



EFFECT OF FOUR ENDODONTIC CHELATING AGENTS (SMEAR CLEAR, 1% PHYTIC ACID, 0.5% PHYTIC ACID AND Q-Mix) ON PUSH-OUT BOND STRENGTH OF ROOT DENTIN.

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ABSTRACT

Aim: The present study aims at evaluating the push-out bond strength of dentin-sealer interface after final irrigation done with chelating agents- Smear clear, 0.5% Phytic acid 1% phytic acid and Q-Mix 2 In 1 where phytic acid can be a potential adjuvant to EDTA as a root canal chelating agent. **Materials And Methods:** 60 single-rooted permanent mandibular premolars freshly extracted were collected and randomly distributed into four groups, (15 in each). After denudation of crown portion of the teeth below the cement-enamel junction leaving the remaining root working length at 15 mm biomechanical preparation was done. Based on the irrigating solution used they are divided into four experimental groups as follows; Group I, Group II, Group III and Group IV followed by obturation with AH plus sealer. From the experimental groups, 2mm thick specimens at coronal, middle, and apical thirds of each root slices were obtained and they were subjected to pushout bond strength using a universal testing machine to assess the maximum failure load. **Results:** Statistically, data were analyzed by one-way variance analysis (ANOVA) and p value < (0,005), considered as significant. Group II (0.5 % phytic acid), III phytic acid (1 % phytic acid), irrespective of concentration, showed the strongest bond strength in all the regions having statistically significant results ($P < 0.05$). **Conclusion:** Phytic acid shows to be an efficient and biocompatible alternative as a chelating agent.

KEYWORDS : Phytic acid, Push-out bond strength, QMix 2in 1, Smear layer.

INTRODUCTION:

Endodontic treatment is successful when the root canal system is thoroughly prepared chemomechanical along with obturation three-dimensionally, sealing the areas formerly occupied with the canal substitutes.^[1] Primary goal of chemomechanical endodontic preparation is to completely eliminate critical tissues, necrotic debris, and microorganisms from the root canal to eradicate any irritant or bacterial substrate.^[2] During chemomechanical preparation, a 1 to 2 mm thick amorphous and porous layer (smear layer) formed on the root-dentinal walls, smear layer contains both inorganic and organic compounds of dentin debris, fragments of odontoblastic processes, necrotic pulp tissues, bacteria and their by-products.^[3] Smear layer should be eliminated with supplementary chemicals to better intracanal medication diffusion and sealer adaption to the dentinal walls. The most efficient method for eliminating the smear layer right now is irrigation, which uses various chelating agents to wash away loose, necrotic, and contaminated materials before reaching further into the root canals and apical tissues.^[4]

Sodium hypochlorite (NaOCl) 0.5% to 5.25% is routinely used irrigating solution in dissolving the organic portion of the smear layer and bacteria that come into direct contact with the irrigant.^[5,6] Smear layer containing inorganic matter in deepest portion of the dentin and dentinal tubules cannot be removed adequately by solution alone. It is necessary to combine it with chelating agents that act on both smear layer

and inorganic matter. While the function of removing the smear layer has been mostly contested, endodontic literature on the antibacterial action of irrigants suggests that using EDTA and NaOCl together is more effective than using NaOCl alone when testing bacterial survival after numerous sessions.^[7]

The chelating agent ethylenediaminetetraacetic acid (EDTA) is specifically manufactured from ethylenediamine, formaldehyde, and sodium cyanide. Dentine calcium ions react with EDTA to generate soluble calcium chelates within 5 minutes, dentin is decalcified to a depth of 20-30 μ m.^[8]

SmearClear (SybronEndo, Orange, CA) composed of 17% EDTA solution with cetrimide (a quaternary ammonium compound), as well as a proprietary surfactant (polyoxyethyleneisooctylcyclohexyl ether) which lowers surface tension and improve endo-canalary cleaning capacity.^[9]

Agents like EDTA are likely to cause significant harm and hinder the healing process to the periapical tissues.^[9] Since EDTA is not easily biodegradable, there have been some worries concerning leaks of this irrigant into the periapical region, and its extrusion outside of the root canal should be prevented.^[8]

These circumstances need the use of an alternative to EDTA in the removal of the smear layer, and phytic acid was

considered as a more novel biocompatible chemical.

Since phytic acid (IP6, inositol hexakisphosphate) has multiple negative charges, it is an efficient chelator of multivalent cations like calcium (Ca²⁺), magnesium, and iron, which are acidically soluble but precipitate in the neutral pH. Phytic acid is a major storage form of phosphorus in plant seeds and is extracted from rice bran.^[10] IP6's pharmacological effects are dependent on both its ability to bind to specific proteins and chelate multivalent cations like Ca, which results in the formation of the very insoluble Ca-phytate salt.^[11]

When compared to Phosphoric acid or EDTA, it demonstrated greater biocompatibility to pulpal- and osteoblast-like cells. Due to its ability to chelate with Ca²⁺, IP6 has been demonstrated to be a useful agent for removing the smear layer. IP6-treated dentinal tubules were open, intertubular collagen was visible, dentin surfaces were devoid of smear layer and smear plugs.^[10]

It has been suggested as a potential substitute for EDTA for effectively eliminating the smear layer with the least amount of damage to osteoblast cells. IP6's unique structure and properties make it suitable for a range of dental applications, such as endodontic irrigants and dental adhesives.^[12,13]

An antibacterial endodontic irrigation solution for removing smear layers is QMix 2in1 (Dentsply Tulsa Dental, Tulsa, OK), it includes the components such as EDTA, chlorhexidine, detergent, and water. The QMix's chemical composition prevents precipitation from happening when combined with CHX in EDTA or NaOCl.^[14]

The AH Plus sealer system has two pastes, that is based on the AH 26. Because of its good biocompatibility, decreased tooth staining, faster setting time, little formaldehyde release, long-term dimensional stability, reduced solubility, apical sealing capabilities, low cytotoxicity, least weight loss in the solubility tests and, micro retention to root, this is the most widely utilized material.^[15]

The primary goal of this study is to assess phytic acid's chelating potential by evaluating the pushout bond strength of teeth treated with phytic acid and various irrigating solutions. The final determinant of the success of root canal treatment is proper seal of the canal walls, according to multiple studies, 17% EDTA is effective in eliminating the smear layer and increasing push-out bond strength; nevertheless, demineralization of root dentin and periapical toxicity of EDTA leads to the search for a possible alternative chelating agent.

In this study, previously considered efficient 17% EDTA is compared with a naturally available plant seed extract Phytic acid for efficient smear layer removal, to increase pushout bond strength, and to minimize previously compromised properties such as demineralization of root dentin and periapical toxicity, making the study novel.

Methodology:

Sample Size Calculation:

Sample size: no of samples (specimens required by each group in the study was estimated based on the information available from the study conducted by Ravikumar J, Bhavana V, Thatimatla C, Gajjarapu S, Reddy SG, Reddy BR(2014)

The non-centrality parameter, 14.2, was used to statistically compute the sample size using the g power programme.

n: Sample size per group

The final sample size would be 15 in each subgroup.

The findings will be shown as the mean and standard deviation.

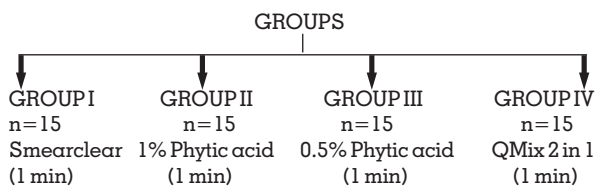
One-way analysis of variance will be used to compare the continuous variables (ANOVA).

Specimen Selection:

Sixty single-rooted permanent mandibular premolars were extracted for periodontal reasons, cleaned, and preserved in normal saline. Inclusion criteria involved a single, completely developed, straight root, teeth with similar morphology, similar length, without any cracks, fractures, non-carious, and haven't received any restorative or endodontic treatments. exclusion criteria involve teeth with decayed, fractured, calcified, accentuated curvature, resorptions evaluated radiographically in the mesio-distal and buccolingual directions.

Distribution Of The Specimens:

The present study was kept double-blind to eliminate possible biases. The groups were coded as Group I, II, III, and IV. A person other than the operator who was not part of the trial did the randomization. Computer-generated random codes with a permuted block randomization scheme were generated with a Research randomizer form version 4. The samples were allocated to different groups as per the randomization sequence. Randomization was done such that each group contain 15 specimens (n=15).



Specimen Preparation:

Decoronation [Figure.1] was completed with a diamond disc under water cooling, with the remaining root working length set at 15 mm using a 10-size K-file. The Flexicon#40/.06 rotary file system was used for biomechanical preparation. Irrigation began with 5% NaOCl, followed by 5 ml of distilled water for 10 seconds.



[Figure 1]: Decoronated Samples

METHOD:

Group I (n=15): After cleaning and shaping, all the samples are treated with Smearclear to eliminate smear layer for a minute, then washed with distilled water. All samples were dried using absorbent paper points [Figure.2a].

Group II (n=15): After cleaning and shaping, all the samples

are treated with 1% Phytic acid to eliminate smear layer for a minute, then washed with distilled water. All samples were dried using absorbent paper points [Figure.2b].



[Figure.2a]: Smear Clear



[Figure.2b]: Phytic acid 1%

Group III (n=15): After cleaning and shaping, all the samples are treated with 0.5% Phytic to eliminate smear layer for a minute, then washed with distilled water. All samples were dried using absorbent paper points [Figure.2c].



[Figure.2c]: Phytic acid 0.5%

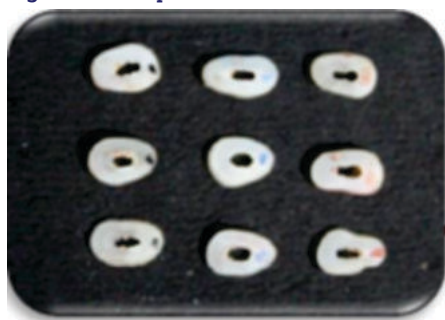
Group IV (n=15): After cleaning and shaping, all the samples are treated with Q MIX- 2 in 1 to eliminate smear layer for a minute, then washed with distilled water All samples dried using absorbent paper points [Figure.2d].



[Figure.2d]: QMIX 2in 1

Obturation processes were carried out by cold lateral compaction with gutta-percha points and AH plus sealer. To guarantee the full set of the sealer, the samples from the four groups were kept in the incubator at 37°C for a week.

Sectioning Of The Samples:



[Figure.3]: Tooth Slices Of 2mm

After the sealer had been completely set, 2mm thick slices were taken from the coronal, middle, and apical thirds of each root using a diamond disc under water cooling, and the first slice of each third was chosen for the push-out test [Figure.3]. The slices are kept on plastic moulds designed for push-out bond strength. Metallic plungers of 1mm, 0.5mm & 0.3mm are

attached to the universal testing machine for pushout bond strength determination. Push-out tests were carried out using a Universal Testing Machine with a crosshead speed of 1 mm/min. The pushout bond strength in MPa was estimated by dividing the maximum load in newtons by the cross-section of the bonded contact.

Statistical Analysis:

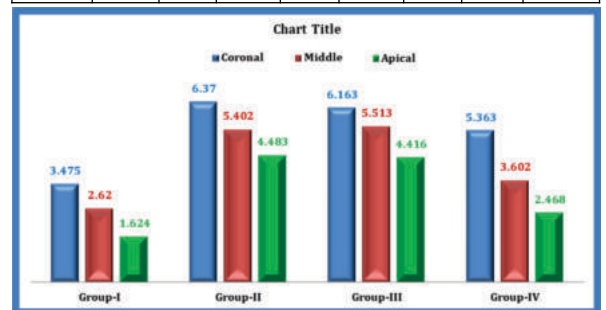
Statistical analysis of the outcomes are from the software SPSS statistics 21.0. The differences between the mean beginning and end values of the groups were statistically analyzed. The statistical study utilized One Way Analysis of Variance (ANOVA) to compare the various group values (p value). Statistical significance was regarded to be shown by a P value of 0.05.

RESULTS:

The experimental groups [Table.1], [Graph.1] showed a difference between the groups in the in-vitro analysis of the coronal, middle, and apical areas that was statistically significant (p<0.005).

[Table.1]: Mean Push-out Bond Strength Of All The Groups With Standard Deviation In Coronal, Middle And Apical 1/3rds.

Group	Coronal		Middle		Apical		F-value	P-value
	Mean	SD	Mean	SD	Mean	SD		
Group-I	3.475	0.524	2.620	0.336	1.624	0.400	70.48	<0.001
Group-II	6.370	0.751	5.402	0.383	4.483	0.352	47.99	<0.001
Group-III	6.163	0.647	5.513	0.420	4.416	0.405	46.21	<0.001
Group-IV	5.363	0.442	3.602	0.834	2.468	0.384	92.18	<0.001



Group I (n=15): Smear clear

Group II (n=15): 1% Phytic Acid

Group III (n=15): 0.5% Phytic Acid

Group IV (n=15): Q Mix- 2 In 1

[Graph.1]: Bar graph representing the mean values of pushout bond strength of all groups in coronal, middle, apical 1/3rd.

GROUP I had the lowest values in the coronal, middle, and apical thirds, with mean values of (3.475, 2.62, and 1.624) correspondingly. The values of pushout bond strength were reduced from the coronal, middle, and apical thirds of the root. GROUP IV had the lowest values in the coronal, middle, and apical thirds, with mean values of (5.363, 3.602, 2.468) correspondingly. The values of pushout bond strength were reduced from the coronal, middle, and apical thirds of the root.

With mean values of 6.370, 5.402, and 4.483 in Group II and (6.163, 5.513, 4.416) in Group III, respectively, Groups II (1% phytic acid) and III (0.5% phytic acid), regardless of concentration, showed the maximum push-out bond strength at (coronal, middle, and apical) root thirds. The values of pushout bond strength reduced from the root's coronal, middle, and apical thirds.

The greatest values in the coronal third were obtained with groups II (1% phytic acid) with a mean value of 6.37 and III (0.5% phytic acid) with a mean value of 6.16, while the lowest values were seen with group I (smear clear) with 3.47.

Group III had the greatest values in the middle third, followed by Group II with values of 5.513 and 5.402, while Group I had the lowest values with a value of 1.642.

Group II had the highest values in the apical third, followed by Group III, with values of 4.483 and 4.416, respectively. Group I had the lowest results, with a value of 1.624.

When all groups were examined at the coronal, middle, and apical areas, the coronal third had the strongest bond strength compared to the middle and apical thirds, while the apical third had the lowest values.

The apical third of the phytic acid group, regardless of concentration, had the strongest bond strength when compared to smear clear, QMix 2in1 and are statistically significant with findings ($P < 0.05$).

DISCUSSION:

A study could be considered novel if it is the first of its kind to investigate a particular aspect of the irrigating solution, if it uses a new or unique method for testing the solution, or if it provides new insights or findings that challenge previous understanding in the field. Even a little amount of EDTA solution extruded through the apical constriction caused permanent decalcification of the periapical bone and may have an impact on the neuroimmune regulatory systems. Even at quantities lower than the 10% used in endodontics, EDTA inhibits the binding of vasoactive interstitial peptides to macrophages. In accordance with Mohannad Issa Michael Nassar (2015), EDTA, a pollutant that is synthetic and not easily biodegradable, can have some cytotoxic effects on osteoblast cells if it protrudes over the apical foramen, which will impede the healing of the periradicular region following root canal therapy. So due to biocompatibility issues and cytotoxicity when accidentally extruded beyond the apex into the periapical area, it shows some adverse effects.^[12,16,17] Other agents which can remove the smear layer efficiently are used in the current study. The present study is considered novel as it is a pioneer study to compare the different concentrations of phytic acid (0.5%, 1%) effect on the root dentin along with other chelating agents Smear clear, Q-MIX 2 in 1 which were promising irrigants which are in use.

In the current investigation, Smear clear used contains cationic surfactants which reduce surface tension and fluid viscosity, increasing the permeability of dentinal tubules to irrigating solutions. Smear clear allows the chelating solution to reach the full depth of the canals while also providing antibacterial and fungicidal characteristics.^[18,19]

Surfactants in smear clear had no effect on binding strength when compared to 17% EDTA. The use of surfactants does not modify the qualities of EDTA-based formulations, and smear clear was shown to be as efficient as EDTA in removing smear layer residue from the dentinal root wall.^[18] As a result, smear clear was chosen for this investigation. But in this study the ability of EDTA to remove the smear layer did not improve when surfactants were added, despite the fact that they could reduce surface tension and increase permeability in the apical region. This indicated that decreasing surface tension could not increase EDTA's ability to chelate, and these results may be explained by the fact that Smear clear had the lowest values when compared to other groups.^[11] IP6's pharmacological actions such as its ability that chelate multivalent cations like Ca, generating salts like Ca-phytate, with relatively poor solubility, and better binding properties to proteins. When compared to pulp tissue and osteoblast-like

cells, it demonstrated greater biocompatibility than Phosphoric acid or EDTA.^[10]

IP6 can be a potential chelating agent to replace EDTA in removing the smear layer based on the features like its capacity to bind with Ca^{2+} and cross-linking with collagen which mechanically strengthens the dentin surfaces which were with dentinal and intertubular collagen open, free of smear plugs and smear layer.^[6] The acidity of a 1% IP6 solution was around 1.2, which helped with Ca^{+2} extraction, its acidity and chelation action combined make it an excellent smear layer remover.^[17]

IP6 effectively removed the smear layer and smear plug while slightly etched the dentin. Despite this sight etching impact, IP6 had the strongest binding strength when compared to the PA and EDTA groups. The insoluble compound hydroxyapatite's increased binding strength to dentin may give some support for the exposed collagen. Second, IP6 functions as a cross-linker, which can mechanically reinforce collagen and prevent it from collapsing. The inclusion of IP6 in the culture media had no effect on the viability or morphology of the bacteria. IP6 has been shown to act as both an iron chelator and a phosphate supply for cells in cell culture. By attaching to iron, a metal that catalysis the generation of hydroxyl radicals, IP6 shields cells against oxidative damage.^[11,12] Regardless of concentration, the Phytic acid group outperformed the Smear clear and Q-Mix 2 in 1 groups in terms of removing smear layers and permitting sealer penetration into dentinal tubules. In this study, there was no significant difference in pushout bond strength values between Group II (1% Phytic acid) and Group III (0.5% Phytic acid).

The inclusion of a surface active agent in QMiX is justified by its capacity to reduce solution surface tension and promote wettability. It also allows for improved irrigant penetration into the root canal. Based on the number of fully opened dentinal tubules,^[20] QMiX eliminated smear layer as efficiently as 17% EDTA, can be used as a final irrigant before the obturation because of its antimicrobial efficacy and these factors might have influenced Q-Mix 2in1 to perform better than smear clear.^[20]

AH Plus has been proven in numerous trials to have a stronger binding strength than most other sealants. The improved binding strength of the AH Plus in exposed amino groups in collagen is by establishing covalent connections between the collagen and resin. Properties like flowability and increased epoxy-based resin sealer's polymerization time allow it to penetrate deep into the dentinal tubules, presumably adding to the sealer's stated binding strength by enhancing the mechanical interaction between the sealer and the dentin.

The greatest bond strength is produced by lateral condensation type of obturation with AH plus and gutta percha.^[21] The pushout test is extensively used in experimental endodontics. The strength of root canal filler materials is evaluated in root canals. The test conditions were compared to clinical scenarios in which the tested materials were placed directly into built canals with a natural canal morphology and tubule arrangement. The load is applied perpendicular to the dentinal tubules, replicating clinical pressures; the test produces more pure shear forces and less stress on the bonding surface during sample preparation than typical tensile and shear bond testing.^[19] When all the groups were compared in this investigation the phytic acid group at the coronal third showed the strongest bond strength having statistically significant ($P < 0.05$) results (table 1).

Firstly ability of phytic acid the optimum bond strength is caused by eliminating the smear layer with just a minor

chelating effect on the root dentin. As a result, there was no substantial depth of demineralization, which may have permitted greater resin monomer infiltration into the dentin. Second, IP6 a natural cross-linker to demineralized dentinal collagen fibrils, it's robust and decreased prone to collapse, allowing for improved inter-fibrillar space preservation and monomer impregnation improving the resin-dentin bond strength. Finally, the ability of IP6 to attach to both protein and calcium, establishing a ternary complex (Protein-Ca-Phytate), as well as IP6 and collagen's interaction with dentin, generating a ternary complex (Collagen-Ca-Phytate), improves resin-dentin binding strength.^[10,22]

The reduction in binding strength in a coronal-apical direction, which is consistent with our study, may be explained by assuming that the number of dentinal tubules in the coronal apical region is significantly lower than in the cervical and mid-root dentin. Because of the irregularly shaped dentinal tubules in the secondary dentin and the presence of cementum apically on the root canal wall, adhesive penetration in the apical root dentin is lower than in the coronal dentin.^[23,24]

CONCLUSION:

Limitation:

The specimens were not examined for the remaining debris and smear layer coverage after irrigation with the test groups and the antimicrobial properties of the Phytic acid.

Clinical Significance:

Within the confines of this in-vitro investigation, the experimental group's Phytic acid push-out bond strength was shown to be larger than the other groups. This shows Phytic acid can be a potential alternative, effective and biocompatible chelating agent and can be a potential alternative to EDTA further requires a microscopic examination of the root dentin bonding interface which was treated with Phytic acid.

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