

Antimicrobial Efficacy of Obturating Materials used In Deciduous Teeth-An in vitro study

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ABSTRACT

Preservation of deciduous tooth with non-vital pulp has been a major problem for pediatric dentistry. In such cases, the preferred treatment modality is pulpectomy.

The objective of pulpectomy is complete removal of necrotic and irreversibly infected pulp of an affected tooth but in spite of chemomechanical preparation and copious irrigation of canal, there are chances of failure of pulp therapy due to entrapped microorganism in the canal space owing to the tortuous and complex nature of the root canal system and change in their morphology with resorption.

AIM: The aim of the study was to assess the antimicrobial efficacy of different obturating materials used in deciduous teeth.

MATERIAL AND METHOD: In the present study, antimicrobial efficacy of Metapex, Zinc Oxide Eugenol (ZOE), Calcium hydroxide with chlorhexidine, and Endoflas were assessed and compared with each other.

RESULTS: Intergroup comparison at 24 hours (Tukey's test) revealed significant difference amongst all the groups. Endoflas had higher zone of inhibition as compared to all the other groups except group treated with Zinc Oxide Eugenol.

CONCLUSION: The antimicrobial activity of obturating materials according to results obtained from the present study can be summarized as follows:

Endoflas > ZOE > Calcium hydroxide with chlorhexidine > Metapex

KEYWORDS: Calcium hydroxide, pulpectomy, antimicrobial efficacy, obturating material

INTRODUCTION

The primary goal of pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues^[1]. Generally, all the bacteria that present in the oral cavity have the ability to invade the pulp space during and after pulp necrosis in primary teeth. *E. faecalis* being a highly resilient and persistent organism plays an important role in the etiology of recurrent periradicular lesions post endodontic treatment in spite of making a small fraction of the flora in an untreated canal^[4].

The objective of pulpectomy is complete removal of necrotic and irreversibly infected pulp of a tooth affected by caries, traumatic injuries or other causes, so that the tooth remains asymptomatic and functional in the oral cavity till it exfoliates normally^[2,3].

Endoflas is a resorbable paste obtained by mixing a powder containing iodoform, zinc oxide, calcium hydroxide, barium sulphate and liquid consisting of eugenol and paramonochlorphenol.

ZOE has been traditionally used as an obturating material in the primary dentition^[5] and was the first obturating material to be recommended for primary teeth. However, its rate of resorption is slower than that of deciduous teeth^[6,7,8]. Moreover, it can irritate the periapical tissue, can cause necrosis of bone and cementum and may alter the path of eruption of succedaneous tooth^[9].

Calcium hydroxide, which was introduced to dentistry by Hermann in 1930, has been known to promote healing in many clinical situations. It has been used as an obturating material for primary teeth alone and also in association

with iodoform^[11,12]. Calcium hydroxide can be used with various vehicles such as distilled water, glycerine, chlorhexidine etc.

The present study was under taken to find out the efficacy of various obturating materials used in deciduous teeth.

MATERIALS AND METHOD

This in vitro study was carried out in department of pedodontics and preventive dentistry in collaboration with the department of pathology and microbiology. With an overall sample size of 68.

PREPATION OF BROTH AND BACTERIAL GROWTH: The antimicrobial activity of obturating materials used in primary teeth against E.Faecalis was evaluated in the study by agar diffusion method. The standard bacterial strain of E.Faecalis was obtained from KGMU, Lucknow, Uttar Pradesh. The purity of test strain was confirmed using the gram's stain. Only 0.37 grams of Brain Heart Infusion broth was added to 10ml of distilled water and mixed by gently shaking of container. This mixture was sterilized by autoclaving for 15 minutes at 121° C and 15lb pressure and then allowed to cool at room temperature. The bacterial stain was inoculated in BHI broth and incubated at 37°C for 24 hours. Following incubation, the cultures were centrifuged at 3000rpm for 10 minutes. The supernatant liquid was discarded and the precipitate containing micribial cells was separated from the base of the test tube. The precipitate containing microbial cell was re-suspended in saline and turbidity of this culture suspension was adjusted until it was equivalent to the no.1.

PREPARATION OF CULTURE MEDIUM: Mueller Hinton agar was added to 400ml of distilled water and mixed by gently shaking the container. This mixture was sterilized by autoclaving for 15 minutes, the liquid was cooled to room temperature. In a laminar flow chamber, this liquid medium was poured in 20 petri dishes of size 90mm and allowed to set.

INOCULATION OF BACTERIAL STRAIN ON CULTURE MEDIA: Each agar plate with 20ml of Mueller Hinton agar was inoculated with 0.1ml microbial suspension using sterile swab. The 3 wells (4mm of depth X 6mm of diameter) were made in each of the agar plates.

PLACEMENT OF OBTURATION MATERIALS AND INCUBATION: A sterile spatula and glass slab was used for mixing the obturating materials in creamy consistency. Each freshly mixed experiment obturating material was placed in 10 wells of different petri dishes. These agar plates were then incubated at 37°C under anaerobic conditions in an incubator using microaerophilia. An anaerobic indicator tablet was placed in the jar to monitor oxygen contamination of the environment.

MEASURING THE SIZE OF ZONE OF INHIBITION: A lack in bacterial colonization was observed for each obturating material. It was indicated by growth inhibitory zones around each obturating material. The most uniform diameter segment of the zone of inhibition was determined in millimeters by measuring the shortest distance between the outer margin of the well and initial microbial growth after 24 hours of incubation.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS version 15.0. The analysis of variance (ANOVA) and post- Hoc Tests (Turkey-HSD) were performed to know the effect of each variable and to reveal the statistical significance. The values were represented in number (%) and mean SD.

GROUP DISTRIBUTION

GROUP 1- Endoflas FS (manufactured by Sanlor Lab, Colombia)

GROUP 2- Zinc Oxide + Eugenol (manufactured by Septodont, India)

GROUP 3- Cavipac + Dento chlor (manufactured by Pyrex Polykem, India)

GROUP 4- Metapex (manufactured by Meta Biomed Co. Ltd, Korea)

TABLE NO.-1 Materials are divided in various groups

S.NO.	Group	Material used	No. of samples	Percentage of distribution of material
1	Group 1	Endoflas	17	15.7
2	Group 2	ZOE	17	15.7
3	Group 3	Ca(OH) ₂ + Chlorhexidine	17	15.7

4	Group 4	Metapex	17	15.7
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RESULTS: In this study a total 68 samples were assessed for antimicrobial efficacy against *E. Faecalis*. Antimicrobial efficacy assessment was done at 24 hours time intervals in terms of mean zones of inhibition. At 24 hours time intervals, maximum inhibition was seen in Group 1 (Endoflas) followed by Group 2 (ZOE), Group 3 (Ca(OH)₂ + chlorhexidine and Group 4 (Metapex)

TABLE NO.-2 Intergroup comparison of mean zones of inhibition in different groups at 24 hours time intervals (ANOVA)

S.NO.	GROUP	MEAN	SD
1	1	4.99	1.10
2	2	4.15	0.92
3	3	3.20	0.31
4	4	2.30	0.35

Where F(ANOVA) is 66.004
p-value is < 0.001

On the basis of above evaluation the following order of mean zone of inhibition was observed in different groups. Endoflas > ZOE > Calcium Hydroxide + Chlorhexidine > Metapex

DISCUSSION: The preservation of primary teeth helps in esthetics, phonetics, mastication and social acceptance. Pulpectomy is indicated when pulp has become irreversibly infected or necrotic due to caries, trauma or other causes [2]. The aim of pulp therapy in primary teeth is to preserve the tooth in functional stage until their exfoliation as the teeth act as natural space maintainer, so that the permanent teeth may have adequate space for aligning themselves [13,14].

The optimal success of pulp therapy, substances with antimicrobial properties are advocated as root filling material in deciduous teeth. Bacterial re-entry and growth of residual microorganism may be prevented by incorporation of antimicrobial of antimicrobial components into obturating materials [15].

The success rate of root canal treatment can be lowered significantly if there is presence of *E. faecalis* in pulp canal system at the time of obturation [10]. Mostly importantly, *E. faecalis* efficiently and rapid colonizes dentinal tubules compared to other species and therefore is particularly difficult to eradicate [16]. It represents a standard against which the antimicrobial action of a medicament should be tested [16,17].

In the present study, endoflas, calcium hydroxide with chlorhexidine, were tested as there is very sparse literature and studies present regarding them, as an obturating material in primary teeth, whereas ZOE and metapex are regularly used in dental practice for obturation of primary teeth.

Endoflas FS showed marginally better, antimicrobial activity than ZOE obturating material probably due to incorporation of known bactericidal agents such as iodoform [18]. Iodoform acts by the liberation of iodine. It is believed that iodine, which is an oxidizing agent, can irreversibly oxidize and thus, inactivate essential metabolic compounds like proteins, nucleotides and fatty acid resulting in cell death, but the exact mode of action is not fully known [19]. Similar results have been reported by Gopikrishna et al., and Kayaoglu et al [20,21].

Eugenol based root canal obturating material (Endoflas and ZOE) showed highest antimicrobial activity as compared to the non-eugenol containing in the present study. The antimicrobial effect of eugenol based obturating material was mainly attributed to the action of eugenol. Eugenol acts on micro-organism by causing protein denaturation rendering it non-functional [18]. The results of the various studies performed by Gomes et al., Markowitz et al., and Saggat et al., also confirmed that eugenol containing obturating materials were more superior in inhibiting the microorganisms [18,22,23].

Among the different preparations of calcium hydroxide, calcium hydroxide with chlorhexidine showed the best result followed by freshly prepared mix of calcium hydroxide and iodoform while metapex showed minimum inhibitory action. The possible reason could be that, chlorhexidine gluconate is a broad spectrum antimicrobial agent and is effective against bacteria and fungi [23-27]. Thus, antimicrobial activity of calcium hydroxide increases when mixed with chlorhexidine [14]. Pabla et al., in their study reported lesser antimicrobial activity of metapex against aerobic and anaerobic bacteria in comparison to ZOE, KRI paste and MAISTO paste [14]. However, Garcia-Godoy and Nurko studied the effectiveness of metapex in the root canal treatment of primary teeth and reported treatment

to be deemed successful as clinically the tooth was painless, with no pathological mobility, healthy gingiva and no fistulation^[1,11].

It was observed from the present study that eugenol based root canal obturating material particularly endoflas showed the highest antimicrobial activity against *E. faecalis* at 24 hours time intervals.

The rationale for performing this invitro study is to offer information to clinicians about antimicrobial efficacy of different root canal obturating materials used in primary tooth against *E. faecalis*.

LIMITATION: Limitation of this study could be that the antimicrobial efficacy of obturating materials has been evaluated in vitro conditions which may be modified in a clinical set up due to presence of dentin and serum. A large samples size are required to evaluate the antimicrobial efficacy of root canal obturating materials.

CONCLUSION: Within the limitations of the study, all materials produced zones of microbial growth inhibition against the tested microorganism *E. faecalis*. The mean zone of inhibition in different groups at different time intervals, endoflas showed maximum mean zone of inhibition followed by ZOE. The mean zones of inhibition can be summarized as follows.

Endoflas > ZOE > Calcium Hydroxide + Chlorhexidine > Metapex

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