Original Article

Effect of Three Different Smear Layer Removal Agents on the Push-Out Bond Strength of an Endodontic Sealer: An *In Vitro* Study

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Abstract

Aim: The aim of this study was to evaluate the effect of smear layer removal agents 0.2% chitosan, 7% maleic acid, and 0.6% chitosan maleate on the push-out bond strength of AH Plus sealer to root dentin. **Materials and Methods:** Thirty single-rooted mandibular premolar teeth were decapitated and enlarged using Gates Glidden drills up to #3. Three slices with 2-mm thickness were obtained from the middle third of each root. After standardized irrigation using 5.25% sodium hypochlorite, the roots slices were randomly divided into three groups (n = 10) Group 1: 0.2% chitosan, Group 2: 7% maleic acid, and Group 3: 0.6% chitosan maleate. Final irrigation regimen was carried out and the root slices were, then, dried and AH plus sealer applied. Each root section was, then, subjected to a compressive load through a universal testing machine until sealer dislocation occurred. Bonded interface area was calculated. **Results:** The data were collected and statistically analyzed. **Conclusion:** Removal of smear layer with 0.6% chitosan maleate as a final rinse enhanced the adhesive ability of AH plus sealer, followed by 0.2% chitosan and 7% maleic acid. The new chelating solution 0.6% chitosan maleate gave highest push-out bond strength values when compared to 0.2% chitosan and 7% maleic acid.

Keywords: Chitosan, Maleic acid, Chitosan maleate, AH plus sealer, Smear layer removal, Bond strength.

Introduction

Successful endodontic therapy depends on chemomechanical preparation of the root canal system as well as three-dimensional obturation that provide complete sealing of the spaces previously occupied by the canal contents.[1]

Studies show that currently used methods of instrumentation, especially rotary instrumentation techniques, produce a smear layer that covers root canal walls and the openings to the dentinal tubules.

Smear layer can hinder the penetration of intracanal medicaments and sealers.[2] When the smear layer is removed from the root canal wall, endodontic sealers penetrate to the dentinal tubules and increase adhesion to the root canal dentin.[3]

Among the different endodontic irrigants which have been introduced over the decade, the alternate use of sodium hypochlorite (NaOCl) and ethylene diaminetetra acetic acid (EDTA) has been considered as the accepted protocol in irrigation.[4]

Access this article online

Website: www.jrad.co.in

DOI: https://doi.org/10.53064/jrad.2022.13.6.287

This conventional irrigation protocol has the following demerits: (a) NaOCl is associated with cytotoxicity, allergic reaction, unpleasant taste, and adverse effects on periradicular tissues; and (b) EDTA causes erosion, leading to decreased dentin micro hardness, as well as negatively influencing the bonding of sealer to dentin.[5,6] Due to such demerits, a safer and more effective alternative to this combination is desirable.

Maleic acid is a mild organic acid[7,8] with smear layer removal ability. It is biocompatible than EDTA and has antibacterial property.[9]

Chitosan is a natural polysaccharide[10] with excellent biocompatibility, no toxicity, high bioactivity, biodegradability,

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Received: Oct, 01, 2022; **Accepted:** Oct, 16, 2022; **Published:** Oct, 30, 2022

How to cite this article: Shreya MA, John NK, Kuruvilla A, Babu BS, Mathew RA, Nisha C, Pardhe ND. Effect of Three Different Smear Layer Removal Agents on the Push-Out Bond Strength of an Endodontic Sealer: An *In Vitro* Study. J Res Adv Dent 2022;13(6):13-17.

selective permeability, and adsorption capacity.[11,12] It has a broad spectrum of antimicrobial activity[13] and high chelating properties.[14]

A new irrigation solution 0.6% chitosan maleate was introduced which is a combination of chitosan and maleic acid that are proven to be excellent smear layer removal agents in recent literature. And to assess its smear layer removal ability, we conducted an *in vitro* study initially comparing it with that of the conventional irrigant NaOCl + EDTA.

The results showed that the alternate application of NaOCl and EDTA caused too much erosion and degradation of the dentin as compared to the newer irrigation solution 0.6% chitosan maleate at the apical level of the root canals (Figures 1 and 2).

Epoxy resin-based sealer such as Ah Plus sealer (Dentsply, Germany) represents the gold standard in clinical practice and the reference material for other types of sealers in laboratory and clinical research.[15-17] AH Plus sealer have been widely accepted due to their good physical properties, sealability, adequate biological performance, and microretention to root dentin.[18,19]

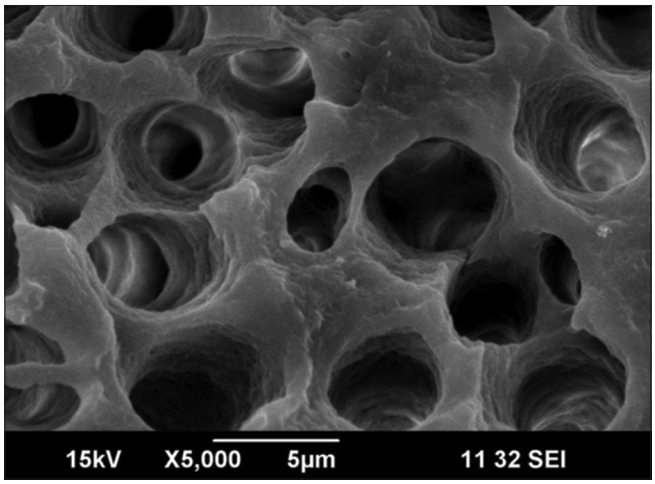

Figure 1: NaOCl + EDTA (apical)

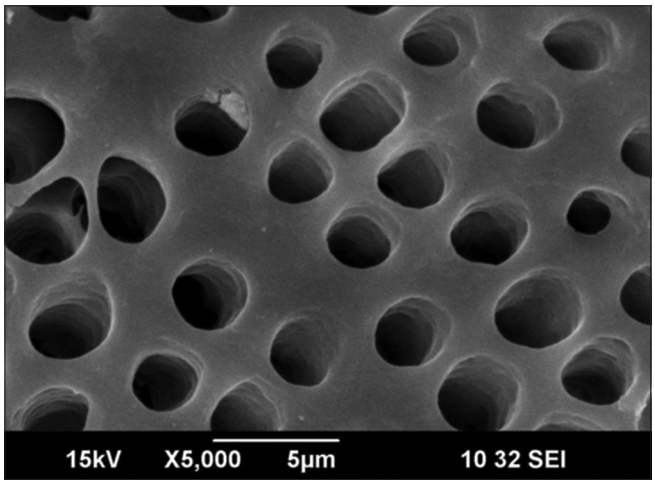

Figure 2: 0.6% chitosan maleate (apical)

Hence, in this study, we have evaluated and compared the efficacy of smear layer removal agents 0.2% chitosan, 7% maleic acid, and 0.6% chitosan maleate on the push-out bond strength of AH Plus sealer to root dentin.

Materials And Methods

Thirty single-rooted human mandibular premolar teeth with single canal were selected and decapitated to a standard root length of 16 mm. The decapitated roots were enlarged using gates Glidden drills up to size #3 (Mani drills #1–3, Japan). Three slices with 2 mm thickness were obtained from the middle third of each root (Figure 3).

After standardized irrigation using 5.25% NaOCl (Unibiosys Lab, Cochin, India), each of three slices from the same root were randomly assigned to have its smear layer removed by one of the three chelating agents: Group 1 using 0.2% chitosan (Sigma Aldrich, India), Group 2 using 7% maleic acid (Nice Chemical (P) Ltd, India), and Group 3 using 0.6% chitosan maleate.

Final irrigation procedure was carried out with distilled water (IDS Denmed Water Distiller, India) for 1 min, followed by 5.25% NaOCl for 1 min and distilled water for 1 min. The root slices were, then, dried with absorbent paper points (Diadent Group International, Korea) and placed on a glass plate. All the slices were filled with AH Plus (Dentsply, Germany) sealer and allowed to set completely (Figure 4).

Push-out assay

Loading was performed on a universal testing machine at a cross head speed of 0.5 mm/min until sealer dislocation occurred. Bonded interface area was calculated (Figure 5).

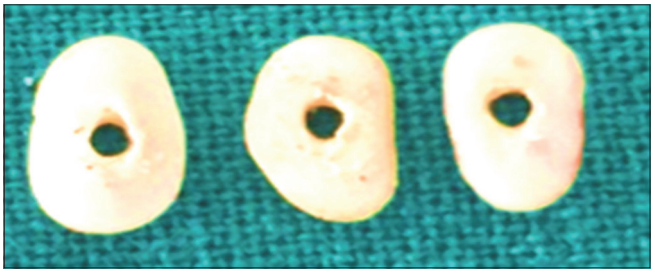

Figure 3: Root slices of 2 mm thickness

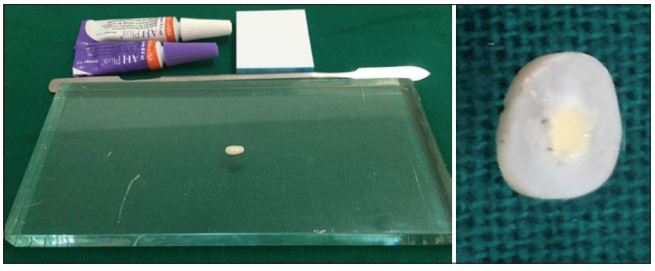

Figure 4: Application of AH Plus sealer on the root slice

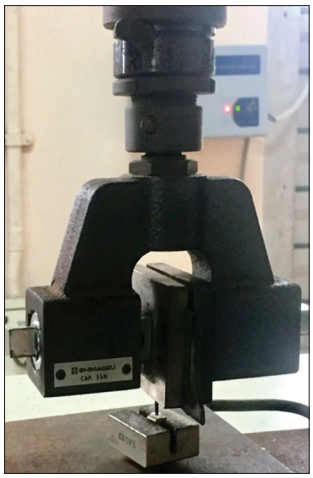

Figure 5: Loading performed on a universal testing machine

Table 1: Mean and standard deviation of test groups

Groups	Mean (nm^2)	SD
Group 1: 0.2% Chitosan	3.4838	1.70828
Group 2 :7% Maleic acid	3.3064	1.25349
Group 3: 0.6% Chitosan Maleate	3.9596	1.86937

Table 2: Comparison of Variance for the groups

Groups	Df	F	Sig
N	2	0.406	0.670
Between groups	25		
Within groups			
mm	2	0.639	0.536
Between groups	25		
Within groups			
N/mm^2	2	0.406	0.670
Between groups	25		
Within groups			

Results

The new chelating solution 0.6% chitosan maleate was found to give the highest push-out bond strength values when compared to 0.2% chitosan and 7% maleic acid. However, the results were not statistically significant (Tables 1 and 2).

Discussion

Smear layer is defined as a surface film of debris retained on dentin and other surfaces after instrumentation with either rotary instruments or endodontic files; it consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components, and retained irrigant.[20] According to Brannstrom and Perez-Heredia *et al*., smear layer feeds microorganisms and helps them colonize.[21,22] According to Kokkazs *et al*.[23] and Tuncer and Tuncer[24] smear layer prevents the penetration of sealer into dentin tubules.

According to Zehnder, the alternate application of EDTA and NaOCl is commonly used for the effective removal of smear layer from the root canal system.[25] Baumgartner and Mader in 1987, reported that when EDTA and NaOCl solutions were alternately applied to uninstrumented root canal wall, dentine showed an eroded appearance with enlarged tubular diameter.[26] Gorduysus *et al*. demonstrated an excessively aggressive effect of this combination on the root canal walls that could cause too much erosion and degradation of the dentin.[27] Calt and Serper also reported dentinal erosion when irrigated with 10 ml of 17% EDTA solution followed by 10 ml of 5% NaOCl. Furthermore, when used in combination with EDTA, NaOCl is inactivated with the EDTA remaining functional for several minutes.[28]

To overcome these drawbacks, many new irrigants and irrigant combinations were developed. Hence, in this study, a new combination irrigant 0.6% chitosan maleate was introduced which was prepared by dissolving 0.6% chitosan powder in 100 ml of 7% maleic acid.

AH Plus sealer was used in this study, because AH Plus sealer is considered as the gold standard among all the other root canal sealers and has shown to have the highest bond strength to root dentin.[29]

Studies have proven that AH Plus sealer has highest push-out bond strength when compared to calcium silicate-based sealers MTA Fillapex and Total fill BC sealer.[30] AH Plus sealer has shown the highest push-out bond strength compared to UDMA-based sealers Melike *et al*.[31] AH Plus sealer also has the highest push-out bond strength values than Endo Sequence BC sealer.[32]

High bond strength of AH Plus sealer is due to the low polymerization stress of the sealer and its long-term dimensional stability.[30] AH Plus sealer forms covalent bond with open epoxide rings to exposed amino groups present in dentin collagen network.[33]

Bond strength testing is a popular method to determine the effectiveness of adhesion between endodontic materials and the tooth. This method of testing is less sensitive to small variations among specimens and to the variation of stress distribution during load application.[31] Goracci *et al*. expressed that push-out technique was more reliable and precise than the micro-tensile technique for measurement of bond strength to dentin and should be run to suit the convenience of providing specimens and statistical analysis. Providing 2-mm thick specimens eliminated the probability of non-uniform stress distribution.[34]

7% maleic acid was considered as the irrigant of choice in this study due to its better smear layer removal ability and lesser cytotoxicity.[9] Smear layer removal ability of maleic acid was significantly better than EDTA.[35-37] The efficacy of EDTA decreases over time due to decrease in pH, but maleic acid being highly acidic has a better demineralizing effect, especially in apical third of the root canal system, within a shorter period of time.[38] Ballal *et al*. reported that 7% MA applied for 1 min was more efficient than 17% EDTA in the removal of smear

layer from the apical third of the root canal system.[36] Results of this study also showed that removal of smear layer with maleic acid as a final rinse enhanced the adhesive ability of AH Plus sealer.[39] Similar results were obtained by studies done by Ravikumar *et al*. who proved that removal of smear layer with maleic acid as a final rinse enhanced the adhesive ability of AH Plus sealer, followed by EDTA, citric acid, and MTAD.[7] It was also proven that 7% maleic acid produces maximum surface roughness on root canal walls as compared to 17% EDTA that might be responsible for greater micromechanical retention of the sealer with the dentin.[8]

Thus, 7% maleic acid removes the smear layer completely, creating efficient microretention between the dentinal tubules and the AH Plus sealer responsible for greater bonding.[40]

Chitosan is obtained by deacetylation of chitin, found in crab and shrimp shells. In addition, the production cost of chitosan is considered to be low, making its use ecologically and economically attractive.[41] It is found to possess excellent chelating property for various metal ions in acidic conditions.[14]

Two models explaining about the chelating property of chitosan have been reported in the literature. First is the bridge model based on the theory that two or more amino groups of a chitosan chain will bind to the same metal ion.[42] The second model supports the theory that only one of the amino group of the chitosan chain will bind to the metal ion.[43]

0.2% chitosan for 3 min can efficiently remove smear layer.[44] 0.2% chitosan removed smear layer effectively from the middle and apical third.[45] Studies have shown that 0.2% chitosan is also found to be more efficient than 17% EDTA, 10% maleic acid and MTAD in smear layer removal,[46] and 0.2% chitosan had better smear layer removal than Q-Mix 2 in 1 and smear clear.[47] The result of this study is in accordance with a study conducted by Bayram *et al*. who also proved that chitosan significantly increased the bond strength of all root canal sealers.[48]

Hence, this study has proved that the bond strength of AH Plus sealer is differently affected by different final irrigating solutions. It has shown that the new irrigation solution 0.6% chitosan maleate which is a combination irrigant of chitosan and maleic acid to have superior properties to that of the individual irrigant solutions 0.2% chitosan and 7% maleic acid.

From the results obtained from the present *in vitro* study 0.6% chitosan maleate, a new combination irrigant as a final rinse showed better results and enhanced the adhesive ability of AH Plus sealer, followed by 0.2% chitosan and 7% maleic acid.

Conclusion

Within the limitations of the study, 0.6% chitosan maleate gave better push-out bond strength values than 0.2% chitosan and 7% maleic acid.

0.6% chitosan maleate can be considered as an effective irrigant in Endodontics as it serves as an alternative chelating agent with positive effects on the bonding of root canal sealers. However, further studies are needed to investigate in detail its physical, chemical, and biological properties.

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