Original Article

An *in vitro* comparative evaluation of the effect of three intracanal medicaments – chlorhexidine gel, triple antibiotic paste, and calcium hydroxide paste on the push-out bond strength of MTA Plus, Biodentine, and calcium-enriched mixture

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ABSTRACT

Aim: Caries or traumatic injuries affecting young permanent teeth during root development usually result in an open apex which is highly challenging to treat. The aim of this study was to evaluate the push-out bond strength of apical plugs of Mineral Trioxide Aggregate (MTA) Plus, Biodentine, and calcium-enriched mixture (CEM) after premedication with chlorhexidine, triple antibiotic paste (TAP), and calcium hydroxide.

Methods: Fifty-four extracted intact anterior teeth were decoronated and 3 mm was sectioned from the apex. The canals were rendered parallel using #80 K-files and #3, #4 Peeso reamers to mimic an open apex situation. The samples were divided into 3 groups containing 18 samples each for the three intracanal medicaments. After premedication, the samples were stored for 2 weeks at 37 C, following which the canals were cleaned using #80 K-file along with ethylenediaminetetraacetic acid and sodium hypochlorite irrigation. The samples were further divided into three subgroups, each containing six samples for the three calcium silicate cement used in this study. They were later subjected to push-out bond strength testing.

Results: Regardless of the type of intracanal medicament used, Biodentine had significantly higher bond strength than MTA Plus and CEM. The highest push-out bond strength results were obtained in samples premedicated with chlorhexidine. Compared to TAP and calcium hydroxide ($Ca(OH)_2$), this value was statistically significant ($P < 0.001$).

Conclusion: Within the limitations of this study, Biodentine showed the highest push-out bond strength as compared to MTA Plus and CEM. Chlorhexidine may be superior to calcium hydroxide and TAP medicaments when used for apexification procedures with calcium silicate cement.

Keywords: Biodentine, calcium hydroxide, calcium-enriched mixture, chlorhexidine, MTA plus, push-out bond strength, triple antibiotic paste

INTRODUCTION

Challenges in endodontic treatment of immature pulpless teeth include problems in achieving complete debridement and optimal sealing of the root canal system.[1] The most common treatment option for inducing apical closure is by apexification using calcium hydroxide.[2]

In recent times, calcium silicate cement have evolved as viable alternatives to calcium hydroxide apexification procedures.[3] The most widely used calcium silicate cement is Mineral Trioxide Aggregate (MTA) (Dentsply, Tulsa Dental Specialties,

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Johnson City, TN, USA). It has excellent sealability, bioactivity, and biocompatibility. However, it has handling difficulties and a prolonged setting time. In order to overcome these drawbacks, several modifications of MTA have been developed.[4]

MTA Plus (Prevest DenPro, Jammu, India) is a new calcium silicate-based cement which can be used for apexification. It has adequate radiopacity, rapid setting time, and a high early strength.[5]

Some of the more recent modifications of MTA include Biodentine (Septodont, France) and calcium-enriched mixture (CEM) (Yektazist Dandan, Iran). Biodentine has excellent biocompatibility and bioactivity along with high strength and rapid setting.[6] CEM is a modification of MTA containing calcium silicate, calcium phosphate, calcium sulfate, calcium carbonate, calcium chloride, and calcium oxide. Its particle size is smaller than that of MTA, and it exhibits good sealing ability and regenerative potential.[7-9]

During one-step apexification procedures with calcium silicate cement, disinfection of the canal should be achieved with minimal root canal instrumentation as the dentinal walls are already very fragile. Hence, it is necessary to use suitable intracanal medicaments prior to placement of the barrier to ensure proper healing. The most commonly used intracanal medicament is calcium hydroxide. Various other medicaments such as triple antibiotic paste (TAP) and chlorhexidine have also been recommended for root canal disinfection during apexification procedures.[10-12]

Typically, medicament pastes are removed by the use of an endodontic K-file, followed by copious irrigation with sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA). In case of open apices, complete elimination from the apical region is practically impossible due to the fragility of this region. This raises concern whether traces of the medicament may interfere with the placement and setting of the barrier material.[10]

Therefore, the aim of the present study was to check the effect of residual medicament used during apexification (calcium hydroxide paste, chlorhexidine gel, and TAP) on the adhesive strength of three new calcium silicate-based cement (Biodentine, MTA Plus, and CEM).

The null hypothesis is that none of the intracanal medicaments have any effects on the push-out bond strength of tricalcium silicate cement.

METHODOLOGY

Fifty-four maxillary extracted anterior teeth, free of cracks, caries, and restorations were used in this study. The teeth were cleaned using a periodontal scaler to remove any soft tissue remnants and calculus and stored in 0.5% thymol solution.

Preparation of samples

The collected teeth were decoronated and 3 mm of the root apex was sectioned and sealed with wax. The length of the roots was standardized as 10 mm. The canals were prepared using hand files up to size #80 K-files and the walls were made parallel using #3, #4 Peeso reamers. The samples were then divided into 3 groups of 18 samples each for the 3 intracanal medicaments, following which calcium hydroxide (RC Cal, Prime Dental, India), TAP (metronidazole, ciprofloxacin, and doxycycline mixed with propylene glycol, Cash Pharmacy, Bangalore), and chlorhexidine gel (Gluco-CHeX 2% gel, Cerkamed, Poland) were placed in the prepared canals.

The specimens were stored at 37°C in 100% humidity for 2 weeks after which the medicaments were removed using a #80 K-file along with EDTA and sodium hypochlorite irrigation. The groups were further divided into three subgroups, each containing six specimens.

Calcium silicate cement (MTA Plus [Prevest DenPro Limited], Biodentine [Septodont], and CEM [Bionique Dent]) were placed inside the sectioned specimens using a hand plugger. After 1 week, the samples were subjected to push-out bond strength testing (universal testing machine, crosshead speed of 1 mm/min).

RESULTS

For Biodentine samples, the mean push-out bond strength value was 16.76 ± 6.99 MPa, for the CEM group, it was 3.98 ± 1.40 MPa, and for MTA Plus, it was 5.49 ± 1.85 MPa. The difference among these groups was found to be statistically significant ($P < 0.001$) [Table 1].

Table 1: Estimated marginal mean push-out bond strength between the calcium silicate cement: Biodentine, calcium-enriched mixture, and Mineral Trioxide Aggregate Plus

Calcium silicate cement	*n*	Mean±SD	95% CI for mean		*F*	*P*
			Lower	Upper		
Biodentine	18	16.76±6.99	13.29	20.24	48.580	<0.001*
CEM	18	3.98±1.40	3.28	4.68		
MTA Plus	18	5.49±1.85	4.57	6.42		

*Statistically significant ($P<0.001$); SD: Standard deviation; CI: Confidence interval; CEM: Calcium-enriched mixture; MTA: Mineral trioxide aggregate

When the effect of the three intracanal medicaments on push-out bond strength of the tested calcium silicate cement was compared, CHX sample exhibited a mean value of 11.36 ± 9.77, the $Ca(OH)_2$ group of 8.54 ± 5.69, and the TAP group of 6.33 ± 4.02 [Table 2].

Statistical method

Two-way analysis of variance (ANOVA) test was used to compare the mean push-out bond strength to observe the possible interaction between different calcium silicate cement and intracanal medicaments. One-way ANOVA test followed by Bonferroni's *Post hoc* analysis was used to compare the mean push-out bond strength between different calcium silicate cement and intracanal medicaments.

DISCUSSION

The barrier material used during apexification should provide an excellent seal while resisting displacement under functional loading. Thus, bond strength to root dentin is an important factor for long-term clinical success. This property can be evaluated using various tests including tensile, shear, and push-out bond strength. The push-out test has been demonstrated to be a reliable, efficient, and practical method to assess the bond strength of apical barrier materials to root canal dentin.[13]

Maxillary anterior teeth were selected for the present study as they are single rooted and are most commonly involved in traumatic injuries in children leading to immature root closure. The protocol employed for mimicking an open apex situation was according to the methodology of Nagas *et al*. and Berkhoff *et al*.[10,14]

While calcium hydroxide has been the gold standard intracanal medicament, 2% chlorhexidine and TAP are recently gaining popularity. Hence, these three medicaments were chosen for the present study.

Chlorhexidine is a broad-spectrum antimicrobial agent with low cytotoxicity and added substantivity. 2% chlorhexidine gel has been recommended as an intracanal medicament in immature nonvital teeth as it has no adverse effects even upon contact with periapical tissues.

Table 2: Estimated marginal mean push-out bond strength between intracanal medicaments: Chlorhexidine, $Ca(OH)_2$, and triple antibiotic paste

ICM	*n*	Mean±SD	95% CI for mean		*F*	*P*
			Lower	Upper		
CHX	18	11.36±9.77	6.50	16.23	18.607	<0.001*
$Ca(OH)_2$	18	8.54±5.69	5.72	11.37		
TAP	18	6.33±4.02	4.33	8.33		

*Statistically significant (P<0.001); CHX: Chlorhexidine; TAP: Triple antibiotic paste; CI: Confidence interval; SD: Standard deviation

TAP was introduced by Topçuoğlu *et al*. as a medicament for disinfection of infected root canals. It is a combination of three antibiotics, namely, metronidazole, minocycline, and ciprofloxacin. Metronidazole is a nitroimidazole compound which is antimicrobial again protozoans and bacteria. Minocycline is bacteriostatic and shows activity against Gram-positive and Gram-negative bacteria. Since minocycline causes discoloration of tooth structure, it has been replaced with doxycycline, which has a similar antibiotic spectrum. Ciprofloxacin is a synthetic fluoroquinolone, exhibiting high antibacterial activity against Gram-negative bacteria. TAP is therefore an effective medicament against both Gram-positive and Gram-negative anaerobic bacteria seen commonly in endodontic infections.[1,11] Several clinicians also favor its use as a medicament during apexification.

The recommended duration of intracanal medication in open apex cases is 2 weeks as studies have reported that beyond this period, there is a significant decrease in the microhardness of dentin.[15] Hence, this protocol was followed in the present study.

The most common method to remove intracanal medicaments prior to placement of the apical barrier material is manual irrigation with EDTA and sodium hypochlorite along with gentle use of a size #80 K-file. This was the technique employed in the present study.[10]

Irrespective of the technique employed so far, no method has been able to completely eliminate residual intracanal medicaments from open apex situations.[10] Therefore, the aim of the present study was to check the effect of the residual medicament on the adhesive strength of three new calcium silicate-based cement (Biodentine, MTA Plus, and CEM), as it is crucial that these cement should resist the condensation pressure of root canal obturation materials.

The three calcium silicate cement were manually condensed as it was demonstrated to be a superior method than ultrasonic condensation by Aminoshariae *et al*.[16] Push-out bond strength testing was done after 1 week of storage as this is reported to increase the bond strength values.[17]

From the results of the present study, it is clear that chlorhexidine medication gave the maximum push-out bond strength for all the tested calcium silicate cement followed by calcium hydroxide paste while TAP gave the least values. Based on this, the null hypothesis was rejected. The superior

performance of chlorhexidine is attributed to its minimal effect on dentin demineralization and microhardness.[18]

Calcium hydroxide did not significantly affect the bond strength of CEM and MTA Plus. The high alkaline pH of calcium hydroxide has been reported to improve the bonding properties of calcium silicate cement. It has been reported that MTA Plus reacts with residual calcium hydroxide resulting in improved marginal adaptation.[19]

In contrast, prior application of calcium hydroxide lowered the bond of Biodentine to a significant extent. A previous study reported a slight erosion of dentinal tubules after medication with calcium hydroxide as a factor in reducing the bond strength of Biodentine.[6] This could also be a likely reason for the findings of the present study.

Of the three medicaments tested, TAP gave the least bond strength. TAP has a higher diffusion and retention within the dentin making it difficult to eliminate. Its high acidity (pH 2.9 ± 0.1) causes significant erosion of dentinal tubules. It has been reported that it consistently decreases dentin microhardness. As a result, dentin is rendered softer which may affect the push-out bond strength of calcium silicate cement. The findings of the present study corroborate the results of previous studies.[6,20]

Among the three calcium silicate cement, Biodentine demonstrated the highest bond strength compared to MTA Plus and CEM. The smaller particle size and uniform components may be a factor in better interlocking of Biodentine within the dentin. This allows for better penetration within the dentinal tubules forming tag-like structures leading to a micromechanical anchor.[21] The results of the present study also revealed Biodentine exhibited the highest push-out bond strength when in contact with 2% chlorhexidine. This is in accordance with Guneser *et al*. who observed under SEM that contact with chlorhexidine showed the normal globular crystalline structure of Biodentine without any alteration.[22]

MTA Plus demonstrated significantly lower bond strengths than Biodentine. It is primarily composed of tricalcium silicate and bismuth oxide. It is similar in composition to MTA except that the particle size is finer to make it more reactive. Hence, its response could also be considered similar to MTA explaining the findings of the present study.[23]

According to the results, CEM demonstrated the least push-out bond strength and was affected by all the three intracanal medicaments. This is in accordance with the findings of Sobhnamayan *et al*.[24] They observed that the exposure of CEM to both alkaline and acidic pH decreases its bond strength. The alkaline pH influences the early hydration of the setting cement and may cause the breakdown of the calcium silicate gel. The same authors also observed that acidic pH decreased push-out bond strength of CEM. SEM analysis showed the development of porous substances and lack of needle-like crystals. Adhesion between CEM and dentin walls as well as formation of hydroxyapatite crystals are likely to be delayed. (adhesion of the cement to dentin walls, as well as delay the hydroxyapatite crystal formation).[11]

Finally, to sum up the findings of the present study, it may be inferred that when calcium silicate cement are used for apexification, premedication with chlorhexidine may be a better option instead of calcium hydroxide or TAP. Of the three calcium silicate cement tested, Biodentine demonstrated the highest bond strength.

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Conflicts of interest
There are no conflicts of interest.

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