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 Ia (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 Ia 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 faecalis*, Triclosan, Zinc oxide eugenol.

*International Journal of Clinical Pediatric Dentistry* (2021): 10.5005/jp-journals-10005-1960

**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.³ 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.³ 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. 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 was used to evaluate the antibacterial potential. The growth media used was tryptone soya agar (TSA). 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).

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.

**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...
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.
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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)

| 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 Ila (0.00)***            |
| Group Iia    | 18.33 | 2.02  |          |        | Ia vs II (0.00)**             |
|              |       |       |          |        | Ia vs Ila (0.00)**            |
|              |       |       |          |        | II vs Ila (0.28)**            |

*Post hoc Tukey’s test, **p < 0.05 Significant, ***p < 0.01 Highly significant

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.

REFERENCES

1. Reddy S, Ramakrishna Y. Evaluation of antimicrobial efficacy of various root canal filling materials used in primary teeth: a microbiological study. J Clin Pediatr Dent 2007;31(3):193–198. DOI: 10.17796/jcpd.31.3173.14061424(2578).
2. Praveen P, Anantharaj A, Karthik V, et al. A review of the obturating material for primary teeth. SRM Univers J Dent Sci 2011;2(1):42–44.
3. Chawla HS, Setia S, Gupta N, et al. Evaluation of a mixture of zinc oxide, calcium hydroxide, and sodium fluoride as a new root canal filling material for primary teeth. J Indian Soc Pedod Prev Dent 2008;26(2):53–58. DOI: 10.4103/0970-4388.41616.
4. Queiroz AM, Nelson-Filho P, Silva LA, et al. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex and EndoREZ. Braz Dent J 2009;20(4):290–296. DOI: 10.1590/S0103-64402009000400005.
5. Barja-Fidalgo F, Moutinho-Ribeiro M, Oliveira MA, et al. A systematic review of root canal filling materials for deciduous teeth: is there an alternative for zinc oxide–eugenol? ISRN Dent 2011;2011:367318. DOI: 10.5402/2011/367318.
6. Nudera WJ, Fayad MI, Johnson BR, et al. Antimicrobial effect of triclosan and triclosan with Gantrez on five common endodontic pathogens. J Endod 2007;33(10):1239–1242. DOI: 10.1016/j.joen.2007.06.009.
7. Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. ASDC J Dent Child 1987;54(1):30–34.
8. Kriplani R, Thosar N, Baliga MS, et al. Comparative evaluation of antimicrobial efficacy of various root canal filling materials along with aloe vera used in primary teeth: a microbiological study. J Clin Pediatr Dent 2013;37(3):257–262. DOI: 10.17796/jcpd.37.3.j62u53q230048x5.

9. Sainulabdeen S, Neelakantan P, Ramesh S, et al. Antibacterial activity of triclosan incorporated glass ionomer cements—an in vitro pilot study. J Clin Pediatr Dent 2010;35(2):157–161. DOI: 10.17796/jcpd.35.2.9674752725Sn608x.

10. Jha M, Atil SD, Sevekar S, et al. Pediatric obturating materials and techniques: A review. J Contemp Dentis 2011;1(2):27–32. DOI: 10.5005/jcd-1-2-27.

11. Lin LM, Pascon EA, Skribner J, et al. Clinical, radiographic, and histologic study of endodontic treatment failures. Oral Surg Oral Med Oral Pathol 1991;71(5):603–611. DOI: 10.1016/0030-4220(91)90371-i.

12. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002;13(2):171–183. DOI: 10.1177/154411130201300207.

13. Molander A, Reit C, Dahlén G. The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. Endod Dent Traumatol 1999;15(5):205–209. DOI: 10.1111/j.1600-9657.1999.tb00775.x.

14. Sundqvist G, Figdor D. Life as an endodontic pathogen. Ecological differences between the untreated and root-filled root canals. Endodontic Topics 2003;6(1):3–28. DOI: 10.1111/j.1601-1546.2003.00054.x.

15. Shantiaee Y, Dianat O, Janani A, et al. In vitro evaluation of the antibacterial activity of three root canal sealers. Iran Endod J 2010;5(1):1–5.

16. Balouiri M, Sadiki M, Ibnoussa SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 2016;6(2):71–79. DOI: 10.1016/j.jpha.2015.11.005.

17. Kaiwar A, Nadig G, Hegde J, et al. Assessment of antimicrobial activity of endodontic sealers on Enterococcus faecalis: an in vitro study. World J of Dent 2012;3(1):26–31. DOI: 10.5005/jp-journals-10015-1123.

18. Navit S, Jaiswal N, Khan SA, et al. Antimicrobial efficacy of contemporary obturating materials used in primary teeth - an in-vitro study. J Clin Diagn Res 2016;10(9):ZCD9–ZC12. DOI: 10.7860/JCDR/2016/21883.8426.

19. Kothari A, Langalia A. Comparative evaluation of the antimicrobial activity of different endodontic sealers on Enterococcus faecalis – an in vitro study. NJIRM 2013;4(3):121–127.

20. Cobankara FK, Altinöz HC, Ergani O, et al. In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod 2004;30(1):57–60. DOI: 10.1097/00004770-200401000-00013.

21. Fuss Z, Charniaque O, Pilo R, et al. Effect of various mixing ratios on antibacterial properties and hardness of endodontic sealers. J Endod 2000;26(9):519–522. DOI: 10.1097/00004770-200009000-00008.

22. Kaplan AE, Picca M, Gonzalez MI, et al. Antimicrobial effect of six endodontic sealers: an in vitro evaluation. Endod Dent Traumatol 1999;15(1):42–45. DOI: 10.1111/j.1600-9657.1999.tb00748.x.