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Research Article

A COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFICACY OF MTA AND BIODENTINE AGAINST ENTEROCOCCUS FAECALIS WHEN COMBINED WITH CHLORHEXIDINE, TRICLOSAN AND TRIPLE ANTIBIOTIC PASTE

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A R T I C L E I N F O

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MTA, Biodentine, agar diffusion method, enterococcus faecalis.

A B S T R A C T

Aim: The aim of this study was to evaluate the antibacterial efficacy of MTA and Biodentine against Enterococcus faecalis when combined with 2% Chlorhexidine, 2.5% Triclosan and 1.5% Triple antibiotic paste.

Materials & methods: The antimicrobial activities of the freshly mixed cements were evaluated by the agar diffusion method on Enterococcus faecalis.

The inoculums were prepared by making a direct brain heart infusion broth suspension of the isolated colonies from an overnight growth on blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to 0.5 McFarland opacity standards. A sterile cotton swab dipped and streaked 3 times over the entire sterile agar surface, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculums. Enterococcus faecalis were inoculated on blood agar plates.

Wells were prepared on the medium with the help of puncher of 4mm diameter and 4 mm depth and then immediately filled with freshly prepared test materials. The test materials were manipulated in accordance with the manufacturer's instructions in aseptic condition. After pre diffusion of the test materials for 2 hours at room temperature, all the plates were incubated at 370c in an incubator directly.

A blinded, independent observer then evaluated the plates at 24h, 48h, and 72h. Microbial inhibition zones were measured with a precision ruler.

Results: Efficacy between MTA and Biodentine were done by using independent "t" test for normally distributed data or Mann-Whitney test for non-normally distributed data.

A p-value less than 0.05 will be considered as significant. Antibacterial efficacy of MTA and Biodentine with experimental group showed greater inhibition zone than the control group. Group with triple antibiotic paste showed largest inhibition zone

Conclusions: Antibacterial efficacy of MTA and Biodentine enhanced with incorporation of antibacterial agents. Biodentine has greater antibacterial properties than MTA.

INTRODUCTION

The fundamental aim of endodontic treatment is to prevent or cure apical periodontitis. It is well documented that bacterial infection of the root canal is the primary cause of apical periodontitis. [1] Teeth with apical periodontitis, bacteria invade and colonize the entire root canal system, and treatment is directed toward the elimination of micro-organisms from the root canal system and prevention of re-infection.[2] Facultative anaerobic micro-organisms such as *E. faecalis, Staplylococcus aureus, and Candida albicans* are considered to have the highest resistance in the oral cavity, with the potential to cause failure of root canal treatment.[3] Success of endodontic treatment not only depends on sealing of root canal to prevent future contamination of root canal system but also elimination of infected tissues and micro-organisms as re-infection of root canal after treatment is not desirable.[4]

Therefore, an ideal root-end filling material should not only be dimensionally stable, radiopaque, non-reasonable, non-toxic, and biocompatible but also bactericidal or bacteriostatic and should provide an impervious seal.

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ProRoot MTA is marketed as gray- and white-colored preparations, both of which are composed of 75% Portland cement clinker, 20% bismuth oxide, and 5% gypsum by weight. MTA is a powder is made of fine hydrophilic particles, that in presence of water form a colloidal gel which solidifies and forms hard cement within approximately 4 hrs. Although MTA has excellent biocompatibility, it has a delayed setting time, poor handling characteristics with.[5]

Recent introduction of Biodentine have high mechanical properties with excellent biocompatibility and bioactive behaviour. Biodentine is based on calcium silicate based material, and it is packaged in single use capsules that contain powder and liquid.[6]

Both MTA and Biodenitne widely used for crown and root dentin repair, indirect and direct pulp capping, pulpotomy, repair of perforations or resorptions, apexification, and root-end fillings.[7]

The present study aims to evaluate the antimicrobial activity of MTA and Biodentine with the addition of 2% CHX, Triclosan, Triple antibiotic paste against E. faecalis.

MATERIALS AND METHOD

MTA (Pro Root Mta, Dentsply Tulsa Dental Specialties) and Biodentine (Septodont) has be used along with Antimicrobial agents including CHX (SmaartPharmaceuticals, Jalgaon, Maharashtra. India), Triclosan (V2 ChempharmaPvt. Ltd. Hyderabad. Telangana. India) and Antibiotics (ciprofloxacin, minocycline and metronidazole) (Aurobindo pharmaceuticals, Hyderabad. Telangana. India).

Preparation of the antibacterial cements

MTA (PRO Root MTA, Dentsply Tulsa Dental Specialties) and Biodentine (Septodont) mixed with saline as the control.
In experimental groups, MTA (PRO ROOT MTA, Dentsply Tulsa Dental Specialties) and Biodentine (Septodont) mixed with Antimicrobial agents including 2%CHX (Smaart Pharmaceuticals, Jalgaon, Maharashtra. India), 2.5% Triclosan (V2Chempharma Pvt. Ltd. Hyderabad. Telangana. India) and 1.5% Antibiotics (ciprofloxacin, minocycline and metronidazole) (Aurobindo pharmaceuticals, Hyderabad. Telangana. India).

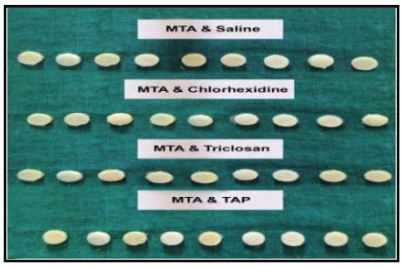


Fig 1 MTA experimental samples

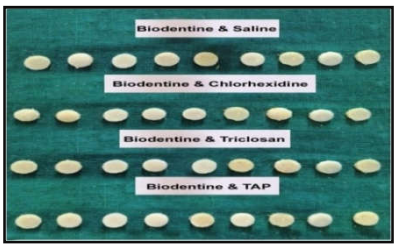


Fig 2 Biodentine experimental samples

Antimicrobial Activity Screening Tests

The antimicrobial efficacy of set specimens were evaluated against pure strains of E. faecalis (*MTCC* 439) obtained from microbial type culture collection (MTCC) Chandigarh, with agar diffusion tests. Briefly, with some modifications, the strains stored at -20°C. After that E. faecalis strains were cultured on blood agar (Hi Media Pvt. Ltd., Mumbai, Maharashtra, India) at 37°C for 24 hours.

Single colonies from the agar plate was transferred into BHI broth (Hi Media Pvt. Ltd., Mumbai, Maharashtra, India) and incubated at 37°C, for 24 hours. Suspension of the strains prepared in Phosphate Buffered Solution at a concentration of $1.5x10^8$ organisms/ml by using the McFarland 0.5 turbidity tube. Suspensions were flood-inoculated onto the surface of 24 agar plates. Plates were air dried by leaving the E. faecalis strain at 37°C for 15 minutes. In each agar plate 3 wells were prepared of diameter of 4 mm and thickness of 4 mm with the help of puncher.

The set disc-shaped experimental specimens (4 mm in diameters, 4 mm thick) were prepared. After setting at room temperature for 30 minutes, the experimental specimens were placed into BHI agar plates.

After incubation at 37°C for 24 hrs, 48hrs and 78hrs inhibition zones around the specimens were measured. The sizes of the inhibition zones were calculated by subtracting 4 mm (diameter of wells) from the average diameter of the zones for each specimen and control.

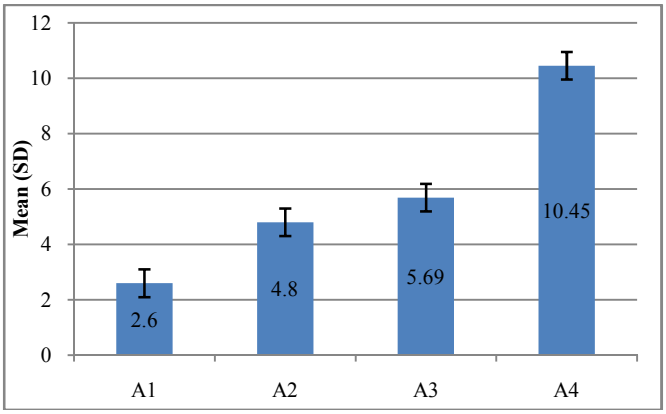
RESULT

Table 1 Comparison of antibacterial efficacy (size of inhibition zones in mm) of MTA/A and biodentine/B with different material using unpaired t test

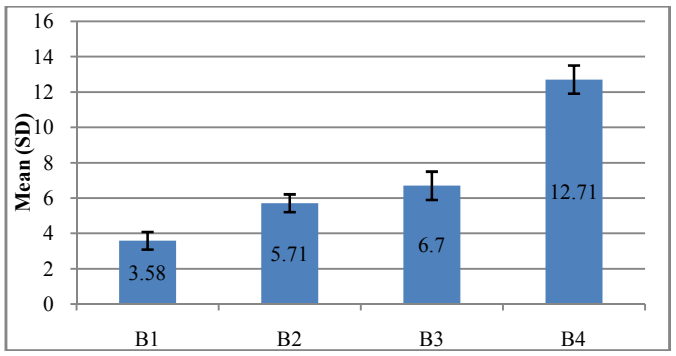
MTA/A		BIODENTINE/B		t value	p value
A1	2.60 (0.5)	B1	3.58 (0.5)	4.381	<0.001**
A2	4.80 (0.5)	B2	5.71 (0.5)	3.957	<0.001**
A3	5.69 (0.5)	B3	6.70 (0.8)	3.124	0.006*
A4	10.45 (0.5)	B4	12.71 (0.8)	6.682	<0.001**

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

Graphical representation



Graph 1 Comparison of antibacterial efficacy (size of inhibition zones in mm) of different groups using ANOVA test with *MTA*/A



Graph 2 Comparison of antibacterial efficacy (size of inhibition zones in mm) of different groups using ANOVA test with Biodentine/B.

DISCUSSION

Development and progression of pulpal and periapical diseases and endodontic failure has been associated with various micro-organisms. Varieties of disease-causing micro-organisms have been isolated from the infected root canals e.g. *Enterococcus, Actinomyces, Propionibacterium, Yeasts, Streptococcus* etc.[8] Facultative anaerobic micro-organisms such as *Enterococcus faecalis*, *Staplylococcus aureus*, *and Candida albicans* are considered to have the highest resistance in the oral cavity, with the potential to cause failure of root canal treatment.[9]

MTA had no antimicrobial activity, but did cause some effects on facultative bacteria.10 The antibacterial effect of MTA against organisms could be because of its high pH or release of diffusable substance(s) into the growth medium.[11]

Biodentine, an endodontic repair material, possesses several advantageous properties that include good sealing capability, biocompatibility, and antibacterial activity. However, study conducted by Venkayla *et al*.[12] concluded that the antibacterial activity of Biodentine is comparable to other Ca-based cements, but it is more dependent on strain type.[13]

CHX, a broad-spectrum antibacterial agent, has shown its effectiveness on various microbes, particularly against both *E. faecalis* and *C. albicans*. CHX penetrates into bacteria and exerts toxic effects through disturbance in their membrane charges. Additionally, CHX may induce reactive oxygen species production in the alkaline environment. The production of reactive oxygen species may inhibit E. faecalis growth because of the destruction of the cell wall and the plasma membrane mediated by nitric oxide.[14]

According Jedrychowski *et al*.[15] the antibacterial effects observed by the addition of chlorhexidine were dependent upon the concentration of the disinfectant added to GICs. But higher concentrations had resulted in poor physical properties of the restorative materials. This was the main reason for choosing a concentration of 2% CHX.

Similarly triclosan and triple antibiotic paste shows efficacy against gram positive and gram negative microorganism. According McDonnell G *et al*. triclosan exhibits particular activity against gram-positive bacteria. Its efficacy against gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane.[16]

Jadhav *et al*., in their case series mixed equal proportions of the components of Triple antibiotic paste with distilled water to a thick paste consistency. They found successful revascularization of immature maxillary anterior teeth. Shaik J *et al*., reported that TAP + saline combination has shown better antimicrobial effect against *E. faecalis* when compared with calcium hydroxide and saline combination.[17]

In our study MTA and biodentine was mixed with 2% chlorhexidine, 2.5% triclosan and 1.5 % triple antibiotic paste to enhance the antibacterial properties of MTA and biodentine. Agar plate diffusion was the method of choice for this study because the process is relatively inexpensive and can be performed rapidly and easily with a large number of specimens. However, there are also some limitations with this test method.[18]

In this study MTA and Biodentine when mixed control group (saline) showed some antibacterial properties that is (2.60 ±.5) and (3.58 ±.5) when compared to other group. According Vineeta *et al*.[4] both MTA and biodentine has some limited anti-microbial effects and might be attributed to high pH. *E. faecalis* can survive in extreme alkaline environments up to pH of 11.1. Perhaps the inherent, persistent alkalinity of Biodentine is just enough to overwhelm the *E. faecalis*.[4]

With chlorhexidine, both group showed (4.80 ±.5) mm and (5.71±.5) mm inhibiton zone with MTA and Biodentine respectively. When compared with control group it showed greater inhibition zone. Result came according to the study done by Vinneta N *et al*., addition of chlorhexidine to Biodentine showed larger inhibition zone 8-26 mm compared with doxycycline.[4]

With triple antibiotic paste both group showed highest inhibition zone that is MTA (10.45 ± 0.5)mm and Biodentine (12.71 ± 0.8)mm than other experimental group. When compared with MTA and Biodentine, Biodentine showed greater antimicrobial efficacy than MTA. This result came according to the study conducted by Vankayala B *et al*.[11] They concluded that antimicrobial action of Biodentine on all the microorganisms tested was superior to that of MTA and GIC, showing a mean inhibition zone of 3.2 mm. In this study Biodentine showed greater inhibition zone than MTA in all experimental group.

The result of the current study demonstrated that MTA and Biodentine with triple antibiotic shows highest inhibition zone. And Biodentine shows greater antimicrobial efficacy than MTA.

CONCLUSION

MTA with triple antibiotic paste showed highest inhibition zone than control and experimental group.

Similarly Biodentine showed highest inhibition zone with triple antibiotic paste.

When compared with MTA, Biodentine showed highest inhibition zone among all groups.

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