

An In-vitro Analysis of the Antimicrobial Efficacy of a Novel Obturating Material for Primary Teeth

Lavanya Govindaraju¹, Ganesh Jeevanandan¹, Vishnu Priya Veeraraghavan²,
Mohamed El-Sherbiny^{3,4}, Ghala Basem Binshafi³, Shouq Khalid alkharij³,
Elaf Abdulrahmam Alhazza³, Rania A . Fouad^{3,5}, Ateya Megahed Ibrahim^{6,7}

1. Department of Pediatric and Preventive Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences. Saveetha University. Chennai.
2. Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University. Chennai.
3. Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Saudi Arabia.
4. Department of Anatomy, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
5. Department of Medical Biochemistry, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
6. College of Nursing, Prince Sattam bin Abdulaziz University Alkharj, Saudi Arabia.
7. Department of Family and Community Health Nursing, Faculty of Nursing, Port Said, University, Egypt.

Abstract

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

Different concentrations of the Novel Obturating Material was prepared using Calcium hydroxide, Zinc oxide cement and Metronidazole. It was tested for antimicrobial efficacy against *S.mutans* and *E.faecalis* using MHA for 24 hours at 37 ° C. The zone of inhibition was measured in millimetres after 24 hours. The values were subjected to statistical analysis. Maximum zone of inhibition against *S.mutans* was seen at 100 microliters with statistical significance at 3% 70-30 concentration and no difference was noted against *E.faecalis*.

Comparison in between the groups revealed 1% 70-30 concentration of the Novel Obturating material at 100 microliters showed greater inhibition zone against *S.mutans* and no difference was noted against *E.faecalis*. 1% Metronidazole at 70-30 (calcium hydroxide- Zinc oxide) concentration of the Novel Obturating material at 100 microliters is effective against *S.mutans* and *E.faecalis*.

Experimental article (J Int Dent Med Res 2024; 17(1): 110-114)

Keywords: Child dentistry, Deciduous teeth, Novel, Obturating material, Pulpectomy.

Received date: 12 January 2024

Accept date: 11 March 2024

Introduction

The vindication for failure of the endodontic treatment in primary teeth even after an adequate and efficient chemo-mechanical

preparation is attributed to the torturous morphology of the primary root canals which can retain the infectious micro-organisms within the canal leading to persistent infection. This demands for an obturating material with good antimicrobial properties for obturation in primary teeth.

Zinc oxide eugenol, widely used root canal filling material in primary teeth has shown good antimicrobial property, nevertheless the detrimental effects of Eugenol should not be unintended.¹⁻² By the same token, calcium hydroxide and iodoform based cements also has its own downside, which substantiates the need for an ideal obturating material with maximal antimicrobial property and minimal toxicity.³⁻⁵

Studies have shown that the root canal infections in primary teeth are predominantly due to anaerobes and gram positive bacteria. The

*Corresponding author:

Lavanya Govindaraju

Reader Department of Pediatric and Preventive Dentistry
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Saveetha University. Chennai-77
E-mail: glaavuu@gmail.com

Vishnu Priya Veeraraghavan

Professor Department of Biochemistry
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Saveetha University. Chennai-77
E-mail: vishnupriya@saveetha.com

most predominately isolated organism from the primary root canal infections is *S.mutans*, whereas the secondary endodontic infections were majorly encompassed of *E.faecalis*.⁶⁻⁷

Hence, a novel root canal filling material effective against both *S.mutans* and *E.faecalis* would be an ideal obturating material in primary teeth. The aim of the present study was to gauge the antimicrobial efficacy of different concentrations of a newly developed root canal filling material composed of calcium hydroxide, Zinc oxide and metronidazole against *S.mutans* and *E.faecalis*.

Materials and methods

The present microbiological study was designed as an in vitro study and the clearance was obtained from the institutional review board. Preparation of the Obturating material:

700mg of calcium hydroxide powder and 300mg of Zinc oxide powder was measured using a digital weighing machine. The exact amount of the above said powders were mixed to form 70-30 concentration of the mixture, which was kept in magnetic stirrer for 1 hour. 400mg of Metronidazole tablet was crushed into powder using motor and pistle. 1% of Calcium hydroxide - Zinc oxide - Metronidazole powder was prepared by mixing 2mg of metronidazole powder to 198mg of the calcium hydroxide - zinc oxide mixture. Similarly 2% and 3% Calcium hydroxide - Zinc oxide - 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 exact amount of the powders were dispensed by using a digital weighing machine. The samples were mixed with 2ml of distilled water measured with micropipette, placed in vortex for 15 minutes and then in Mixer for 24 hours.

Preparation of the culture medium:

Agar well diffusion method was employed to evaluate the antimicrobial activity of the different concentrations of the novel obturating material against *S.mutans* and *E.faecalis*. Mueller Hilton Agar (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. 1%,

2% and 3% of the Novel obturating material were loaded at 25, 50, 100 microliters and were incubated at 37 ° C for 1 day. The diameters of the inhibition zone was measured in millimetres after 24 hours using vernier calliiper. (Figure 1 and 2)

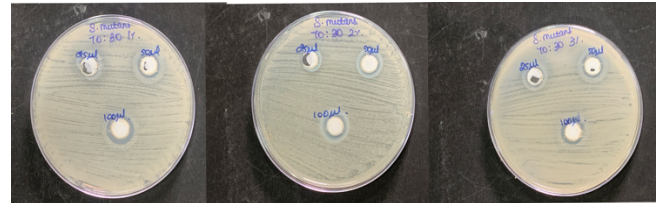


Figure 1. Zone of inhibition observed for *S.mutans* after 24 hours at different concentration of the Novel Obturating material.

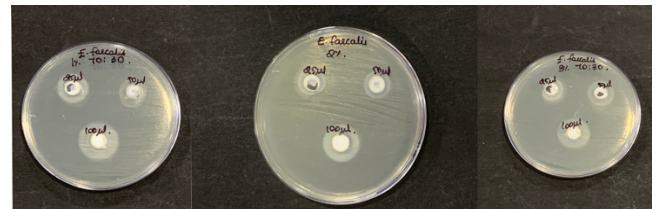


Figure 2. Zone of inhibition observed for *E.faecalis* after 24 hours at different concentration of the Novel Obturating material.

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

Statistical analysis performed showed that the maximum zone of inhibition against *S.mutans* was seen at 100 microliters with statistical significance at 3% 70-30 concentration of the Novel Obturating material and no difference was noted with regards to *E.faecalis*. (Table 1 and 2) Comparison in between the groups shows that 1% 70-30 concentration of the Novel Obturating material at 100 microliters shows greater inhibition zone against *S.mutnas* and no difference was noted with regards to *E.faecalis*. (Table 3 and 4)

1% 70-30 concentration	Mean	Std. Deviation	P value
25 MICROLITRE	12.60	4.93	0.51
50 MICROLITRE	14.40	3.29	
100 MICROLITRE	15.40	0.55	
2% 70-30 concentration			
25 MICROLITRE	12.40	3.29	0.69
50 MICROLITRE	13.60	2.19	
100 MICROLITRE	14.00	1.00	
3% 70-30 concentration			
25 MICROLITRE	10.80	1.09	0.01*
50 MICROLITRE	12.60	0.55	
100 MICROLITRE	14.60	0.55	

Table 1. Comparison of Different concentrations of the Novel obturating material against streptococcus mutans.

25 MICROLITRE	Mean	Std. Deviation	P value
1%	12.60	4.93	0.78
2%	12.40	3.29	
3%	10.80	1.09	
50 MICROLITRE			
1%	14.40	3.29	0.94
2%	13.60	2.19	
3%	12.60	0.55	
100 MICROLITRE			
1%	15.40	0.55	0.04*
2%	14.00	1.00	
3%	14.60	0.55	

Table 3. Comparison between 1%,2%,3% 70-30 concentration of the Novel Obturating material against streptococcus mutans at 25,50,100 microlitre.

1% 70-30 concentration	Mean	Std. Deviation	P value
25 MICROLITRE	9.00	0.00	-
50 MICROLITRE	9.00	0.00	
100 MICROLITRE	9.00	0.00	
2% 70-30 concentration			
25 MICROLITRE	9.00	0.00	-
50 MICROLITRE	9.00	0.00	
100 MICROLITRE	9.00	0.00	
3% 70-30 concentration			
25 MICROLITRE	9.00	0.00	-
50 MICROLITRE	9.00	0.00	
100 MICROLITRE	9.00	0.00	

Table 2. Comparison of Different concentrations of the Novel obturating material against E.Faecalis.

25 MICROLITRE	Mean	Std. Deviation	P value
1%	9.00	0.00	1.00
2%	9.00	0.00	
3%	9.00	0.00	
50 MICROLITRE			
1%	9.00	0.00	1.00
2%	9.00	0.00	
3%	9.00	0.00	
100 MICROLITRE			
1%	9.00	0.00	1.00
2%	9.00	0.00	
3%	9.00	0.00	

Table 4. Comparison between 1%,2%,3% 60-40 concentration of the Novel Obturating material against E.faecalis at 25,50,100 microlitre.

Discussion

With substantial evidence that an ideal root canal filling material in primary teeth should have maximum of antibacterial properties, in the present study a Novel Root Canal Filling material was developed taking this into consideration. The combination of Zinc oxide Eugenol and calcium hydroxide/ iodoform paste is considered as the material of choice for root canal treatment in primary teeth.⁸⁻⁹ Nevertheless, the detrimental effects of eugenol and iodoform is overlooked. The former can cause irritation to the periapical region, necrosis of the bone and cementum.¹⁻²

While the latter can cause yellowish discolouration and accelerated resorption of the primary teeth.⁴⁻⁵ Considering the above effects, the novel root canal filling material for primary teeth was developed by substituting eugenol and iodoform, which are known for its antibacterial properties with Metronidazole to the calcium hydroxide and zinc oxide powder (70-30 concentration). 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

The antimicrobial efficacy of this novel root canal filling material at different concentrations of the metronidazole was evaluated using Agar well diffusion method as this method of evaluating the antimicrobial efficacy was more reliable and easy to perform.¹¹ Also, the antimicrobial efficacy was tested against *S.mutans* and *E.faecalis* as these organisms are reported to be predominantly associated with primary and secondary infections of the primary teeth.⁶⁻⁷

The zone of inhibition obtained against *S.mutans* in the present study was greater at 100 microliters, whereas no difference was noted in the inhibition zone against *E.facecalis*. Also, the zone of inhibition against *S.mutans* at 100 microliters was statistically greater with 1% metronidazole added to 70-30 concentration of calcium hydroxide and Zinc oxide mixture and it was all the same against *E.faecalis*. Under similar methodology, the obturating material with 50-50 and 60-40 concentration of calcium hydroxide and zinc oxide showed better antibacterial properties against both *Strep mutans* and *Entero faecalis* with 2%

metronidazole at 100 μ l.¹²⁻¹⁵ Similarly, at 80-20 (calcium hydroxide-zinc oxide) concentration of the Obturating material, 3% metronidazole at 100 μ L showed better antimicrobial activity against both *Strep mutans* and *Entero faecalis*.¹⁶ Further studies needs to conducted to evaluate the safety of this concentration of the novel root canal filling material for clinical applicability.

Conclusion

The formulation obtained with 1% Metronidazole at 70-30 (calcium hydroxide- Zinc oxide) concentration of the Novel Obturating material at 100 microliters showed better antimicrobial activity against both *S.mutans* and *E.faecalis*.

Declaration of Interest

The authors report no conflict of interest.

Acknowledgments

The work was supported by the White lab , Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University - project number (SDC/2024/105), Chennai, India.

The work was also supported by the Researchers Supporting program, AlMaarefa University, Riyadh, Saudi Arabia and via funding from Prince Sattam bin Abulaziz University project number (PSAU/ 2024/R/1445) and AlMaarefa Riyadh, Saudi Arabia.

References

1. Spedding RH. Incomplete resorption of resorbable zinc oxide root canal fillings in primary teeth: report of two cases. ASDC J Dent Child 1985; 52(3):214-6.
2. Hendry JA, Jeansonne BG, Dummett Jr CO, Burrell W. Comparison of calcium hydroxide and zinc oxide and eugenol pulpectomies in primary teeth of dogs. Oral Surge Oral Med Oral Pathol 1982; 54(4):445-51.
3. Nurko C, Ranly DM, Garcia-Godoy F, Lakshmyya KN. Resorption of a calcium hydroxide/iodoform paste (Vitapex) in root canal therapy for primary teeth: a case report. Pediatr dent 2000;22(6):517-20.
4. Moskovitz M, Tickotsky N, Ashkar H, Holan G. Degree of root resorption after root canal treatment with iodoform-containing filling material in primary molars. Quintessence Int 2012;43(5).
5. Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. ASDC J Dent Child 1987;54(1):30-4.
6. Schleifer KH, Kilpper-Bälz R. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. Int J Syst Evol Microbiol 1984; 34(1):31-4.

7. Ryan KJ, Ray CG. Medical microbiology. McGraw Hill, 2004; 4(370).
8. Najjar RS, Alamoudi NM, El-Housseiny AA, Al Tuwirqi AA, Sabbagh HJ. A comparison of calcium hydroxide/iodoform paste and zinc oxide eugenol as root filling materials for pulpectomy in primary teeth: A systematic review and meta-analysis. Clin Exp Dent Res 2019;5(3):294-310.
9. Ravindran V, Jeevanandan G, Veeraraghavan VP, El-Sherbiny M, Alnamly JM, Ayad NS, Alsaleebi N, Ibrahim AM. Comparative Evaluation of Physical and Antimicrobial Properties of Doxycycline Incorporated Formulation of Mineral Trioxide Aggregate-An In-Vitro Study. Journal of International Dental and Medical Research. 2023 Oct 1;16(4):1501-9.
10. Weir CB, Le JK. Metronidazole. [Updated 2021 Jun 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK539728/>
11. Kriplani R, Thosar N, Baliga MS, Kulkarni P, Shah N, Yeluri R. Comparative evaluation of antimicrobial efficacy of various root canal filling materials along with aloe vera used in primary teeth: a microbiological study. Clin Pediatr Dent 2013; 37(3): 257-62.
12. Govindaraju L, Jeevanandan G. Evaluation of the antimicrobial efficacy of different concentrations of a novel root canal filling material for primary teeth - An *in vitro* study. Dent Res J 2023;20:20.
13. Govindaraju L, Jeevanandan G. Antimicrobial efficacy of a novel obturating material used in primary teeth - An *In vitro* Study. J Popul Ther Clin Pharmacol 2023;30(6):e149-56.
14. Govindaraju L, Jeevanandan G. Development of an Advanced Material for Obturation in Primary Teeth Based on the Microbiological Analysis of the Antimicrobial Efficacy of Various Concentrations: An *In Vitro* Study. Int J Clin Pediatr Dent 2023;16(6):772-5.
15. Govindaraju L, Jeevanandan G, Maganur PC, et al. Antimicrobial Efficacy of a Novel Obturating Material with and without Iodoform: A Microbiological *In Vitro* Study. World J Dent 2024;15(1):68-71.
16. Govindaraju L, Jeevanandan G. Comparative evaluation of the antimicrobial efficacy of different concentrations of the novel root canal filling material for primary teeth: A microbiological study. J Int Oral Health 2023;15:84-8.