



Antimicrobial efficacy of a Novel Obturating Material used in Primary teeth - An In Vitro Study

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ABSTRACT

Introduction : Managing primary teeth with pulpectomy is a strenuous procedure and the chances for re-infection or failure of the endodontic treatment is more due to the complex and varied morphology of the primary root canals. This necessitates the need for an ideal obturating material for primary teeth with maximal of antibacterial properties. The aim of the present study was to evaluate the antimicrobial efficacy of different concentrations of the Novel Obturating Material against *S.mutans* and *E.faecalis* in primary teeth.

Materials and Method : Different concentrations of the Novel Obturating Material was prepared using Calcium hydroxide, Zinc oxide cement and Metronidazole and was tested for its antimicrobial efficacy against *S.mutans* and *E.faecalis* using Muller Hilton Agar (MHA). 1%,2%,3% of the obturating material were loaded at 25, 50, 100 microliters and were incubated for 24 hours at 37 ° C. The zone of inhibition was measured in millimetres after 24 hours. The values were entered in Microsoft Excel spreadsheet and subjected to statistical analysis.

Results: No statistically significant difference with 1% and 3% 50-50 concentration of the Novel obturating material at 25,50 and 100 microiliters against *S.mutans*. However at 2% 50-50 concentration, 100 microliters shows significantly greater zone of inhibition against *S.mutans*. With regards to *E.Faecalis*, at 1% and 3 %, there was statistically greater zone of inhibition seen at 100 microliters.

Conclusion : 2% 50-50 concentration of the Novel Obturating Material (2% metronidazole in equal parts of zinc oxide and calcium hydroxide) at 100 microlitres appears to be most effective against both *S.mutans* and *E.faecalis*. Hence, this novel root canal filling material can be a promising alternative to an ideal root canal filling material in primary teeth exhibiting long term antimicrobial action within the primary root canals, increasing the success rate.

Keywords: *Material, vitro, study*

INTRODUCTION

Management of primary teeth with irreversible pulpitis is challenging and pulpectomy is the only option to treat and preserve the primary teeth in its position to properly guide the eruption of permanent teeth. A recent study depicts that the majority of the children requiring pulpectomy were around 5 years of age. (1) However, the complexity of the root canal morphology in primary teeth cumbers an effective canal preparation, making the success of the procedure depend on the obturating material used. (2,3,4)

Zinc oxide Eugenol served as the only obturating material for primary teeth up till 2008. (5) The disadvantages of the Zinc oxide Eugenol cement paved way for calcium hydroxide and Iodoform based cements. (6,7) Regardless of its tendency to resorb at a faster rate than the tooth, the combination of calcium hydroxide and iodoform paste is the primarily used obturating material. Pulpectomy, despite being the commonest procedure in paediatric dental practice has no ideal or close to ideal obturating material. Hence the search for a better obturating material in primary teeth continues.

An ideal obturating material in primary teeth should be non-inflammatory, non irritating, antimicrobial, radio-opaque, easy to insert and remove. Also, it should adhere well to the canal walls, should be insoluble in water, should be resorbable at the rate of the tooth, should not set to a hard mass and should not discolour the tooth. (8,9) Considering the aforesaid criteria, development of a Novel Root Canal filling material with Zinc oxide cement, Calcium hydroxide and Metronidazole was undertaken. Calcium hydroxide and Zinc oxide was mixed at 50:50 concentration respectively as the evidences demonstrates that zinc oxide has the tendency to resorb slowly while calcium hydroxide resorbs much faster than the resorption rate of the tooth. The combination of these materials can overcome the disadvantages of one material over the other. 1%, 2%, 3% of metronidazole - an antimicrobial agent was added to 50:50 concentration of Calcium hydroxide and Zinc oxide, and was checked for antimicrobial efficacy. Metronidazole is known to be effective against anaerobic facultative micro-organisms.

The mechanism of action involves diffusion of the agent into the organism, inhibition of the protein synthesis, consequently resulting in the cell death of the susceptible organisms.(10)

In general, all the micro-organisms present in the oral cavity can invade the pulpal space. *S.mutans* and *E.faecalis* are the most commonly encountered micro-organisms in the root canals of primary teeth with pulpal necrosis. Studies have shown that the root canal infections in primary teeth are predominantly due to anaerobes and gram positive bacteria. The most predominately isolated organism from the primary root canal infections is *S.mutans*, whereas the secondary endodontic infections were majorly encompassed of *E.faecalis*.(11,12) Our team has extensive knowledge and research experience that has translate into high quality publications.(13-22). Hence, the present study was conducted to find out the antimicrobial efficacy of different concentrations of the Novel Obturating Material against *S.mutans* and *E.faecalis* in primary teeth.

MATERIALS AND METHOD

The present study was conducted as an in-vitro study to compare the anti-microbial efficacy of different concentrations of the Novel Obturating material for primary teeth.

Preparation of the Obturating material

300mg of Zinc oxide powder was mixed with 300mg of calcium hydroxide powder and was kept in magnetic stirrer for 1 hour. 400mg of Metronidazole tablet was crushed into powder using motor and pistle. 1% Zinc oxide - Calcium hydroxide - Metronidazole powder was prepared by mixing 2mg of metronidazole powder to 198mg of the zinc oxide- calcium hydroxide mixture. Similarly 2% and 3% Zinc oxide- Calcium hydroxide - Metronidazole powder was prepared by mixing 4 and 6 mg of metronidazole powder to 196 and 194mg of zinc-oxide- calcium hydroxide mixture respectively. The samples were mixed 2ml of distilled water, placed in vortex for 15 minutes and then in Mixer for 1 day.

Preparation of the culture medium

Mueller Hilton Agar (MHA) was utilised to determine the antibacterial activity of the different concentrations of the novel obturating material against *S.mutans* and *E.faecalis*. The Oral pathogens were collected from the Microbiology lab, Saveetha Dental College and hospital, Chennai. MHA was prepared and sterilised at 121 degree Celsius for 15 minutes. The media was poured into 30 sterilised plates (15 for *s.mutans* and 15 for *E.faecalis*) and was allowed for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The obturating material with different concentrations were loaded at 25, 50, 100 microliters and were incubated for 24 hours at 37 ° C. The zone of inhibition was measured in millimetres after 24 hours of incubation by measuring the shortest distance between outer margin of the well and initial microbial growth. (Figure 1 and 2)

Statistical analysis

Data were entered in Microsoft Excel spreadsheet and analysed using SPSS software (IBM SPSS Statistics, Version 20.0, Arming,NY: IBM Corp.). Descriptive statistics were used for data summarisation and presentation. Friedman test and Kruskal Wallis test were used to compare the different concentrations of the Novel obturating materials against *S.mutans* and *E.faecalis*.

RESULTS

The results of the present study show that at 1% and 3% 50-50 concentration of the Novel obturating material, there is no statistically significant difference at 25,50 and 100 microiliters against *S.mutans*. However the zone of inhibition was maximum at 100 microliters. At 2% 50-50 concentration, 100 microliters shows statistically significant greater inhibition zone against *S.mutans*. (Table 1 -3)

With regards to *E.Faecalis*, at 1% and 3 %, there was statistically greater zone of inhibition seen at 100 microliters. No statistically significant difference was noted at 2% 50-50 concentration, however the inhibition zone was greater at 100 microliters. (Table 4-6). The novel obturating

material shows maximum anti-microbial property at 100 microliters against both *S.mutans* and *E.faecalis*.

Comparison between the groups, shows no significant difference between 1%, 2% and 3% 50-50 concentration at 25, 50 and 100 microliters against both *S.mutans* and *E.faecalis*. (Table 7,8) Though 1% concentration of the novel material shows greater inhibition zone against *S.mutans* at 100 micro-litres, only at 2% concentration the inhibition zone was greater against *E.faecalis*.

DISCUSSION

An ideal obturating material in primary teeth should have maximal antimicrobial properties to prevent re-infection and also to compensate for the inadequate cleansing during canal preparation owing to the complex, ribbon shaped, tortuous primary root canal morphology. Eugenol based cements which includes Zinc Oxide Eugenol cement and Endoflas are known for its highest anti-microbial activity.(23) However, its detrimental effects on permanent teeth cannot be neglected. Iodoform based cements are also said to have better antimicrobial activity.(24) But again, Iodoform is an irritant and can cause yellow discolouration. Thus, arises the need for a better obturating material in primary teeth with little or no harm to the surrounding tissues. That being the case, in the present study a Novel Obturating material was developed with Zinc oxide, calcium hydroxide and Metronidazole as its components. Different concentrations of the metronidazole (1%,2%,3%) was added to equal parts of Zinc oxide and Calcium hydroxide and was evaluated for the antimicrobial efficacy.

In the present study, the antimicrobial efficacy of the Novel Obturating Material was tested against *S.mutans* and *E.faecalis*. *S.mutans* is the most prevalent bacterial species isolated from the oral cavity and the root canals of primary teeth, while eradication of *E.faecalis* has been reported to determine the success rate of the root canal treatment and also serves as a standard for testing the antimicrobial efficacy of any root canal medicament. (25,26,27)

Against *S.mutans*, in the present study it was eminent that the 1%, 2% and 3% of the Novel obturating material was effective at 100

microliters with 2% 50-50 concentration having a statistical significance ($p=0.02$). The Novel Obturating material was also statistically effective against *E.faecalis* at 100 microlitres with 1% and 3 % 50-50 concentration ($p= 0.05, 0.01$). This shows that the Novel Obturating material, irrespective of the concentration of the Metronidazole is effective against *E.faecalis* and *S.mutans* at 100 microliters.

On comparing between groups at 25, 50 and 100 microliters, there was no statistically significant difference noted in the zone of inhibition (Table 7,8). At 100 microliters, for *S.mutans*, the Zone of inhibition was greater at 1% concentration, whereas for *E.faecalis*, the zone of inhibition was greater at 2% concentration. Hence 2% metronidazole would be more effective against both *S.mutans* and *E.faecalis*.

The composition of the Novel Obturating Material was chosen based on a systematic review which concluded that the combination of Zinc oxide Eugenol and calcium hydroxide/ iodoform paste is the material of choice for root canal treatment in primary teeth. (28) Hence in the present study, the detrimental agents (eugenol and iodoform) were excluded and the remaining agents (zinc oxide, calcium hydroxide) were incorporated along with a potent antimicrobial agent - metronidazole as it is known to be effective against facultative anaerobes.

Agar well diffusion method was used in the present study as this method of evaluating the antimicrobial efficacy was more reliable and easy to perform.(12) Also, *S.mutans* and *E.faecalis* were selected as the test organisms as they are reported to be predominantly associated with primary and secondary infections of the primary teeth. (11,12) The present study was conducted in-vitro conditions, further in -vivo studies can justify the use and antimicrobial efficacy of the Novel Obturating material. Further In-vitro studies are also needed to check if there are any alteration in the physical properties of the obturating materials due to the addition of metronidazole. Also more studies are needed to evaluate the safety of this concentration of the novel root canal filling material for clinical applicability.

CONCLUSION

2% 50-50 concentration of the Novel Obturating Material (2% metronidazole in equal parts of zinc oxide and calcium hydroxide) at 100 microlitres appears to be most effective against both *S.mutans* and *E.faecalis*. With further studies on its safety and applicability, this novel root canal filling material can be a promising alternative root canal filling material in primary teeth.

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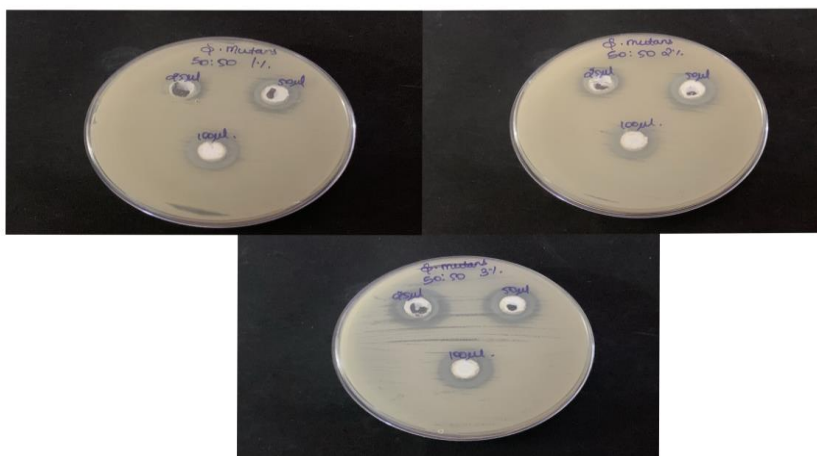


FIGURE 1: Zone of inhibition observed for *S.mutans* after 24 hours at different concentration of the Novel Obturating material

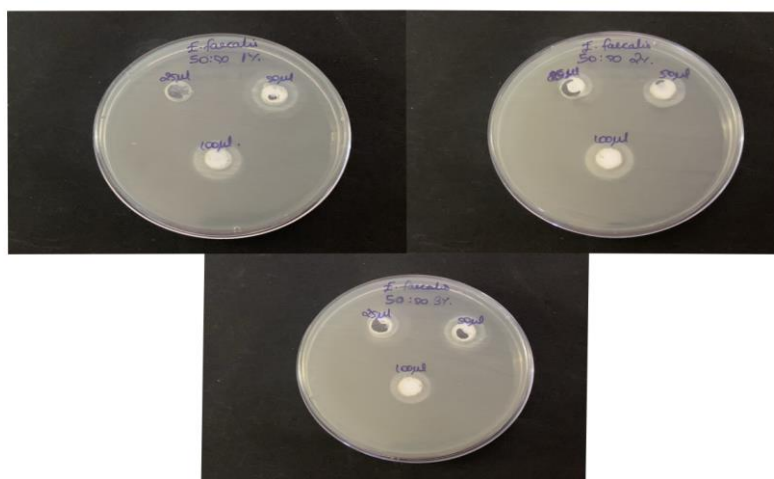


FIGURE 2: Zone of inhibition observed for *E.faecalis* after 24 hours at different concentration of the Novel Obturating material

TABLE 1 : Comparison of 1% 50-50 concentration of the Novel obturating material against streptococcus mutans

	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	15.60	6.50	8.58	0.14
50 MICROLITRE	16.80	7.52		
100 MICROLITRE	19.20	5.54		

TABLE 2 : Comparison of 2% 50-50 concentration of the Novel obturating material against streptococcus mutans

	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	13.00	4.00	7.60	0.02*
50 MICROLITRE	13.80	5.01		
100 MICROLITRE	15.40	4.09		

TABLE 3 : Comparison of 3% 50-50 concentration of the Novel obturating material against streptococcus mutans

	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	14.80	6.57	0.40	0.81
50 MICROLITRE	15.00	5.47		
100 MICROLITRE	16.00	3.67		

TABLE 4 : Comparison of 1% 50-50 concentration of the Novel obturating material against E.Faecalis

1% E.Faecalis	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	10.60	2.19	6.00	0.05*
50 MICROLITRE	13.20	3.83		
100 MICROLITRE	14.20	4.76		

TABLE 5 : Comparison of 2% 50-50 concentration of the Novel obturating material against E.Faecalis

2% E.Faecalis	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	12.00	3.00	5.60	0.06
50 MICROLITRE	12.40	3.13		
100 MICROLITRE	14.80	5.54		

TABLE 6 : Comparison of 3% 50-50 concentration of the Novel obturating material against E.Faecalis

3% E.Faecalis	Mean	Std. Deviation	Friedman test value	P value
25 MICROLITRE	10.20	1.09	8.58	0.01*
50 MICROLITRE	12.20	3.03		
100 MICROLITRE	13.40	3.13		

TABLE 7: Comparison between 1%,2%,3% 50-50 concentration of the Novel Obturating material against streptococcus mutans at 25,50,100 microlitre

25 MICROLITRE	Mean	Std. Deviation	P value
1%	15.60	6.50	0.72
2%	13.00	4.00	
3%	14.80	6.57	

50 MICROLITRE			
1%	16.80	7.52	0.75
2%	13.80	5.01	
3%	15.00	5.47	
100 MICROLITRE			
1%	19.20	5.54	0.42
2%	15.40	4.09	
3%	16.00	3.67	

TABLE 8: Comparison between 1%,2%,3% 50-50 concentration of the Novel Obturating material against E.faecalis at 25,50,100 microlitre

25 MICROLITRE	Mean	Std. Deviation	P value
1%	10.60	2.19	0.57
2%	12.00	3.00	
3%	10.20	1.09	
50 MICROLITRE			
1%	13.20	3.83	0.51
2%	12.40	3.13	
3%	12.20	3.03	
100 MICROLITRE			
1%	14.20	4.76	0.87
2%	14.80	5.54	
3%	13.40	3.13	