RESEARCH ARTICLE

Evaluation of the Antibacterial Activity of Triclosan-incorporated Root Canal Filling Materials for Primary Teeth against *Enterococcus faecalis*

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Abstract

Aim and objective: To compare the antibacterial activity of root canal filling materials namely zinc oxide eugenol and Endoflas FS with or without the incorporation of Triclosan.

Materials and methods: The study consisted of four groups, with 15 samples in each group: group I (zinc oxide eugenol paste), group Ia (zinc oxide eugenol paste + 2.5% Triclosan), group II (Endoflas FS), and group IIa (Endoflas FS + 2.5% Triclosan). A double layer agar well diffusion test was used to evaluate the antibacterial activity against *Enterococcus faecalis*. The zones of microbial inhibition were measured at the end of 24 hours, 6th day, and 29th day.

Results: On intergroup comparison, the difference in the antibacterial activity was found to be highly significant ($p < 0.001$). Among the various groups evaluated, group IIa showed the highest antibacterial activity against *E. faecalis* followed by group II, group Ia, and the least activity being shown by group I throughout the experimental periods. On intragroup comparison at different time intervals, a maximum zone of inhibition was seen at 24 hours with a p value < 0.05 in all the tested groups.

Conclusion: Incorporation of 2.5% triclosan into zinc oxide eugenol and Endoflas FS enhanced the antimicrobial activity of both the root canal filling materials with lasting antimicrobial activity even at the end of the 29th day.

Clinical significance: The antimicrobial efficacy of a root canal filling material is an ideal requirement, which will help in combating the residual microflora present in the root canal system following chemomechanical preparation. The addition of an antimicrobial agent such as triclosan to the root canal filling materials, enhances their antimicrobial efficacy significantly and thus, rendering the pulpectomy-treated tooth with a better prognosis.

Keywords: Antimicrobial activity, Endoflas FS, *Enterococcus fecalis*, Triclosan, Zinc oxide eugenol.

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Introduction

Pediatric dentistry has been offering comprehensive care for children for ages. A pulpectomy is one of the specialized forms of dental treatment in pediatric dentistry rendered to the primary tooth to retain them, till the time they exfoliate.[1] The main objective of pulpectomy is the total elimination of microorganisms from the root canal and the prevention of subsequent reinfection. This is achieved when a good chemomechanical preparation of the root canal system is followed by impervious obturation with a suitable obturating material.[2]

Thus, among the various factors properties of the obturating material used also play a key role in determining the prognosis of the pulpectomy-treated tooth. Due to the inability in achieving complete debridement of primary root canals owing to their anatomic complexities, one of the requirements expected from root canal filling materials is antimicrobial property.[3] The antimicrobial property of the obturating materials help in eliminating the residual pathogens, which persist in the root canal even after cleaning and shaping while neutralizing their toxins, thereby minimizing the possibility of reinfection.[4] It is therefore important to study the antimicrobial spectrum of the root canal filling materials used in primary teeth.

Although zinc oxide-eugenol cement (ZOE) has been the commonest choice as a root canal filling material for deciduous teeth, concerns about its limited antimicrobial activity keep the search still on for a suitable alternative.[5] Endoflas FS is one of the recent root canal filling materials which is an iodoform-based resorbable paste. It contains components similar to that of Vitapex, with the addition of zinc oxide and eugenol. Endoflas FS can disinfect dentinal tubules and difficult-to-reach accessory canals that cannot be disinfected or cleaned mechanically.[2] However, the

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long-lasting antimicrobial activity of this root canal filling material over a period of time has not been proven.

On the other hand, triclosan is a broad-spectrum antimicrobial agent active against gram-positive and gram-negative bacteria as well as some fungi and viruses. Although it is most often used for antisepsis of the skin and other surfaces, incorporation of triclosan into the medical devices and dentifrices has well established its effective intraoral use too.[6] Aim of the present study was to comparatively evaluate the antibacterial activity of the root canal filling materials for deciduous teeth such as zinc oxide eugenol and Endoflas FS when incorporated with 2.5% triclosan. The null hypothesis for the study was set as there will not be any difference in the antibacterial activity of the root canal filling materials for deciduous teeth such as zinc oxide eugenol and Endoflas FS with or without 2.5% triclosan.

Materials and Methods

This study was an *in vitro* intergroup comparative study, which was initiated after obtaining ethical clearance from the Institutional Ethics Committee. It consisted of four groups: group I: zinc oxide and eugenol paste (SS White, Rio de Janeiro, RJ, Brazil), group Ia: zinc oxide + 2.5% Triclosan, group II: Endoflas-FS (Sanlor laboratories, Colombia), and group IIa: Endoflas-FS + 2.5% Triclosan.

The obtained sample size per group was 15 and to detect differences among the means at 0.05 significance level.

Preparation of the Experimental Root Canal Filling Materials

0.625 g of triclosan (Acuro Organics Ltd, New Delhi) was weighed in an electronic weighing machine and was mixed with 25 g of zinc oxide and 25 g of Endoflas FS separately. These powders were triturated in a glass mortar and pestle in increments to get a uniform smooth mixture that represented group Ia and group IIa.

Evaluation of the Antibacterial Efficacy

The antibacterial activity of the tested root canal filling materials was tested against *Enterococcus faecalis* (ATCC 35550). *Enterococcus faecalis* inoculum was adjusted to the turbidity of 0.5 McFarland standards. A double layer agar well diffusion assay[4] was used to evaluate the antibacterial potential. The growth media used was tryptone soya agar (TSA).[7]

In each culture plate, five standardized wells (10 × 4 mm) were punched using a sterile hollow tube device. Bacterial inoculation was made over the agar surfaces with 0.5 mL of the bacterial suspension. The tested root canal filling materials were freshly mixed according to the manufacturer's instructions to obtain a paste-like consistency, which were then placed into the respective wells on each plate (Fig. 1). Plates were then incubated in an incubator at 37°C for 24 hours. Following 24 hours, the diameters of the circular inhibition zones produced around the specimens were measured in millimeters using a digital Vernier caliper. The zones of inhibition produced were measured at three different points and the mean value was recorded as day 1 value (Fig. 2).[8]

After measurement of the inhibition zone, all samples were removed aseptically and rinsed with sterile deionized water to remove any attached bacteria. Each sample was then stored in sterilized deionized water until day 6. On the 6th day, new agar plates were prepared. Five standardized wells were punched into this new agar plate along with bacterial inoculation with 0.5 mL of the bacterial suspension. The specimens were taken out from the deionized water, placed into the new wells, and then incubated at 37°C for 24 hours. The inhibition zones around the specimens were measured and recorded in millimeters as day 7 value. After performing the measurements, each sample was removed and stored in the sterilized deionized water until day 29. The procedure was repeated with the fresh agar plates inoculated with microorganisms on the 29th day for obtaining inhibition zone dimension of day 30.[9]

Statistical Analysis

Repeated ANOVA was used for simultaneous multiple group comparison followed by *post hoc* Tukey's test for group-wise comparison.

Results

At the end of 24 hours, a statistically significant difference in the antibacterial property was observed between all the groups ($p < 0.001$). The highest zone of inhibition was observed in group IIa followed by group II and group Ia least being in group I (Table 1). Even at the end of the 6th day and 29th day, while a similar trend of antibacterial activity was seen ($p < 0.001$), pairwise comparison between group I and group Ia showed statistically no significant difference (Tables 2 and 3). Pairwise comparison also revealed

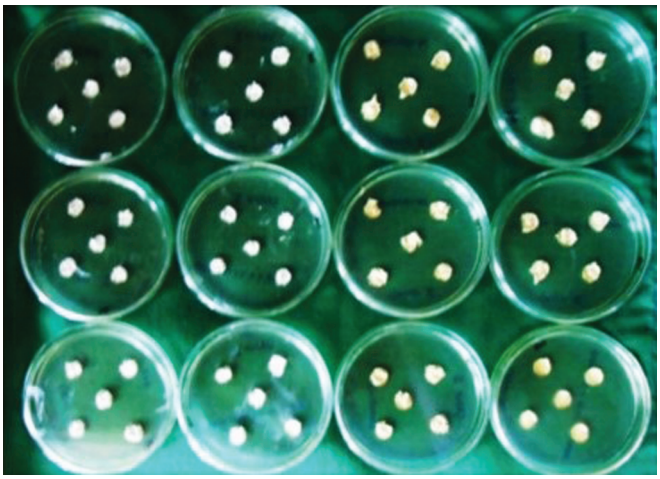

Fig. 1: Tryptone soya agar plates with all the experimental materials in the respective wells

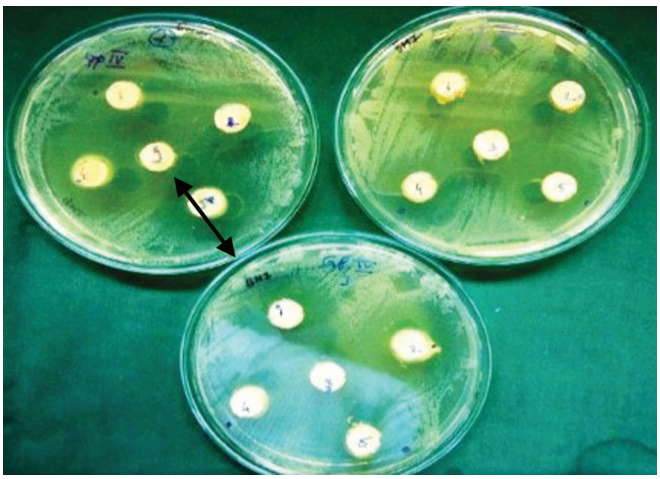

Fig. 2: Measurement of zone of inhibition in millimeters

Table 1: Descriptive statistics of intergroup comparison of the zone of inhibition (in mm) among different groups at the end of 24 hours (1st-day value)

	24 hours				
Groups	*Mean*	*SD*	*F*	*p*	*Group-wise comparisons^ <0.001*
Group I	16.13	1.60	117.19	0.00**	I vs Ia (0.00)**
Group Ia	21.47	1.77			I vs II (0.00)**
Group II	24.67	2.44			I vs IIa (0.00)**
Group IIa	31.67	3.16			Ia vs II (0.002)**
					Ia vs IIa (0.00)**
					II vs IIa (0.00)**

^*Post hoc* Tukey's test, *$p < 0.05$ Significant, **$p < 0.01$ Highly significant

Table 2: Descriptive statistics of intergroup comparison of the zone of inhibition (in mm) among different groups at the end of the 6th day (7th-day value)

	7th day				
Groups	*Mean*	*SD*	*F*	*P*	*Group-wise comparisons^ <0.001*
Group I	14.4	1.64	94.55	0.00**	I vs Ia (0.07)
Group Ia	16.53	1.36			I vs II (0.00)**
Group II	20.93	2.28			I vs IIa (0.00)**
Group IIa	27.60	3.44			Ia vs II (0.00)**
					Ia vs IIa (0.00)**
					II vs IIa (0.00)**

^*Post hoc* Tukey's test, *$p < 0.05$ Significant, **$p < 0.01$ Highly significant

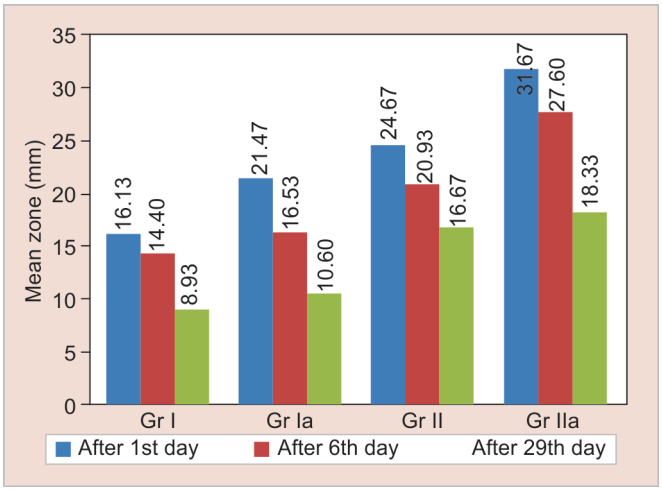

Fig. 3: Variations in zone of inhibition (in mm) at different time intervals

statistically no significant difference between group II and group IIa at the end of 29th day.

When intragroup comparison was done at different time intervals using repeated measures of ANOVA, the zones of inhibition produced after 24 hours, 6th day, and 29th day were in the decreasing order. This difference seen between various time intervals was statistically significant for all the groups ($p \leq 0.001$) except for group I. Group I showed no significant difference statistically when the antimicrobial activities shown after 24 hours and at the end of the 6th day were compared ($p = 0/06$) (Fig. 3).

Discussion

While the literature has shown that periapical lesions heal at a higher rate in teeth with negative root canal bacterial cultures obtained at the time of canal filling in comparison with those with positive cultures,[10] it is also been concluded that the part of the root canal space often remains untouched during chemomechanical preparation regardless of the technique and instruments employed.[11] Love and Jenkinson,[12] Molander et al.,[13] Sundqvist and Figdor[14] reported the presence of microorganisms in areas such as isthmus, ramifications, deltas, irregularities, and dentinal tubules even after thorough chemomechanical debridement of the root canal system.

Hence, obtaining a hermetic seal of the root canals with a root canal filling material that possesses an excellent antimicrobial property is critical for endodontic success. Thus, the present study was conducted to evaluate if there is an enhanced antimicrobial activity when a broad-spectrum antimicrobial agent namely triclosan was incorporated into zinc oxide eugenol and Endoflas FS.

It is advised to test the dental materials immediately after mixing, once the final chemical setting stage has been reached. This is because of the formation of various temporary or permanent by-products during the setting reaction which may influence the original results.[15] Thus in the present study, the freshly prepared root canal filling materials were placed into agar plates. The agar diffusion method was used in our study to evaluate the antibacterial efficacy as it has been widely used to test the antimicrobial activity of dental materials and medicaments. The advantages of this method include simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results. Moreover, it demonstrates a good clinical correlation.[16] However, this procedure is influenced by two factors: the material's microbial toxicity as well as the materials affinity and diffusibility in the culture medium. A material that easily diffuses will produce larger zones of inhibition of bacteria.[15]

The results of the present study showed Endoflas FS has better antimicrobial properties when compared with zinc oxide

Table 3: Descriptive statistics of intergroup comparison of the zone of inhibition (in mm) among different groups at the end of the 29th day (30th-day value)

	7th day				
Groups	*Mean*	*SD*	*F*	*p*	*Group-wise comparisons^ <0.001*
Group I	8.93	2.60	49.33	0.00**	I vs Ia (0.28)
Group Ia	10.60	3.23			I vs II (0.00)**
Group II	16.67	2.02			I vs IIa (0.00)**
Group IIa	18.33	2.02			Ia vs II (0.00)**
					Ia vs IIa (0.00)**
					II vs IIa (0.28)**

^*Post hoc* Tukey's test, *$p < 0.05$ Significant, **$p < 0.01$ Highly significant

eugenol. This is in accordance with a study done by Kaiwar et al.[17] and Navit et al.[18] who attributed the superior antibacterial efficacy of Endoflas to the presence of both Eugenol and Iodoform in its composition. Iodoform acts by the liberation of iodine. It is believed that iodine, which is an oxidizing agent, can irreversibly oxidize and thus, inactivate essential metabolic compounds like proteins, nucleotides, and fatty acids resulting in cell death.[18] Eugenol, a phenolic compound acts on microorganisms by protein denaturation whereby the protein becomes non-functional.[17] However, the *in vitro* study by Kothari and Langalia[19] concluded that there is no significant difference in the antimicrobial activity between zinc oxide eugenol and Endoflas FS on *E. faecalis*.

Another important finding of the present study 2.5% triclosan incorporated Endoflas (group IIa) and zinc oxide eugenol (group 1a) showed higher antimicrobial activity compared with their plain counterparts (group II and group I, respectively). The highest antibacterial activity was shown by Triclosan incorporated Endoflas, the lowest being shown by plain zinc oxide eugenol.

Triclosan is a broad-spectrum antimicrobial agent active against gram-positive and gram-negative bacteria as well as some fungi and viruses. Keeping this in mind, Nudera et al.[6] conducted a study to evaluate, the antimicrobial effect of triclosan and triclosan with Gantrez on five common endodontic pathogens and concluded triclosan with or without the addition of the copolymer Gantrez S-97 might emerge as a valuable antimicrobial agent for use in endodontic treatment. In a pilot study conducted by Sainulabdeen et al.[9] evaluating the antibacterial activity of glass ionomer cement incorporated with different concentrations (0.5, 1.25, and 2.5%) of a non-releasing bactericide triclosan against two common cariogenic bacteria—*Lactobacillus acidophilus* and *Streptococcus mutans*, triclosan at a concentration of 2.5% was more effective than at lower concentrations. So, in our study, triclosan at a concentration of 2.5% was incorporated into the experimental group.

In the present study, the antimicrobial activity of all the tested root canal filling materials gradually decreased with time, the highest being at the end of 24 hours and the lowest being at the end of the 29th day. The endodontic sealers have shown to give the greatest antimicrobial effects immediately after spatulation, following which there is a gradual loss in antibacterial effect over time which can be supported with the results obtained by Cobankara et al.,[20] Fuss et al.,[21] and Kaplan et al.[22]

Based on the results of the present study, we recommend the use of 2.5% triclosan with zinc oxide eugenol or Endoflas as root canal filling materials in highly infected primary teeth requiring pulpectomy to improve the success of the endodontic therapy. Further studies evaluating the same under *in vivo* conditions may help in substantiating the obtained results of this study.

Conclusion

The following conclusions can be drawn from the present study:

- The root canal filling pastes had different inhibitory effects on *E. faecalis* which gradually decreased with time.
- The incorporation of 2.5% triclosan significantly enhanced the antimicrobial activity of zinc oxide eugenol and Endoflas.

Clinical Significance

The antimicrobial efficacy of a root canal filling material is an ideal requirement, which will help in combating the residual microflora present in the root canal system following chemomechanical preparation. The addition of an antimicrobial agent such as triclosan to the root canal filling materials, enhances their antimicrobial efficacy significantly and thus, rendering the pulpectomy-treated tooth with a better prognosis.

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