single rooted teeth with single canals were used. All teeth were decoronated at 12mm length from the apex. Complete chemo-mechanical preparation was done using NaOCl as conventional irrigation protocol. Teeth were randomly divided into 3 groups (n = 7 in each) according to the final irrigation protocol as follows: Group (A) final irrigation with 17% EDTA for 5 minutes followed by CPP-ACP remineralizing solution for 10 minutes, Group (B) final irrigation with 17% EDTA for 5 minutes, and Group (C) final irrigation with normal saline for 5 minutes. Each root specimen was cut horizontally at 3 different levels (coronal, middle, apical). Vicker's Microhardness Testing was done at three different horizontal depths from the canal lumen (25µm, 50µm, 100µm).

Results: The results showed the highest values of microhardness for group A, followed by group C and B respectively. There was a significant difference in microhardness values between group A and the other groups at the middle third of the root, whereas the difference at coronal and apical thirds was insignificant. In addition, the values of microhardness increased significantly at deeper depths of dentin away from the canal lumen towards cementum in all groups.

Conclusion: Final irrigation with CPP-ACP remineralizing solution may improve mechanical properties of dentin including microhardness, especially after EDTA irrigation for smear layer removal.

Keywords: Smear Layer; Demineralization; Remineralization; Mechanical Properties; Microhardness; Endodontic Irrigation

Introduction

Endodontic treatment aims to remove all sources of infection within the complex root canal system. This is obtained by multiple successive steps that end up with a clean, well-shaped canal, free of necrotic tissue and suitable for receiving a three dimensional seal.

Subsequent to chemo-mechanical preparation, a residual smear layer is formed on the canal walls. This amorphous layer contains organic and inorganic substances, microorganisms and toxins, fragments of odontoblastic processes, and tissue debris [1]. Its generation is inevitable during root canal instrumentation, with the risk of influencing the sealing ability of the sealer-dentin interface and increasing the potential for microbial survival and reproduction. Moreover, it may limit the penetration of irrigants and medicaments into the dentinal tubules. Therefore, removal of smear layer is usually preferred [2].

Current methods of smear layer removal include the use of chelating agents in conjunction with other irrigants to eliminate effectively such a layer. The use of sodium hypochlorite (NaOCl) has a sufficient role in dissolving the organic component, but un-

fortunately it has a very limited action against the inorganic part. Hereby, the critical role of chelating agents is evident, in which they react with the calcium ions in dentin, converting them to soluble calcium chelates [2,3]. Most chelating solutions are based on Ethylenediaminetetraacetic (EDTA) in various concentrations.

Unfortunately, using these solutions alternatively for 5 minutes alters the Calcium: Phosphate (Ca: P) ratio by removing calcium ions from hydroxyapatite crystals in dentin at approximate depths of 20-30µm [4]. This change in ratio affects the original proportion of organic to inorganic components, thus altering dentin's microhardness, solubility, permeability, flexural strength and surface roughness[2,4,5]. Furthermore, it was found that EDTA reduced dentin microhardness to greater extent compared to other solutions regardless of the application time and total amount of the chelating agent [2]. Such changes could influence the adhesive properties of root dentin and decrease root strength and fracture resistance [5,6].

To overcome these alterations in the mechanical properties of dentin, the concept of remineralization was introduced. It is a repair mechanism that aims to restore the mineral content of the tooth structure in ionic forms to the hydroxyapatite crystal lattice [7]. Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) is a milk protein derivative that acts as a biomimetic remineralizing agent [7]. GC Tooth Mousse is a commercially available CPP-ACP that can be used for dentin and enamel remineralization in cases of dentin hypersensitivity, enamel demineralization and initial caries lesions [8].

Although there are many studies that discussed the effect of remineralization of coronal dentin on its microhardness and mineral content [9-11]. To our knowledge, there is lack of studies that investigate the effect of remineralization of root dentin after using chelating agents on its microhardness.

Materials and Methods

The protocol of this in-vitro study was reviewed and approved by the ethics committee (EC), Faculty of Dentistry, Cairo university, with respect to scientific content compliance with applicable rehearse and human subjects and regulation. Approval number was (4-11-20).

Twenty-one freshly extracted single rooted teeth were collected from dental clinic of National Diabetes and Endocrinology Institute in Cairo and Oral Surgery Department, Faculty of Dentistry Cairo University.

Specimens' preparation

Teeth were decoronated at a standardized distance of 12mm from the apex with low-speed sectioning disc. Apical patency was checked with size 15 k-file, until the tip was just visible in the apical foramen. The working length was standardized for 11mm.

Complete chemo-mechanical preparation was done using M3-Pro+ Gold Rotary system (United Medical Group - China) until file size 35 taper 4%. NaOCl 5.25% (5ml) was used as irrigation between every subsequent file, with 30-gauge side vented needle. The teeth were randomly assigned in three groups according to the final irrigation protococl as following

- **Group A (EDTA/CPP-ACP):** Final irrigation with EDTA 17% for 5 min followed by irrigation with CPP-ACP remineralizing agent for 10 min.
- **Group B (EDTA alone):** Final irrigation with EDTA 17% for 5 min.
- **Group C (normal saline):** Final irrigation with normal saline (0.9% NaCl) for 5 min.

Remineralizing solution was prepared by diluting the GC Tooth Mousse (RECALDENT™ GC Corporation, Japan) to 10 times with distilled water [12]. One ml of Tooth Mousse was added to 10 ml of distilled water and mixed thoroughly with spatula until complete homogeneous solution was obtained.

Root specimens were mounted on acrylic blocks and sectioned transversely using a water-cooled precision saw at 3mm, 6mm, and 9mm from the apex respectively to obtain specimens from the apical, middle and coronal thirds of the root. The specimens were polished using silicon carbide discs of decreasing abrasiveness (400, 600, 800, 1200 grit), polished with aluminum oxide paste, cleaned with distilled water after polishing and kept immersed in distilled water to avoid dentin dehydration.

Microhardness testing

Microhardness of dentin (for all groups) was measured by Vickers microhardness tester. Three indentations were done on each sectioned specimen. All specimens from all levels were exposed to 100g load for 10 seconds (100g/10sec) at three different depths from the canal lumen: 25µm, 50µm, and 100µm respectively (Figure 1). The results were expressed as Vickers Hardness numbers (VHN) for all readings.

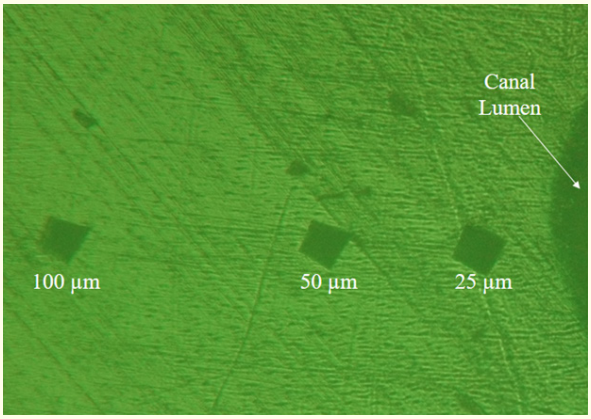

Figure 1: Microscopic image of the three indentations performed at distance of 25, 50, and 100 µm respectively from the canal lumen (dark shadow at the right side is the canal lumen).

Statistical Analysis

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution and using Shapiro-Wilk test. Data showed parametric distribution so one-way ANOVA followed by Tukey's post hoc test was used for intergroup comparisons and repeated measures ANOVA followed by Bonferroni post hoc test was used for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

Results

The mean and standard deviation values of Vicker's microhardness are shown in tables 1-2. Regarding the effect of final irrigation protocol, there was a significant difference between different groups ($p = 0.004$). The highest value was found in EDTA/CPP-ACP (37.10 ± 7.64) followed by saline (34.55 ± 7.80) while the lowest value was found in EDTA (32.77 ± 6.24). Post hoc pairwise comparisons showed EDTA/CPP-ACP to have a significantly higher value than EDTA ($p < 0.001$) (Table 1 and Figure 2).

Micro-hardness (mean ± SD)			P-value
EDTA/CPP-ACP	EDTA	Saline	
37.10 ± 7.64^{A}	32.77 ± 6.24^{B}	34.55 ± 7.80^{AB}	0.004*

Table 1: Mean, Standard deviation (SD) values of micro-hardness for different irrigation protocol0073.

Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

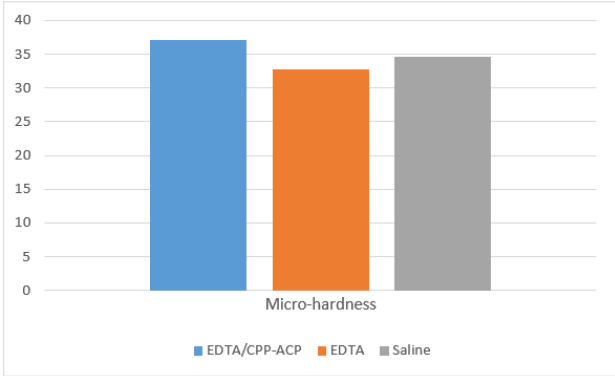

Figure 2: Column chart showing average micro-hardness for different types of irrigation protocols.

Taking in consideration the level of the root, there was a significant difference between the groups at the middle third of the root only, at distances 25µm, 50µm, and 100µm ($p = 0.025, 0.037, 0.019$ respectively) (Table 2 and Figure 3).

Discussion

Smear layer removal dedicates the use of chelating agents that affect the mechanical properties of dentin by decreasing the mineral content of the dental hard tissues. Dentin microhardness stands for the ability of dentin to resist local deformation after removal of a specific load. It can be correlated to other mechanical properties of dentin such as Young's modulus, flexural strength, and fracture resistance [13]. In addition, it has a positive correlation with the inorganic content of the tissue, thus considered as an indirect

Distance from canal lumen	Root section	Micro-hardness (mean ± SD)			p-value
		EDTA/CPP-ACP	EDTA	Saline	
25 µm	Coronal	32.56 ± 4.66^{A}	28.23 ± 3.66^{A}	27.51 ± 2.78^{A}	0.053ns
	Middle	33.70 ± 7.23^{A}	26.24 ± 3.13^{B}	26.16 ± 4.88^{B}	0.025*
	Apical	31.64 ± 8.25^{A}	28.94 ± 6.05^{A}	32.16 ± 7.74^{A}	0.689ns
50 µm	Coronal	36.46 ± 6.91^{A}	33.24 ± 1.92^{A}	35.90 ± 5.88^{A}	0.500ns
	Middle	38.19 ± 6.87^{A}	30.30 ± 4.34^{B}	34.07 ± 4.02^{AB}	0.037*
	Apical	36.11 ± 8.72^{A}	33.91 ± 7.33^{A}	34.77 ± 8.18^{A}	0.878ns
100 µm	Coronal	43.97 ± 8.19^{A}	40.36 ± 3.76^{A}	40.86 ± 6.90^{A}	0.546ns
	Middle	42.29 ± 6.44^{A}	34.97 ± 3.25^{B}	41.74 ± 4.20^{A}	0.019*
	Apical	39.01 ± 3.83^{A}	38.74 ± 5.19^{A}	37.74 ± 9.30^{A}	0.929ns

Table 2: Mean, Standard deviation (SD) values of micro-hardness for different types of irrigation protocols within other variables. Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$).

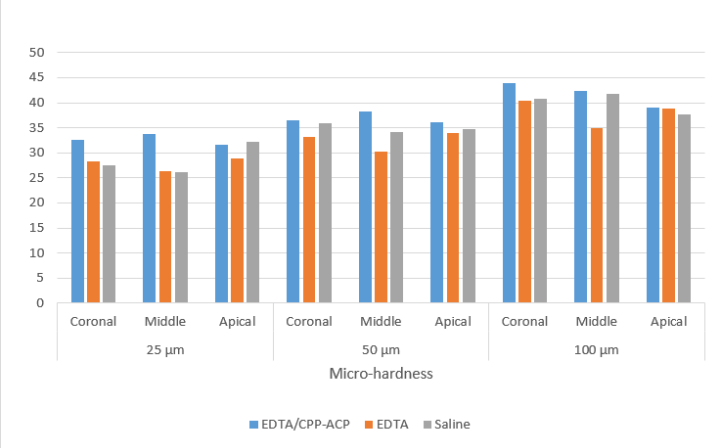

Figure 3: Column chart showing average micro-hardness for different types of irrigation protocols.

indicator of the degree of mineral saturation. Moreover, changes in microhardness values suggest compositional and structural alterations in dentin. However, dentin microhardness is affected by other factors including dentinal tubules density, location in the tooth, distance from the canal lumen, amount of peritubular and intertubular dentin present, tubules direction and degree of dentin sclerosis [14]. In fact, it was found that the mechanical properties of dentin are mainly achieved from the intertubular dentin rather than peritubular dentin. In other words, the majority of changes in dentin microhardness reflect alterations in intertubular dentin [15].

It is well recognized that the density of dentinal tubules decreases by moving apically along the root. Lo Giudice., *et al.* (2015) [16] found that the tubular surface area drops sharply from around 15.47 ± 7.06 μm^2 at coronal third, to around 3.033 ± 2.43 μm^2 api-

cally. Simultaneously, the diameter of each tubule decreases upon moving towards the apical third of the root with narrowing in the tubule lumen. As a result, the amount of peritubular dentin decreases apically, opposed by increase in intertubular dentin. In a similar manner, the dentinal tubules narrow by moving away from canal surface towards the cementum, accompanied by decrease in peritubular dentin and increase in intertubular dentin [17]. These tubules extend from the pulp in a perpendicular direction and run continuously in an oblique direction until reach cementum [18]. Apical dentin has the least tubule density with some areas showing absence of tubules. It has distinctive mineralized tissue exhibiting high degree of sclerosis [19].

Correlating root dentin ultrastructure to microhardness, previous studies have demonstrated that microhardness decreases wherever moving towards the pulp, whereas increases wherever moving away from the pulp towards either enamel or cementum. Liu., *et al.* (2002) [18] suggested that the oblique direction of dentinal tubules within the middle area of root dentin between pulp and cementum is responsible for improved mechanical properties of dentin at that zone. Kinney., *et al.* (1996) [20] found that microhardness of intertubular dentin close to the pulp was significantly less than intertubular dentin far from the pulp. This can explain the difference in microhardness at different distances from the canal lumen in this study.

Results showed an overall significant decrease in dentin microhardness values after final irrigation with 17% EDTA for 5 minutes, when compared to normal saline. This result agrees with previous studies that evaluate the effect of EDTA on root dentin microhardness [21-26]. However, the reduction in microhardness varies between different levels of the root (coronal, middle, and apical), and different depths from the canal lumen (25µm, 50µm, 100µm). These differences can also be explained in the context of dentin ultrastructure. Although the difference was insignificant in the coronal third, there was still a reduction in microhardness at depths 50 µm and 100 µm. The presence of great amount of peritubular dentin coronally close to the pulp could limit the minerals loss caused by EDTA. Peritubular dentin is highly mineralized when compared to intertubular dentin and is more resistant to mineral loss [27]. Therefore, peritubular dentin is less susceptible to the demineralization action of EDTA. Unlike the middle third where there was a significant reduction in microhardness. The relatively greater amount of intertubular dentin could be responsible for serious reduction in Ca/P content. As mentioned earlier, intertubular dentin is considered to be the main determinant of mechanical properties [15], thus, any change in the mineral content of intertubular dentin is supposed to alter microhardness dramatically.

At the apical third, the reduction in microhardness was insignificant. The presence of high degree of dentin sclerosis with great amount of mineralization limited the effect of EDTA [19,28]. The chelating action would be more evident if EDTA was used for a longer time. In addition, the narrow diameter of the canal at the apical third limits adequate delivery of the irrigant till the apex, especially if conventional manual irrigation technique was used [29]. Consequently, the chelating agent had limited ability to remove smear layer, but also had less negative effect on dentin microhardness.

In general, the results revealed a significant increase in microhardness values after final irrigation with the CPP-ACP solution for 10 minutes, when compared with either normal saline or EDTA. The remineralization procedure promoted by CPP-ACP was successful in improving the microhardness of EDTA treated dentin, or even restoring microhardness to its original values before EDTA treatment. Furthermore, most values exceeded their original counterparts in normal saline group. This can be explained by the ability of CPP-ACP to restore the mineral content of dentin and improve the mineral/matrix ratio, thus improving mechanical properties. These results agree with a previous study that evaluated the effect of CPP-ACP on eroded dentin [11] and another one that evaluated the effect of remineralization on EDTA treated dentin microhardness [12].

However, increases in microhardness values were not equally significant at different zones of root dentin. Although there was an increase in microhardness coronally to an extent that exceeded the original value, the difference was insignificant at all distances from canal lumen (25µm, 50µm, 100µm). Similarly, the apical third showed a slight increase in microhardness indicating insignificant difference. On the contrary, the middle third values revealed a significant increase at all depths from canal lumen, and exceeded significantly not just the EDTA group, but also the saline group. These results seem logical and coincide with the variations of dentin ultrastructure discussed previously.

It is suggested that intertubular dentin undergoes higher degrees of remineralization than peritubular dentin. Gu., *et al.* (2010)

[30] evaluated remineralized peri and intertubular dentin and found that remineralized peritubular dentin contained very small nanocrystals of hydroxyapatite (5-10nm), whereas intertubular dentin consisted of larger mineral platelets (50nm). Toledano., *et al.* (20104) [31]. stated that demineralized intertubular dentin provides a well preserved and highly organized collagen scaffold that allows deposition of continuous crystalline platelets. Unlike peritubular dentin which provides a delicate scaffold that disrupts easily. Taking in consideration the various distribution of intertubular dentin in the root, the previous studies justify the findings of this study, in which the maximum remineralization occurred in the middle third of the root where the amount of intertubular dentin is the highest. On the other hand, the presence of larger amount of peritubular dentin coronally could have a role in limiting remineralization capacity.

Conclusion

Within the limitations of this in-vitro study, it can be concluded that final irrigation with the remineralizing solution CPP-ACP can improve microhardness of previously EDTA treated root dentin.

Conflict of Interest

The authors declare no conflict of interest.

Source of Funding

The whole research was self-funded by the first author.

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