

Effect of Etidronic Acid, Chitosan and EDTA on Microhardness of Root Canal Dentin

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Original Research Article

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Article History

Received: 13.04.2018

Accepted: 25.04.2018

Published: 15.04.2018

DOI:

10.21276/sjodr.2018.3.4.5



Abstract: Complete cleaning of the root canal system requires the elimination of organic and inorganic debris including the smear layer. Endodontic irrigation alone is unable to ensure adequate removal of deeply entrenched bacteria in the dentinal tubules. Use of a chelating agent in conjunction with endodontic irrigants is necessary. The most commonly used chelating agent is EDTA. However, it has certain disadvantages when used with Sodium hypochlorite like reduction in dentin microhardness and lesser antimicrobial activity. The search for alternatives to EDTA has brought up newer alternatives like Etidronic acid and Chitosan solution. Clinical studies have proved that these are equivalent to EDTA in certain actions during endodontic treatment. This study aims to compare the changes in dentin microhardness seen when cleaning and shaping of the root canal system is done using EDTA, Etidronic acid and 0.2% Chitosan solution. Results of this study showed that both Etidronic acid and 0.2% Chitosan had comparable effects on dentin microhardness which were similar to EDTA.

Keywords: Chelating ability, Chitosan, Dentin microhardness, EDTA, Etidronic acid, Vickers hardness number.

INTRODUCTION

Biomechanical preparation is one of the most important aspects of endodontic treatment. It involves removal of infected organic and inorganic debris and shaping of the root canal system so as to receive a filling material. This is accomplished by means of endodontic instruments and irrigating solutions. Endodontic irrigants need to have certain ideal characteristics to be effective during the treatment.

No single irrigant possesses all the required properties and hence there is a requirement of combinations and adjunctive aids to improve its efficacy. Whenever instrumentation is done, dentin is cut into small particles of mineralized collagen matrix. This spreads over the surface of the root canal system and is called Smear layer [1]. Particle size ranges from 0.5-15 micron. Complete removal of the debris laden smear layer ensures cleansing of attached microbiota and their toxins from the dentinal tubules [2].

Sodium Hypochlorite (NaOCl) is the most widely used endodontic irrigant and it is used in concentrations from 0.5 to 6%. It has proven antimicrobial activity and tissue dissolving ability. However, when used as a standalone irrigant, it does not remove the smear layer to the full extent [3]. There is a need for an agent which can act in conjunction with NaOCl to remove the smear layer and this is fulfilled by Chelating agents [2]. The term 'Chelate' originates from the Greek word 'Chele' (crab claw). These are stable complexes of metal ions with organic substances due to ring shaped bonds [4]. These agents act on both dentin and the smear layer, leading to exposure of

collagen and reduction in dentin microhardness. This will in turn increase penetration of irrigant into the dentinal tubules thereby improving disinfection. It also reduces dentin microhardness, thereby enhancing the action of endodontic instruments, especially in narrow canals [4].

A liquid solution of Ethylene diamine tetraacetic acid (EDTA) was first employed as a chelating agent. It is also available in gel form, in concentrations ranging from 15-20%. It reacts with Calcium in dentin to form soluble chelates. There have been conflicting reports on the efficacy of EDTA, with some claiming dentin decalcification upto depth of 20-30µ in 5 min while others claim it is ineffective in the apical third [5].

HEBP (1-hydroxyethylidene- 1, 1-bisphosphonate), known as Etidronic acid or Etidronate in pharmacology, is an osteoporotic drug. It is also used in metal industry for anticorrosive effect and to prevent rancidification and oxidation of fatty acids. It has been found to have the ability to chelate metallic ions. It has been suggested as potential alternative to EDTA [6].

Chitosan is a natural polysaccharide obtained from deacetylation of chitin, which is obtained from the shells of crabs and shrimps. It is endowed with properties of biocompatibility, biodegradability and bioadhesion. It has significant antibiofilm efficacy. Studies have found that it has a high chelating capacity for different metallic ions [7].

The objective of this study was to evaluate and compare the effect of three different chelating agents, 20% Etidronic acid, Smear Clear (17% EDTA solution), 0.2% Chitosan nanoparticles solution on microhardness of root canal dentin. The null hypothesis was that all the chelating agents will have the same effect on dentin microhardness.

MATERIALS AND METHODS

Freshly extracted teeth with straight roots extracted for periodontal and orthodontic reasons were collected and those with curvature of roots more than 15 deg, those with root caries and with fluting of roots were discarded. A total of 47 single rooted teeth were finally selected. The selected teeth were decoronated at cement-enamel junction with a diamond disc and cervical preflaring was performed with Gates Glidden drills (Mani, Japan). Working Length (WL) was established with a #10 K file (Mani, Japan) by measuring the length at the point where the tip of the file emerges from the apical foramen. The WL was kept 0.5mm less than this measurement.

Cleaning and shaping was done with Protaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) upto F3 size. Irrigation was done with 2ml 3% Sodium Hypochlorite [NaOCl] (Parcan, Septodont India, Raigad, Maharashtra) at each change of file with a total time of 30 seconds for each flush. Final irrigation was done with 20ml 3% NaOCl. Grooves were prepared along long axis of roots with a diamond disc,

taking care to see that the canal space was not infringed upon. The grooved roots were cleaved longitudinally with a chisel and mallet to separate them into two halves.

The convex cemental root surfaces were flattened using a high speed tapered diamond abrasive point so that adequate wetting of the dentin surfaces is possible. Dentin on the sectioned root surfaces were abraded at 45deg angle for better polishability. The specimens were embedded in dental stone for ease in performing the microhardness testing procedure.

The block sets were randomly assigned to 3 groups of 15 teeth each according to final irrigating solution. Two teeth were kept as controls (n=2).

- Group I- Etidronic acid (n=15) [Sigma Aldrich, Missouri, USA]
- Group II- 17% EDTA (n=15) [Smear Clear, Sybron Endo]
- Group III – 0.2% chitosan solution. This was prepared by mixing Chitosan nanoparticles with 1% acetic acid. (n=15)

50µl of each chelating agent was placed with micropipette on the polished surface of root dentin for 5 min following which it was washed off with saline. Indentations with Vickers microhardness tester were made at 1000µ, 1200µ and 1400µ from orifice and measured. The tester was used under 40 x magnifications with 100g load and 15 sec dwell time. The average lengths of the two diagonals were used to calculate the microhardness value [Fig 1]. The representative hardness value for each specimen was obtained as the average of the results for the three indentations. Obtained data was subjected to statistical analysis using ANOVA test and inter group comparison was done by post hoc Tukey’s.

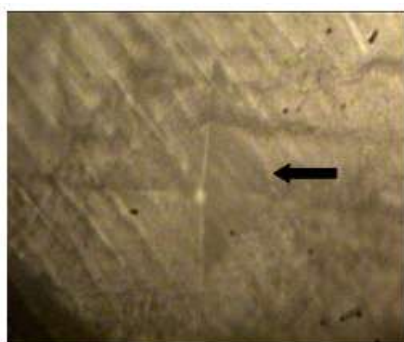


Fig-1: Diagonal obtained during Vickers testing of sample

Table-I: Mean values of VHN for each group at three different levels

	Group I (Etidronic acid)			Group II (EDTA)			Group III (Chitosan)		
Hardness value (VHN)	1000 µ	1200 µ	1400 µ	1000 µ	1200 µ	1400 µ	1000 µ	1200 µ	1400 µ
Mean value	60.5633	58.81	57.5533	61.6240	60.5820	59.5427	62.0373	60.2620	59.7380
Std Deviation	2.51321	2.28179	2.38966	3.48694	2.88291	2.40389	3.58827	3.69499	3.36364

RESULTS

EDTA had better reduction of dentin microhardness when compared with 0.2% Chitosan and Etidronic acid at 1000 μ , 1200 μ and 1400 μ (Table 1) but it was statistically insignificant ($p < 0.05$). 0.2% Chitosan and Etidronic acid had comparable effect on dentin microhardness at 1000 μ , 1200 μ and 1400 μ ($p < 0.05$). Hardness of Control sample estimated was 87.80 VHN at 1000 μ , 85.11 VHN at 1200 μ & 84.9 VHN at 1400 μ .

DISCUSSION

NaOCl is one of the most widely used endodontic irrigants. It has certain advantages like tissue dissolving ability, wide spectrum antimicrobial activity especially against Enterococcus, Actinomyces and Candida [8]. In a study on infected dentin blocks, NaOCl was found to eliminate *E faecalis* in 15 min at a concentration of 0.25%. Necrotic tissue in the root canal system can be dissolved even with lower concentrations [9]. However, penetration of NaOCl into dentin tubules and lateral canals depends on removal of the smear layer and necrotic dentin, thereby allowing access to the deeply entrenched bacteria. Hence, uses of adjunctive aids like chelating agents come into the picture [2].

EDTA was one of the earliest chelating agents and was introduced in 1957 [2]. These react with dentin and create a stable calcium complex comprising of dentin debris, smear layer and calcific deposits. These can be easily removed by instrumentation so that disinfection of the root canal system can be aided. EDTA retains its Ca complexing ability when mixed with NaOCl but reduces the amount of Chlorine in NaOCl. This results in reduced tissue dissolving capacity (upto 4%) [5]. Short term use of NaOCl after the use of EDTA results in strong erosion of canal wall dentin. It has been found that dentin is decalcified upto depth of 20-30 μ m in 5 mins [7].

The recommended pH for chelating action of EDTA should be around 7.3. During their action, the release of the acid takes place by removal of calcium from dentin and replacing it with hydrogen. The efficacy of EDTA decreases with time which is a potential disadvantage. Also, the reaction of the acid with hydroxyapatite affects the microhardness of dentin [10]. Hülsmann and Hahn demonstrated that EDTA solutions demineralized dentin up to a depth of 50 μ m per canal wall [11].

Etidronic acid (HEBP) has little short term action on the action of NaOCl. Also, demineralization with HEBP is significantly slower than that of 17% EDTA [12]. HEBP-calcium chelation from root canals depends on the concentration of HEBP in solution. With 20% HEBP solution, the amount of calcium ion complexes removed from the root canals was found to be similar with 17% EDTA or 10% citric acid [13]. In this study, 20% HEBP has been used. HEBP maintains antimicrobial properties of NaOCl solution. It also has

antimicrobial properties of its own which is an added advantage. One of the beneficial actions of HEBP is that it optimized bonding quality of resin based sealer during obturation [6].

Chitosan acts with its functional phosphate groups reacting with dentin Ca ions leading to formation of Ca phosphate layer. It improves resistance of dentin to degradation by Collagenase and previous studies have showed that irrigation with 0.2% chitosan for 3 mins effectively removed smear layer [14]. Chelating effect is greater than that of 10% citric acid [14]. In our study, 1% acetic acid was used to prepare 0.2% Chitosan solution. This solution also acts on inorganic portion of smear layer, helping its removal during instrumentation.

According to the results obtained, all the agents had negative effect on dentin microhardness. All the agents had better effect at 1400 μ . This could be due to the reducing thickness of dentin layer towards the apex. EDTA had better reduction of dentin microhardness when compared with 0.2% Chitosan and Etidronic acid at 1000 μ , 1200 μ and 1400 μ but it was statistically insignificant ($p < 0.05$), thus disproving the null hypothesis. 0.2% Chitosan and Etidronic acid had comparable effect on dentin microhardness at 1000 μ , 1200 μ and 1400 μ .

CONCLUSION

Disadvantages of the commonly employed chelating agent, EDTA when used with NaOCl can be minimized by using 0.2% Chitosan solution and Etidronic acid. 0.2% Chitosan solution has a reduced contact angle which enables it to penetrate better into the dentin to improve its effects. Therefore, 0.2% Chitosan solution and Etidronic acid can be thought of as alternatives to EDTA when used as a chelating agent when used along with NaOCl.

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