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Evaluation and Comparision of Antimicrobial Activity of MTA to Antioxidant Mix Used As a Pulpotomy Agent- In Vitro Study

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Abstract

Introduction: Pulpotomy technique is most widely accepted clinical procedure for treating primary teeth with coronal pulp inflammation caused by caries with no involvement of the radicular pulp.

Aim and Objective: Evaluation andComparision of antimicrobial activity of MTA to Antioxidant Mix used as a pulpotomy agent- In vitro study

Materials and Methods: The test materials i.e MTA and antioxidant mix were manipulated strictly in accordance with the manufacturer's instructions. The antimicrobial activity of this material is evaluated by the agar diffusion method against two reference strains: *E. faecalis* (ATCC 29212), *S. mutans* (ATCC25175). Each material is evaluated at concentrations suggested by the manufacturer. Bacteria is diluted to obtain a suspension of approximately 5 × 108 colony forming units/ml, in sterile Trypticase Soy Broth (TSB). Microbial strains were confirmed by colony forming units and growth characteristics. *E. faecalis* is inoculated with sterile cotton swabs onto Meuller-Hinton agar plates and *S. mutans* is inoculated onto blood agar media. Wells 4 mm in diameter and 4 mm deep are prepared on plates with a copper puncher, and immediately filled with freshly manipulated test materials. After prediffusion of the test materials for 2 h at room temperature, all the plates are incubated at 37°C and evaluated at 24 h. Microbial inhibition zones are measured with a 0.5 mm precision ruler and the results are expressed as the mean and standard deviation. To compare the differences among MTA and antioxidant mix data are

analyzed statistically by one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) post-hoc test, using Statistical Package for Social Sciences (SPSS) software version 21 (SPSS Inc, Chicago, IL, USA).

Conclusion: MTA has better antimicrobial efficacy whereas Antioxidant mix as a new pulpotomy agent though biocompatible and cost effective than any other commercially available medicament does not show any antibacterical activity.

Introduction

Natural primary teeth are considered as best space maintainers; hence every effort should be directed to preserve these teeth as far as possible. Dental caries still, is the most widespread dental disease seen in children (Ruth Holt et al, 2000) and has been the main threat to the overall integrity of primary dentition[1]. The mere extractions of these carious teeth result in many complications, such as difficulty in chewing, loss of normal occlusion and inappropriate arch space[2]. Hence the restoration and maintenance of carious primary teeth is one of the most important goals in pediatric dentistry. They should be restored such that they are in a functional state until their normal exfoliation. Depending on the extent of inflammation and involvement of pulp, various kinds of pulpal therapies are indicated, that is, pulp capping, indirect pulp therapy, pulpotomy or pulpectomy. If a tooth with a carious lesion remains untreated or, is inadequately treated, a bacterial invasion of the coronal pulp will occur originating an inflammatory response at that level. At this stage the inflammation is confined to the coronal space, but if the affected tissue is removed and the entrance to the root canals is covered with an appropriate agent, the remaining tissue is capable of recovering (Pallares et al, 2010)[3]. Therefore, removal of coronal pulp is an accepted procedure for treating primary and permanent teeth (McDonalds & Avery, 2004)[4]. According to AAPD guidelines 2009 Pulpotomy is performed in a primary tooth with extensive caries but without evidence of radicular pathology when caries removal results in a carious or mechanical pulp exposure[5]. It is a conservative therapy performed to remove the inflamed coronal pulp tissues followed by application of an effective and compatible bactericidal medicament which encourages the tissue in the root canals to remain vital[6]. Pulpotomy procedure can be categorised according to different treatment approaches for example –devitalization using formocresol and electro surgery, where the intent is to destroy the radicular pulp; preservation where the remaining radicular pulp is preserved with the use of glutaraldehyde and ferric sulphate and regeneration of the radicular pulp by stimulation of a dentinal bridge with the use of calcium hydroxide, Mineral trioxide aggregate and bone morphogenetic protein[7].

Mineral trioxide aggregate (MTA) a relatively newer material has been introduced to dentistry in 1995 by Torabinejad who had suggested it for endodontic root filling. It is composed of tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide. It also contains oxides of iron, magnesium and bismuth which is added for radiopacity purpose (Lewis, Salako et al, 2003)[8]. It is biocompatible, has high sealing ability, ability to form dentinal bridge and can cause regeneration of cementum and periodontal ligament. It also has the ability to stimulate cytokine release from bone cells, so it has the capacity to actively promote hard tissue formation (Eidelman et al, 2001). Recently, its use is extended to pulpotomy in primary teeth[9]. It is biocompatible, bactericidal, promotes regeneration of the original tissues when comes in contact with dental pulp tissue. Its sealing, dentinogenic and osteogenic properties have made it a material of choice in various clinical setup. In past few

years, most of the literature has focused on the regenerative material. However, the current scenario is about safety pulpotomy medicaments regarding toxicity and potential carcinogenicity. Alternative to all these medicaments, researcher had showed interest toward the wound healing phenomenon as a pulpotomy medicament. Very few researcher had concentrated on wound healing for restoring the anatomical continuity of damaged pulp tissue and disturbed functional status of the radicular tissue Free radicals which are released during inflammation get inactive by antioxidant before they attack human cells. Humans have generated highly complex antioxidant systems (enzymes or nonenzymes), which work synergistically and in combination with each other to prevent cells or organs against free radicals, so elimination of these free radicals enhances the healing process and repairs the remaining radicular tissue. As major scientific safety concerns have been raised about popular pulpotomy medicaments regarding toxicity and potential carcinogenicity[10,11] search for an alternative ideal pulpotomy agent is on till dateAppropriate method, of wound healing, is essential for the restoration of anatomical continuity of damaged tissue and disturbed functional status of the radicular tissue.

Wound healing processes are well‑organized, biochemical and cellular events, leading to the growth and regeneration of injured radicular tissue in a special manner.[12,13] Healing of wounds involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured radicular tissue.[14] Antioxidants counter the excess proteases and reactive oxygen species (ROS) often formed by neutrophil accumulation in the wounded area and protect protease inhibitors from oxidative damage.[15] Fibroblasts and other cells may be killed by excess ROS and tissues will be made less flexible, so antioxidant substances will reduce the possibility of these adverse events occurring. All these facts and overall antioxidant effects appear to be important in the successful treatment of wound healing.

In literature, MTA has showed to be the most successful medicament for pulpotomy. Hence, the present study is done to evaluate and compare the antibacterial efficacy of antioxidant mix and MTA.

Material and Methods

Fig 1- OXYNUT 40 (Antioxidant mix) and MTA (Rootdent)

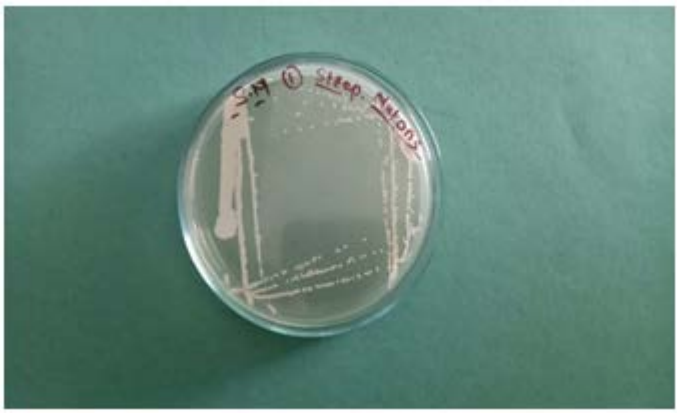

Fig 2- S. mutans

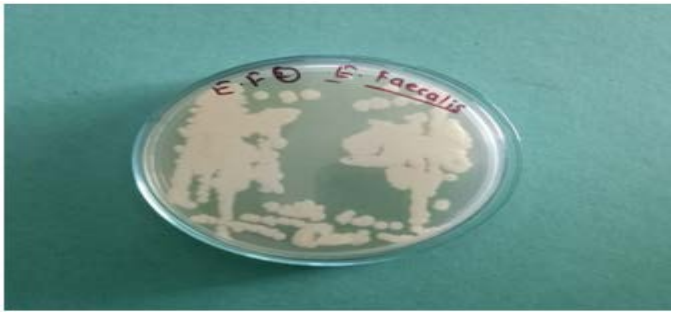

Fig 3- Strains of E.Faecalis

The materials evaluated in this study were MTA(Rootdent) AND Antioxidant mix (oxyfruit 40/oxynut). The test materials were manipulated strictly in accordance with the manufacturer's instructions. The antimicrobial activity of this materials is evaluated by the agar diffusion method against two reference strains: *E. faecalis* (ATCC 29212), *S. mutans* (ATCC25175).

Each material is evaluated at concentrations suggested by the manufacturer. Bacteria is diluted to obtain a suspension of approximately 5×10^8 colony forming units/ml, in sterile Trypticase Soy Broth (TSB). Microbial strains were confirmed by colony forming units and growth characteristics. *E. faecalis* is inoculated with sterile cotton swabs onto Meuller-Hinton agar plates and *S. mutans* is inoculated onto blood agar media. Wells 4 mm in diameter and 4 mm deep are prepared on plates with a copper puncher, and immediately filled with freshly manipulated test materials. After prediffusion of the test materials for 2 h at room temperature, all the plates are incubated at 37°C and evaluated at 24 h. Microbial inhibition zones are measured with a 0.5 mm precision ruler and the results are expressed as the mean and standard deviation. To compare the differences among MTA and antioxidant mix data are analyzed statistically by one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) post-hoc test, using Statistical Package for Social Sciences (SPSS) software version 21 (SPSS Inc, Chicago, IL, USA).

The agar plate diffusion test is used for the cultures, including saline solution as a negative control.Indicator strains are grown in Brain Heart Infusion Agar.In each sterilized petri dish, a base layer of 15 ml BHI agar mixed with 300ml of each inoculum is prepared.After solidification of culture medium three wells were made in each plate and completely filled with one of the testing materials.

Zones of bacterial inhibition and material diffusion were photographed and measured in millimeters (mm), using a digital caliper (Mitutoyo, São Paulo, SP, Brazil). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well. Antibacterial tests were repeated three times to confirm the homogeneity of the results. Results were expressed as means and subjected to statistical non-parametric tests of Kruskal–Wallis and Mann–Whitney (Wilcoxon rank-sum-tests) at the significance level of 5%.

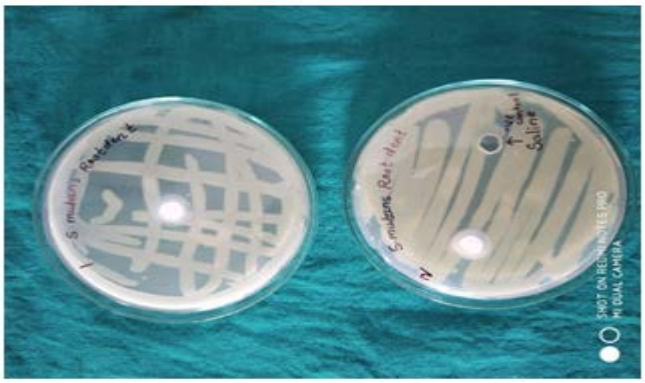

Fig 4 a- inhibition zone of rootdent on S.mutans

Fig 4 b- inhibition zone of rootdent on E.Faecalis

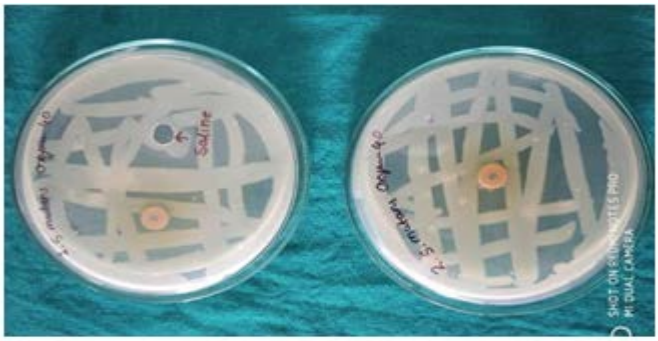

Fig 5 a- inhibition zone of oxynut 40 on S.mutans

Fig 5 b- inhibition zone of oxynut 40 on E.faecalis

Results

The mean values and standard deviations of the inhibition zones for each material according to the bacteria strain are shown in Table 1. Antibacterial activity was only considered to have occurred when a true inhibition zone was present, whether associated or not with the diffusion zone.

Group -A Zone of inhibition when treated with - Root dent

Group -B Zone of inhibition when treated with – Oxy - 40

S. No	E. Fecalis	S. Mutans
1	00.00 mm	00.00 mm
2	00.00 mm	00.00 mm
3	00.00 mm	00.00 mm
4	00.00 mm	00.00 mm
5	00.00 mm	00.00 mm
6	00.00 mm	00.00 mm
	No zone of Inhibition observed	

Table 1

MATERIALS	BACTERIA			
	S.MUTANS		E.FAECALIS	
MTA	Mean	SD	Mean	SD
	14.317	0.7653	15.100	1.2083
ANTIOXIDANT MIX	0.0	0.0	0.0	0.0

Means of inhibition zones diameters (mm) and SD values observed on inoculated agar plates after 48 h according to experimental material and bacteria strain.

It can be observed that MTA material showed antibacterial activity, and they were statistically different from the negative control against bacteria studied strains. Whereas antioxidant mix showed no zone of inhibition again S.mutans and E. faecalis strains culture.

Discussion

In this study, the antimicrobial activity of ANTIOXIDANT MIX, and MTA cement were investigated. MTA, due to showing good clinical and radiographic results and being biocompatible.

The microorganisms studied in this study are Gram-positive aerobic bacteria strains, which are part of the aciduric phase of the dental caries progression. Dental caries is resulted by imbalance of the de-/remineralization process where strains of *S. mutans* may be dominant.[16] Around 30-50% of the microorganisms present in the caries lesions are represented by *S. mutans*.[17] Different in vitro methods have been used to study antibacterial activity of dental materials.[18,19] Although differences in the agar medium, capacity of diffusion of the materials, bacterial strains, and cell density may interfere in the formation of inhibition zones around the materials studied, the agar plate diffusion test was selected in this study to be the method of antimicrobial activity widely used.[20,21,18]

The present study revealed that the diameter of the inhibition zone varied according to the material and microorganism tested. The results showed that MTA had significant antimicrobial activity whereas antioxidant mix had no antibacterial activity. This result suggests that MTA which is a popular choice nowadays contains more potent antibacterial inhibitors whereas antioxidant mix has no antibacterial agents present in it. However, clinically and radiographically antioxidant mix (95%) showed

impressive result, especially considering the long-term follow-up period of 6 and 12 months[22]

Elimination of microorganisms from infected root canals always remains a challenging task. The chances of successful root canal treatment are significantly superior if microbes are eradicated effectively before the root canal system is obturated. However, tenacious existence of micro-organisms in the canal leads to treatment failure.[23]

Enterococcus faecalis is most frequently isolated from persistent apical periodontitis [24]and has many virulence factors. It remains viable and is more resistant to endodontic treatment with the capability to invade the dentinal tubules and adhere to collagen in the presence of human serum, thus acting as a pathogen in failed endodontic treatment. [25]

Using ADT Torabinejad et al. examined the antibacterial effect of some root end filling materials and concluded that MTA had no antibacterial activity against E. faecalis, S. aureus and Bacillussubtilis and there was not any effect on the strict anaerobic bacteria.[26]

Possible explanation for the success rate observed in this study may be because antioxidants counter the excess proteases and free radicals often formed by neutrophil accumulation in the wounded area and protect protease inhibitors from oxidative damage. Fibroblasts and other cells may be killed by excess ROS and tissues will be made less flexible, so antioxidant substances will reduce the possibility of these adverse events occurring. Elimination of ROS may be an important strategy in improving healing of the radicular pulp in the present study. Antioxidant along with Vitamins such as A, [27] B, and C [28] and zinc [29]have been used on the assumption that they might play an important role in the inflammatory phase, proliferative phase, wound remodeling, including reorganization of new collagen fibers in radicular tissue healing. Presence of interlacing nerve fibers and connective tissue in initial SEM samples show the biocompatibility of the material and its ability in tissue repair and regeneration.

Estrela *et al*., demonstrated that MTA had no antimicrobial activity against *E. faecalis*, but this study proved its antimicrobial efficacy against *E. faecalis*[30]However, available nutrients, level of oxygen tension, incubation period, methods of evaluation, and different laboratory set-ups employed; influenced the study results.

The MTA setting process is based on the reactions of anhydrous cement compounds with water (hydration reaction), producing a calcium silicate hydrate of low alkalinity, which dissociates in CH molecules. Thus, MTA, after setting, may be considered as CH surrounded by a silicate matrix.[31] MTA has shown clinical and radiographic results compatible to FC,[32,33,34,35] and higher than CH.[33,36] In addition, MTA presents other ideal properties as its high sealing ability, biocompatibility, capacity to stimulate dentin neoformation and regeneration of periodontal tissue.[33,34]

According to the results of the present study, MTA presented antimicrobial activity notably against *S. mutans and E.Faecalis* than antioxidant mix.

Conclusion

MTA has better antimicrobial efficacy whereas Antioxidant mix as a new pulpotomy agent though biocompatible and cost effective than any other commercially available medicament does not show any antibacterical activity.

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