

Original Research

Comparative evaluation of intracanal medicaments on sealing ability of MTA and Hydroxyapatite apical plug in simulated immature teeth – An in vitro study.

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Abstract

Background: Immature teeth must be managed by apexification as there is no definite apical stop. Use of intracanal medicaments prior to apexification affect the sealing ability of apical plug. Present study was done to compare the effect of various intracanal medicaments: Metapex, Chlorhexidine digluconate, Curcuma longa, and Triple antibiotic paste (TAP) on the sealing ability of MTA and Hydroxyapatite as an apical plug.

Methodology: 130 single-rooted teeth were divided into two experimental groups (n=60 each) [Group I: MTA; Group II: Hydroxyapatite], and two control groups (n=5 each) [Group IIIA: MTA; Group IIIB: Hydroxyapatite] based on the apical plug used. Experimental groups were subdivided, based on intracanal medicaments [Group IA and IIA – Metapex; Group IB and IIB - 2% Chlorhexidine digluconate, Group IC and IIC - Curcuma longa, Group ID and IID - TAP]. After biomechanical preparation, simulation of immature apex was done using peeso-reamer. Intracanal medicaments were placed, and study samples were incubated for 7 days. Then all medicaments were removed using H-files, followed by retrograde apical placement, obturation, and restoration. Samples were subjected to dye penetration test, followed by clearing procedure. Apical microleakage was assessed using stereomicroscope at 40X. Data collected was subjected to statistical analysis using SPSS version 20.0 (p=0.05).

Results: Apical dye penetration was minimum in control group, followed by Group IB (0.17±.46) < IIB (0.53±.57) < IIC (0.90±.96) < IID (0.93±.59) < IC (1.83±.83) < ID (1.91±.81) < IIA (2.13±.81) < IA (2.31±.73). Intragroup comparison for apical microleakage was statistically significant (p<0.001), whereas intergroup comparisons were statistically significant except for group IA v/s IIA, IB v/s IIB, IC v/s IIA, ID v/s IIA.

Conclusion: Hydroxyapatite showed better sealing ability than MTA with all intracanal medicaments. Sealing ability was minimal with Metapex, followed by Turmeric, TAP, and 2% Chlorhexidine.

Keywords: Mineral Trioxide Aggregate; Hydroxyapatite; Metapex; Chlorhexidine Digluconate; Curcuma Longa; Triple Antibiotic Paste.

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Introduction

Aim of endodontic treatment is to remove bacteria and their by-products from the root canal system and to create a tight seal that prevents reinfection.[1] Successful endodontic therapy in an immature tooth is challenging, as it does not have a definite apical stop to achieve complete debridement and to limit the obturation.[2] If the pulp has become necrotic, as a result of trauma or other insults in an immature root, apexification is the treatment of choice. [3] Various materials used for apexification include dentinal chips, Portland cement, Hydroxyapatite, Biodentine, and Mineral Trioxide Aggregate (MTA). [2] MTA has gained popularity in single visit apexification; as it has good sealing ability, high degree of biocompatibility, low cytotoxicity, antimicrobial properties, ability to set in the presence of moisture, shorter treatment time, and induction of hard tissue deposition peri-radicularly. [4] Hydroxyapatite is one of the most biocompatible and bioactive materials formed of nano-sized particles like the apatite crystals of the tooth enamel morphology, crystal structure, and crystallinity. [5].

However, for successful endodontic therapy, various intracanal medicaments are used. They eliminate bacteria from the root canal system, prevent bacterial proliferation between appointments, and act as a physicochemical barrier, thus preventing root canal infection and nutrient supply to the remaining bacteria. [1] Various intracanal medicaments are available in the market, out of which Calcium hydroxide is most used. *Metapex* is a silicon oil-based calcium hydroxide paste, containing 38% iodoform and barium sulfate as a radiopacifier. It is widely accepted in dentistry due to its affectivity.[6] Other medicaments, such as 2% *Chlorhexidine digluconate*, were introduced to increase the antibacterial effect of intracanal medications and to eliminate microorganisms associated with persistent infection and treatment failure. [2] A herbal alternative, *Turmeric (curcumin longa)*, is a principal curcuminoid of the popular Indian spice turmeric.[7] The curcumin, a phenolic compound, has shown bactericidal, antioxidant, anti-inflammatory, and antimicrobial properties, which may prove to be a boon in dentistry. [8] As the root canal infections are polymicrobial, consisting of both aerobic and anaerobic bacterial species, a single antibiotic paste may not be effective in canal disinfection. Use of biocompatible *triple antibiotic paste (Ciprofloxacin, Metronidazole, Amoxicillin)* with potent antimicrobial properties is recommended for sterilizing the root canal.[9]

In immature teeth, the apex presents with two morphological variations: either a blunderbuss apex or a parallel-to-convergent apex. Conventional endodontic treatment is not indicated in both types of apices, as it is nearly impossible to attain an appropriate apical seal that can control the extrusion of obturating material. Thus, it is important to assess the efficacy of materials being used as apical plug; and further, it is imperative to evaluate the effect of intracanal medicaments, being used during the endodontic procedure, on the sealing ability of apical plug materials. In present study, sealing ability was assessed using the linear dye penetration method and clearing technique. We prefer to use clearing technique over tooth sectioning, as penetration of dye can be assessed in three dimensions, thus enabling the reading of maximum extent of dye. Thus, the present study was conducted to evaluate and compare the effect of intracanal medicaments: *Metapex* [calcium hydroxide with iodoform paste]; 2% *Chlorhexidine digluconate*; *Curcuma longa*, and *Triple antibiotic paste* on the sealing ability of MTA and Hydroxyapatite as an apical plug.

Materials and Methods:

The present study was conducted in the Department of Pediatric and Preventive Dentistry from the period of August 2022 to November 2024. A total of 130 single-rooted extracted teeth with single root canals having closed apices were included in the study. Teeth with developmental anomaly, external and internal resorption, calcified canals, extra canals, or curved canals were excluded from the study. Teeth were stored in 0.05% thymol crystal solution, till they were used further for the study.

All the study samples were decoronated to obtain a standardized length of 15mm. Endodontic access cavities were prepared using endo access burs, followed by root canal preparation using the crown-down technique with Protaper rotary instruments after determining the working length using RVG. The canals were irrigated using 2ml of 5.25% sodium hypochlorite and a final flush of 17% EDTA to remove the smear layer, followed by rinsing the canals with normal saline. To simulate the anatomy of teeth with immature apices, Peeso Reamers between #1 and #4 were introduced into the canals until #4 passes freely out of the apex.

The study samples were randomly divided into two experimental groups (n=60 each) [Group I: MTA and Group II: Hydroxyapatite] and two control groups (n=5 each) [Group IIIA: MTA; Group IIIB: Hydroxyapatite] based on the apical plug used. The experimental groups were further divided into four subgroups based on intracanal medicaments [Group IA and IIA- Metapex; Group IB and IIB - 2% Chlorhexidine digluconate; Group IC and IIC - Curcuma longa (Turmeric), Group ID and IID - Triple antibiotic paste (TAP)] used (n=15 each).

200 grams of ground turmeric powder was boiled in 500 ml of distilled water to prepare an aqueous turmeric extract. Triple antibiotic paste (TAP) was made with the combination of Ciprofloxacin 500mg tablets (4 tablets) + Metronidazole 400mg tablets (5 tablets) + Amoxicillin 500mg capsules (4 capsules). The antibiotic powder was then mixed with sterile saline on a glass slab (20 mg each mixed with 1 ml of sterile saline) to form TAP, after which it was placed into the root canal. The access cavities were then sealed with temporary restorative material and were stored at 100% humidity at 37°C for 7 days in an incubator.

After 7 days of incubation, all the medicaments from the experimental groups were removed with stainless steel H-files #15 to #25 along with 2.5 ml of 5.25% sodium hypochlorite irrigation, followed by final irrigation with 5 ml of normal saline. Root canals were then dried with paper points. Retrograde MTA and Hydroxyapatite apical plugs were placed upto 4 mm apical portion of the root (with simulated open apex). Samples were then wrapped in wet gauze and stored at 100% humidity at 37°C in an incubator for 24 hours to ensure the complete setting of apical plug materials. Root canals were then obturated using lateral condensation technique, and access cavity was sealed with nanohybrid composite restorative material.

Roots of study samples were coated with two layers of nail varnish, leaving 4 mm of the root from apex. All teeth were then subjected to dye penetration test by immersing them in 2% methylene blue dye for 24 hours. Then all study samples were subjected to clearing procedure as described by Robertson D *et al.* [10] Clearing technique involves soaking of teeth in 5% nitric acid solution for 72 hours. Acid was changed every 24 hrs and stirred every 8 hrs. Teeth are then dehydrated using ascending grades of 60 to 100% alcohol rinses. Finally, teeth were rendered transparent after dipping them in a clearing agent (xylene). Cleared samples were then graded for dye penetration under a Stereomicroscope at 40X magnification using an ocular micrometer. All readings were evaluated by a single observer in triplicate at a one-week interval, to avoid eye fatigue. The mean of all readings was taken, and the data obtained was subjected to statistical analysis using SPSS version 20.0 (Chicago, USA) with a level of significance at $p=0.05$.

Results:

The intra-observer reliability for all the study groups was analyzed using Intraclass Correlation Coefficient (ICC). According to ICC, values of all groups showed almost perfect agreement (>0.8). (Table 1).

Table 1: Intra-observer reliability of all the study groups for triplicate study

Groups	No. of readings	Mean	SD	ICC
IA	3	2.313	0.728	0.976
IB	3	0.173	0.459	0.993
IC	3	1.835	0.829	0.987
ID	3	1.913	0.807	0.982
IIA	3	2.131	0.810	0.983
IIB	3	0.529	0.568	0.981
IIC	3	0.904	0.964	0.992
IID	3	0.933	0.593	0.976
IIIA	3	0	0	1
IIIB	3	0	0	1
Explanatory notes: SD – Standard Deviation; ICC – Intraclass Correlation Coefficient				

The intragroup comparison for apical microleakage was analyzed using one-way ANOVA statistical analysis, and it was found to be statistically significant (p -value < 0.001). (Table 2).

Table 2: Intragroup comparison between subgroups in Group I and II using one way ANOVA statistical analysis

Groups	Mean	SD	F value	p-value	Status
IA	2.313	0.728	25.89	$< 0.0001^*$	Significant
IB	0.173	0.459			
IC	1.835	0.829			
ID	1.913	0.807			
IIA	2.131	0.810	12.87	$< 0.0001^*$	Significant
IIB	0.529	0.568			
IIC	0.904	0.964			
IID	0.933	0.593			
Explanatory notes: F value – Coefficient ANOVA Statistical Analysis; p-value – Level of Significance					

The intergroup comparison of apical microleakage between two experimental groups was done using paired t-test and was found to be statistically significant ($p < 0.001$), except for group IA v/s IIA, IB v/s IIB, IC v/s IIA, ID v/s IIA. (Table 3)

Table 3: Intergroup comparison between two experimental groups

Comparing group	Mean difference	t-value	p-value
IA VS IIA	0.182	0.647	0.523*
IA VS IIB	1.784	7.483	0.001**
IA VS IIC	-3.218	4.517	0.001**
IA VS IID	1.38	5.692	0.001**
IB VS IIA	-1.958	8.145	0.001**
IB VS IIB	-0.356	1.888	0.069*
IB VS IIC	-0.731	2.652	0.013**
IB VS IID	-0.760	3.925	0.005**
IC VS IIA	-0.296	0.989	0.331*
IC VS IIB	1.306	5.033	0.001**
IC VS IIC	0.930	2.836	0.008**
IC VS IID	0.902	3.427	0.002**
ID VS IIA	-0.218	0.738	0.466*
ID VS IIB	1.384	5.432	0.001**
ID VS IIC	1.009	3.108	0.004**
ID VS IID	0.980	3.790	0.001**
Explanatory notes: t-value – Coefficient paired t-test			
*p>0.05 is insignificant; **p<0.05 is statistically significant			

The intergroup comparison of apical microleakage values between two experimental groups and control groups was found to be statistically significant except for group IB v/s IIIA, IB v/s IIIB, IIB v/s IIIA, and IIB v/s IIIB. (Table 4)

Table 4: Intergroup comparison between two experimental groups with control group

Comparing group	Mean difference	t-value	p-value
IA VS IIIA	2.310	6.976	<0.0001*
IA VS IIIB	2.310	6.976	<0.0001*
IB VS IIIA	0.173	0.828	0.413**
IB VS IIIB	0.173	0.828	0.413**
IC VS IIIA	1.830	4.860	0.001*
IC VS IIIB	1.830	4.860	0.001*
ID VS IIIB	1.910	5.205	<0.0001*
ID VS IIIA	1.910	5.205	<0.0001*

IIA VS IIIA	2.130	5.777	<0.0001*
IIA VS IIIB	2.130	5.777	<0.0001*
IIB VS IIIA	0.528	2.045	0.056**
IIB VS IIIB	0.528	2.045	0.056**
IIC VS IIIA	0.904	2.059	0.054*
IIC VS IIIB	0.904	2.059	0.054*
IID VS IIIA	0.933	3.455	0.002*
IID VS IIIB	0.933	3.455	0.002*
*p<0.05 is statistically significant; **p>0.05 is insignificant			

The results of present study showed that apical microleakage was maximum in Group IA, followed by Group IIA, Group ID, Group IC, Group IID, Group IIC, Group IIB, Group IB, and Group III (Group IIIA and IIIB; Control group). Apical dye penetration was minimum in control group, followed by Group IB ($0.17\pm.46$) < IIB ($0.53\pm.57$) < IIC ($0.90\pm.96$) < IID ($0.93\pm.59$) < IC ($1.83\pm.83$) < ID ($1.91\pm.81$) < IIA ($2.13\pm.81$) < IA ($2.31\pm.73$).

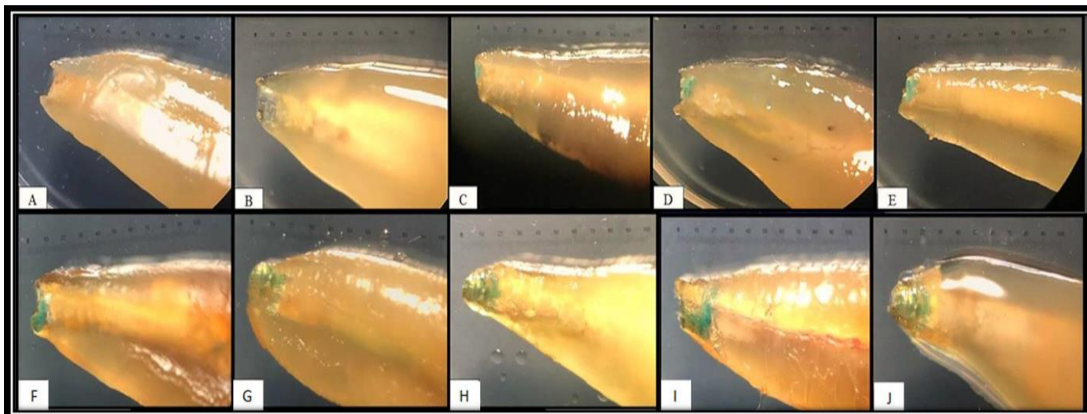


Figure 1: Apical microleakage assessment by dye penetration method for A) Group IIIA; B) Group IIIB; C) Group IB; D) Group IIB; E) Group IIC; F) Group IID; G) Group IC; H) Group ID; I) Group IIA; J) Group IA.

Discussion:

Among intracanal medicaments, calcium hydroxide is the most widely used, but it is not effective against *E. faecalis*, which is one of the most frequently recovered bacteria in cases of failing root canal treatment.[11] However, Metapex (Calcium hydroxide + Iodoform) is a silicon oil based material, its oily and viscous vehicle prolongs the duration of action of the medicament, and iodoform improves the antibacterial properties of the material. According to Cwikla SJ *et al.* [11], metapex efficiently removed *E. faecalis* from the root canal system and was the most effective dentinal tubule disinfectant as compared to calcium hydroxide with potassium iodide and calcium hydroxide mixed with water. Hence, in the present study, metapex was preferred as an intracanal medicament over calcium hydroxide.

2% chlorhexidine digluconate is a cationic bisbiguanide, successful in the elimination of microorganisms associated with persistent infection and treatment failure, and this is mainly due to its property called substantivity. [12] Curcumin (diferuloylmethane), being the main bioactive component of turmeric,

inhibits the assembly of a protein-filamenting temperature-sensitive mutant Z (FTSZ) protofilaments and increases the GTPase activity of FTSZ. The perturbation of the GTPase activity of FTSZ assembly is lethal to bacteria.[13] Also, curcumin in aqueous preparations exhibits a phototoxic effect against Gram-positive and Gram-negative bacteria.[13] Hence, in our study, we preferred *Curcuma longa* (aqueous turmeric extract) as an intracanal medicament. TAP was originally developed by Hoshino *et al.* [14] with the combination of ciprofloxacin, metronidazole, and minocycline. But the existence of minocycline had shown the potential for crown discoloration. Thus, potential alternatives were developed like amoxicillin, arestin, or cefaclor. In our study, minocycline was replaced by amoxicillin in the formulation of triple antibiotic paste.

For in-vitro studies, several methods have been used to create an open apex, including over instrumentation with large files or retrograde application of NiTi rotary files or apical root resections. [2] The disadvantages of these methods are that they create a round apical foramen that may not resemble the clinical situation, as in immature teeth. As in most of the studies found in literature, the canals were instrumented with Peeso reamers to simulate a standardized divergent open apex as used in the study by Zhabuwala MS *et al.*, [4] Nikhil V *et al.*, [15] and Nabavizadeh M *et al.*, [16]. Thus, to simulate immature apices, we introduced peeso reamers between #1 to #4 into the canal until peeso reamer #4 passed freely out of the apex.

For the process of apexification in an immature tooth, an apical plug is required to ensure an adequate apical seal. According to Bani M *et al.* [17], 4 mm apical plug of MTA gave satisfactory results against apical leakage, than an apical plug of 1-2 mm. Hence, in the present study, a 4 mm apical plug was placed in simulated immature teeth. To evaluate apical microleakage, various methods are used, like light microscopic method, fluid filtration, dye penetration method, and scanning electron microscope. [18] In the present study, we used dye penetration method, being relatively simple and not requiring complex equipments.[18] For evaluating apical microleakage, assessment of linear dye penetration can be done either by sectioning of teeth or using clearing technique. In the present study, clearing technique was preferred over tooth sectioning, as dye penetration can be assessed in three dimensions, which enables the reading of maximum extent of dye.

We observed that apical dye penetration was maximum in MTA+Metapex combination, followed by Hydroxyapatite+Metapex; MTA+TAP; MTA+Curcuma longa; Hydroxyapatite+TAP; Hydroxyapatite+Curcuma longa; Hydroxyapatite+2% Chlorhexidine digluconate; and least with MTA+2% Chlorhexidine digluconate. In the present study, maximum dye penetration was seen in groups using metapex as intracanal medicament. In accordance to our research, studies by Kim SK *et al.*, [19] Shrivastava AA *et al.*, [2] also found that calcium hydroxide medicated root canals showed significantly more apical leakage, as it cannot be removed completely from the apical 4 mm of root canal and its residue is likely to interfere with the sealing ability of filling materials leading to microleakage. TAP showed comparatively less microleakage than metapex, but more than turmeric and chlorhexidine intracanal medicaments. Nabavizadeh M *et al.* [16] reported that TAP significantly reduced the sealing ability of MTA apical plug. This could be attributed to its effect on the chemical structure of root dentin, surface changes, superficial collagen degradation and demineralization. The authors stated that more than 80% of the medicament could not be cleaned from the canal because it penetrates deeper inside the dentinal tubules. Hence, it could have shown more microleakage than turmeric and 2% chlorhexidine.

Turmeric (*Curcuma longa*) showed less microleakage as compared to metapex and TAP, but more than 2% chlorhexidine digluconate. The turmeric solution was more viscous, and hence it might have adhered to the dentinal walls, leaving behind the residual turmeric extract, resulting in increased microleakage than CHX. However, its viscosity was less than TAP and metapex, thereby causing less microleakage. In the present study, 2% chlorhexidine digluconate showed the least microleakage among experimental

groups. In accordance with the present study, Shrivastava AA *et al.* [2] concluded that chlorhexidine digluconate, when used as an intracanal medicament, showed less microleakage than calcium hydroxide and did not affect the sealing ability of apical plug used in their study. The authors stated that it could be due to its unique property called substantively, that is the ability to be adsorbed in the dentin and gradually released over time, which differs from calcium hydroxide, in which the residue is left on the dentinal tubules, affecting the sealing ability of material, thus causing comparatively more microleakage than chlorhexidine.

In the present study, effects of these intracanal medicaments on sealing ability of MTA and hydroxyapatite apical plug were evaluated, which demonstrated that the hydroxyapatite apical plug showed better apical sealing ability as compared to MTA. In accordance with the present study, Brandell DW *et al.* [20] concluded that apical plugs with hydroxyapatite had more hard tissue formation, a greater amount of apical closure, and less inflammation compared to demineralized dentin and dentin chips. The authors also stated that increased levels of fibrous connective tissue with numerous fibroblasts and coarse bundles of collagen were seen in the periapical region. In the present study, hydroxyapatite granules were crushed into fine powder, which increases the surface area of the material and hence their wettability, thus creating a thick and strong apical plug, which might have improved the adaptability of hydroxyapatite to the dentinal walls.

Thus, in our study, Hydroxyapatite apical plug showed comparatively better sealing ability than MTA apical plug, with all intracanal medicaments except 2% chlorhexidine digluconate, with which the sealing ability of MTA was better than Hydroxyapatite. This may be due to the reason that the apical plug formed by MTA with chlorhexidine is better than the complex formed by hydroxyapatite with chlorhexidine, thus showing better results. The sealing ability was minimal with metapex, followed by turmeric, triple antibiotic paste, and 2% chlorhexidine digluconate.

Our research has the strength of evaluating two important biomimetic materials for sealing ability as apical plugs in apexification. We also assessed four intracanal medicaments that are commonly used for endodontic therapy. Our study is one of the few studies in literature that assessed the impact of four different medicaments on the sealing ability of apical plugs. Besides strengths, the present study has limitations of being conducted on a small sample size. The results of the study cannot be extrapolated to *in-vivo* situations, but they do permit reasonable comparisons. There is a need to study the applicability of these materials in clinical conditions. Further studies could be done using techniques like the light microscopic method, scanning electron microscopic method, or fluid filtration method to better quantify the amount of microleakage.

Conclusion:

The present study concluded that the hydroxyapatite apical plug showed comparatively better sealing ability than the MTA apical plug with all intracanal medicaments, except for 2% chlorhexidine digluconate. The sealing ability was minimal with metapex, followed by turmeric, triple antibiotic paste, and 2% chlorhexidine digluconate. Hence, hydroxyapatite apical plug could be used as an alternative to MTA for apexification in pediatric patients, with water-based intracanal medicaments giving better results.

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