An In vitro Comparative Analysis Of The Influence Of Various Chelators Used As Final Irrigants On The Bond Strength Of An Epoxy resin-Based Sealer And A Bioceramic Sealer

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Abstract

Background: This study compared the bond strength of two sealers after employing various chelating agents as final rinses

Materials and Methods: Ninety extracted mandibular premolars were sectioned at the level of the cementoenamel junction. Following the working length determination and biomechanical preparation, the specimens were randomly divided into three groups based on the chelating agent used as final rinses: Group I **-** 17% ethylene diaminetetraacetic acid, Group II **-** 1% peracetic acid and Group III - 0.2% chitosan. In all three groups, 5 ml of the chelating solution was used for 1 minute. Then, they were further sub-grouped on the basis of the endodontic sealer used for obturation of the root canal (AH Plus/Bio-C bioceramic sealer). Bond strength was measured using a Universal Testing Machine and fracture mode was determined using a stereomicroscope. Data were analyzed using two-way ANOVA test, Tukeys post-hoc test and Chi-square test ($P < 0.05$).

Results: The highest push-out bond strength was of Bio-C bioceramic sealer after treatment with 0.2% chitosan (2.92±0.46) while the least was of AH Plus sealer after 17% EDTA treatment (2.12±0.21). Mixed failure (both adhesive and cohesive) was commonly found in AH Plus sealer while Bio-C bioceramic sealer mostly had a cohesive type of failure.

Conclusion: Within the limitations of this study, conclusion drawn is that chelating agents studied in the present study influence the bond strength of endodontic sealers when used as final rinses.

Key Words: Chelating agents, Bioceramic sealer, AH Plus sealer, Bond-strength testing.

Introduction

Contemporary endodontictechniques produce 'smear layer' encompassing mineralized, unmineralized contents, bacterial by-products and necrotic tissues.[1]Smear layer forbids complete conformation of sealers to dentin. Various methods like chemicals, ultrasonics and lasersare used to eliminate it.[2]Literature advocates the use of chelating solutions as an irreplaceable regime of irrigation. But, they have been found to interfere with adherence of the endodontic sealers to the root dentin.[2]Therefore, presentinvitro study determines the effect of chelating agents (as final rinses) on the adhesion of AH Plus sealer and Bio-C bioceramic sealer to radiculardentin and assesses failure modes of the same.

Methodology:

Sample collection and selection: Ninety human mandibular premolar teeth, recently extracted, were collected and washed under tap water to remove the blood stains. Then samples were stored in 5.25% Sodium Hypochlorite (NaOCl) for nearly two hours to clean the remnants of periodontal tissues from the root surface. The hard deposits were scaled with a periodontal curette. Then the specimens were stored in 0.1% thymol solution for two weeks since it has no effect on the mineralized and unmineralized components of teeth and on the dentin permeability. Fully formed mandibular premolars with single root and single canal and root curvature between 0°-10° were included in the study. The carious teeth or teeth with previous restoration or root canal treatment or cracks, root fracture, open apices, internal resorption, external resorption, calcification and developmental anomalies were rejected.

Sample Preparation: The selected specimens (n = 90)were decoronated at the height of the cementoenamel junction with the aid of a diamond disc using a low-speed handpiece and water as a coolant to achieve a standardized root length of 15 mm using a digital vernier caliper. Round bur No #BR 46 (Mani Inc., Japan) was used to prepare the access cavity on each specimen. Further, the working length was determined by inserting a #10 K-file (Mani Inc., Japan) into the canal till the root apex and 1 mm was then subtracted from the above-noted length.

Root canal preparation: #10 and 15 K-files (Mani Inc., Japan)were inserted into the canal for attaining the initial patency. 3 ml of 5.25% NaOCl solution(Prime Dental Products Private Limited, Pune, India) was used for intermittent irrigation.This was followed by cleaning and shaping of canals with ProTaper Gold rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland), working on the crown-down technique. All the canals were cleaned and shaped till #F3/.09upto the working length.

Specimens Grouping: After root canal preparation, random distribution of the samples was done into three groups (n = 30) according to the chelating solution used(as final rinsing solution).:

Group I - 5 ml of 17% EDTA (Prevest DenPro Limited, Jammu and Kashmir, India) was used for irrigation for 1 minute.

Group II - The root canals were irrigated with 5 ml of 1% peracetic acid (Prime Laboratories, Hyderabad, India) for 1 minute.

Group III - 5 ml of 0.2% chitosan{prepared by dissolving 0.2 gm of low molecular weight chitosan (Sigma Aldrich, Missouri, United States) in 100 ml of 1% acetic acid and agitating the mix in a magnetic stirrer for 2 hours} was used to irrigate the canal for 1 minute. The irrigating solutions were conveyed with a 27-gauge needle in a vertical direction to reach the walls of the canals properly.Final flushing of the canals wasdone with 5 ml of distilled water to mask the remaining quantity and effect of the chelating agents being tested. Sterile paper points were used for the complete drying of the canal.

Obturation: Additionally, the subgrouping of each group was done on the basis of the endodontic sealer used for obturation (n = 15). Single-cone obturation technique was used with both the AH Plus sealer or Bio-C bioceramic sealer.

Subgroup A (IA/IIA/IIIA) (AH Plus subgroup): The AH Plus sealer is dispensed in the form of two pastes. The pastes were dispensed in equal quantities on a paper pad and mixed to form a homogenous, creamy mix. With a pumping movement, the selected master gutta-percha cone #F3 layered with the sealer was inserted into the canal, removed from the canal, and again coated with a layer of sealer to insert into the canal till the established working length.

Subgroup B (IB/IIB/IIIB) (Bio-C subgroup): Bio-C bioceramic sealer is available as a pre-mixed form in a syringe along with intracanal tips. The syringe’s tip was placed upto the coronal-third part of the canal. The sealer was then smoothly disbursed while withdrawing the tip from the root canal. A thin layer of sealer was further coated on selected master gutta-percha cone #F3 which was then introduced into the canal till the full working length.
Post-obturation periapical radiograph of each specimen was taken. The temporary filling material (Waldent Den Temp)sealed the canal orifices and the sealer used for obturation was allowed to set by storing the specimens at a temperature of 37˚C in the presence of 100% humidity for 7 days.

Push-out Bond Strength: Thirty 2.00 ± 0.05 mm thick slices per subgroup were obtained by sectioning the middle part of each root perpendicularly to the tooth’s long axis and the final thickness was confirmed by the digital vernier caliper having 0.01 mm accuracy. Then, in a universal testing machine (UTM) with the help of a 0.7 mm cylindrical plunger the loading of the filling material (placed on the upper compartment of UTM)was performed in an apical-coronal direction at a speed of 0.5 mm/min. The push-out force was enforced till failure of the bond was recorded which was reflected by the dislodgement of the filling material from the interference of the coronal surface. The maximum force required to displace the filling material was noted by dividing the load by the surface area. i.e, Push-out bond strength (MPa) = Maximum failure load (in N)/ surface area of adhesion (in mm^2)

Analysis of Failure Modes: Following the assessment of push-out bond strength, each slice of the root was analysed using a 40x magnification stereomicroscope to determine failure modes. Corresponding to the classification by Skidmore et al, the noted bond failure modes were:[3]

- Type 1: Adhesive failure (occurring at the junction of dentin-dentin).
- Type 2: Cohesive failure (occurring within the dentin or sealer interface).
- Type 3: Mixed failure (combination of above-mentioned failures).

Statistical analysis: The statistical analysis of the data was done using SPSS version 11.5. The concise data was distributed normally as confirmed by Shaprio-Wilk test (*P*<0.05). For intergroup comparison, a two-way analysis of variance (ANOVA) (Table 1) and Tukeys post-hoc test was used (Table 2) and assessment for the failure types was done using the Chi-square test (*P*< 0.05) (Table 3).

Results:

The present study demonstrated that the highest push-out bond strength was of Bio-C bioceramic sealer after treatment with 0.2% chitosan (Group IIIB) while the least was of AH Plus sealer after 17% EDTA treatment (Group IA). Also, the push-out bond strength of both sealers is different and the mean for Bio-C bioceramic sealer is more than AH Plus sealer.A mixed mode of failure was predominantly present in all AH Plus sealer groups,while Bio-C bioceramic sealer reported cohesive type of failure commonly.

Table 1: Intergroup comparison using Two-way ANOVA:

	Sum of squares	df	Mean square	F	*P*-value
Between chelating agents	1.238	2	1.8510	4.321	0.002*
Between sealers	1.127	1	1.248	3.112	0.001***
Between chelating agents and sealers	1.315	2	2.459	5.216	0.001***

Table 2: Multiple comparisons of various groups by Tukey's post-hoc test

Group (I)	Group (J)	Mean Difference (I-J)	Standard Error	*P*- value	95% confidence limit	
					Upper	Lower
IA	IIA	-0.51	0.41	0.01**	-0.89	-0.35
	IIIA	-0.39	0.22	0.02*	-0.50	-0.10
	IB	-0.6	0.10	0.01**	-0.30	-0.05
	IIB	-0.72	0.13	0.03*	-0.90	-0.15
	IIIB	-0.80	0.21	0.01**	-0.45	-0.20
IIA	IA	0.51	0.41	0.01**	0.70	0.30
	IIIA	0.11	0.21	0.01**	0.50	0.05
	IB	-0.10	0.32	0.02*	-0.20	-0.06
	IIB	-0.22	0.13	0.03*	-0.60	-0.10
	IIIB	-0.30	0.21	0.02*	-0.41	-0.20
IIIA	IA	0.39	0.22	0.03*	0.50	0.15
	IIA	-0.11	0.21	0.01**	-0.60	-0.02
	IB	-0.21	0.13	0.01**	-0.35	-0.10
	IIB	-0.33	0.21	0.02*	-0.60	-0.29
	IIIB	-0.41	0.23	0.01**	-0.70	-0.20
IB	IA	0.60	0.10	0.02*	0.30	0.02
	IIA	0.10	0.32	0.01**	0.40	0.05
	IIIA	0.21	0.13	0.01**	0.30	0.12
	IIB	-0.12	0.22	0.01**	-0.34	-0.02
	IIIB	-0.2	0.12	0.02*	-0.8	-0.1
IIB	IA	0.72	0.13	0.01**	0.95	0.15
	IIA	0.22	0.13	0.02*	0.58	0.14
	IIIA	0.33	0.21	0.01**	0.49	0.17
	IB	0.12	0.22	0.01**	0.34	0.8
	IIIB	-0.08	0.25	0.03*	-0.018	-0.02
IIIB	IA	0.80	0.25	0.01**	0.95	0.32
	IIA	0.30	0.21	0.02*	0.53	0.11
	IIIA	0.41	0.23	0.01**	0.69	0.14
	IB	0.20	0.12	0.02*	0.21	0.02
	IIB	0.08	0.25	0.01**	0.18	0.01

Table 3: Comparison of modes of failure between different groups using the Chi-square test

Group	Failure mode	%	*P*-value
Group IA	Adhesive	16	0.01**
	Cohesive	14	0.01**
	Mixed	70	0.02*
Group IIA	Adhesive	15	0.01**
	Cohesive	6	0.01**
	Mixed	79	0.02*
Group IIIA	Adhesive	17	0.01**
	Cohesive	11	0.02*
	Mixed	72	0.02*
Group IB	Adhesive	8	0.01**
	Cohesive	87	0.02*
	Mixed	5	0.01**
Group IIB	Adhesive	9	0.01**
	Cohesive	88	0.02*
	Mixed	3	0.02*
Group IIIB	Adhesive	8.8	0.02*
	Cohesive	89.5	0.01**
	Mixed	2	0.01**

Discussion:

The successfulness of endodontic therapy is predominantly determined by the binding of root canal filling materials to the root dentin. It eradicates the effluence of irritants into periradicular tissues and resists the dislodgement forces acting during condensation of permanent restorative materials.[4]The bonding between endodontic sealers and the walls of the canal is crucial in both static and dynamic conditions so as to eradicate the spaces, not allowing the transfer of fluids and microbes from the dentin to the root canal filling material and vice-versa. It ensures maintenance of the sealer-dentin alliance during various operative and restorative procedures yielding the mechanical stresses.[5]A multitude of factors like formation of smear layer during biomechanical preparation may inhibit the diffusion of irrigation agents and sealers into the tubular dentin. The smear layer was first noted by Eick JD et al (1970).However, McComb D et al(1975) first disclosed presence of a smear layer on the instrumented root dentin surface.[6]They described composition of this layer as shavings of dentinal cuttings, residual processes of odontoblasts, remnants of pulp and a biofilm of microbes. [6] A plethora of views were mentioned regarding the maintenance or elimination of the smear layer during biomechanical preparation. Some investigators advocate root canal preparation without smear layer removal as it seals the radicular dentinal tubules acting as a barrier. It restrains the microbialentry into the tubules of dentin.[7]However, many arguments have been presumed to eradication of smear layer before obturation as it acts as substratum for the survival of microorganisms, resulting in various future infections; prohibits the effect of intracanal medicaments by resisting their way into the tubular dentin; restricts the adequate adherence of root canal sealer to the dentinal surface, thus, compromising the satisfactory seal formed hence, increasing the probability of post-obturation microleakage.[7] Sodium hypochlorite (NaOCl), introduced by Henry Drysdale Dakin is considered a benchmark among the endodontic irrigants owing to its antimicrobial efficacy, ability to act on necrosed as well as vital pulp tissue, organic debris and biofilm. Nevertheless, it has no effect on the mineralized component of the smear layer.[8]Therefore, adjunctive use of biocompatible acids or chelating solutions is recommended to allow adequate disinfection of the root canal by elimination of both portions of the smear layer. 17% ethylene diaminetetraacetic acid(EDTA) was used in the present study with pH = 7.5. It results in better smear layer removal at this concentration and p H.[9]Also, sequence of using NaOCl followed by EDT A favours the opening of the dentinal tubules facilitating the elimination of debris, thereby, filling of lateral canals by the sealer.[9]But EDTA lacks antimicrobial characteristics. Therefore, the use of peracetic acid (PAA) is advocated for collective smear layer removal and root canal disinfection. The acetic acidin the peracetic acid is liable for dissolving smear layer.In the current study, 1% PAA (final rinse) was selected as it efficiently removes smear layer. At higher concentrations, it decreases the hardness of dentin by facilitating enhanced calcium loss from the dentinal walls.Also, NaOCl and 1% PAA have a synergistic effect on smear layer removal and pulp tissue dissolution.[10]Chitosan interacts with the mineralized portion of the smear layer and the metallic ionsresulting in the formation of complexes called chelates. The hydrophilic characteristics of chitosan favours deeper infiltration into dentinal tubules as it closely contactsroot canal dentin.[11]Presently there are two models to interpret the process of chelation. The first one 'the chemical chain bridge model' advocates the attachment of two or more amino groups of chitosan molecule to a similar metal ion during the process of chelation. The second version 'the hook or free-arm model' emphasizes the involvement of only one amino group in the attachment to the metal ion.[11] 0.2% chitosan was used in this study because it removes smear layer with minimal erosion of dentinal tubules. The combination of NaOCl and chitosan was selected as chitosan' spossess a tendency to inhibit bacterial adherence and formation of biofilm on the dentinal surface.[11]The application of all the chelating agent was done for 1 minute. This is the optimum time for adequate chelation. If time extended above 1minute reckless erosion and enhanced demineralization have been reported.[12]Similar effects were seen for peracetic acid. The use of 0.2% chitosan for more than 1 minute resulted in the increased diameter of tubular dentin as heavy erosion of the dentinal surfaceis observed.[12]The chelating solutions affect the structural and chemical constituents of the dentin, and alter the penetration properties of endodontic sealers. Thus, the usage of chelating solutions for the final rinsing of the canals has always been a subject of concern for researchers asthey influence adherence of the sealers to the dentinal surface.In the present study, two commercially available endodontic sealers having varied adhering properties were studied. AH Plus sealer, an epoxyresin-based, creates a covalent bond with the radicular dentin and pervades into the tubules as the epoxide ring is opened.[13] Bio-C bioceramic sealer is a premixed ready-to-use, injectable calcium-aluminosilicate paste. It is hydraulic in nature and sets if moisture is present, lead to high dimensional stability and minimal shrinkage.[13]For measuring the strength of adherence between dentin and root canal filling materials, different bond strength tests have been a preferred approach.[14] Tests include micro-tensile strength testing, shear strength and push-out strength testing.The current study used the push-out bond strength test to evaluate the adhesiveness of AH Plus sealer and Bio-C bioceramic sealer to radicular dentin. This method is less sensitive, more reliable, easily employed, reproducible, simple to interpret, and generates forces parallel to the dentin-sealer interface.[14] But certain factors such as the position of the sample, variation in root canal diameter and size of plunger influence the push-out testing. In the current study, these shortcomings were overcome by standardizing the thickness of the root sections to avoid uneven distribution of stress and prevent debonding. Under experimental situation, the results revealed that each chelating agent (17% EDTA, 1% peracetic acid and 0.2% chitosan) significantly affected the push-out strength of the sealers (AH Plus and Bio-C bioceramic sealer). This is in conformance with studies conducted by Jain G et al.[15]Conversely, results contradicted Carvalho et al who reported that chelating agents did not influence the dislodgement resistance in respective sealers.[16] Bio-C bioceramic sealer showed higher push-out strength as compared to AH Plus sealer irrespective of the irrigation protocol followed. This may be attributed to the synthesis of hydroxyapatite during setting, a chemical bond amid the sealer and dentinal wall leading to the creation of a 'mineral infiltration zone'.[17]Further bonding efficacy is increased by low shrinkage during setting, the small size of particles and the low contact angle allows it to spread easily ensuring adequate hermetic seal.[17]For the Bio-C bioceramic sealer, the impact of chitosan was maximum at the push-out bond strength followed by peracetic acid and the least of EDTA. This may be because of the hydrophilicity of the chitosan that enabled the sealer to move deeply into the dentinal tubules and abundance of free hydroxyland amino groups favoured the ionic reaction with the calcium ions present in the dentin.[10]The peracetic acid exposes the collagen fibres present in the dentinal matrix and thus sealer has chances of dentin hybridization and more bond strength of the sealer.The reason behind the least push-out bond strength in EDTA may be the baffling action of EDTA on the apatite crystals synthesized during the setting of the Bio-C sealer. Additionally, the continuous formation of chelates by calcium ions from the sealer interfered with the adhesion of the sealer. These conclusions are in compliance with Agarwal S et al.[18] In the case of AH Plus sealer, the samples irrigated with peracetic acid had the highest mean push-out bond strength followed by chitosan and EDTA. Peracetic acid removes the collagen layer (destroyed by NaOCl) and exposes the healthy layer of tubular dentin, thus, enhances the adhesion of the sealer. Its acidity prevents the reprecipitation of calcium ions for adequate demineralizing effect responsible for the maximum retention of sealer in the samples obturated with AH Plus sealer.[19]The decreased bond strength in groups irrigated with chitosan than those with peracetic acid is because of absence of a surface roughening effect thus lesser penetration of the sealer.Moreover, chelating effect of chitosan opened the dentinal tubules later acted as stress raisers for specific areas,thus resulting in the failure of the adhesive interface.The lower demineralization ability and lack of surfactant effect in the case of EDTA were responsible for the low wettability and increased pH reduces the breakdown of hydroxyapatite, thus decreasing the strength of adhesion of AH Plus sealer to the radicular dentin.[19]The literature states an interrelationship between dislodgement resistance and the mode of failure.[20]If the dislodgement resistance is more, fracture is expected to occur inside the sealer (cohesive). Since stereomicroscope is a non-invasive method, it was used to analyse the failure type.All the specimens were evaluated using stereomicroscopic techniques following the push-out test, to determine the failure modes. AH Plus sealer revealed the mixed type of bond fracturepredominantly while cohesive failure was more common in Bio-C bioceramic sealer irrespective of the chelating solution used as a final rinse in both the subgroups.

AH Plus sealer penetrates the tubular dentin. There is a layer of filler particles with diameter larger than dentinal tubules. The presence of a resin-depleted layer and interface enrichedwith filler particles favoured existence of a mixed mode of fracture.[21] Bio-C bioceramic sealer showed mainly cohesive failure due to the breakdown of the sealer-dentin interface as the dislodgement resistance increased after the application of force. The presence of a mineral infiltration zone at the sealer-dentin interface resulted in minimal gap creation, forming a complete bond. These observationsare consistent with the results by Bayram et al.[22]

Limitations of the present study: Since the roots of the premolar teeth may show a certain degree of curvature, the perfect horizontal sectioning of the specimens perpendicularly to the long axis may be hindered, thus affecting the push-out bond testing. The use of bioceramic-coatedgutta-percha would have enhanced the bond strength. Additionally, certain invivoconditions may impact the clinical outcome. Therefore, invivo studies are important for evaluating the achievement of AH Plus sealer and other bioceramic sealers using various chelating solutions as the final rinse.

Conclusion:

Considering the limitations of the present study, it can be concluded that chelating agents when employed for final rinsing of root canal before obturation, influence the bond strength of endodontic sealers.

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