Evaluation of Antimicrobial Efficacy of Various Root Canal Filling Materials Used in Primary Teeth: A Microbiological Study

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The primary goal of endodontic treatment in primary teeth is to eliminate infection, and to retain the tooth in a functional state until their normal exfoliation time without endangering the permanent dentition or the general health of the child. The complexity of the pulp canal system in primary teeth presents a discerning problem for chemo-mechanical preparation. One of the factors determining the success of endodontic treatment in infected primary teeth is the sealing material that should encompass among other factors a potent bactericidal effect and the capacity to resorb along with the roots of primary teeth.

This study evaluated the antimicrobial effectiveness of 5 root canal filling materials and a negative control agent against 23 strains of bacteria isolated from infected root canals of primary molar teeth and 3 non standard bacterial strains using agar diffusion assay. The materials were Zinc oxide and Eugenol (ZOE), Zinc oxide-Eugenol and Formocresol (ZOE+FC), Calcium hydroxide and sterile water (CAOH+H2O), Zinc oxide and Camphorated phenol (ZO+CP), Calcium hydroxide and Iodoform (Metapex) and Vaseline (Control).

All the materials except Vaseline showed varied antimicrobial activity against the test bacteria. The zones of inhibition were ranked into 4 inhibition categories based on the proportional distribution of the data. All the 26 bacterial isolates were classified under 4 groups based on Aerobic/Anaerobic and Gram positive/Gram negative. Statistical analysis was carried out to compare the antimicrobial effectiveness between materials tested with each of the bacterial groupings. ZOE+FC produced strong inhibition against most bacteria when compared to ZOE, ZO+CP and CAOH+H2O. Metapex and Vaseline were found to be non inhibitory

Key words: *antimicrobial, root canal, primary teeth, zinc oxide, calcium hydroxide, Camphorated phenol, Calcium hydroxide, Iodoform*

J Clin Pediatr Dent 31(3):195-199, 2007

INTRODUCTION

Maintenance of the integrity of the primary dentition until the normal exfoliation period is vital for proper development and maturation of the child, growth of the facioskeletal complex to its full potential, to its normal occlusion and with good esthetic qualities.[1] The teeth with pulpal and periapical problems, specifically the primary teeth, should be retained until their normal exfoliation because their premature loss may lead to undesirable aberrations in the future dentition.

Root canals harbor different types of microorganisms and provide an ideal culture media for bacterial growth. The micro flora of the root canal has been studied over many years. Tomie-Karovie and Jelinek[2], Edward and Nord[3] demonstrated that root canal infections of primary teeth are usually polymicrobial in nature. With the advent of modern technology, now it is known that the predominant pathogens in the root canal are obligate anaerobes. The next predominant group being the facultative anaerobes followed by the aerobes. The complexity of the root canal configuration in the primary molars prevents thorough debridement through mechanical instrumentation.

Tronstad[4] stated that success of endodontic therapy depends on the reduction or elimination of the bacteria from the root canals. Among the ways of reducing or eliminating bacteria are: Adequate root canal debridment, antimicrobial irrigants and antibacterial filling materials

According to Grossman[5] an ideal root canal filling material should be bactericidal at least not encourage bacterial growth. In search of an ideal obturating material for a primary tooth, over the years, a number of materials were tried. The antimicrobial properties of these filling materials used in primary teeth contribute significantly to the clinical success of endodontic therapy by inhibiting residual bacteria not removed by mechanical debridement.[6]

The aim of this study is to evaluate the antimicrobial efficacy of five root canal filling materials viz., Zinc oxide and eugenol, Zinc oxide-eugenol with formocresol, Zinc oxide with camphorated phenol, Calcium hydroxide with sterile water, and Calcium hydroxide with Iodoform (Metapex) against the microorganisms isolated from

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infected primary teeth.

MATERIALS AND METHODS

Microbial specimens were obtained from 20 infected primary molar teeth with pulp pathology and with abscesses or sinus tracts from the pediatric patients who had attended the Department of Pedodontics. Selection was done at random, irrespective of the cause of pulpal or periapical pathology in patients ranging from 4-8 years of age. Teeth employed in the study met the following criteria.[7]

1. Contained at least one necrotic canal.
2. An abscess, sinus tract or obvious interradicular radiolucency present.
3. Antibiotics not received by the patient 4 weeks prior to sampling
4. Did not have resorbing roots or broken crowns

Specimen collection/ Inoculation/ Isolation/ Identification

The entire procedure was carried out under strict aseptic conditions. No endodontic procedure was performed before collection of sample, so as to avoid disturbing the root canal flora. The involved tooth was isolated with rubber dam and field of operation (exposed tooth, clamp & rubber dam) was disinfected with two applications of 30% hydrogen peroxide followed by 5% tincture of iodine.[8] The tooth and the operating field were re-disinfected after caries removal. Sterile paper points were inserted into the accessible root canals and left over for 60 sec. The paper point was immediately rolled on a plate of brucella blood agar enriched with hemin (5mg/ml) and mendione (1mg/ml). This plate was placed in an anaerobic Gen Bag (Biomérieux marcy-1 Étoile/France) and examined after 48hrs of incubation.

Multiple paper points were also inoculated into thioglycollate broth supplemented with vitamin k and hemin (Fig-2). After two hours of incubation aerobic subculture was done on 5% sheep blood agar, chocolate agar, Mueller-Hinton agar and MacConkey agar and incubated at 37ºc in an atmosphere containing 5% Co^2 for 48 hrs.

Thioglycollate broth was further incubated for 7 days to provide a backup source of material when there was no growth on agar plates. The aerobic and anaerobic isolates obtained were identified based on their morphology in a gram stain smear, colony characteristics and species was identified using Mini API system (Biomérieux, Analytical Profile Index, France).

Test Filling Materials

5 test filling materials and one control material were tested in this study.

1. Zinc oxide mixed with eugenol (ZOE) (DPI, Mumbai)
2. Zinc oxide eugenol mixed with Formocresol (ZOE + FC) (DPI, Mumbai ; Vishal Pharma, Ahmedabad)
3. Zinc oxide mixed with Camphorated Phenol (ZO + CP) (DPI, Mumbai ; Vishal Pharma, Ahmedabad)
4. Calcium hydroxide mixed with sterile water (CAOH + H2O) (Deepti Dental Products, Ratnagiri)
5. Metapex (CAOH + Iodoform) (Meta Dental Co. Korea)
6. Vaseline (Control)

The powder and liquid ratio of all test filling materials were standardized according to the formula given by Tchaou *et al*[7] and are summarized in Table 1. An electronic balance and a digital micropipette were used to measure the exact amount of powder and liquid to be dispensed. The filling materials were spatulated on a dry pre sterilized glass slab using a cement spatula at room temperature, just before it is assayed for Agar diffusion assay. The mixture is back loaded into a sterile 2ml syringe and kept ready.

Agar Diffusion Assay

Sensitivity testing was done by the standard Agar diffusion method.[9] Actively growing broth cultures of the organisms with turbidity adjusted to 0.5 McFarland standard were used. The media used for broth cultures were Brain Heart Infusion broth (BHI) for aerobes and thioglycollate broth for anaerobes. Using a sterile swab the entire surface of the agar plate was swabbed 3 times to ensure even distribution of the inoculum. Mueller-Hinton agar was used for aerobes and Brucella blood agar supplemented with vitamin k and hemin for anaerobic organisms.

3 filling materials were tested in each plate. After the agar plates were dried, 3 wells of 4 mm diameter and 3 mm deep were made in the agar plates using sterile agar puncher and filled with the test materials. The diameter of the zones of inhibition in millimeter around the filling materials was measured after 16-24 hrs for aerobic isolates and after 48 hrs for anaerobic isolates. The experiment was repeated thrice for each isolate and two observers measured zones independently. Mean zone of inhibition for each filling material and bacterial isolate combination was then calculated from 6 measurements.

RESULTS

All the 20 microbial samples obtained from infected primary teeth demonstrated polymicrobial infection. Only those strains which can be isolated purely were included in the study. A total number of 26

1	**ZOE**	Zincoxide 1 scoop 0.2 g	Eugenol 7 drops 0.07 c.c	
2	**ZOE + FC**	Zincoxide 1 scoop 0.2 g	Eugenol 6 drops 0.06 c.c	Formocresol 2 drops 0.02 c.c
3	**ZO + CP**	Zincoxide 1 scoop 0.2 g	Camphorated phenol 8 drops 0.08 c.c	
4	**CAOH + Water**	Calcium hydroxide 1 scoop 0.17 g	Sterile water 10 drops 0.1 c.c	
5	**Metapex**	Commercial product		
6	**Vaseline**	Commercial product		

TABLE 1: Powder and liquid ratio's of test filling materials

Group - 1 : Facultative / Aerobic gram-positive

1. Streptococcus angiousus
2. Streptococcus salivarius
3. Streptococcus sangius (strain-1) (s1)
4. Streptococcus sangius (strain-2) (s2)
5. Streptococcus mitis (strain-1) (s1)
6. Streptococcus mitis (strain-2) (s2)
7. Streptococcus oralis (strain-1) (s1)
8. Streptococcus oralis (strain-2) (s2)
9. Streptococcus oralis (strain-3) (s3)
10. Streptococcus oralis (strain-4) (s4)
11. Streptococcus oralis (strain-5) (s5)
12. Streptococcus oralis (strain-6) (s6)
13. Staphylococcus aureus
14. Enterococcus faecalis
15. Enterococcus hirae
16. Gamella morbillorum
17. Leuconostoc spp (strain-1) (s1)
18. Leuconostoc spp (strain-2) (s2)

Group - 2 : Facultative / Aerobic gram-negative

19. E. coli (strain-1) (s1)
20. E. coli (strain-2) (s2)
21. Pseudomonas aeurginosa
22. Pantoea spp

Group - 3: Anaerobic gram-negative

23. Peptostreptococcus micros (strain-1) (s1)
24. Peptostreptococcus micros (strain-2) (s2)

Group - 4: Anaerobic gram-negative

25. Prevotella oralis (strain-1) (s1)
26. Prevotella oralis (strain-2) (s2)

TABLE 2: Grouping of bacterial isolates

pure bacterial strains [23 isolated from primary teeth, 2 strains of E.coli and 1 strain of *Pseudomonas aeurginosa* (Non-standard strains obtained from Dept. of Microbiology)] were employed in the experimental procedure. The isolated bacterial strains were divided into 4 groups based on aerobic or anaerobic, gram positive or gram negative and are shown in Table 2. The mean zone of inhibition of 6 test filling materials against 26 bacterial isolates is shown in Table 3. Measurements of inhibitory zones were ranked arbitrarily into the following four categories according to the proportional distribution of the data set.[7] 1. No inhibition (No) 2. Weak inhibition (W) 3. Medium inhibition (M) 4. Strong inhibition (S)

Rank	Range of zone diameters (mm)	% of data set represented *	Frequency (N=156)
NO	0	42.2	67
WEAK	0.1 - 11.5	17.9	28
MEDIUM	11.5 - 19.7	19.2	30
STRONG	> 19.7	19.9	31

TABLE 4: Ranking scheme for microbial inhibition

Zone size categories and proportions of data represented in each category are represented in Table 4. The inhibition results of 6 test filling materials against 26 bacterial strains according to the ranking scale are represented in Table 5.

Statistical Analysis

Statistical analysis was carried out by one-way ANOVA using software SPSS version 10.0 with post-hoc tests to compare the statistical difference of antimicrobial effects between materials tested with each of the four bacterial groupings (Aerobic gram-positive, Aerobic gram-negative, Anaerobic gram-positive, Anaerobic gram-negative). A semi qualitative comparison was made with the understanding that the results could not be completely transferable to the in vivo situation as there was difference of diffusion in the agar media for various materials.

Statistically significant grouping of inhibitory effects of materials against the categories of microbial species i.e. aerobic gram positive, aerobic gram negative, anaerobic gram positive, anaerobic gram negative revealed the following

1. **Group-I** (Aerobic Gram positive): ZOE + FC was most inhibitory, ZO + CP, ZOE, CAOH + H2O were less inhibitory, Metapex and Vaseline were non-inhibitory
2. **Group-II** (Aerobic Gram negative): ZOE + FC was most inhibitory, ZO + CP, ZOE, CAOH + H2O were less inhibitory, Metapex and Vaseline were non-inhibitory
3. **Group-III** (Anaerobic Gram positive): ZOE + FC, ZO + CP were most inhibitory, Metapex and ZOE were less inhibitory, CAOH + H2O and Vaseline were non-inhibitory
4. **Group-IV** (Anaerobic Gram negative): ZOE + FC, ZO + CP, ZOE were most inhibitory, CAOH + H2O, Metapex and Vaseline were non-inhibitory

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
ZOE	12.5	14.5	11.9	10.3	10.6	6.6	10	11.8	12.1	0	15.5	10	16	0	0	25	0	10.7	18.7	17.8	0	13	14	18.6	20.5	28
ZOE + FC	32.6	13.8	10.5	27.3	0	27.5	30.1	31.6	29.3	29.5	37.5	29.1	31.5	22.3	28.5	30	0	26	21.5	20.3	17.1	21.8	30.5	24	27.8	31.3
CAOH + Water	11.1	11.3	11.5	10.5	11.5	9.6	11	12	10.3	12.1	13	11	11	0	0	0	10.6	10	0	7	10.6	6.6	0	0	0	0
ZO + CP	11	5.5	11.1	14	0	11.6	12	13.5	12.3	12.6	13.8	11.6	12	0	13.2	12	0	12.1	11.3	10	23	13.8	21.5	20.3	25.8	26
Metapex	0	0	0	0	0	0	0	0	0	0	0	0	40.6	0	0	0	4.3	0	0	0	0	0	0	23.6	0	0
Vaseline	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 3: Zones of inhibition (mm) of 6 test filling materials against 26 microorganisms
[1-26: Microorganisms according to the list given in Table 2]

	ZOE	ZOE+FC	CAOH+H2O	ZO+CP	Metapex	Vaseline
1. Streptococcus angiousus	M	S	W	M	No	No
2. Streptococcus salivarius	M	M	W	W	No	No
3. Streptococcus sangius (strain-1) (s1	M	W	W	W	No	No
4. Streptococcus sangius (strain-2) (s2	W	S	W	M	No	No
5. Streptococcus mitis (strain-1) (s1)	W	No	W	No	No	No
6. Streptococcus mitis (strain-2) (s2)	W	S	W	M	No	No
7. Streptococcus oralis (strain-1) (s1)	W	S	W	M	No	No
8. Streptococcus oralis (strain-2) (s2)	M	S	M	M	No	No
9. Streptococcus oralis (strain-3) (s3)	M	S	W	M	No	No
10. Streptococcus oralis (strain-4) (s4)	No	S	M	M	No	No
11. Streptococcus oralis (strain-5) (s5)	M	S	M	M	No	No
12. Streptococcus oralis (strain-6) (s6)	W	S	W	M	No	No
13. Staphylococcus aureus	M	S	W	M	S	No
14. Enterococcus faecalis	No	S	No	No	No	No
15. Enterococcus hirae	No	S	No	M	No	No
16. Gamella morbillorum	S	S	No	M	No	No
17. Leuconostoc spp (strain-1) (s1)	No	No	W	No	No	No
18. Leuconostoc spp (strain-2) (s2)	W	S	W	M	W	No
19. E. coli (strain-1) (s1)	M	S	No	W	No	No
20. E. coli (strain-2) (s2)	M	S	No	M	No	No
21. Pseudomonas aeurginosa	No	M	W	S	No	No
22. Pantoea spp	M	S	W	M	No	No
23. Peptostreptococcus micros (strain-1)	M	S	No	S	No	No
24. Peptostreptococccus micros (strain-2)	M	S	S	S	S	No
25. Prevotella oralis (strain-1) (s1)	S	S	No	S	No	No
26. Prevotella oralis (strain-2) (s2)	S	S	No	S	No	No

No-No inhibition S-Strong inhibition M-Medium inhibition W-Weak inhibition

TABLE 5: Inhibition results of 6 test filling materials against 26 microorganisms according to the ranking scale

DISCUSSION

In spite of many preventive measures available to reduce the incidence of caries, premature loss of pulpally involved primary teeth remains a common problem. Pulpectomy is one of the specialized forms of dental treatment designed to retain the primary tooth as a functional unit in the dental arch. The root morphology of the primary teeth does change continuously and a better understanding of the anatomy of primary roots before endodontic therapy is essential.

Various studies have shown the changes occurring in root canals of primary teeth by reproducing pulp canal system in various materials such as vulcanite rubber and acrylic resin.[10] Primary root resorption causes the position of the apical foramen to change continuously. Simultaneously secondary dentin is deposited within the pulp canal system. This deposition produces variations and alterations in the number and size of the root canals, as well as appearance of many small connecting branches or fins between the facial and lingual aspects of the canal. Continued deposition of dentin within the root will divide into separate canals, which complicates the endodontic therapy. Microorganisms and their end products are considered the major cause of pulp and periapical pathosis. The main objective of endodontic therapy is to eliminate microorganisms from the pulp canal system and prevent subsequent reinfection.

Even if several filling materials have been used thru the years,the most common ones are Zinc oxide eugenol, Iodoform and Calcium hydroxide. Generally after a good filing and irrigation, the final outcome of this depends on the quality of the root canal filling material, which can neutralize any remaining pulp tissue and microorganisms. However most authors had used a fixative at some stage of the pulpectomy procedure, either before filling the canals as an intracanal dressing or integrated into the filling material itself in the form of silver nitrate, formocresol or iodoform all of which have fixative properties. The filling material most commonly used for primary teeth has been ZOE either alone or with a fixative and the success rate ranges from 65-86%.[11,12] In this study ZOE exhibited weak inhibitory effect against Group-I test organisms, medium inhibitory effect against Group-II and Group-III and strong inhibitory effect against Group-IV test organism respectively. Broisman *et al*[13], Cox *et al*[1], Grossman[5], Pupo *et al*[14], Rahmat[15], Canalda and Pumarola[16] and Pumarola *et al*[17] agree that the sealers with a ZOE base are those that have greater inhibitory effect against the microorganisms found in root canals. The antimicrobial effect of ZOE is attributed to the

eugenol content of the material. Cox *et al*[1] demonstrated that zinc oxide powder had no inhibitory effect and the addition of eugenol to zinc oxide retarded the growth of only the gram-positive organisms. The inclusion of zinc acetate as a setting accelerator inhibited both gram-positive and gram-negative bacteria. They demonstrated that the inhibitory effects of ZOE could be greatly enhanced by the addition of formocresol or paraformaldehyde.

In the present study ZOE + FC exhibited strongest antimicrobial activity against most of the test organisms which is on par with Cox *et al*[1], Thomas *et al*[18], Pupo *et al*[14], Seow[19], Stuart *et al*[20] & Tchaou *et al*[21]. Formocresol has for the past five decades been the most widely used as a medicament for pulpotomy in primary teeth. Studies have shown it to have adequate fixative properties highly bactericidal in nature.[11,22,23] However much concern has arisen over the mutagenic and carcinogenic potential of formaldehyde containing products, the cytotoxic effect of formocresol and the possible diffusion of this substance into surrounding and systemic tissues. Animal studies have confirmed that formocresol does appear systemically after its use as a pulpotomy agent and that cell injury may occur in these systemic tissues.[24,25,26,27,28]

Paradoxically, Spanberg *et al*[29] stated that level of free formaldehyde is very low in human tissues due to its rapid metabolism and its half life in humans is approximately 1 hr. When considering the high rate of exposure and tolerance of mammals to formaldehyde, the added load of few milligrams of formaldehyde in a root canal sealer is negligible from a toxicological point of view. They further stated that the undesirable effect of formaldehyde in an endodontic sealer should not be discussed as a general toxicity problem, as low exposure to formaldehyde is rather insignificant.

CAOH+H2O exhibited weak antimicrobial activity against Group-I and Group-II test organisms but failed to inhibit anaerobic bacteria. Similar results were reported by Difiore *et al*[30], Abdulkader *et al*[31], Siqueria and Gonclaves[32] through their studies using agar diffusion assay. They demonstrated that CAOH associated with an inert substance (Distilled water, Saline, Glycerin) was ineffective against several obligatory and facultative anaerobic bacteria. The weak inhibitory effect of CAOH +H2O in the agar diffusion assay can be explained by the fact that blood or buffers present in the agar media might have neutralized CAOH, a phenomenon that may also occur in vivo where blood and buffering systems are present.[21,33,34]

Iodoform has long been advocated as an antiseptic in the treatment of pulpless teeth.[35] In 1928 Walkoff[36] introduced Iodoform as a root canal filling material in primary teeth. Iodoform proved to be a potent bactericidal, non-irritant radiopaque material.[37] Dominguez and Solano[38] reported that when combining pure Iodoform with calcium hydroxide powder, excellent results were obtained based on clinical, radiographic and histological evaluation.

In the studies done by Ninomiya *et al*[39], Tchaou *et al*[21] and Pabla *et al*[40], CAOH + Iodoform exhibited no antibacterial activity against most pure cultures in agar diffusion tests. In the present study Metapex was found to be ineffective against all microorganisms except *Leuconostoc spp, Staphylococcus aureus* and *Peptostreptococcus micros* (s2). The weak activity may be partially explained by the fact that CAOH an ingredient of Metapex has been demonstrated to interfere with the antiseptic capacity of dyadic combinations of endodontic medicaments.[19]

Most of the studies related to antimicrobial activity of the root canal filling materials had been done using standardized bacterial strains (ATTC-American Type Culture Collection). Very few studies were reported in the dental literature exclusively on bacterial strains isolated from infected primary teeth.[21,40,41] The mean zone of inhibition of a material/species combination in this study cannot be compared with previous studies because of the variability's in bacterial strains, culture media, culture conditions and powder & liquid ratio of the test filling materials. Based on the results of this study ZOE + FC was found to have superior antimicrobial activity against most of the test organisms, followed by ZOE, ZO + CP, CAOH +H2O in the descending order. Metapex and Vaseline showed minimal and no inhibition respectively.

CONCLUSION

The following conclusions were drawn from this study:

1. The root canal flora of infected primary molar teeth is polymicrobial in nature
2. All the test filling materials showed varied antimicrobial activity against the microorganisms tested
3. ZOE+FC combination was found to have superior antimicrobial activity against most of the microorganisms followed by ZOE, ZO+CP, CAOH+H2O in descending order.
4. Metapex (CAOH+Iodoform) and Vaseline showed minimal or no inhibition
5. Although the incorporation of formocresol in ZOE mixture produced significant inhibition against the test organisms, it should be clinically correlated with toxic effects produced by this medicament.

It is difficult to draw conclusions based on *in vitro* evaluation of antimicrobial activity with isolated bacteria. It is well known that endodontic infections are mixed with complex floral interactions. The effect of test filling materials against a single strain may not be effective against a mixed variety of infection. The use of artificial media also plays an important role in determining the experimental results. Even the principle utilized to determine the antimicrobial activity i.e. Agar diffusion assay has got its own limitations. It is possible that different results might have obtained if other methods of testing antimicrobial activity i.e. Agar dilution method, Direct contact test etc. were employed. Microbial interactions in the oral cavity should be considered before concluding the ideal test results. *In vivo* studies are required to state the specific antimicrobial activity and merits and demerits of any of the test filling material.

ACKNOWLEDGEMENTS

The author's express their deep sense of gratitude to Dr. Uma Bala. P, Assoc. Professor & Dr. V. Lakshmi, Additional Professor & Head, Dept. of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, INDIA, for extending all the facilities, sparing their valuable time & guiding at every step in the microbiological procedures. The author's also expressing their sincere thanks to Dr. D. Mahesh Kumar, Dr. P. Vinay Kumar, Dr. Garima Yeluri & Mrs. Sirisha for their help in this study & in preparing this manuscript.

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