Original Article

Evaluation of calcium ion release from apical plugs formed by Biodentine and MTA with and without incorporation of triple antibiotic powder and modified triple antibiotic powder (cefaclor) using atomic absorption spectrophotometry – An *in vitro* study

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Abstract

Introduction: Apexification procedure with Mineral trioxide aggregate (MTA) and Biodentine as apical plugs along with the incorporation of medicaments such as silver zeolite, chlorhexidine, and triple antibiotic powder (TAP) is a new area of research that is gradually gaining momentum in dentistry.

Aim: The study aimed to evaluate and compare the calcium released from the apical plugs formed by MTA and Biodentine with and without incorporation of 2% TAP and 2% modified triple antibiotic powder (mTAP).

Materials and Methods: Ninety single-rooted teeth were randomly divided (n = 15) into two experimental groups with three subgroups (n = 5) each based on the composition of apical plugs (4 mm) as follows: Group A: Biodentine – Subgroup 1: Biodentine, Subgroup 2: Biodentine + 2% TAP, and Subgroup 3: Biodentine + 2% modified TAP and Group B: MTA – Subgroup 1: MTA, Subgroup 2: MTA + 2% TAP, and Subgroup 3: MTA + 2% modified TAP. Each sample tooth was then immersed in 10 mL of deionized water. Evaluation of calcium release was done on days 7, 15, and 30 using an atomic absorption spectrophotometer. Data were analyzed using a one-way analysis of variance and a Tukey's *post hoc* test.

Results: Calcium ion release was maximum for Biodentine compared to MTA and was greater with materials incorporated with TAP and mTAP than materials alone at days 7, 15, and 30.

Conclusion: The incorporation of 2% TAP and 2% mTAP resulted in increased calcium ions released from MTA and Biodentine which helps in faster apexification.

Keywords: Apexification; atomic; calcium ions; spectrophotometry; triple antibiotic paste

INTRODUCTION

Open apex teeth in need of endodontic treatment present several challenges to clinicians. A thin blunderbuss apical

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portion of the root canal with fragile dentinal walls might increase susceptibility to fracture during cleaning and shaping. Along with this, there is an increased risk of extruding material into the periradicular tissues. These problems can be treated by apexification procedure.[1,2]

Traditionally, the material of choice for the apexification procedure was calcium hydroxide owing to its antimicrobial properties and ability to stimulate hard tissue formation.[1] "Although calcium hydroxide[3] has shown evidence of successful results, there have been some associated drawbacks also." Limitations of calcium hydroxide apexification procedure include extended treatment time (9–24 months), need for multiple appointments, lack of patient compliance, and increased susceptibility to root fracture.[1]

The advent of one-step or two-step apexification procedure with the use of hydrated calcium silicate types of cement has largely overcome the above-mentioned drawbacks found in association with calcium hydroxide.[3] The major advantage associated with these cement is decreased duration of treatment and predictable results.[1] Calcium silicate based cement (CSC's) such as Mineral trioxide aggregate (MTA) and Biodentine are biocompatible, antimicrobial with superior sealing ability.[4]

The two most frequently used HCSC for apical plug formation are MTA and Biodentine.[2,4]

The drawbacks of using MTA as a root canal filling material include difficult handling characteristics, discoloration potential, and long setting time. When compared with MTA, Biodentine depicts better handling characteristics and sealing ability, lack of discoloration, and shorter setting time of 12 min, thereby alleviating the need for two-step apexification and risk of bacterial contamination.[5-8] Further, the mechanical properties of Biodentine were comparable to those of natural dentine.[9] Due to its improved material properties, Biodentine has a distinct advantage over its closest alternatives in the treatment of teeth with an open apex.[10,11]

Another potential challenge in immature teeth is to ensure the disinfection of polymicrobial root canals. Several *in vitro* studies[12-15] conducted in the past demonstrated enhanced antimicrobial activity of HCSC as an apical plug when mixed with different medicaments such as 2% chlorhexidine and silver zeolite powder. Considering the diversity of root canal microflora and the possibility of bacterial resistance to a single antibiotic, the use of multiple antibiotics has been advocated to overcome the resistance and disinfection with polymicrobes.[16]

To date, the most effective combined drug to overcome the resistance of bacterial strains is triple antibiotic paste (TAP).[16] Several studies[14,16,17] support the superior efficacy and healing ability of TAP when compared to calcium hydroxide. The most important drawback associated with TAP is discoloration.[16]

Therefore, modified triple antibiotic paste (MTAP) with different medicament substitutes for minocycline, such as amoxicillin and cefaclor (a member of the second-generation cephalosporins suggested by Thibodeau and Trope), has been used to counter the above-mentioned drawbacks.[14,16]

Calcium ion release is a prerequisite for HCSC as it imparts bioactive, osteoinductive, osteoconductive, and biomineralizing properties to these cement.[4,6]

In this *in vitro* study, the incorporation of TAP and modified triple antibiotic powder (cefaclor) was done in MTA and Biodentine. Then, the study was assessed for calcium ion release from apical plugs formed by MTA and Biodentine using atomic absorption spectrophotometer.

MATERIALS AND METHODS

The study included 90 intact caries-free freshly extracted human single-rooted teeth which were examined for the absence of root fracture, preexisting external defects, or cracks on the root surface.

Sample preparation for the study was initiated by access opening with endodontic access bur no. 2 (Dentsply Maillefer, Switzerland). The patency was checked and the working length was determined using a 25 mm #10 K-file (Dentsply Maillefer, 108 Switzerland). Biomechanical preparation was done with hand K-files such that the apical part was prepared up to size 40 K and coronal flaring was done by size 80 K using 15% ethylenediaminetetraacetic acid (EDTA) (Glyde, Dentsply Maillefer, Ballaigues, Switzerland). Root canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) since this is the safe concentration for use in open apex cases between each instrumentation.[1] After completing biomechanical preparation, alternate irrigation with EDTA and NaOCl was done. This ensured the complete removal of the smear layer. Final irrigation with saline was done to ensure the complete elimination of NaOCl from canals. Root canals were dried with paper points as a final step.

Teeth were measured using a digital caliper and were decoronated through the cementoenamel junction, and simulation of the open apex was done by resecting apical 2 mm of root with the aid of a diamond disc on a rapidly rotating handpiece. All samples were standardized to have a 12 mm root length.

Subsequently, all root samples were randomly divided into two experimental groups that is Group A: Biodentine and Group B: MTA. Then, each group was subdivided into three

subgroups based on the composition of apical plugs (4 mm) as follows ($n = 15$):

Group A: Biodentine:
- Subgroup 1: Biodentine
- Subgroup 2: Biodentine + 2% TAP
- Subgroup 3: Biodentine + 2% modified TAP.

Group B: MTA:
- Subgroup 1: MTA
- Subgroup 2: MTA + 2% TAP
- Subgroup 3: MTA + 2% modified TAP.

After root sample preparation, 2% TAP (ciprofloxacin + metronidazole + doxycycline) and 2% modified triple antibiotic powder (mTAP – mTAP prepared by replacing doxycycline of conventional TAP with cefaclor) were prepared and stored in a sterile airtight container.

For apical plug formation in Subgroup 1 of respective groups, Biodentine and MTA were strictly manipulated according to the manufacturer's instruction and a 4 mm apical plug was formed using endodontic pluggers.

While preparing apical plugs using Biodentine and MTA, 2% by weight of TAP and 2% of mTAP were mixed with propylene glycol in Subgroup 2 and Subgroup 3, respectively. A 4 mm apical plug was prepared using endodontic pluggers.

After the formation of apical plugs, nail varnish coating [Figure 1] was applied on an external surface of the root except at apical 2 mm. This is to standardize the evaluation of calcium ion release only through the apical end of the root canal. Coronal orifices were then sealed with a temporary gutta-percha.

Following the final setting time of Biodentine and MTA, all samples were stored in 10 mL of deionized water at 37°C. Fifteen samples from each subgroup were then subdivided into $n = 5$ by three different testing time intervals (7, 15, and 30 days) and were subsequently evaluated for calcium ion release at respective time intervals using atomic absorption spectrophotometer [Figure 2].

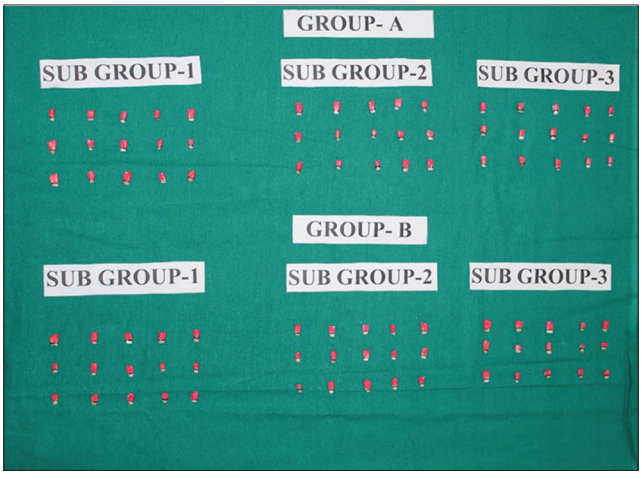

Figure 1: Prepared samples of each group

RESULTS

The data for the present study were entered into Microsoft Excel 2007 and analyzed using the IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. (Armonk, NY: IBM Corp). The descriptive statistics included mean and standard deviation. The level of significance for the present study was fixed at 5%.

The intergroup comparison for the difference of mean scores between independent groups was done using the one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis.

Tukey's range test is a single-step multiple comparison procedure and statistical test. It can be used on raw data or in conjunction with an ANOVA (*post hoc* analysis) to find means that are significantly different from each other. Tukey's test compares the means of every treatment to the means of every other treatment; that is, it applies simultaneously to the set of all pairwise comparisons and identifies any difference between two means that is greater than the expected standard error.

Apical plugs formed by MTA, Biodentine with and without incorporation of TAP, and modified triple antibiotic powder exhibited calcium release over 30 days of the study period.

One-way ANOVA [Table 1] showed that at all tested time intervals, i.e., 7th, 15th, and 30th days, Biodentine + mTAP demonstrated the highest calcium ion release and MTA revealed the least calcium ion release. ANOVA further revealed that among the experimental materials, incorporation of either TAP or modified triple antibiotic powder demonstrated greater calcium ion release when compared to materials alone at every periodic evaluation.

Tukey's *post hoc* test [Table 2] depicted a statistically significant difference at all tested periods in calcium ion release while comparing MTA and Biodentine + mTAP groups.

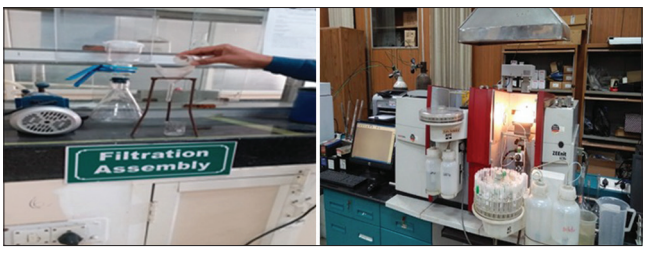

Figure 2: Atomic absorption spectrophotometry

DISCUSSION

The management of nonvital immature teeth necessitates the simulation of the apical barrier. One-step apexification with contemporary calcium silicate cement such as MTA and Biodentine has now become a gold standard for apexification procedures owing to their potential advantages over calcium hydroxide.[1,7,11] The high rate of calcium release and fast formation of apatite may well explain the role of calcium silicate biomaterials as a scaffold in apexification. Hence, MTA and Biodentine were selected in our present study.[4]

Another endodontic challenge associated with young permanent teeth is ensuring proper debridement of polymicrobial root canal infection. Various attempts have been made in the past to enhance the antimicrobial efficacy of apical plugs by incorporating medicament additives in them such as chlorhexidine, silver zeolite, doxycycline, and 2% TAP.[14,15,17] Similarly, 2% TAP and 2% mTAP were incorporated in MTA and Biodentine to form apical plugs.

Table 1: Analysis of variance

Materials	7th day (mean)	15th day (mean)	30th day (mean)
MTA	16.35±4.52	8.17±4.53	13.87±7.39
Biodentine	20.19±2.46	16.06±14.75	15.31±14.09
Biodentine + TAP	27.08±9.95	23.10±10.39	28.81±11.28
MTA + TAP	22.37±3.81	21.69±11.72	17.01±6.86
MTA + mTAP	26.91±7.21	34.51±6.78	39.58±22.21
Biodentine + mTAP	31.81±19.39	43.79±29.42	43.23±12.61
F	1.633	3.525	4.614
P	0.190 (NS)	0.016 (significant)	0.004 (significant)

NS: Nonsignificant, MTA: Mineral Trioxide aggregate, TAP: Triple antibiotic powder, mTAP: Modified TAP

Calcium ion release is cardinal for calcium silicate-based cement to exhibit biointeractive (ion-releasing) and bioactive (apatite-forming) properties.[4] Hence, the following study was undertaken to evaluate calcium ion release from MTA and Biodentine and also to whether the incorporation of TAP and modified triple antibiotic powder impacts their calcium release potential.

Ninety single extracted human teeth instead of polyethylene tubes that were used in various past *in vitro* studies were chosen to simulate the clinical situation and to standardize the release of calcium only from the apical one-third of the root canal.[18] This methodology was following a study conducted by Mapara *et al*.[19]

Similar to Latif and Al Qasi[20] in the present study, deionized water with neutral pH was chosen for immersion of prepared samples. This is to prevent the effect of storage medium (deionized water) on the calcium ion release of the prepared samples. Then, deionized water was evaluated under atomic absorption spectrophotometer for each sample separately at a time interval of 7 days, 15 days, and 30 days.

Numerous methods have been described in the past to measure calcium ion release which include atomic absorption spectrophotometry, fluorometry, colorimetry, EDTA titration method, flame photometry, and Ca ion-selective electrode.[21] According to Robertson and Marshall,[21] measuring calcium ion release using atomic absorption spectrophotometer was most accurate compared to other methods due to the potential advantages of being highly

Table 2: *Post hoc* analysis

	MTA	Biodentine	Biodentine + TAP	MTA + TAP	MTA + mTAP	Biodentine + mTAP
MTA						
7th day	-	0.539	0.094	0.338	0.099	0.019
15th day	-	0.421	0.135	0.174	0.012	0.001
30th day	-	0.867	0.091	0.715	0.006	0.002
Biodentine						
7th day	-	-	0.274	0.726	0.285	0.071
15th day	-	-	0.473	0.566	0.068	0.008
30th day	-	-	0.125	0.842	0.009	0.003
Biodentine + TAP						
7th day	-	-	-	0.451	0.978	0.45
15th day	-	-	-	0.885	0.248	0.042
30th day	-	-	-	0.177	0.217	0.102
MTA + TAP						
7th day	-	-	-	-	0.468	0.138
15th day	-	-	-	-	0.196	0.031
30th day	-	-	-	-	0.014	0.005
MTA + mTAP						
7th day	-	-	-	-	-	0.434
15th day	-	-	-	-	-	0.346
30th day	-	-	-	-	-	0.671
Biodentine + mTAP						
7th day	-	-	-	-	-	-
15th day	-	-	-	-	-	-
30th day	-	-	-	-	-	-

P<0.05 is significant. MTA: Mineral Trioxide aggregate, TAP: Triple antibiotic powder, mTAP: Modified TAP

specific for calcium, permitting large sample testing and little preparation required of the sample.[22]

Hence, the same methodology is followed in the present study for calcium ion release. Then, calcium ion release was evaluated at 7, 15, and 30 days. Similar to AL-Hyali,[23] the period of evaluation of calcium ion release from calcium silicate cement was at 7, 15, and 30 days, respectively. This periodic evaluation was justified by Gandolfi *et al*.[24] and Jacinto *et al*.[15] as hydration of calcium silicate cement yield calcium hydroxide as one of their by product. which further dissociates into calcium and hydroxyl ions. These calcium and hydroxyl ions responsible for alkalinizing and antimicrobial properties respectively. Calcium silicate materials like Ca(OH)2 types of cement possess early (up to 15–30 days) antimicrobial/bacteriostatic action related to their alkalinizing activity. Calcium silicate cement release Ca ions for a long time after setting and are the only family of materials ensuring a continuous release in contact with moist dentin and biologic fluid.[4]

In our present study, we have evaluated calcium ion release from HCSC by incorporating 2% TAP and 2% mTAP containing cefaclor. Results demonstrated that in all tested time intervals, incorporation of 2% TAP and 2% mTAP containing cefaclor increased the net amount of calcium release (43.23 ± 12.61) from study groups when compared to other groups containing materials (MTA and Biodentine) (13.87 ± 7.39) alone.

Similar results were observed by Pawar *et al*.[25] and Mapara *et al*.[19] that the incorporation of 2% TAP resulted in greater calcium ions release from MTA, Biodentine, and EndoSequence Root Repair when compared to study groups containing material only (MTA, Biodentine, and EndoSequence Root Repair).

The justification put forward by Rajasekharan *et al*.[26] and Yavari *et al*.[27] was that certain factors associated with HCSC, such as their composition, setting reaction, and change in pH of the environment during setting, vary the number of calcium ions released by them. Triple antibiotic paste has an acidic pH (2.9) which increases the solubility of tricalcium silicate cement.[26,27] This possibly explains increased calcium ion release from cement.

Further, when compared with 2% mTAP containing cefaclor, the tetracycline group of antibiotics tends to chelate with calcium ions, thereby decreasing the net amount of free calcium ions available.[28] Subsequent replacement of tetracycline with amoxicillin or cefaclor in triple antibiotic paste increases the net amount of free calcium ion available.

In our present study, Biodentine (15.31 ± 14.09) released more calcium ions than MTA (13.87 ± 7.39) at all tested time intervals. Aprillia *et al*.,[29] Kumari *et al*.,[30] Han and Okiji,[4] and Rakesh *et al*.[31] compared Ca2+ ion release from bioactive materials MTA Angelus® and Biodentine, and then, the authors concluded that Biodentine showed the highest calcium ion release.

The justification put forward by Gandolfi *et al*.[32] and Grech *et al*.[33] for the above results are based on the fact that calcium ion release from calcium silicate cement depends on a multitude of factors which include their composition, structure, solubility, and particle size.

The addition of calcium chloride in the liquid component of Biodentine results in a faster setting, thereby increasing calcium ion release by increasing calcium hydroxide formation and from unreacted calcium chloride. Apart from this, Biodentine exhibits smaller particle size and greater solubility. Biodentine® forms calcium phosphate particles that measure <1 μ, which results in a more compact surface layer when compared with MTA Angelus, which has a larger particle size (diameter: 1–5 μ).[29]

Apexification materials should be densely packed. A 4 mm apical plug thickness exhibits superior sealing ability and fracture resistance.[34,35]

The limitation of this study is that the bioactivity of apical plugs following the incorporation of TAP and mTAP was evaluated. Parameters such as physical and mechanical properties of HCSC when mixed with TAP need further evaluation in futuristic studies.

CONCLUSION

The clinical significance of this study is that in cases of immature teeth with pulp necrosis, medicaments like TAP can be incorporated in apical plugs. This has the advantage of enhancing both antimicrobial and bioactive properties of MTA and Biodentine. However, due to limited research, further *in vivo* studies are required to support the above evidence in the clinical scenario.

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Conflicts of interest
There are no conflicts of interest.

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