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Comparative Evaluation Of Microhardness Of Root Dentin After Irrigation With Different Chelating Agents – An Invitro Study

Dr.V. Pavithra Devi [1], Dr. Vinoo Subramaniam[2], Dr. Vinay Kumar. G[3], Dr. Sibi Swamy[4], Dr. S.Surya[5], Dr. K. Abinaya[6]

[1]*postgraduate Student, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*
[2] *Professor, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*
[3]*hod, Professor, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*
[4]*reader, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*
[5]*senior Lecture, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*
[6]*senior Lecture, Department Of Conservative Dentistry And Endodontics, Rvs Dental College And Hospital, Coimbatore, Tamil Nadu, India*

Abstract:
Background*: Chelating agents used for removing the smear layer shows reduction in root dentin microhardness and can impact the sealer's adhesion to root dentin walls. Reduced dentin microhardness weakens the root structure, resulting in the fracture of the endodontically treated tooth. In this study, chelating agents from natural sources have been used to assess the root dentin microhardness. There are no previous studies comparing 10% Glycolic acid and 0.2% Chitosan as final irrigants regarding root dentin microhardness. The aim of this study is to evaluate the microhardness of root dentin after irrigation with different chelating agents utilizing Vickers microhardness analysis.*
Materials and Methods*: Thirty freshly extracted mandibular premolars were decoronated at the cementoenamel junction. Teeth were instrumented and irrigated with 3% sodium hypochlorite and distilled water. The roots were split into two halves with a chisel to obtain 60 samples, and they were embedded in acrylic resin. 60 samples were randomly divided into four groups, and their baseline microhardness was evaluated at coronal, middle and apical third. Then the specimens were soaked in test irrigants for 3 minutes, based on their assigned groups. Group I - Distilled water, Group II - 17% ethylenediaminetetraacetic acid (EDTA), Group III - 10% Glycolic acid, and Group IV - 0.2% Chitosan. The samples were washed with distilled water, and microhardness was evaluated.*
Results*: Group II-17% EDTA showed the highest mean reduction in root dentin microhardness (31.18), followed by group IV- 0.2% chitosan (23.84), group III - 10% glycolic acid (13.99), and group I -Distilled water (4.32). Among the tested chelating agents, 10% GA showed the least microhardness reduction at all the three levels of root dentin.*
Conclusion: *Glycolic acid (10%) is a promising final irrigant for removing the smear layer, without compromising the root dentin mechanical properties.*
Key Word*: Chitosan, Glycolic acid, Microhardness*

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I. Introduction

The success of root canal treatment depends on balancing biological goals and mechanical objectives[1]. During root canal instrumentation, a layer of granular material forms on the root dentin, known as the "smear layer." Sharvan et al. stated that thorough elimination of the smear layer prior to obturation, facilitates intimate adhesion of the sealer to the root dentin surface and augments the antibacterial effectiveness of root canal irrigants[2-3].

Irrigation is the only means to reach areas inaccessible to mechanical instruments to remove the smear layer[4] The smear layer cannot be completely removed by any of the presently available root canal irrigants. An alternative use of sodium hypochlorite (NaOCl) and a potent chelating agent is recommended for complete removal of the smear layer[5].

During endodontic procedures NaOCl, a halogenated compound, is commonly employed for root canal irrigation. It has antimicrobial action and the ability to dissolve the pulpal remnants and organic components of dentin, but lacks smear removal efficacy[6].

The most commonly utilized chelating agent for removing the smear layer is EDTA. Baumgartner and Mader showed that sequential use of EDTA and NaOCl resulted in peritubular and intertubular erosion, which may increase the risk of vertical tooth fracture[7]. This may be attributed to the alternating action of EDTA, which demineralized the inorganic portion and NaOCl, which dissolved the organic part of dentin[7]. In addition, EDTA interacts with NaOCl and reduces the available free chlorine, thus affecting the dissolution and antibacterial ability of NaOCl[8].

Therefore, there is a need to identify an effective chelating agent from natural sources that removes the smear layer without affecting the root dentin's mechanical properties.

Glycolic acid (GA) belongs to the group of alpha-hydroxy acids and is derived from sugar cane, beetroots, grapes, and other sweet vegetables. GA was initially utilized to prepare the dentin surface for restorative procedures. Subsequently, research explored its potential as an irrigating solution with varying concentrations ranging from 5 to 17%. Bello et al. found that 10% GA effectively removed the smear layer, comparable to EDTA and Citric acid but with lower cytotoxicity. Furthermore, GA exhibited superior antibacterial properties against Enterococcus faecalis compared to EDTA and Citric acid. Additionally, GA induced minor changes in the apatite/collagen ratio of dentin without impacting the flexural strength of dentin[9-10].

Chitosan is a natural polysaccharide, a non-toxic cationic biopolymer usually obtained by alkaline deacetylation of chitin. It has a chelating capacity and antimicrobial effects[11].

Microhardness was assessed to indirectly evaluate mineral content and structural changes caused by chelating agents on radicular dentin and their subsequent impact on the hard tooth structure, such as root fracture, adhesion, and sealing ability to the root canal wall[12].

There are no previous studies comparing the natural chelating agents, (10% GA and 0.2% chitosan) with proven smear removal efficacy regarding root dentin microhardness. Hence, in this study, we compared the effects of different final irrigating solutions: 17% EDTA (Desmear), 10% glycolic acid (Molychem), and 0.2% chitosan (Panvo Organics) on root dentin microhardness.

II. Material And Methods

Subjects & selection method: 30 freshly extracted, single rooted mandibular premolars, for orthodontic reasons was collected from the department of Oral and Maxillofacial Surgery, RVS Dental college and Hospital Sulur.

Inclusion criteria:
30 single rooted mandibular premolars extracted for orthodontic treatment.

Exclusion criteria:
Teeth with two canals
Open apex
Caries
Cracks

Preparation of tooth specimens:

All the samples were decoronated, and the root length was standardized to 16 mm. After gauging with the #10K file, the working length was estimated to be 1 mm short of the apical foramen with the #15K file.The canals were instrumented in a standardized crown-down manner using rotary ProTaper Gold (Dentsply, Maillefer, Switzerland) at 250 rpm up to the F3 file according to the manufacturer's instructions. Irrigation was performed with 5 mL of 2.5% NaOCl between each file, and final irrigation with 5-mL distilled water was performed to remove any residue[10].

A diamond disk was used at low speed to make longitudinal grooves on the buccal and lingual surfaces of each root without reaching the canal. The roots were then split into two halves with a chisel to obtain 60 samples. The specimens were mounted in autopolymerizing acrylic resin (DPI), with the root canal facing upwards. The specimen surfaces were polished using silicon carbide paper in the following order: 400, 800, and

1200 grit under cold distilled water. Finally, samples were washed with distilled water and dried with an aspiration cannula[10].

60 samples were randomly divided into 4 groups each containing 15 samples.
- GRP 1(Control) –Distilled Water (pH 6.80)
- GRP 2 - 17% EDTA (pH 7.17)
- GRP 3– 10% Glycolic acid (pH 4)
- GRP 4- 0.2 % Chitosan (pH 5)

Microhardness evaluation

Before application of the solutions, baseline microhardness values of the specimens in each group were measured with a Vickers microhardness tester (Mitutoyo Corporation, Yokohama, Kanagawa, Japan) using a 200 g load and a dwell time of 10 seconds. Three distinct indentations were created 0.5 mm from the root canal wall on the cervical, middle, and apical regions of the root dentin in each specimen using Vickers diamond indenter [13].

Irrigation protocol

- According to the group allocated, the specimens were completely immersed in the test solutions for 3 minutes. After this period, samples were washed with 5 ml of distilled water and dried with an aspiration cannula.
- Following treatment, the post-treatment microhardness values of the specimens in each group were measured with Vickers microhardness tester. Three indentations were made at the symmetrical points of the previous ones on the opposite side of the root canal[13].

Statistical analysis

Statistical analysis was performed using software SPSS version 24.0. For comparing baseline and post-treatment Vickers microhardness data was carried out using the student t-test. Intragroup comparisons between the four groups were carried out using one-way analysis of variance (ANOVA).

III. Result

The pretreatment and posttreatment Vickers microhardness values (mean ± standard deviation) for all tested specimens at the cervical, middle, and apical levels are listed in the Table 1.

The mean % reduction for all the groups is represented in Table 2. Mean reduction in Vickers microhardness value for different groups is represented in Graph 1

All the chelators on application, reduced the root dentin microhardness at all the levels. Although the percentage reduction in microhardness was less at the apical third than at the cervical and middle third, with statistically significant difference ($P < 0.05$).

Group II-17% EDTA showed the highest reduction in root dentin microhardness, followed by group IV- 0.2% chitosan, group III - 10% glycolic acid, and group I -Distilled water. Among the tested chelating agents, 10% GA showed the least microhardness reduction at all the three levels of root dentin.

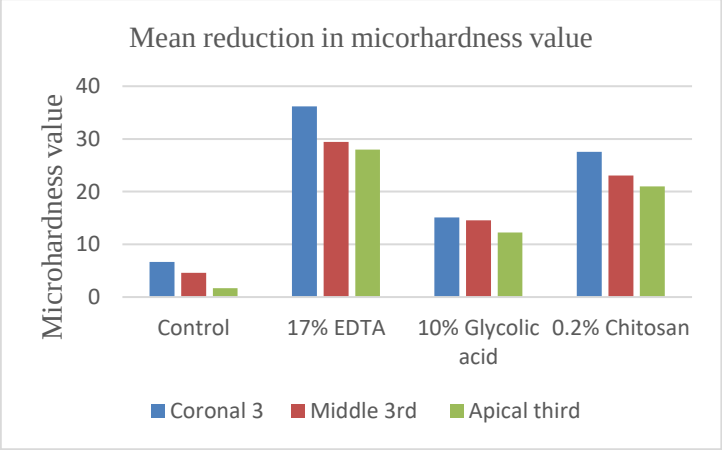
Table 1 - Mean pretreatment and posttreatment Vickers microhardness value of radicular dentin at different levels.

Groups	Cervical third			Middle third			Apical third		
	Before Mean ±SD	After Mean ±SD	Mean reduction	Before Mean ±SD	After Mean ±SD	Mean reduction	Before Mean ±SD	After Mean ±SD	Mean reduction
Control	**63.28 ± 2.29**	**59.04 ±3.26**	**6.68**	**65.03 ± 3.57**	**62.02 ± 2.47**	**4.63**	**67.02 ± 3.15**	**65.90 ± 6.40**	**1.67**
17% EDTA	**64.33 ± 2.69**	**41.06 ±1.66**	**36.16**	**67.69 ± 2.68**	**47.77 ± 4.27**	**29.42**	**69.87 ± 2.72**	**50.33 ± 2.12**	**27.96**
10% Glycolic acid	**63.566 ±2.14**	**53.94 ±2.98**	**15.13**	**66.10 ± 2.40**	**56.46 ±2.29**	**14.58**	**67.31 ±2.81**	**59.04 ±3.26**	**12.28**
0.2% Chitosan	**64.74 ±2.60**	**46.92 ±3.71**	**27.53**	**67.48 ±2.78**	**51.93 ±2.18**	**23.03**	**68.84 ±3.27**	**54.40 ±2.29**	**20.98**

Table 2- Mean reduction of Vickers microhardness values of root dentin for each group after immersion in test groups

Groups	Mean reduction	P value
Control	4.32	< 0.05
17% EDTA	31.18	
10% glycolic acid	13.99	
0.2% chitosan	23.84	

Graph 1- Mean reduction in Vickers microhardness value for different groups.



IV. Discussion

Chelating solutions reduce the mineral and non collagenous protein components of the dentin, leading to surface softening, and they also impact the sealer's adhesion to root dentin walls by changing the calcium to phosphorus ratio[13-14]. Pawlicka found that chelators used to remove the smear layer may alter the root dentin microhardness[15]. The reduction in root dentin microhardness can be unfavorable because it may induce reduction in root dentin elasticity, and flexural strength, compromising tooth fracture resistance[16].

For the evaluation of microhardness, Vickers microhardness tester was chosen over the Knoop hardness tester because of its in-depth ability to evaluate surface changes in dental hard tissues[17]. For the evaluation of reduction in hardness, standardization was done by estimating the pretreatment hardness of every sample and then comparing it with the posttreatment hardness.

According to Pashley *et al.*, there is an inverse relationship between dentin microhardness and tubular density. Carrigan *et al*. showed that tubule density decreased from cervical to apical dentin[18]. Based on these findings, since root dentin microhardness varies at different levels, indentations were made 0.5 mm from the root canal wall in the cervical, middle, and apical thirds of the radicular dentin.

Calt and Serper found that during cleaning and shaping procedures, the final irrigants were effective at a contact time of 3 minutes and even increasing the contact duration did not result in superior smear layer removal outcomes[7]. So, in this present study, the time of action of the final chelating agents was fixed at 3 minutes.

After immersion in the chelating solution, all the specimens showed a reduction in microhardness, which indicates the potent direct effects of these chemical solutions on the components of dentin structure[13]. Intragroup comparison of tested solutions shows a significant reduction in microhardness at all three levels. However, the percentage reduction was found to be least at the apical level ($P > 0.05$). This could be attributed to the composition of the apical region, such as dentin sclerosis, the low content of non-collagenous protein, and cementum-like tissue. In addition to this, there is less penetration and limited contact time for chelating solutions in the apical third[19].

In this study, among the tested chelating agents, mean dentin microhardness reduction was significantly greater in the 17% EDTA (31.18) followed by 0.2% chitosan (23.84), and 10% glycolic acid (13.99) which showed the least reduction in microhardness.

The chelating mechanism of EDTA significantly reduced dentin microhardness, which was in accordance with De Deus et al. This is attributed to the substantial loss of mineral content and hydroxyapatite in the intertubular dentin, which negatively impacts the hardness of the human dentin structure[20].

Glycolic acid is an organic, biodegradable acid with a low molecular weight and pH stability. Its chelating efficiency is attributed to its acidic pH and surface tension. In this study, 10% GA resulted in a

significantly lesser microhardness reduction. This was in accordance with a previous study by Bello et al., who noted that reductions in microhardness and changes in collagen/apatite ratios are associated with glycolic acid concentration, and it was proven that 10% GA is the most appropriate concentration with effective smear removal efficacy[10].

Chitosan is a natural polysaccharide that possesses chelating capacity for various metal ions. In this study, 0.2% Chitosan resulted in lesser reduction in microhardness than 17% EDTA, which was in accordance with a previous study by Ratih *et al.,* who reported that 0.2% Chitosan nanoparticles had a smear removal efficacy similar to 17% EDTA but demonstrated higher microhardness and lower surface roughness[21-22].

There are two theories attributed to its chelating mechanism. The first is the bridge model, in which two or more amino groups on the chitosan chain bind the same metal ion. According to the second theory, just one of the amino groups on the chitosan chain is bonded to the metal ion[21]. Chitosan and metal ion complexes were formed due to ion exchange, chelation, and adsorption[23].

In the present research, chitosan was dissolved in 1% acetic acid to form the solution. A previous study by Silva et al. concluded that the chelating ability of the chitosan solution is higher when solubilized in acetic acid[24]. It could be speculated that the chelating effect observed in this study would be due to the acid and not because of chitosan. However, previous research indicated that 5% acetic acid had a lower efficiency than 15% EDTA for decreasing dentin microhardness, eliminating the smear layer, and chelating calcium ions in the root canal. As a result, the impact of chitosan on dentin microhardness is mostly related to the material and not to the acid[25].

In this present study, all the test irrigating solutions showed a reduction in root dentin microhardness compared with the control group. However, 10% glycolic acid showed the highest mean root dentin microhardness, which was in accordance with previous studies.

V. Conclusion

According to the results obtained in the study, it was concluded that 10% Glycolic acid is a promising alternative as a final irrigant in endodontic therapy due to its effective smear layer removal without altering the dentin mechanical properties

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