

Comparative Evaluation of Push-out Bond Strength of Conventional Mineral Trioxide Aggregate, Biodentine, and Two Novel Antibacterial-enhanced Mineral Trioxide Aggregates

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ABSTRACT

Aim: To evaluate the push-out bond strength of two newly modified mineral trioxide aggregates (MTAs) with conventional MTA and biodentine.

Materials and methods:

Material preparation: Two commercially available bioactive bioceramics: Group I: Mineral trioxide aggregate; Group II: Biodentine; and two newly formulated modified MTAs: Group III: Doxycycline incorporated MTA formulation; Group IV: Metronidazole incorporated MTA formulation was used in the present study. All the test materials were then carried using a plastic instrument to the desired experimental design.

Teeth sample preparation: A total of 120 teeth samples were collected and divided into four groups of test materials with 30 teeth samples per group. Single-rooted permanent teeth, that is, incisors were collected and stored in saline until the study was performed. Sectioning of the teeth into 2.0 ± 0.05 -mm thick slices was performed perpendicular to the long axis of the tooth. The canal space was instrumented using Gates Glidden burs to achieve a diameter of 1.5 mm. All four prepared materials were mixed and placed in the lumen of the slices and placed in an incubator at 37°C for 72 hours.

Push-out test and bond failure pattern evaluation: The push-out test was performed using a universal testing machine. The slices were examined under a scanning electron microscope (SEM) at 40× magnification to determine the nature of bond failure. All the collected data were recorded and statistically analyzed.

Results: The mean push-out bond strength was found to be the highest for group II (37.38 ± 1.94 MPa) followed by group III (28.04 ± 2.22 MPa) and group IV (27.83 ± 1.34 MPa). The lowest mean push-out bond strength was noticed with group I (22.89 ± 2.49 MPa). This difference was found to be statistically significant ($p = 0.000$). Group I samples had the predominantly adhesive type of failure (86.4%), while group II samples showed the cohesive type of failure (94.2%). Both the modified MTAs (groups III and IV) primarily showed mixed types of failures.

Conclusion: Both the antibacterial-enhanced MTAs had better pushout bond strength compared to conventional MTA but did not outperform biodentine. Hence, it could serve as a substitute for conventional MTA due to its augmented physical properties.

Clinical significance: Carious pulp exposure and nonvital open apices pose a critical challenge to pediatric dental practitioners. In such circumstances, maintaining the vitality of pulp and faster healing would help in a better prognosis. Novel MTAs without any cytotoxic components, and enhanced antibacterial contents with augmented physical properties can help in treating such clinical conditions.

Keywords: Antibacterial-enhanced mineral trioxide aggregate, Biodentine, Dental, Disease, Doxycycline, Metronidazole.

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INTRODUCTION

Decades since its introduction, the conventional mineral trioxide aggregate (MTA), has been continuously modified and/or upgraded to suit the clinicians' needs. Reduced cytotoxicity, significant sealing ability, better biocompatibility, and ability to induce the formation of dentin make MTA suitable for use in pulp therapies including pulp capping and apexification procedures.¹ Recent studies have shown that tricalcium aluminate, being one of the major constituents in the conventional MTA that is responsible to accelerate the initial cement hydration, has shown to be having cytotoxic properties that compromise the biocompatible nature of the cement. Authors have suggested eliminating tricalcium silicate and replacing the material with other biocompatible chemicals can improve the physical properties and biological properties of the cement.²

Efforts to enhance the antimicrobial efficacy of this bioactive cement have involved the use of agents such as chlorhexidine

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gluconate, tetracycline, nitric oxide-releasing compounds, calcium hydroxide, and, more recently, the incorporation of fluorohydroxyapatite and calcium fluoride.³⁻⁵ While there has been an improvement in antimicrobial activity, these modifications often result in a compromise of the cement's physical properties.⁶ Metronidazole, a broad-spectrum nitroimidazole, has demonstrated lethality against gram-positive anaerobes such as *Enterococcus faecalis*.⁷ Attempts to combine metronidazole with glass ionomer cements and antibiotic intracanal medicaments have yielded varied results.^{7,8} Another antimicrobial, doxycycline, a hydroxyl derivative of tetracycline, has been used as an adjuvant in irrigants and intracanal medicaments over the past decade.⁹ A literature search reveals only one identified study that concludes doxycycline did not significantly enhance the antimicrobial activity of the final set of biodentine cement.¹⁰

Based on the use of such bioactive bioceramic cements, it is necessary to assess the marginal adaptations and push-out bond strength that tends to evaluate the material's resistance to dislocating forces and its ability to bond with dentin.^{11,12} Testing push-out bond strength permits specimen standardization and replicates clinical stress thereby making it more reliable and practical for assessment.^{13,14}

Based on current published research, we were not able to find published data comparing the push-out bond strength of calcium silicate-based cements with added antimicrobial agents. Therefore, the purpose of the current study was to evaluate the push-out bond strength of conventional MTA, biodentine, doxycycline incorporated MTA and metronidazole incorporated MTA. The null hypothesis was that there was no difference in the push-out bond strength between the materials tested.

MATERIALS AND METHODS

Study Design

The current study was an *in vitro* design performed at the dental material research facility of a private dental institute between July 2023 to September 2023. The study design, method of experimentation, and the composition of the material used were approved by the members of the institutional ethical committee. The null hypothesis for the present study was that the four bioceramic cements used would not have any difference in the push-out bond strength.

Material Preparation

The commercially available materials available in the dental market which were used in the present study were as follows:

- Group I: Mineral Trioxide aggregate was obtained from Angelus (Londrina, PR, Brazil) packed as powder-liquid formulation was dispensed as recommended by the manufacturer (3:1) on a mixing pad. The powder was completely hydrated by the liquid until the mix turned to be thick, like a putty-like consistency.
- Group II: Biodentine was obtained from Septodont (Saint Maur des Fossés, France) packed as powder-liquid formulation was dispensed as recommended by the manufacturer. Five drops of the liquid were poured into the capsule containing the powder and mixed using a mechanical triturator (Dentsply Maillefer) for roughly 30 seconds. Once the mix was complete, it would have a thick consistency like putty.

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Table 1: Composition of newly formulated MTAs in groups III and IV used in the current study

Group III	
Powder	Weight % (wt%) for every 100 mg of powder
Tricalcium silicate	60
Dicalcium silicate	20
Calcium fluoride	5
Calcium sulphate	5
Calcium carbonate	4
Zirconium oxide	1
Liquid	Concentration
Calcium chloride	20%
Doxycycline	5%
Group IV	
Powder	Weight% (wt%) for every 100 mg of powder
Tricalcium silicate	60
Dicalcium silicate	20
Calcium fluoride	5
Calcium sulfate	5
Calcium carbonate	4
Zirconium oxide	1
Liquid	Concentration (%)
Calcium chloride	20
Metronidazole	1

The newly formulated modified and enhanced MTAs that were used in the present study were:

- Group III: Doxycycline incorporated MTA—the novel composition of MTA in this group comprises tricalcium silicate, dicalcium silicate, calcium carbonate, calcium sulfate, and calcium fluoride as the base powder components. Tricalcium silicate and dicalcium silicate, the core components, were synthesized in the laboratory following the manufacturing process recommended by Moon HJ *et al.*² Doxycycline and calcium chloride in powder form were sourced from TCI Chemicals (India) Pvt. Ltd. For a 5% concentration, doxycycline was separately dissolved in 1 mL of distilled water. Calcium chloride was mixed with 1 mL of distilled water to achieve a 20% concentration. Both liquids were combined until a uniform mixture was obtained. The specific composition is detailed in Table 1. Using trial-and-error ratios, 100 mg of the proposed powder content and 40 µL of the liquid component were dispensed on a pad for the mixing process.

After complete hydration of the powder with the liquid, the mixing procedure was continued until a uniform mix with a moldable consistency was achieved.

- Group IV: Metronidazole incorporated MTA—the powder component in this group mirrored the composition provided in group III. Metronidazole, obtained in powder form from TCI Chemicals (India) Pvt. Ltd., was separately dissolved in 1 mL of distilled water to attain a 1% concentration. Calcium chloride, mixed with 1 mL of distilled water, aimed for a 20% concentration. Both liquids were mixed until a uniform mixture was achieved. The specific composition is detailed in Table 1. Following trial-and-error ratios, 100 mg of the proposed powder content and 40 μ L of the liquid component were dispensed on a pad for the mixing process. After the complete hydration of the powder with the liquid, the mixing procedure was continued until a uniform mix with a moldable consistency was obtained.

Teeth Collection

A total of 120 maxillary central incisors were used in the present study. The teeth were extracted from patients who had a noncarious tooth structure with severe periodontitis condition that had a poor prognosis. A signed consent was obtained from the study participants after informing them about the use of the extracted teeth for research purposes. The teeth were immersed in 5.25% sodium hypochlorite to remove any remnant hard and soft tissues before the samples were stored in saline until the study was performed. On the day of performing the study, the teeth were visually examined. The presence of any caries, fractures, and cracks led to the exclusion of the tooth sample. A radiographic examination was also done to exclude any aberrant anatomy, pulp stones, internal resorption, and the presence of more than one root canal.

Teeth Sample Preparation

Teeth were embedded vertically in a rubber mold with the use of a mounting device that ensured orientation along the long axis. Epoxy resin was used for mounting purposes (Vertex Orthoplast; Vertex-Dental, Zeist, The Netherlands). Sectioning of the tooth was performed perpendicular to the long axis of the tooth at the middle third. The teeth were sliced into 2.0 ± 0.05 -mm thick slices using a low-speed diamond disc under continuous water irrigation. A total of 120 root dentin slices ($n = 30$ per group) were obtained.

To standardize the teeth samples used in the study, a single operator instrumented the canal space in each slice using Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland). A complete pass of burs from size 2 to size 6 sequentially was done to achieve a diameter of 1.5 mm. The prepared samples were rinsed using 17% ethylene diamine tetra acetic acid for 3 minutes to remove any smear layer formed during the above-mentioned canal preparation. Finally, the samples were rinsed using saline and paper points were used to absorb any remnant liquid along the canal walls. The samples were randomly divided into four groups based on the materials prepared as mentioned above. All four prepared materials were mixed as per the instructions provided and placed in the lumen of the slices using an MTA carrier or plastic instrument. The material was then condensed using an endodontic plugger. Excess material was removed, and the specimens were wrapped in a piece of wet gauze and kept at 100% relative humidity in an incubator at 37°C for 72 hours.

Table 2: Mean push-out bond strength of the test materials used in the present study

Group	Material	Mean \pm SD (MPa)	p-value
I	MTA	22.89 \pm 2.49 [†]	0.000
II	Biodentine	37.38 \pm 1.94*	
III	Newer doxycycline incorporated MTA formulation	28.04 \pm 2.22* [†]	
IV	Newer metronidazole incorporated MTA formulation	27.83 \pm 1.34* [†]	

*Statistically significant when compared with mean Push-out bond strength of group I. [†]Statistically significant when compared with mean Push-out bond strength of group II. MPa, megapascals; MTA, mineral trioxide aggregate; SD, standard deviation

Push-out Test

The push-out test was performed using a Universal Testing Machine (Instron testing machine; Model 5965, ITW, Massachusetts, USA) as per the methodology suggested by Alsubait et al.¹⁵ The operator was blinded to the material used in the sample. The push-out bond strength was calculated in megapascal (MPa) by using the following formula:

Bond strength (MPa) = Force for dislodgement (N)/Bonded surface area (mm^2)

Bonded surface area = $2 \times p \times r \times h$

where $p = 3.14$ (constant), $r =$ radius of the root canal, and $h =$ thickness of the dentin slice in millimeters.

Bond Failure Pattern Evaluation

Following bond failure while performing the push-out test, the slices were examined under a scanning electron microscope (SEM) at 40 \times magnification to determine the nature of bond failure. The nature of bond failure was categorized as (A) adhesive failure (bond failure noticed at the material-dentin interface); (B) cohesive failure (fracture noticed within the material); and (C) mixed failure, being the combination of the above two failures. The operator examining the slices was blinded to the type of material used for each sample. All the collected data were recorded and statistically analyzed.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 21; SPSS Inc, Chicago, Illinois, USA). To check normal distribution, Shapiro–Wilk normality test was performed. To compare the means of push-out bond strength for all the different materials tested, a one-way analysis of variance (ANOVA) test was performed. A *post hoc* test was performed for intergroup comparisons. A p -value of < 0.05 was considered statistically significant.

RESULTS

The mean push-out bond strength was found to be the highest for group II (37.38 \pm 1.94 MPa) followed by group III (28.04 \pm 2.22 MPa) and group IV (27.83 \pm 1.34 MPa). The lowest mean push-out bond strength was noticed with group I (22.89 \pm 2.49 MPa) (Table 2). Analysis of variance showed a statistical significance among the tested materials rejecting the null hypothesis ($p = 0.000$).

Post hoc tests revealed that there were statistically significant differences when comparing group I with all the other groups and group II with all the other groups. There was no significant difference when comparisons were made between the newly formulated MTAs, that is, groups III and IV.

Regardless of the cement used, all types of failures were noticed during the SEM analysis of the samples. The majority of the samples under group I had the adhesive type of failure (86.4%), while group II samples showed the cohesive type of failure (94.2%). Both groups III and IV showed mixed types of failures (89.3 and 84.9%, respectively) (Table 3). Representative images of the bond failures noticed in each group were provided in (Fig. 1).

Improved bond strength and mixed type of bond failures of both the antibacterial-enhanced MTAs outperform the conventional MTA but cannot surpass the strength and bond of biodentine.

DISCUSSION

One of the ideal requirements of endodontics materials used for apical plug in apexification, repair of perforations, and capping during vital pulp therapy was to resist mechanical stresses during tooth function.¹⁶ This can be determined by evaluating the adhesion of such materials to dentin surfaces using different bond strengths that include tensile, shear, and push out. Push-out bond strength was used in the current study that has been repeatedly assessed

during the past few decades for assessment of similar endodontic materials in clinical practice.^{11,17}

Various attempts have been made to modify conventional MTA to improve compressive strength, setting properties, and antimicrobial properties. One such recent study by Ravindran V and Jeevanandan G involved a modification of conventional MTA with removal of tricalcium aluminate that showed improved compressive strength and better antimicrobial properties.¹⁸ Similar material was used in the present study to check on its adhesive property toward dentine.

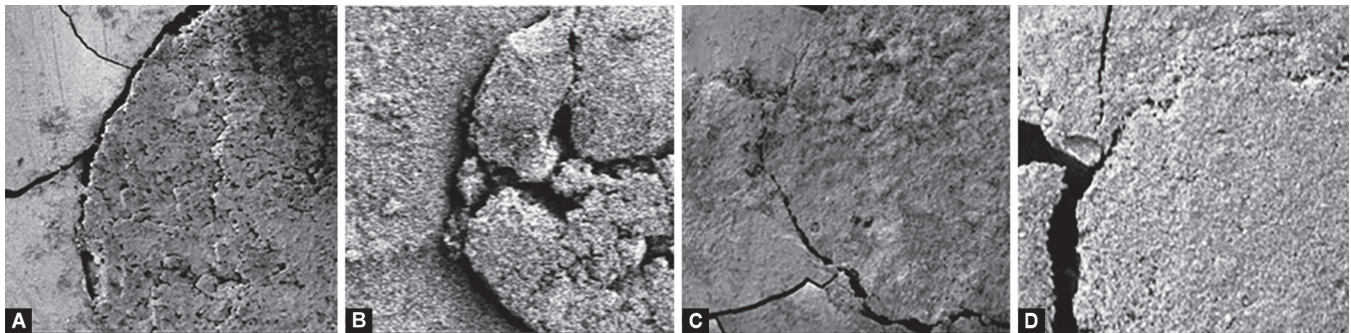
Antimicrobial properties of conventional MTA assessed by various authors showed a low to moderate activity against *E. faecalis* as compared to biodentine, a tricalcium silicate cement.¹⁹ Agents like doxycycline, calcium fluoride, chlorhexidine gluconate, and fluorohydroxyapatite were suggested to improve antimicrobial activity.^{10,20,21} In the present study, 5% doxycycline was added in group IV and 1% metronidazole was added in group V. For the assessment of the push-out bond strength test, a universal testing machine was used which was the predominantly utilized test performed in similar other studies.^{11,17} The samples were tested at an identical elapse of time period, that is, 72 hours, which could minimize the difference in different setting time periods of the varied materials used. This was done to standardize the post-mixing time before performing the bond strength test.

Antibiotics have been a longstanding component in dental practice for disinfection purposes, employed in various forms such as irrigants, intracanal medicaments, and as additives to dental cements.^{7,8} Previous attempts to combine chlorhexidine with dental cements did not yield improvements in the final cement's physical properties and cytotoxicity.⁶ Notably, *E. faecalis*, a highly resistant endodontic pathogen, has demonstrated susceptibility to nitroimidazole antibiotics like metronidazole.⁷ Metronidazole demonstrates bactericidal effects, combating anaerobic microorganisms through the inhibition of nucleic acid synthesis.²² The tetracycline group, including minocycline and doxycycline, has been a traditional component in triple antibiotic pastes.⁹ Tetracycline has bacteriostatic properties, effective against both gram-positive and gram-negative bacteria, by obstructing protein synthesis through its binding to the ribosomal subunit.²² This rationale guided the addition of metronidazole and doxycycline in the current study, revealing superior outcomes compared to conventional MTA.

Table 3: Types of bond failures of the test materials used in the present study

Group	Material	Adhesive (%)	Cohesive (%)	Mixed (%)
I	MTA	86.4	6.9	6.7
II	Biodentine	1.5	94.2	4.3
III	Newer doxycycline incorporated MTA formulation	4.8	5.9	89.3
IV	Newer metronidazole incorporated MTA formulation	6.7	8.4	84.9

MTA, mineral trioxide aggregate



Figs 1A to D: Representative SEM images of bond failures noticed in the materials tested at 40x magnification. (A) Adhesive failure in group I samples; (B) Cohesive failure in group II samples; (C) and (D) Mixed failure in group III and group IV samples, respectively

The results of the present study showed that the antibacterial-enhanced MTAs had better push-out bond strength when compared to conventional MTA. However, biodentine showed the highest push-out bond strength among the materials tested. This result was supported by previous studies which showed similar conclusions.^{17,23–25} Authors have suggested that neither the presence of a smear layer nor the irrigants used affected the push-out bond strength. Also, tag-like structures are formed by biodentine adjacent to root canal dentin which would have enhanced the retention.¹⁵

There were no other studies to compare the complete results of the present study as this was the first study to assess the push-out bond strength of an antibacterial combination of MTA-type cements. However, there are studies to justify the reason behind eliminating TCA and the addition of calcium fluoride. Another study compared BioAggregate, ProRoot MTA, and biodentine which showed lower push-out bond strength of BioAggregate, an aluminum-free cement. The authors suggested that the absence of tricalcium aluminate was the reason behind the lower bond strength of the cement.¹⁵ This was contradicting with the results of the present study as the modified MTAs were aluminum-free. The absence of aluminum did not compromise the bond strength. This could be due to the addition of calcium fluoride to the modified MTAs. Ranjkesh et al. studied the bond strength of another novel fast-setting calcium silicate that showed improved bonding to dentine increased over time which was due to the presence of fluoride.²⁶

The present study also evaluated the type of bond failure that occurred with the materials tested. The majority of conventional MTA samples showed adhesive failure which was supported by the results of Saghiri et al.²⁷ and Shokouhinejad et al.²⁸ but contradicted by the findings of Rahimi et al.²⁹ Different study designs of the aforementioned studies might have provided varied results. Biodentine's cohesive failures in the present study were also in accordance with various other studies^{15,25,29,30} which could be due to the smaller particle sizes and tag-like formations which enhanced the retention at the interface. A mixed type of failure was noticed with the modified MTAs. This could be due to the reduced setting duration before the push-out test was performed.

Based on the literature search, the present study was found to be the only study that assessed push-out bond strength for MTA-based cements with the addition of antibacterial agents. However, this *in vitro* methodology cannot replicate the *in vivo* clinical environment. Based on the findings of the present study, MTA with the addition of doxycycline or metronidazole, did not affect the push-out bond strength of the set cement. Although the antibacterial-enhanced MTAs had comparatively better bond strength when compared to conventional MTA, it could not surpass the strength of biodentine. Due to the faster setting of the modified MTAs as per previous research and better push-out bond strength as per the current study, the antibacterial-enhanced MTAs could serve as an alternative to the conventional MTA in day-to-day clinical practice.¹⁸

CONCLUSION

Both the antibacterial-enhanced MTAs (either doxycycline or metronidazole) did not affect the push-out bond strength of the material. Although these novel materials outperformed the

conventional MTA, they still lagged biodentine. Such antibacterial-enhanced cements can serve as a substitute for conventional MTA due to their augmented physical properties. Hence, potential clinical advantages for the newly formulated antibacterial-enhanced MTAs, while recognizing the superior performance of biodentine.

Ethical Approval

The study design, method of experimentation, and the composition of the material used were approved by the members of the institutional ethical committee (No. SRB/SDC/PhD/Pedo/2022/045).

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