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Evaluation of bond strength of chitosan coated gutta-percha and chitosan incorporated resin- based sealer Bond strength of chitosan modified GP and sealer

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ABSTRACT

Context

Bond-strength of sealers to radicular dentin is essential for a fluid-tight seal ofthe obturation. A material with good bondability to root canal with antimicrobial properties would be desirable.

Aim

Evaluation of push-out bond-strength(PBS) of chitosan-modified gutta-percha(GP)and sealer.

Study design

An in-vitro study was designed following the Checklist for Reporting In-vitro Studies.

Materials and methods

60 freshly extracted single-rooted mandibular first premolars were decoronated at the cementoenamel-junction(CEJ), subjected to root canal treatment, randomized into four groups: (i)Control,(ii)0.2%chitosan-coatedGP+0.2%chitosan-modified AH-Plus sealer(CGP+CAH),(iii)0.2%chitosan-coatedGP+unmodified AH-Plus sealer(CGP+AH) and (iv)uncoated GP+0.2%chitosan-modified AH-Plus sealer(GP+CAH). 1mm thick horizontal sections from coronal, middle and apical thirds were subjected to PBS testing and SEM to analyze bond failure.

Statistical Analysis

PBS was compared with One-way ANOVA and Tukey's HSD Post-hoc Analysis, failure pattern with Chi-squared test.

Results

Mean PBS in coronal, middle and apical thirds was found to decrease in the following order Group 4,Group 1,Group 3 and Group 2. The PBS of Group 4 was found to be significantly higher than other groups. The difference in PBS ofothergroups was insignificant. Intragroup pushout bond-strengthwas greatest in the coronal third followed by the middle and apical thirds with the difference being insignificant. All specimens showed predominantly mixed failure.

Conclusion

Group 4 provided best PBS. Group 2 showed lowest PBS. Better PBS can be achieved with the use of uncoated GP and 0.2% CAH.

Keywords: Gutta-percha, Chitosan, Push out bond, Sealer, Root canal, Endodontic treatment

I. INTRODUCTION

The primary goal of endodontic therapy is the elimination of microbial load from the root canal system through mechanical and chemical means[1,2] and to develop a fluid-tight seal.[3] Chemomechanical preparation of the root canal is achieved by a combination of mechanical instrumentation, irrigation and antibacterial medication.[4] This is followed by the placement of a root canal filling.

Gutta-percha(GP) is biocompatible, dimensionally stable, radiopaque, and easy to remove but requires a sealer to ensure that the intricacies of the root canal system are sealed.[5] Even though predictable clinical results have been obtained with the use of nonbonding root canal sealers [6], a search for sealers that bond to root canal dentin and filling materials continues.

Due to its excellent properties, such as low solubility, less expansion, adhesion to dentin and good sealing ability, AH-Plus is considered the "Gold Standard" among resin-based sealers. It is thixotropic, radiopaque and has good flow.[7] However, it has minimal antimicrobial properties and doesn't bond to the root dentin.

The pulp space can never be rendered completely free of microorganisms; hence, anti-microbial materials provide an added advantage in preventing reinfections. Antibiotic enhanced sealers are presumed to disrupt the microbial environment and maintain bactericidal properties for an extended period of time.

Chitosan is of interest in health-sciences research due to its biocompatible nature and antimicrobial properties.[8]In endodontics, majority of this research pertains to its use as an irrigant. Recent studies have shown good antibacterial activity and improved the physical and mechanical properties with the use of chitosan-coated GP(CGP).[9] There are studies incorporating chitosan in eugenol based sealers[5,10]. However, there are not many studies evaluating the properties of chitosan-modified resin-based sealers. Also, the presence of free amino groups in chitosan[11] may provide an avenue for a better bonding between CGP and the chitosan-modified resin-based sealer. Besides, different combinations of incorporating chitosan either to the GP or resin-based sealer have not been evaluated.

In the context of the above, this study was undertaken to evaluate the PBS of different combinations of incorporating chitosan either to GP or resin-based sealer, to root dentin.

II. METHODS

The study was developed following the recommendations of the Checklist for Reporting In-vitro Studies.[12]The study was approved by the Instititutional Ethcis Committee(Id:43-IRB-2019). Informed consent was taken from all research participants for use of their teeth in this study. 60 freshly extracted(less than 6months old) single rooted mandibular first premolars with single straight canals(verified radiographically)were collected, cleaned and placed in 0.5%chloramine-T and stored at 4–7◦C for a maximum of 6months beforestart of the experiment.

Sample size was estimated to be 60 using GPower v.3.1.9.2considering the effect size to be measured at 45%, power of the study at 80% and the margin of the error at 5%; 15 samples per group.

A 0.2% chitosan solution was prepared and GP points(DiaDent, India), size 30 6% taper, coated with this solution were allowed to dry as per previous study by Cardelle-Cobas A et al. [9] The unmodified AH-Plus sealer(Dentsply Sirona, Germany) was prepared as per the manufacturer's instructions. The modified AH-Plus sealer was prepared by proportionally adding chitosan 0.2% by weight.[13]

A single operator carried out root canal treatment to ensure uniformity. Before canal instrumentation, each tooth was decoronated at the CEJ using a diamond disk. Root lengths were standardized to 12 mm. Canal patency and working lengths were established by inserting a #15 K-file(Mani, Japan) 1 mm short of the radiographic apex. Instrumentation was completed by using Hyflex CM #30 file with 6% taper(ColteneWhaledent, India) to working length. The canals were irrigated with 3% sodium hypochlorite(Prime Dental Products Pvt. Ltd., India) during instrumentation and a final rinse with 17% EDTA(Prime Dental Products Pvt. Ltd., India) for 30 seconds to effectively remove the smear layer. The cleansed and shaped canals were dried with absorbent points and randomly divided based on a computer-generated sequence table into the 4 obturation groups-

(i) Control
(ii) 0.2%chitosan-coated gutta-percha+0.2%chitosan-modifiedAH-Plus sealer(CGP+CAH),
(iii) 0.2%chitosan-coatedgutta-percha+unmodified AH-Plus sealer(CGP+AH) and
(iv) Conventional gutta-percha points+0.2%chitosan-modifiedAH-Plus sealer(GP+CAH).

Passive-fit single-cone obturation protocol, without any type of condensation, was followed. The sealer was coated onto the root canal with the help of the GP point followed by obturation with the GP point coated with sealer to working length. The excess GP was sheared off using a heated ball burnisher. The specimens were stored at 37◦C in dry conditions for 24 hours for the initial set of the sealer.

Each sample was measured lengthwise and evenly divided into three segments: a coronal, middle, and apical segment. These samples were then sectioned using a diamond disk perpendicular to the long axis to obtain 1mm thick sections and subjected to PBS tests using a universal testing machine(Mecmesin/Multitest 10i), at a crosshead

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speed of 1mm/min using a cylindrical plunger. The load upon failure was recorded in Newton and converted to Mega Pascal(MPa). The mode of failure(MOF) of different obturation groups, after PBS tests, was visualized by means of scanning electron microscopy(Carl Zeiss/Ultra 55 SEM) independently by two examiners.

Statistical Package for Social Sciences (Windows Version 22.0 Armonk, NY: IBM Corp.), was used to perform statistical analyses. One-way ANOVA test followed by Tukey's HSD Post hoc Analysis was used to compare the mean PBS between 04 groups. Chi-Squared Test was used to compare the different grades of bond failure interface between different study groups. Level of significance [P-Value] was set at P<0.05

III. RESULTS

1.1. Push-out Bond strength

The mean PBS in the coronal 3rd region(Table 1) was found to be greatest in Group 4 followed by Group 1, Group 3 and Group 2. According to Tukey's post hoc test, the mean PBS in the coronal 3rd region was found to be statistically higher for Group 4 as compared to all the other groups, while a statistical difference in the PBS of Group 2 was found only when compared to Group 1 and Group 4. The difference in PBS of Group 3 was statistically significant only when compared to Group 4.

The mean PBS in the middle 3rd region(Table 1) was found to be greatest in Group 4 followed by Group 1, Group 3 and Group 2. According to Tukey's post hoc test, the mean PBS in the middle 3rd region was found to be statistically higher for Group 4 as compared to all the other groups, while a statistical difference in the PBS of Group 2 was found only when compared to Group 1 and Group 4. The difference in PBS of Group 3 was statistically significant only when compared to Group 4.

The mean PBS in the apical 3rd region(Table 1) was found to be greatest in Group 4 followed by Group 1, Group 3 and Group 2. The mean PBS in the apical 3rd region was found to be statistically higher for Group 4 as compared to all the other groups, while a statistical difference in the PBS of Group 2 was found only when compared to Group 1 and Group 4. The difference in PBS of Group 3 was statistically significant only when compared to Group 4.

Table 1: Comparison of mean push-out bond-strength (in MPa) between 4 groups in different regions using One-way ANOVA Test

Region	Groups	N	Mean	SD	P-Value
Coronal 3rd	Group 1	15	2.387	1.009	<0.001*
	Group 2	15	1.727	0.332	
	Group 3	15	2.334	0.422	
	Group 4	15	4.241	0.756	
Middle 3rd	Group 1	15	2.344	1.014	<0.001*
	Group 2	15	1.700	0.335	
	Group 3	15	2.320	0.421	
	Group 4	15	4.231	0.753	
Apical 3rd	Group 1	15	2.298	0.998	<0.001*
	Group 2	15	1.686	0.338	
	Group 3	15	2.311	0.419	

	Group 4	15	4.224	0.754	

*** - Statistically Significant**

There was no statistically significant difference in the pushout bond-strength values between different regions in each group(Table 2). The PBS was highest in the coronal third followed by middle and apical thirds respectively.

Table 2: Comparison of mean push-out bond-strength (in Mpa) values between different regions in each group using Repeated Measures of ANOVA Test

Group	Regions	N	Mean	SD	P-Value
Group 1	Coronal	15	2.387	1.009	0.49
	Middle	15	2.344	1.014	
	Apical	15	2.298	0.998	
Group 2	Coronal	15	1.727	0.332	0.64
	Middle	15	1.700	0.335	
	Apical	15	1.686	0.338	
Group 3	Coronal	15	2.334	0.422	0.76
	Middle	15	2.320	0.421	
	Apical	15	2.311	0.419	
Group 4	Coronal	15	4.241	0.756	0.77
	Middle	15	4.231	0.753	
	Apical	15	4.224	0.754	

1.2. Mode of Failure

Mixed MOF was found to be the greatest in the coronal, middle and apical 3^{rd}regions(Figure 1a) across all 4 groups.

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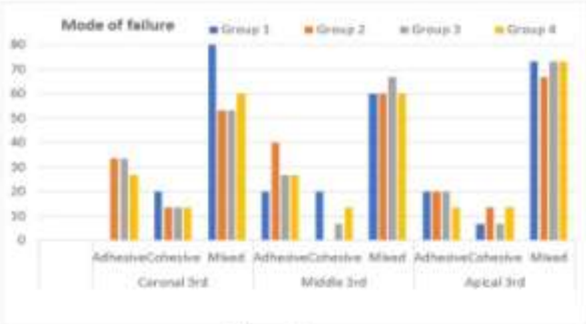

Figure 1a

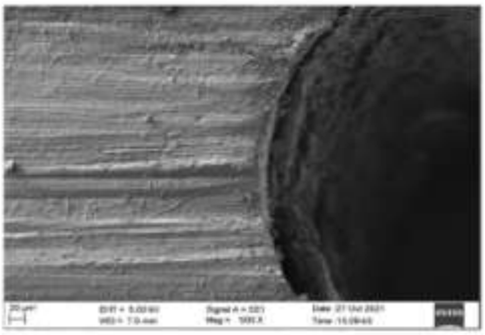

Figure 1b

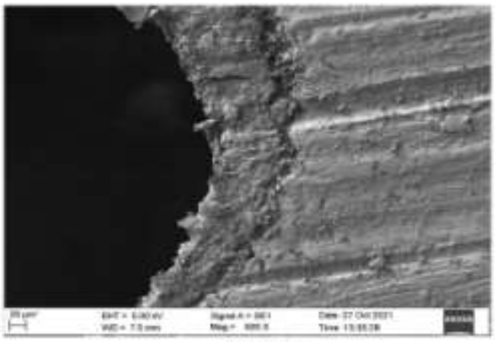

Figure 1c

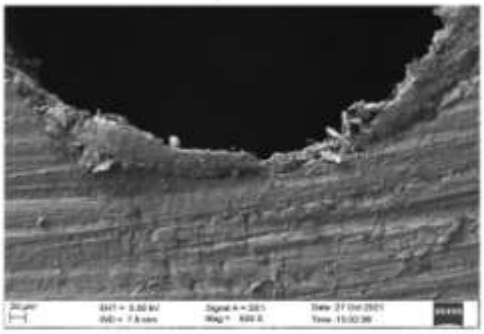

Figure 1d

Figure 1a- Mode of failure across different regions Figure 1b - Adhesive Failure
Figure 1c - Cohesive Failure Figure 1d - Mixed Failure

In the coronal 3rd,Group 1 showed the maximum mixed failures followed by Group 4, and Groups 2 and 3-which showed equal mixed failures. No adhesive failures were found in Group 1 while equal adhesive failures were found in Groups 2 and 3 followed by fewer adhesive failures in Group 4. Maximum cohesive failures were noted in Group 1 followed by an equal percentage in Groups 2 and 4, and the lowest in Group 3.

In the middle 3rd,Group 3 showed the maximum mixed failures followed by Groups 4,2 and 3-which showed equal mixed failures. Adhesive failures were maximum in Group 2 followed by equal adhesive failures in Groups 3 and 4, with the least in Group 1. No cohesive failures were seen in Group 2, while the maximum cohesive failures were noted in Group 1 followed by Group 4 and Group 3.

In the apical 3rd,Group 4 showed the maximum mixed failures followed by Groups 1,3, which showed equal mixed failures, followed by Group 2 with the least. Adhesive failures were maximum in Groups 1,2 and 3, with the least in Group 4. Maximum cohesive failures were seen in Groups 2 and 4 and the least in Groups 1 and 3.

Figures 1b, 1c and 1d show the different modes of failure.

IV. DISCUSSION

Bond-strength of root canal sealers to radicular dentin is important for the integrity of the seal in root canal filling.[14,15] Optimum adhesion requires intimate contact between the adhesive and adherend facilitating molecular interaction and chemical or micromechanical adhesion.[16] An advantage of using CGP with chitosan-modified sealer is the presence of functional amino groups on the surface of chitosan particles. These provide avenues for hydrogen bonding.[11] The results of the present study are discussed below.

Groups 2 and 3, containing CGP, showed lower PBS as compared to groups with uncoated GP when used with conventional AH-Plus as well as chitosan-modifiedAH-Plus. Al-Haddad, Kutty and Aziz found the PBS of resin-coated GP to be lower than that of other groups studied.[17] Another study comparing the bond-strengths of a calcium silicate-based root canal sealer, to dentin or calcium silicate-impregnated GP, with the bond-strengths of an epoxy resin-based sealerfound that the bond of the calcium silicate-based root canal sealer to impregnated GP was not superior to the epoxy resin-based sealer bonded to uncoated GP.[18] However, other studies that evaluated the bond-strength of hydroxyapatite-coated GP, showed improved bond-strength compared to uncoated

GP.[17,19]Findings of the present study appear to be concurrent with findings of majority of the studies on use of coated GP. In absence of contrary studies, it may be concluded that coating the surface of GP with chitosan is not a viable method for improving the bond-strength of GP to sealer.

In Group 4, a significant increase in the bond-strength was noted when CAH was combined with uncoated GP. Elsaka S et al.[20] found incorporation of chitosan solution into RealSeal self-etching primer to be advantageous, since the bond-strength of RealSeal system with chitosan-modified self-etching primer to radicular dentin was not significantly different from the control. Other studies on the bond-strength of antibiotic incorporated resin-sealers have shown either no difference or improved PBS.[21,22] Existing literature showed no degradation of the bond when chitosan and other antibiotic agents are incorporated into sealers. The results of this study have shown improved bond-strength when CAH was used with unaltered GP. This could possibly be explained by the presence of functional amino groups on the surface of chitosan particles which can form covalent linkages with the epoxy groups of the resin-based sealer.[11] However, a lack of literature on this combination of AH-Plus sealer with chitosan calls for further research to validate the findings of this study.

In Group 2, a marked decrease in the bond-strength was noted when CAH was used with CGP. This could be explained by the hypothesis that increasing concentration of chitosan tends to result in formation of large, discrete, aggregated nanoparticles[23], thus degrading the quality of the bond.

Although Group 4 showed significantly better bond-strength compared to Groups 1, 2 and 3, its antibacterial efficacy needs to be studied further. Considering the antimicrobial properties of chitosan and the bond-strengths of Groups 1, 2 and 3 (which were found to be statistically insignificant), the use of either of the experimental groups could be beneficial over conventional obturation materials due to added antimicrobial action. Long term bond-strengthand antimicrobial sensitivity testing will provide further insight on this subject.

The regional PBS was seen to decrease from the coronal to the apical third in accordance with previous studies.[24,25] This could be attributed to the variable and irregular root canal anatomy in the middle and apical thirds. This decrease in bond-strength in the middle and apical thirds could be due to improper adaptation of the GP to the root canal walls, resulting in an increased thickness of sealer between the GP and root canal walls.

Failure mode analysis by SEM showed predominantly mixed failures in all groups indicating a good bond between the sealer and dentin. This study demonstrated that CAH when used with uncoated GP showed significantly improved bond-strength, while CGP when used with AH-Plus sealer or CAH provided no additional benefit in terms of bond-strength.

V. CONCLUSION

Within the limitations of this study, thefollowing conclusions can be put forth:

(a) Group 4 showed a statistically significant higher PBS in the coronal, middle and apical thirds compared to other groups. This suggests a better bond with root dentin when obturation was carried out withuncoated GP points with 0.2% CAH.
(b) There was no significant difference in bond-strength of Groups), 2 and 3.
(c) In terms of bond-strength, addition of chitosan to sealer enhanced the bond-strength but there was no additional benefit of improving the bond by coating the GP with chitosan.
(d) Intragroup comparison showed the PBS to be greatest in the coronal followed by the middle and apical 3rd with no significant difference between the three groups.
(e) Mixed MOF was found to be the greatest across all groups and regions.

While the anti-microbial effect of chitosan-modified sealers and CGP have been studied separately, more complex biofilm models and microbiological tests are needed to further evaluate any advantage a combination of the two might provide. Insight into this would provide valuable information on the viability of using this combination.

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