Influence of different endodontic irrigants on the push-out bond strength of an epoxy-resin based sealer and newly introduced bioceramic sealer to root dentin: An in-vitro study

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Abstract

Aim: The present *in-vitro* study evaluated the influence of different organic and inorganic root canal irrigants used during chemomechanical preparation on the push-out bond strength of an Epoxy-resin based AH Plus sealer and a Bioceramic Endosequence BC sealer.

Materials and Methods: Eighty extracted permanent human mandibular single rooted premolar teeth were used and randomly assigned to four major groups [according to irrigation protocol using sodium hypochlorite (NaOCl) with MTAD (mixture of tetracycline, acid and detergent) or ethylenediaminetetraacetic acid (EDTA) or phosphoric acid or normal saline (NS) as control group]. The root canals were instrumented using rotary nickel-titanium Hyflex® CM file system to size 30/0.06 taper, at the working length, as final shaping and finishing file, following different irrigation protocols, as per group allocation. The samples were further subdivided into two groups, with ten samples each (n=10), based on endodontic sealer; roots were obturated with gutta-percha and test sealers (AH-Plus or Endosequence Bioceramic sealer). The teeth were decoronated and obtained samples of eighty obturated roots of eight groups were cut to obtain 2mm thick coronal root slices (10 root section in each group), using hard tissue microtome. Bond strength of sealers was then measured by subjecting each root section to a compressive load via Instron Universal testing machine. The data were tabulated and statistically analyzed using analysis of variance (ANOVA) and Tukey's HSD along with Bonferroni's post-hoc test and level of significance set at a p value<0.05.

Results: The highest mean push-out bond strength of 5.38 MPa for AH Plus sealer was obtained in Group 2A followed by 3.94 MPa for Endosequence BC sealer (Group 2B), when phosphoric acid was used as chelating agent. However, mean bond strength of AH Plus sealer was significantly decreased ($p<0.01$) to 2.30 MPa when MTAD was used as chelating agent in Group 3A as compared to other groups where EDTA (Group 1A) or phosphoric acid (Group 2A) was used. Moreover, the use of chelating agents for removal of smear layer yield significantly high mean bond strength values in different groups (Group 1, Group 2, Group 3) as compared to control group (Group 4) where only NS was used ($p<0.01$).

Conclusion: The Bioceramic based Endosequence BC sealer and gutta-percha core combination was not superior in push-out bond strength to AH Plus and gutta-percha core combination. Moreover, use of different organic and inorganic endodontic irrigants influenced the push-out bond strength of both the sealers.

Clinical Significance: In dynamic clinical situation, for longer functional viability of an endodontically treated tooth, adhesion of a sealer to root dentin is necessary to avoid its dislocation during tooth flexure and also even during operative procedures like post space preparation. Adequate measures should be undertaken while using different irrigation protocols bearing in mind that the canal irrigants used during chemomechanical preparation can affect root dentin surface, thereby influencing sealer adhesion.

Keywords: A-H plus sealer, Bioceramic Sealer, MTAD, Push-out bond Strength, Sodium Hypochlorite.

Introduction

The primary function of a tooth is mastication. When the normal structure of a tooth is compromised by traumatic injuries or dental caries involving pulp, endodontic treatment remains the only viable option to maintain the tooth.[1] Endodontic treatment has numerous clinical steps that include not only effective biomechanical instrumentation of root canal but also irrigation with proper disinfecting solutions for dissolution of organic and inorganic matter, producing a debris free surface, and finally achieve a three dimensionally sealed and obturated root canal using an ideal sealer along with gutta-percha.[2,3]

Microorganisms and their by-products present in root canals not only invade anatomical irregularities in the root canal system but also the dentinal tubules.[4] Although mechanical instrumentation is essential for elimination of this root canal infection but it leads to formation of an iatrogenic layer (smear layer) on dentinal walls, primarily made up of inorganic particles of calcified tissue and organic material including bacteria, necrotic tissue, pulp, odontoblastic processes and blood cells.[2,3,5] So, for the removal of this formed smear layer, root canal irrigants which include both organic and inorganic solvents such as organic acids and chelating agents are used.

Smear layer removal is an essential step as its presence inhibits canal filling materials penetration in irregularities of the root canal system and dentinal tubules, preventing complete adaptation of the canal filling materials to the root canal walls.[3,5] As there is no single irrigating solution that alone sufficiently covers all the required functions, so, a combined use of two or several irrigating solutions in a specific sequence is preferred to predictably obtain the goals of safe and effective irrigation.[6]

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most common endodontic irrigants used in root canal disinfection. NaOCl has a long history of successful usage in endodontics and is the most preferred irrigating solution because of it being antimicrobial and antiseptic organic solvent with ability to solubilize tissues and dissolve biofilms, having low surface tension, it easily diffuses through dentinal walls of root canal.[7] However, because of it's inability to dissolve inorganic contents and remove the smear layer, adjunctive use of an acid or chelating agent with such properties is recommended. Goldman M and Goldman LB et al.[8] advocate the use of ethylenediaminetetraacetic acid (EDTA) alongside NaOCl for smear layer removal while Prado M and Gusman H et al.[9] suggested that 37% phosphoric acid could be used for this purpose.

Chelating solutions remove smear layer thereby exposing a large number of dentinal tubules that promotes adhesion between sealer and root canal dentin. In due process, these solutions also extract inorganic elements like calcium ions from surface dentin, and their longer exposures, specifically of EDTA, cause excessive removal of both peritubular and intratubular dentin. As a result of this demineralization, chemical bonding of root canal sealers with dentin is thought to be compromised.[10] Keeping in view of these detrimental effects of chelating agents on dentin surface, MTAD presents a clinically effective biocompatible endodontic irrigating and chelating solution. It has an effective antimicrobial property as well as ability to remove smear layer without causing damage to root canal dentin as compared to EDTA, hence possess no adverse effects on flexure strength and modulus of elasticity of dentin.[11] However, the use of different endodontic irrigants can alter the dentin surface composition; thereby affecting its adhesion with root canal filling materials.[12]

Gutta-percha along with a sealer is most accepted root canal filling material used for obturation to achieve a fluid impervious seal. A sealer not only fills irregularities of root canal walls, apical ramifications but also aids in bonding the gutta-percha to the dentinal walls of prepared canal.[13] Epoxy resin-based sealer like AH Plus have been widely used in endodontic therapy because of its better wettability of dentin and gutta-percha and acceptable physical properties, low solubility, dimensional stability, adequate microretention to dentin and biologic performance.[2] However, a Bioceramic sealer based on calcium phosphate silicate, Endosequence BC Sealer, has been introduced recently, which is claimed to have low toxicity as its composition is similar to white mineral trioxide aggregate (MTA), and according to manufacturer, it uses moisture present within the dentinal tubules to initiate and complete its setting reaction. In addition, its bond strength is superior to many traditionally used sealers like Sealapex and EndoREZ and reported to be equivalent to AH Plus sealer.[14,15]

Several studies have investigated the effect of endodontic irrigants on bond strength of different types of root canal sealers. In the light of their observations, it can be ascertained that root canal irrigants used during chemomechanical preparation can affect dentin surface. Bearing in mind these findings, the aim of the present study was to evaluate the effect of different irrigation protocols using 3% NaOCl with either MTAD for smear layer removal or conventional chelating agents 17% EDTA or 32% phosphoric acid on the sealer / dentin interface bond strength of AH Plus sealer (Dentsply, USA)/gutta-percha and newly introduced Bioceramic Endosequence BC sealer (Brasseler, USA)/gutta-percha. The null hypothesis stated there is no difference in bond strength of epoxy resin and Bioceramic sealers while using different chelating agents.

Materials and Methods

The materials used in the study included sodium hypochlorite (NaOCl) as endodontic irrigant during chemomechanical preparation, followed by ethylenediaminetetraacetic acid (EDTA) or phosphoric acid or MTAD (mixture of tetracycline, acid and detergent) as chelating agents for smear layer removal and finally for root canal obturation, gutta-percha along with two different sealers namely AH-Plus or Endosequence Bioceramic sealer. [Table 1]

Specimen Preparation

In the present *in-vitro* study, eighty permanent human mandibular single rooted premolar teeth, extracted for periodontal or orthodontic reasons, caries free, non-carious lesion free accompanied by normal anatomical form and structure with root canals minimum up to 3mm in cervical diameter and at least 15mm in root length were chosen, cleaned with ultrasonic scaler (Biosonic, Coltene Whaledent, USA) and stored in distilled water (Sadbhavna Chemicals, Gujarat, India) at room temperature, the preferred method of storage with least negative influence as suggested by Strawn SE and White JM *et al*.[16] Access cavity was made using airotor handpiece with an Endo-access bur (Dentsply Maillefer, Switzerland), completed and refined using Endo-Z Bur (Dentsply Maillefer, Switzerland). Endodontic explorer DG-16 (API, Germany) was used to locate the root canal orifice and ISO size 08 K-Flex file (Dentsply Maillefer, Switzerland) was used to establish canal patency. The working length was confirmed and recorded by reducing 1mm from the file length after its emergence through apical foramen. Next, ISO size 15 and ISO size 20 K-file was used to standardize the foramina.

The teeth were divided into four major groups according to irrigation regimen and further into two subgroups each on basis of endodontic sealer used for root canal filling. The root canal of each tooth was shaped by means of rotary nickel-titanium Hyflex® CM file system (Coltene Whaledent, USA) with 0.08 taper/25 size file as orifice widener followed by following file sequence till working length:0.04/20, 0.04/25, 0.06/20, 0.06/25 and 0.06 taper/30 size as final shaping and finishing file. During chemomechanical preparation, before insertion and during instrumentation of each file size, 1ml 3% NaOCl (Vishal Dentocare Ltd., Gujarat, India) was used as chemical auxiliary substance. Between each file, the root canals were irrigated using syringe and 27 gauge needle with 1ml of normal saline (NS) (Nirlife, Nirma Ltd.(Healthcare Division), Gujarat, India) as standard for all groups. After root canal shaping procedure was completed, final irrigation with CanalPro^{TM} EDTA 17% (Coltene Whaledent, USA) (Group 1), 32% phosphoric acid solution (3M ESPE, Germany) (Group 2), MTAD (BioPure Tulsa Dentsply, USA) (Group 3) and NS (Group 4 as control group) was done for 30 seconds to remove the smear layer. Finally, normal saline (NS) was used for final flush, as a standard for all groups, to remove the remaining chelating solution from the canal. [Group allocation summarized in Table 2].

The root canals were dried with paper points (Meta Biomed Co. Ltd., Korea) and later filled with corresponding size gutta-percha cones (Coltene Whaledent, USA) and AH Plus sealer (Dentsply, USA) or Endosequence BC sealer (Brasseler, USA) as per respective subgroups using cold lateral compaction technique; as standardized filling method for all groups. The excess gutta-percha above the orifice level was seared off with hot instrument. All the teeth were radiographed from both the mesiodistal and buccolingual directions to access the quality of root canal filling and verify whether the tridimensional filling showed no presence of voids. The access cavity was filled with Coltosol® F (Coltene Whaledent, USA) and to allow the root canal sealers to set properly, all the samples were kept on moist gauze pads at 37^0C and 100% humidity for 2 weeks as suggested by Prado M and Simao RA et al.[17]

Push-out Bond Strength Assessment

The teeth were decoronated at the cementoenamel junction and root apex with a low-speed straight hand piece (NSK, Japan) and carborandum disc (Dentsply, USA) under water coolant to obtain a standardize root length of 15mm for teeth. Each root was then sectioned horizontally, perpendicular to the long axis, at the coronal section of the root into 2mm-thick slices, using hard tissue microtome; with the 1st slice being discarded and the one with more circular shape of the canal filling material being selected. The thickness of each slice was measured using digital caliper (Insize Co. Ltd., Germany). One slice from each root canal was evaluated. Due to convergence of root canal slice, each was marked on its apical side.

Thereafter, push-out bond test was performed by applying a compressive load on the filling material of sample placed from apical to coronal direction. This resulted in root filling displacement towards the larger root canal diameter, hence eliminating any constriction interference due to root canal taper during test. The load was applied with a custom made stainless steel cylindrical plunger of 1mm diameter that provided the most extended coverage over the filling material without contacting the surrounding dentin of canal wall in an Instron Universal testing machine (Model 3382, Instron Industries, USA), at a cross-head speed of 0.5mm/minute until bond failure occurred. [Fig. 1]

These debonding values (maximum load at which bond failure occurred) were used to calculate push-out bond strength in megapascals (MPa) according to the following formula:

Push-out bond strength (MPa) = Maimum load (N) / Adhesion area (mm^2)

Here, adhesion area was calculated as:-

$$\pi(R+r)[h^2+(R-r)2]^{0.5}$$

Where:- π = 3.14, 'R' is the radius of greatest base of slice, 'r' is the radius of smallest base of slice, and 'h' is the thickness of the slice.

The data were tabulated and statistically analyzed using analysis of variance (ANOVA) and Tukey's HSD along with Bonferroni's post-hoc test to determine whether significant differences in push-out bond strength values existed between and within groups. The selected level of significance was set at a p value<0.05. Analysis was performed on SPSS 19 software (IBM Corporation, Chicago).

Results

The mean and standard deviation values of push-out bond strength (MPa) and descriptive statistics are presented in Table 3 and Table 4 respectively. The highest mean bond strength values was obtained in Group 2A (5.38 MPa) for AH Plus sealer followed by Group 2B (3.94 MPa) for Endosequence BC sealer when phosphoric acid was used for smear layer removal with statistically significant difference (p<0.01) as compared to other groups studied. However, mean bond strength of AH Plus sealer was significantly decreased (p<0.01) to 2.30 MPa when MTAD was used as chelating agent in Group 3A as compared to other groups where EDTA (Group 1A) or phosphoric acid (Group 2A) was used. Moreover, the use of chelating agents for removal of smear layer yield high mean bond strength values in different experimental groups (Group 1, Group 2, Group 3) as compared to control group (Group 4) where only NS was used (p<0.01). Further, in phosphoric acid group (Group 2), statistically significant difference (p<0.01) in bond strength was observed while comparing AH Plus and Endosequence BC sealer.

Table 1: Composition of Endodontic irrigants and sealers used

Materials	Manufacturer	Composition	pH
MTAD	BioPure Tulsa Dentsply, USA	Tetracycline isomer (doxycycline), 4.25% citric acid, 0.5% Tween 80 detergent [polyoxyethylene sorbitan monooleate]	2.15
NaOCl (Sodium Hypochlorite)	Vishal Dentocare Ltd., India	Ionizes in water into Na^+ (sodium) and OCl^- (hypochlorite ion)	11
CanalPro™ EDTA 17%	Coltene Whaledent, USA	Sodium EDTA (ethylenediaminetetraacetic acid)	8.5
32% Phosphoric acid	3M ESPE, Germany	H_3PO_4, water	1
Normal Saline	Nirlife (Nirma Healthcare Division), India	Sodium Chloride 0.9%, water	5.5
AH Plus®	Dentsply, USA	Paste A (Epoxide paste): bisphenol-A, bisphenol-F calcium tungstate, zirconium oxide, silica iron oxide pigment Paste B (Amine paste): Dibenzyldiamine aminoadamantane tricyclodecane-diamine calcium tungstate, zirconium oxide, silica, silicone oil	ND
Endosequence BC sealer	Brasseler, USA	zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents	>12

Table 2: Group allocation of experimental groups according to irrigation protocol and type of endodontic sealer used for obturation

Groups	N	Irrigation protocol	Endodontic sealer used with gutta-percha for obturation
Group 1A	10	3% NaOCl + 17% EDTA	AH Plus
Group 1B	10	3% NaOCl + 17% EDTA	Endosequence BC
Group 2A	10	3% NaOCl + 32% Phosphoric acid	AH Plus
Group 2B	10	3% NaOCl + 32% Phosphoric acid	Endosequence BC
Group 3A	10	3% NaOCl + MTAD	AH Plus
Group 3B	10	3% NaOCl + MTAD	Endosequence BC
Group 4A	10	3% NaOCl + normal saline solution	AH Plus
Group 4B	10	3% NaOCl + normal saline solution	Endosequence BC

N: Sample size

Table 3: Push-out bond strength (Mean ± SD) in MPa of the experimental groups

Groups	AH Plus/gutta-percha (Group A)	Endosequence BC/gutta-percha (Group B)
Group 1	3.61±0.23	2.92±1.35
Group 2	5.38±0.59	3.94±0.53
Group 3	2.30±0.52	3.33±0.83
Group 4	1.74±0.83	1.97±0.88

SD: Standard deviation

Table 4: Significance (p values) of mean difference of push-out bond strength of each sealer between groups (i.e., when comparing different irrigation protocols) by Tukey's HSD test

Irrigation Protocol Comparisons	AH Plus sealer (Group A)		Endosequence BC sealer (Group B)	
	p - value	Tukey HSD Q statistic	p - value	Tukey HSD Q statistic
NaOCl + EDTA (Group 1) **vs** NaOCl + Phosphoric acid (Group 2)	0.001	7.1208	0.088	4.0960
NaOCl + EDTA (Group 1) **vs** NaOCl + MTAD (Group 3)	0.008	5.2553	0.899	1.6488
NaOCl + EDTA (Group 1) **vs** NaOCl + Normal saline solution (Group 4)	0.001	7.5059	0.140	3.8111
NaOCl + Phosphoric acid (Group 2) **vs** NaOCl + MTAD (Group 3)	0.001	12.3761	0.646	2.4471
NaOCl + Phosphoric acid (Group 2) **vs** NaOCl + Normal saline solution (Group 4)	0.001	14.6267	0.001	7.9071
NaOCl + MTAD (Group 3) **vs** NaOCl + Normal saline solution (Group 4)	0.727	2.2506	0.005	5.4599

p-value: Level of significance, HSD: Honestly significant difference, Q: Quantile

Discussion

Adhesion of the root canal filling material to dentinal walls is a very desirable physical property and of paramount importance because it prevents fluid percolation between the spaces of obturation, minimizes the risk of filling detachment from dentin during restorative procedures or masticatory functions,[18,19] ensuring intact seal and maintaining the integrity of sealer-dentin interface without being disrupted for long term clinical success of endodontic treatment.

Adhesion of endodontic sealers to root canal dentin resist dislodgement of filling either through frictional retention or micromechanical adhesion and maintains integrity of sealer-dentin interface.[18] Bond strength tests are used to evaluate adhesion between this crucial interface. Various methods have been employed for testing the bond strength including shear bond strength test and micro-tensile strength test. These test models does not replicate clinical conditions and attempts to closely duplicate them have resulted in complicated models that are difficult to reproduce and interpret.[20] While, the push-out bond test is reproducible and can be interpreted easily. It is more effective with an advantage that it allows root canal sealers to be evaluated even with low bond strength.[21] The bond strength testing using push-out test method generates fracture parallel to the dentin-sealer interface,[22] hence, more clinically reliable and efficient results are obtained that better represents bond strength of a sealer. Therefore, in the present *in-vitro* study we used push-out test to compare the bond strength of AH Plus and Endosequence BC sealer to root dentin.

The higher the bond strength of an endodontic sealer to radicular dentin, the higher is the integrity of sealer-dentin interface.[23] Radicular dentin is not uniform and not only its tubular density decreases from coronal to apical region but also the prepared canal wall surface during chemomechanical preparation can differ widely. These differences are present and vary in each prepared sample, and also vary between sites in the same root sample, according to the level (coronal, middle, or apical),[24] hence, coronal, rather than middle or apical root dentin was used in the present study for better reproducibility.

An adequate adhesion requires close contact between adhesive material and substrate for molecular attraction to facilitate either chemical adhesion or penetration for micromechanical surface interlocking. So, adhesion of endodontic sealers to radicular dentin is mainly influenced by relative surface wetting ability of the intraradicular dentin.[25] Surface treatment of radicular dentin during chemomechanical preparation with various endodontic irrigants induce changes in its structural and chemical composition, altering its solubility and permeability.[12] Therefore, it can be ascertained that bonding of root canal sealers to canal dentin is differently affected by the root canal irrigants.

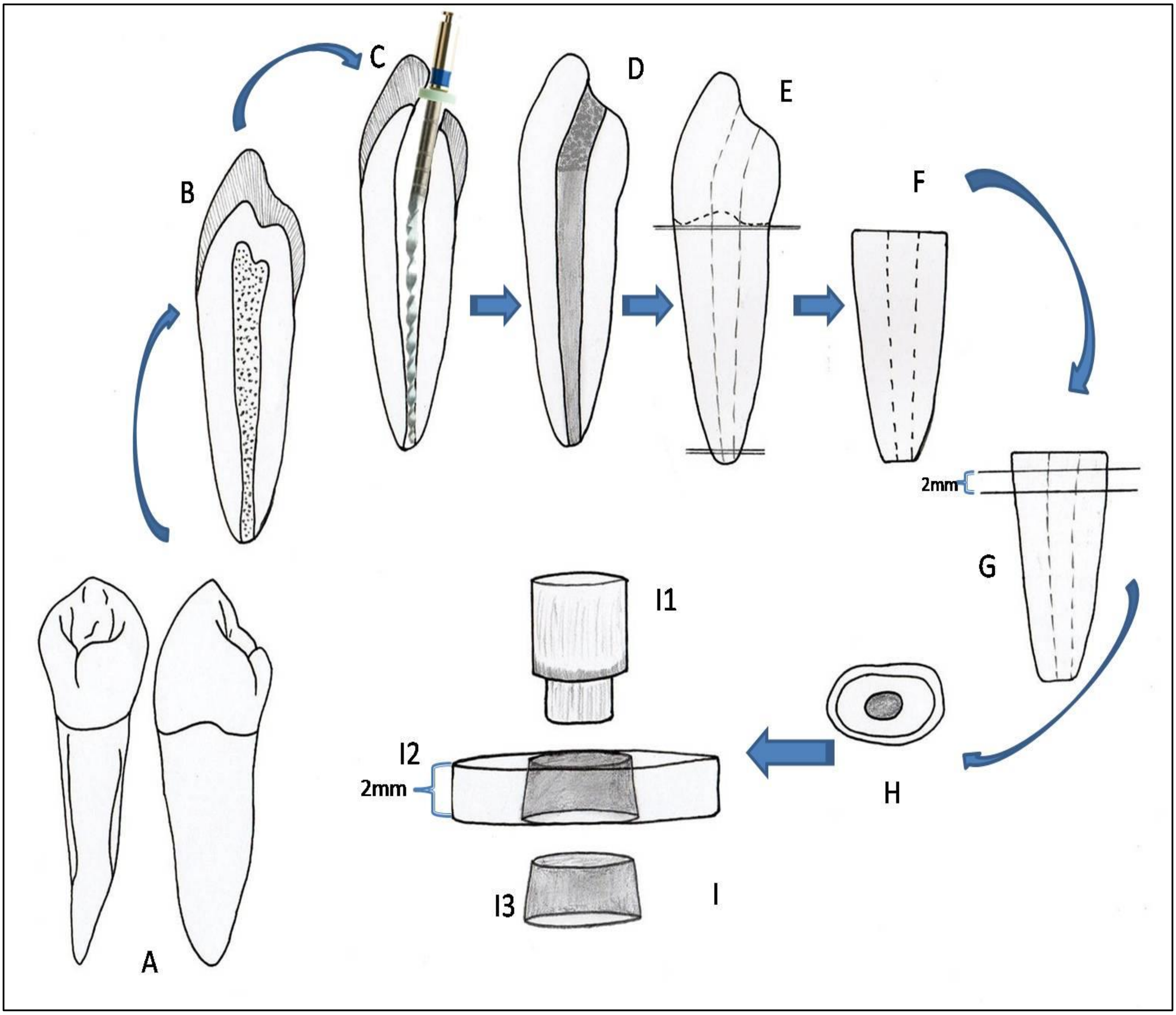

Fig. 1: Schematic diagram of specimen preparation and push-out test. **(A)-** Mandibular premolar teeth. **(B)-** Cross-sectional view of premolar. **(C)-** Access-opening and chemomechanical preparation of root canal space. **(D)-** Obturated root canal using endodontic sealer and gutta-percha with coronal temporary restoration. **(E)-** Tooth sectioned at cemento-enamel junction and apex. **(F)-** Standardized 15mm root length. **(G)-** Coronal portion of Root sectioned to obtain 2mm thick slice. **(H)-** Coronal root sample. **(I)-** Push-out test assembly. **(I1)-** Custom made plunger for load application. **(I2)-** 2mm thick root dentin sample. **(I3)-** Dislodged root canal filling (sealer and gutta-percha).

The effect of different irrigants on bond strength of resin based sealers to dentin has previously been studied, and few researches have evaluated the association of NaOCl with 17% EDTA on bioceramic based sealers.[17] However, none has evaluated effect of MTAD or 32% phosphoric acid on bioceramic sealers. Thus, the main objective of the present *in-vitro* study was to evaluate the effect of NaOCl used during chemomechanical preparation along with chelating agents namely EDTA or phosphoric acid or MTAD, on the bond strength of an epoxy resin based AH Plus and bioceramic based Endosequence BC sealer.

Results obtained within the experimental conditions of the present study indicate that AH Plus sealer (Group 2A) and Endosequence BC sealer (Group 2B) with the use of 3% NaOCl along with 32% phosphoric acid showed the highest mean push-out bond strength of 5.38 MPa and 3.94 MPa respectively, which was statistically significant ($p<0.01$) as compared to other groups studied. The best results obtained with use of 32% phosphoric acid for smear layer removal might be due to higher degree of dentin demineralization. Studies have shown that phosphoric acid is able to remove the collagen layer damaged by NaOCl, exposing the healthy layer and dentinal tubules, allowing greater resin sealer penetration resulting in high push-out bond strength,[9,21] as seen in AH Plus sealer. Further, the use of strong acid such as phosphoric acid, completely removes the inorganic elements of smear layer, exposing the dentin collagen matrix. These exposed collagen fibers enhances the probability of dentin hybridization with hydrophilic sealers,[26] like Endosequence BC sealer, as these sealers possess low contact angle allowing them to spread easily over the canal walls providing adequate adhesion and bond strength.[27] However, detrimental effects, if any, of phosphoric acid on the periapical tissues should be evaluated.

The literatures have suggested the use of NaOCl with 17% EDTA for smear layer removal because it better facilitates exposure of dentin collagen network, rending the dentin substrate more conducive to bonding of resin based sealers, as they can adhere and bond with the organic phase of root canal dentin.[28] In the present study, Group 1 where 17% EDTA was used for smear layer removal, AH Plus sealer (Group 1A) observed higher mean push-out bond strength of 3.61 MPa as compared to 2.92 MPa for Endosequence BC sealer (Group 1B).

The high mean push-out bond strength for AH Plus sealer in Group 1A can be attributed to the use of 17% EDTA which effectively removed smear layer. Previous studies by Hashem AA *et al*.[29] and Neelakantan P *et al*.[28] found that 17% EDTA enhances and facilitates the adhesion of resin based AH Plus sealer, as, complete removal of smear layer by EDTA allows intimate contact of resin sealer with dentin surface, creating an efficient micro-retention due to adequate penetration of resin into the dentinal tubules.[20,30] Further, the higher values of epoxy resin-based AH Plus sealer could also be attributed to its inherent volumetric expansion property that resulted in covalent bond formation between open epoxide ring of sealer and exposed amino groups of root dentin.[31]

Moreover, low mean push-out bond strength of Endosequence BC sealer in Group 1B (2.92 MPa) while using EDTA as compared to Group 2B (3.94 MPa) and Group 3B (3.33 MPa), where phosphoric acid and MTAD was used respectively, might be due to confounding effect of EDTA on apatite formed during setting reaction of the sealer. This sealer is mainly composed of calcium silicate, monobasic calcium phosphate, calcium hydroxide and zirconium oxide similar to white MTA and uses moisture from within the dentinal tubules to initiate setting reaction (hydration process).[32] Although literatures lack information on the influence of chemical irrigants on Endosequence BC sealer, several studies examined there effects on physicochemical properties of MTA.[33,34] Lee YL and Lin FH *et al*.[34] studied adverse effects of EDTA on hydration and micro hardness of MTA and observed that the residual EDTA left behind in root canal dentin after chemomechanical preparation, continue to chelate calcium ions released from MTA during hydration, thereby interfering with the precipitation of hydrated products. These findings could be an explanation for the compromised adhesion of Endosequence BC sealer with dentin.

Further, the mean push-out bond strength of AH Plus sealer was significantly decreased ($p<0.01$) when MTAD was used as chelating agent in Group 3A and found to be 2.30 MPa which was less as compared to other groups where EDTA (Group 1A) or phosphoric acid (Group 2A) was used. Although MTAD is acidic with a pH=2.15 having citric acid among its constituents, resulting in better smear layer removal, along with Tween 80 detergent which enhance the flow and penetration of MTAD deeper into the dentinal tubules, creating 8-12µm deep demineralized dentin zone compared to others, still low bond strength might be due to presence of degradation product which may compromise adhesion.[35] Tay FR *et al*.[35] attributed low bond strength to the presence of red-purple degradation precipitate 4-alpha-12-alphaanhydro-4-oxo-4-deimethylaminotetracycline (AODTC) formed due to oxidation of tetracycline isomer by NaOCl, having high affinity for hydroxyl apatite. Further, Beltz RE *et al*.[36] reported in their investigations that MATD accumulates on dentin tissue reducing the surface area available for adhesion. These findings could explain the decreased bond strength observed in the MTAD group in the present study which is also in accordance to the findings in similar study done by Hashem AA *et al*.[29]

However, the mean push-out bond strength of Endosequence BC sealer in Group 3B was found to be surprisingly higher and was recorded 3.33 MPa as compared to AH Plus in Group 3A when MTAD was used as chelating agent. As earlier mentioned, MTAD consists of a detergent, Tween 80, which increases the intertubular dentin permeability, leading to exposure of collagen matrix and intertubular fluid for increased wettability of sealer,[37] and as literatures show,[32,38] Endosequence BC sealer being hydrophilic, uses moisture present within the dentinal tubules for setting. So exposure of sealer to this surplus intertubular fluid could be a reason for the increased bond strength observed.

According to the results of the present study, sealers in Group 1, Group 2 and Group 3, where a chelating agent was used for smear layer removal, showed statistically significant ($p<0.05$) and higher mean push-out bond strength, as compared to the control group (Group 4) where only NS was used and smear layer was left intact. The cause of increased bond strength in smear layer free groups (Group 1, Group 2, Group 3) has been suggested to be due to more surface contact of dentinal tubules available for sealer penetration, as in corroboration to previous findings by Gencoglu N and Rohani A *et al*.[39] Therefore, the lowest push-out bond strength in control Group 4 (NS) in which smear layer was kept intact is indicative of negative effect of undisturbed smear layer on bond strength of both the experimental endodontic sealers.

Additionally, in control Group 4, epoxy resin sealer AH Plus in Group 4A had a mean bond strength of 1.74 MPa which was less as compared to bioceramic Endosequence BC sealer in Group 4B which had a mean bond strength of 1.97 MPa. This can be attributed to the fact that NaOCl despite of its disinfective properties, being a deproteinizing agent, it can degenerate dentin by collagen dissolution, affecting the resin sealer penetration and hindering the formation of a consistent hybrid layer. Furthermore, NaOCl breaks down into sodium chloride and oxygen that interfere with resin sealer (AH Plus) polymerization, causing strong inhibition at sealer-dentin interface and hence decreasing the bond strength.[19] Whereas, high push-out bond strength of Endosequence BC sealer can be attributed to its true self-adhesive nature as it forms a chemical bond with dentin through hydroxyapatite production during setting reaction.[27]

Conclusion

Within the limitations and experimental conditions of the present *in-vitro* study, it can be concluded that the use of different organic and inorganic root canal irrigants affected the bond strength of epoxy resin-based AH Plus resin sealer and bioceramic Endosequence BC sealer. Smear layer removal with use of phosphoric acid enhanced the adhesion of endodontic sealers followed by EDTA and MTAD. However, use of MTAD had a negative effect on push-out bond strength of epoxy resin-based AH Plus sealer, as compared to other solutions studied. Further, phosphoric acid appears to be a promising alternative to EDTA, provided its efficacy is further investigated with respect to several other properties like biocompatibility with the periapical tissue, effect on chemical composition of root canal dentin and its interaction with endodontic sealers. So, from clinical aspect, for stake of adequate bond between endodontic sealer and root dentin, the results of the present *in-vitro* study may indicate that selection of an appropriate chelating solution, capable of removing the smear layer with minimal adverse effect on root dentin during chemomechanical preparation would be advantageous.

Limitations and Future Scope

In the present study, although two different endodontic sealers that widely differed in their composition as one being a resin based sealer and other being a bioceramic based were assessed only for their bond strength under influence of different irrigation protocols, and adhesion is only one aspect of the endodontic sealer quality. Even then, adhesion is considered one of the most important quality affecting prognosis of an endodontically treated teeth. The present study being *in-vitro* test, so the results obtained from laboratory experimental study cannot be directly co-related to clinical situation and care should be taken when drawing conclusions from the data, as many factors can affect the results obtained *in-vitro*. However, they provide reliable and reproducible means for evaluating prospective endodontic sealers for their clinical success on preset parameters.

Conflicts of Interest: None.

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