

## Original Article

# Evaluation of the antimicrobial efficacy of different concentrations of a novel root canal filling material for primary teeth - An *in vitro* study

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## ABSTRACT

**Background:** The demand for an ideal root canal filling material in primary teeth with maximum antibacterial properties keeps escalating to improve the success rate of root canal treatment in primary teeth. Hence, the present study was conducted to determine the antimicrobial efficacy of the different concentrations of a novel root canal filling material.

**Materials and Methods:** An *in vitro* study was designed to compare the antimicrobial efficacy of different concentrations of the novel obturating material for primary teeth. Different concentrations of the novel obturating material were prepared using calcium hydroxide, zinc oxide cement, and metronidazole and were tested for its antimicrobial efficacy against *Streptococcus mutans* and *Enterococcus faecalis* using Mueller–Hinton Agar for 24 h at 37°C. The zone of inhibition was measured in millimeters after 24 h. The values were entered into a Microsoft Excel spreadsheet and subjected to statistical analysis. Descriptive statistics were used for data summarization 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*.  $P < 0.05$  was considered to be significant.

**Results:** Against *S. mutans* at 1% concentration, the zone of inhibition was statistically greater at 50 µl and at 2% and 3%, the zone of inhibition was greater at 100 µl. Similarly, against *E. faecalis*, the zone of inhibition was statistically greater at 100 µl at 2% and no significant difference was noted at 1% and 3%. Comparison between the groups at 25, 50, and 100 µl shows no significant difference against *S. mutans* between 1%, 2%, and 3% of 60–40 concentration. However, 3% 60–40 concentration at 50 µl shows significantly increased zone of inhibition against *E. faecalis*.

**Conclusion:** Two percentage metronidazole at 60–40 (calcium hydroxide–zinc oxide) concentration of the novel obturating material at 100 µl has better antibacterial properties against both *S. mutans* and *E. faecalis*.

**Key Words:** Child, deciduous, *in vitro* techniques, microbiology, pulpectomy, tooth

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## INTRODUCTION

Root canal treatment is the only option to treat primary teeth with pulpal signs and symptoms.<sup>[1]</sup> However, root canal treatment in primary teeth is a strenuous procedure owing to

the complex and torturous nature of the root canal system. Despite proper canal preparation and irrigation in the primary teeth, the microorganisms

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still remain entrapped leading to failure of the root canal treatment.<sup>[2,3]</sup> Hence, the success of root canal treatment in primary teeth majorly depends on the obturating material used. An ideal root canal filling material in primary teeth should have maximal antibacterial activity and at the same time should resorb at the same rate of the primary teeth without exerting any deleterious effects on the permanent teeth and the periapical tissues.<sup>[4,5]</sup>

Conventionally used root canal filling materials such as zinc oxide eugenol cement, calcium hydroxide, and iodoform paste has its respective disadvantages. The former material has a low resorption rate which in turn results in the deflection of the permanent teeth during its eruption.<sup>[6]</sup> While the latter material resorbs much faster than the primary teeth resulting in a hollow tube effect of the primary root canals serving as a nidus for reinfection.<sup>[6,7]</sup> The above evidence authenticates the need for an ideal root canal filling material to proclaim the success of endodontic treatment in primary teeth.

A novel root canal filling material was developed with zinc oxide, calcium hydroxide, and metronidazole at different concentrations and was checked for antimicrobial efficacy to determine the most efficacious composition. The different concentrations of the novel root canal filling material were checked against *Streptococcus mutans* and *Enterococcus faecalis* as these organisms have been reported to be present in higher concentrations in necrotic primary teeth.<sup>[8,9]</sup> The present study aimed to determine the antimicrobial efficacy of the different concentrations of the novel root canal filling material against *S. mutans* and *E. faecalis* in primary teeth and also to determine the most efficacious composition of the novel root canal filling material.

## MATERIALS AND METHODS

An *in vitro* study was designed to compare the antimicrobial efficacy of different concentrations of the novel obturating material for primary teeth.

### Preparation of the obturating material

Six hundred milligrams of calcium hydroxide powder was mixed with 400 mg of zinc oxide powder to form 60–40 concentration of the mixture and was kept in a magnetic stirrer for 1 h. Four hundred milligrams of metronidazole tablet was crushed into powder using motor and pistle. One percentage of calcium hydroxide–zinc oxide–metronidazole powder was

prepared by mixing 2 mg of metronidazole powder to 198 mg of the calcium hydroxide–zinc oxide mixture. Similarly, 2% and 3% calcium hydroxide–zinc oxide–metronidazole powder were prepared by mixing 4 and 6 mg of metronidazole powder to 196 and 194 mg of zinc oxide–calcium hydroxide mixture, respectively. The samples were mixed with 2 µl of distilled water, placed in the vortex for 15 min, and then in a mixer for 1 day.

### Preparation of the culture medium

The antimicrobial activity of the different concentrations of the novel obturating material against *S. mutans* and *E. faecalis* was determined using Mueller–Hinton Agar (MHA). MHA was prepared and sterilized at 121° C for 15 min. The media was poured into 30 sterilized 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. 1%, 2%, and 3% of the novel obturating material were loaded at 25, 50, and 100 µl and were incubated at 37°C for 1 day. The zone of inhibition was measured in millimeters after 24 h of incubation by measuring the shortest distance between the outer margin of the well and initial microbial growth.

### Statistical analysis

Data were entered in a Microsoft Excel spreadsheet and analyzed using SPSS software (IBM SPSS Statistics, Version 20.0, Arming, NY, USA: IBM Corp.). Descriptive statistics were used for data summarization 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

In the present study with 60–40 concentrations of the novel obturating material, it was seen that at 1%, the zone of inhibition was statistically greater at 50 µl against *S. mutans*. However, at 2% and 3%, the zone of inhibition was greater at 100 µl against *S. mutans*, the former being statistically significant. Similarly, against *E. faecalis*, the zone of inhibition was statistically greater at 100 µl at 2% and no significant difference was noted at 1% and 3% [Table 1].

Comparison between the groups at 25, 50, and 100 µl shows no significant difference against *S. mutans* between 1%, 2%, and 3% of 60–40 concentration. However, 3% 60–40 concentration

at 50 µl shows a significantly increased zone of inhibition against *E. faecalis* [Table 2].

## DISCUSSION

A systematic review comparing zinc oxide eugenol and calcium hydroxide/iodoform paste as root canal filling material for pulpectomy in primary teeth 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.<sup>[10]</sup> Despite the fact that the antimicrobial activity of zinc oxide eugenol is mainly attributed to eugenol, it can cause irritation to the periapical region, necrosis of the bone, and cementum.<sup>[11,12]</sup> Similarly, with iodoform, yellowish discoloration and accelerated resorption of the primary teeth were reported.<sup>[5,13]</sup> Hence, a novel root canal filling material was developed by substituting eugenol and iodoform, which are known for its antibacterial properties with metronidazole to the calcium hydroxide and zinc oxide powder (60–40

concentration). 1%, 2%, and 3% metronidazole were added to 60–40 calcium hydroxide and zinc oxide mixture and were evaluated for its antimicrobial efficacy against *S. mutans* and *E. faecalis* to certify the most efficacious concentration of the novel root canal filling material.

The results of the present study show that against *S. mutans*, at 1% concentration, the zone of inhibition is statistically greater at 50 µl. However, the zone of inhibition was greater for *E. faecalis* only at 100 µl with 1% concentration of the novel root canal filling material. At 2% 60–40 concentration, the zone of inhibition was statistically greater at 100 µl for both *S. mutans* and *E. faecalis*. No significant difference was noted with 3% 60–40 concentration of the novel root canal filling material. These results authenticate that the novel root canal filling material shows better antimicrobial properties at 100 µl. Comparison in between the groups at 100 µl shows no statistically significant difference,

**Table 1: Comparison of different concentrations of the novel obturating material against *Streptococcus mutans* and *Enterococcus faecalis***

<i>Streptococcus mutans</i>			
	Mean (mm)	SD	P
1% 60-40 concentration (µl)			
25	10.20	1.64	0.05*
50	14.80	6.69	
100	14.40	3.50	
2% 60-40 concentration (µl)			
25	11.40	3.29	0.01*
50	12.00	3.68	
100	15.40	2.51	
3% 60-40 concentration (µl)			
25	12.40	3.13	0.21
50	12.80	3.56	
100	13.60	4.09	
<i>Enterococcus faecalis</i>			
	Mean (mm)	SD	P
1% 60-40 concentration (µl)			
25	9.00	0.00	0.13
50	9.00	0.00	
100	9.40	0.55	
2% 60-40 concentration (µl)			
25	9.00	0.00	0.01*
50	9.00	0.00	
100	9.80	0.45	
3% 60-40 concentration (µl)			
25	9.40	0.55	0.13
50	11.20	2.59	
100	10.40	1.52	

\*P<0.05, Statistically significant, SD: Standard deviation

**Table 2: Comparison between 1%, 2%, and 3% 60-40 concentration of the novel obturating material against *Streptococcus mutans* and *Enterococcus faecalis* at 25, 50, and 100 µl**

<i>Streptococcus mutans</i>			
	Mean (mm)	SD	P
25 µl (%)			
1%	10.20	1.64	0.53
2%	11.40	3.29	
3%	12.40	3.13	
50 µl (%)			
1%	14.80	6.69	0.86
2%	12.00	3.68	
3%	12.80	3.56	
100 µl (%)			
1%	14.40	3.50	0.59
2%	15.40	2.51	
3%	13.60	4.09	
<i>Enterococcus faecalis</i>			
	Mean (mm)	SD	P
25 µl (%)			
1	9.00	0.00	0.11
2	9.00	0.00	
3	9.40	0.55	
50 µl (%)			
1	9.00	0.00	0.03*
2	9.00	0.00	
3	11.20	2.59	
100 µl (%)			
1	9.40	0.55	0.43
2	10.40	0.45	
3	9.80	1.52	

\*P<0.05, Statistically significant, SD: Standard deviation

however, at 2% concentration shows a greater zone of inhibition.

In the present study, during the development of the material, the concentration of calcium hydroxide was increased when compared to zinc oxide as calcium hydroxide itself exhibits antibacterial properties by ionic dissociation of calcium and hydroxyl ions while the antibacterial effect of zinc oxide-based materials was majorly due to eugenol. Metronidazole was selected as a substitute to eugenol and iodoform as it is known that the root canal space is majorly invaded by anaerobic bacteria and metronidazole fights against anaerobic facultative microorganisms by diffusing into the organism and inhibiting the protein synthesis causing cell death of the susceptible organisms.<sup>[14]</sup>

## CONCLUSION

From the results of the present study, it can be concluded that 2% metronidazole at 60–40 (calcium hydroxide–zinc oxide) concentration of the novel obturating material at 100 µl has better antibacterial properties against both *S. mutans* and *E. faecalis*.

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### Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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