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Original article

Comparison of the antibacterial effect of experimental primary tooth canal pastes containing octenidine dihydrochloride and calcium hydroxide: An in vitro study

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ABSTRACT

Background: *Octenidine dihydrochloride (OCT) has been used as a canal irrigation agent. However, no research has been done on its use as a primary root canal paste mixed with calcium hydroxide in primary teeth.* ***Purpose:*** *This study aims to determine the in vitro antibacterial effect of an experimental canal sealer prepared using OCT and calcium hydroxide (CaOH) on primary root canal pathogens and compare them to zinc oxide eugenol (ZOE) and iodoform paste (IP).* ***Methods:*** *Staphylococcus aureus (S. aureus), Streprococcus mutans (S. mutans) and Enteroccus faecalis (E. faecalis) were used as microorganisms to determine their antibacterial effect. A disc diffusion test was applied to the strains of impregnated discs with canal sealer. The number of bacteria was standardized and incubated into the Mueller–Hinton growth medium. At the end of the incubation period, the inhibition zones around the discs were measured in millimeters and recorded.* ***Results:*** *When the diameters of the inhibition zones were compared, the experimental canal paste obtained by mixing OCT and CaOH at a ratio of 2:1 was found to have the highest antibacterial effect against S. aureus, S. mutans and E. faecalis, and the primary tooth canal paste containing iodoform, which is used routinely in clinical practice, had the lowest antibacterial effect.* ***Conclusion:*** *As a result of this study, the antibacterial effect of experimental canal sealer containing OCT–CaOH on S. mutans, S. aureus and E. faecalis was found to be considerably higher than the root canal sealer containing eugenol and iodoform.*

Keywords: *calcium hydroxide; octenidine dihydrochloride; root canal treatment; zinc oxide eugenol*
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INTRODUCTION

Primary dentition plays a role of utmost importance in a child's growth and development.[1] One of the most important goals of pediatric dentistry is to ensure that primary teeth are kept in a healthy and functional way until the time of physiological exfoliation. Early loss of primary teeth can cause malocclusion, as well as functional and aesthetic problems. Even if space maintainers made after the early loss of primary teeth are beneficial, they cannot perform as functional teeth. In addition, with the development of the child, the space maintainers need to be renewed at certain intervals.[2] The best way to avoid these problems is to preserve the primary teeth in the arch until the exfoliation is attained at the normal time.[3]

Root canal treatment is recommended to prevent premature loss of primary teeth from necrosis or severe chronic infection, including the root pulp.[4] A wide variety of bacteria causes infection in the root canals of primary teeth. There are many different bacteria in different stages of infection in root canals and in failed canal treatments.[5] The success of endodontic treatment depends on the number of infection-causing microorganisms that can be eradicated from the root canal. The reduction in the number of such microorganisms can be achieved by root canal preparation, irrigation, administration of sterilizing medication, and filling material.[6] Bacterial elimination is provided by mechanical debridement, antibacterial irrigation agents, and root canal filling materials. Bacterial elimination in primary teeth cannot be fully achieved by

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mechanical debridement alone. The main principle of root canal cleaning is that the preparation process should reach and clean the entire surface of the root canal walls.[7] Therefore, the success of endodontic treatment depends on the antibacterial properties of irrigation agents and root canal filling materials used.[8]

Important factors that affect the success of root canal treatment in primary teeth depend on the characteristics of canal filling materials.[9] The ideal root canal filling materials should resorb at a similar rate as the root, have antiseptic properties appear as radiopaque on radiography, not shrink, fill the root canals easily, and be easily removed if necessary. It should not cause any irritation in the permanent tooth germ, inflammation in the tooth surrounding tissues and tooth discoloration.

However, no material used by clinicians today can fully meet all these features. CaOH, ZOE, and IP are materials that have been used for root canal treatment in primary teeth for a long time. Due to some disadvantages in these materials, the search for ideal alternative materials continues.[10]

OCT is a bispyridine derivative developed by the Sterling-Winthrop Research Institute.[11] Owing to its biocompatibility with the mucosa, high antibacterial effect, and lack of side effects, OCT has a wide range of applications, such as the treatment of nail infections and acne, wound antisepsis, and biofilm elimination.[12] In addition, OCT has a wide range of uses in the oral cavity. Recently, OCT has been used as an irrigation agent. Tirali et al.[13] found that OCT at 50% and 100% concentrations showed a superior antimicrobial effect against *Enterococcus faecalis (E. faecalis)* bacteria compared with 5.25% sodium hypochlorite. The antibacterial effect was studied by using *S. aureus* (ATCC 25923), *S. mutans* (ATCC 25175), and *E. faecalis* (ATCC 29212) bacterial strains. These microorganisms are found at high rates in microbiological examinations of root canal infections in primary teeth.[14] To the best of our knowledge, no in vitro studies have compared the antibacterial effects of primary root canal materials (ZOE, IP, and OCT). The aim of this study was to determine the in vitro effects of an experimental canal sealer prepared using OCT and CaOH on primary root canal pathogens and to compare them with the effects of sealers with ZOE and IP.

MATERIALS AND METHODS

In this study, primary tooth canal paste containing IP(Diapex®Plus, DiaDent Group International, Korea) and ZOE (Cavex, Holland), and experimental primary tooth canal paste mixes containing OCT (Octenisan®, Schülke, Germany)-CaOH were used to determine the antimicrobial effect. The experimental pastes had different consistencies due to the difference in powder–liquid ratio. Consistency is required for the resulting experimental canal pastes to be used in injector systems, with hydroxyethyl cellulose as enhancer and dibutyl phthalate as thickener. Experimental canal pastes were prepared by mixing liquid OCT (Octenisan®, Schülke, Germany) and powdered CaOH in the ratio of 1:2, 2:3, 1:1, 3:2 and 2:1 by mass.

After the standard bacterial strains were reproduced in blood agar, the bacterial density was adjusted using the 0.5 McFarland Equivalence Turbidity Standard (bioMerieux® SA, Marcy l'Etoile, France; Figure 1), which corresponds to 1.5 x 108 CFU/mL of bacterial colony in the turbidity standard bacterial suspension and indicates the highest density.[15] For the disk diffusion test, disks with a diameter of 6 mm and a thickness of 0.16 mm produced from filter paper were sterilized and then impregnated equally with the prepared root canal pastes.

The Mueller–Hinton broth (RTA, Kocaeli, Turkey) used in the test was prepared with 38 g of dehydrated medium dissolved in 1 liter of water. After sterilization at 121ºC for 15 minutes, the medium was transferred to petri dishes with a thickness of 4 ± 0.5 mm. *S. aureus* (ATCC 25923),

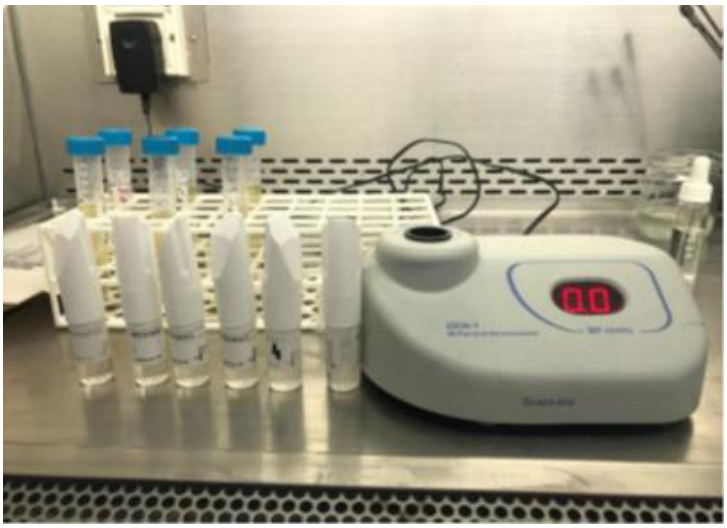

Figure 1. McFarland equivalence turbidity.

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S. mutans (ATCC 25175), and *E. faecalis* (ATCC 29212) suspensions, whose densities were equally adjusted, were cultured on the prepared Mueller–Hinton broth using sterile cotton-tipped swabs. The petri dishes were divided into three equal regions, and the disks belonging to the study groups were placed in the centers of these regions (Figure 2). The broth was allowed to incubate for 24 hours at 37°C. A similar study was taken as a reference when determining the study method.[16]

At the end of the incubation period, the largest diameter of the inhibition zones around the disks was measured in millimeters. The test was repeated three times for each group, and the arithmetic averages of the measurements were calculated. One-way ANOVA was performed to determine whether the canal pastes used had statistically significant differences in antimicrobial effect. When a significant difference was found, Tukey's test was used to statistically identify the groups that caused the difference.

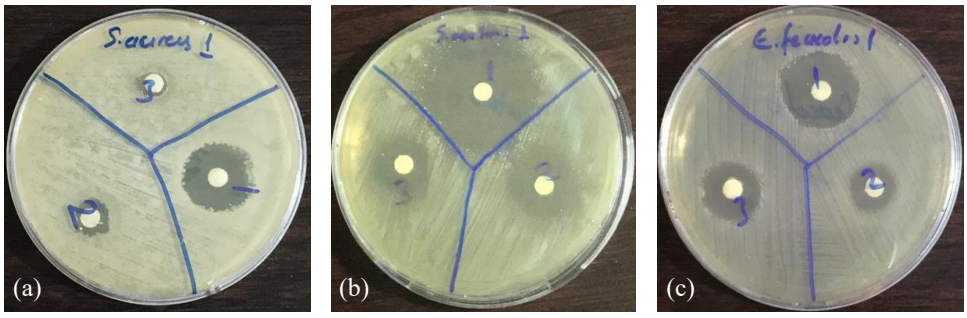

Figure 2. Inhibition zones created by primary tooth canal pastes for (a) *S. aureus;* (b) *S. mutans;* (c) *E. faecalis;* 1:OCT/CaOH=2/1; 2:OCT/CaOH=2/3; 3:OCT/CaOH=1/2.

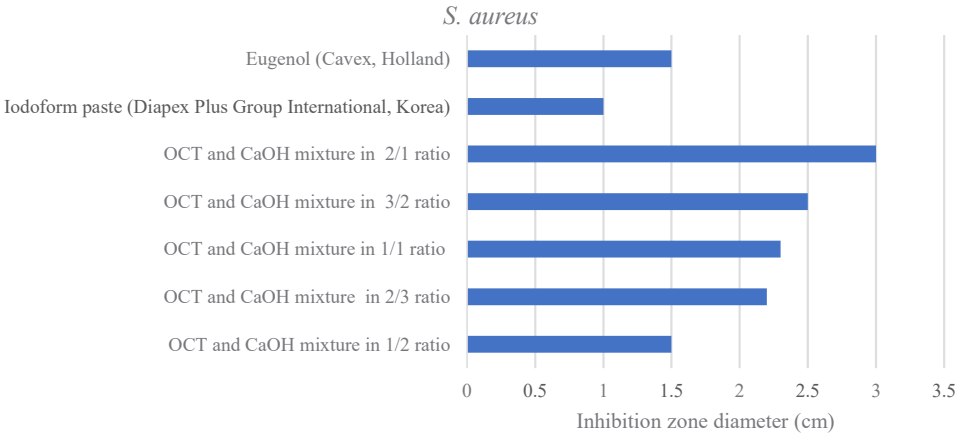

Figure 3. Antibacterial effect against *S. aureus.*

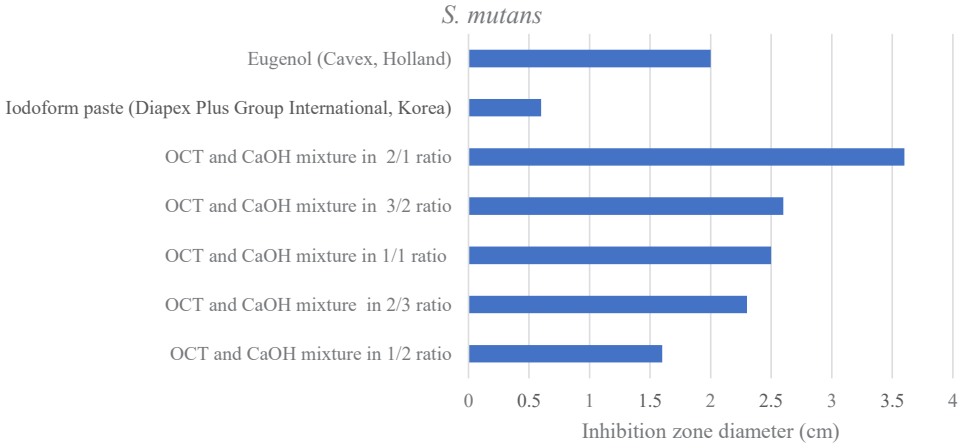

Figure 4. Antibacterial effect against *S. mutans.*

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The statistical analysis for social science (SPSS) version 23.0 (IBM corporation, Ilinois, Chicago, US) was used to analyze the data.

RESULTS

When the diameters of the inhibition zones were compared, the experimental canal paste obtained by mixing OCT and CaOH at a ratio of 2:1 was found to have the highest antibacterial effect against *S. aureus, S. mutans,* and *E. faecalis*, and the primary tooth canal paste containing IP which is used routinely in clinical practice, had the lowest antibacterial effect. (Figures 2, 3, 4, 5). It was found that experimental primary tooth root canal sealer with 2:1 ratio of OCT–CaOH mixture was the highest antibacterial effect against *S. aureus* (Figure 3). In addition, the antibacterial effect increased when the OCT ratio was observed to increase (Table 1). It was found that the antibacterial effect of OCT–CaOH containing experimental primary tooth root canal sealer against *S. mutans* was significantly higher than the antibacterial effect of the primary tooth root canal sealer containing ZOEand IP (Figure 4). Additionally, it was observed that the antibacterial effect increased as the OCT ratio increased (Table 2). It was found that the OCT–CaOH mixture showed the highest antibacterial effect against *E. faecalis* (Figure 5). Additionally, it was observed that the antibacterial effect increased as the OCT ratio increased (Table 3).

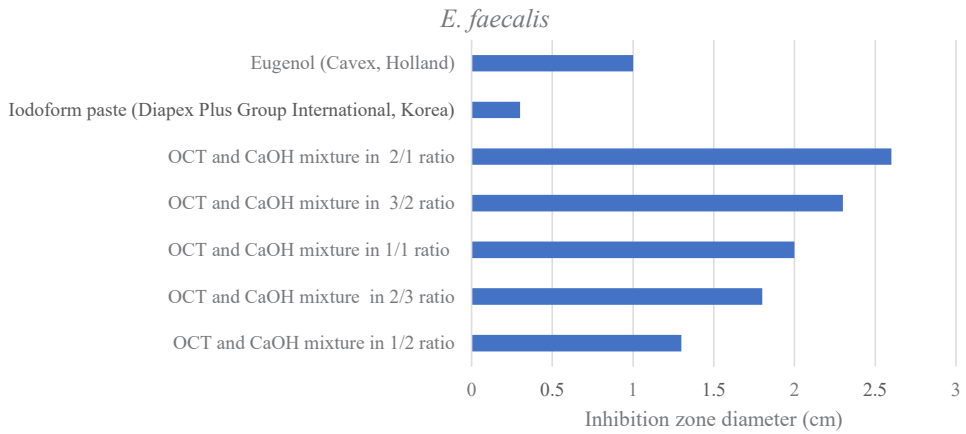

Figure 5. Antibacterial effect against *E. faecalis.*

Table 1. Inhibition zone measurement values created by experimental canal pastes prepared in different ratios for *S. aureus*

S. aureus					
Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values	Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values
1 gr	0.5 gr	3 cm	0.5 gr	1 gr	1.5 cm
1 gr	1 gr	2.3 cm	1 gr	1 gr	2.3 cm
1 gr	1.5 gr	2.2 cm	1.5 gr	1 gr	2.5 cm
1 gr	2 gr	1.5 cm	2 gr	1 gr	3 cm

Table 2. Inhibition zone measurement values created by experimental canal pastes prepared in different ratios for *S. mutans*

S. mutans					
Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values	Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values
1 gr	0.5 gr	3.6 cm	0.5 gr	1 gr	2 cm
1 gr	1 gr	2.5 cm	1 gr	1 gr	2.5 cm
1 gr	1.5 gr	2.3 cm	1.5 gr	1 gr	2.3 cm
1 gr	2 gr	1.6 cm	2 gr	1 gr	3.6 cm

Table 3. Inhibition zone measurement values created by experimental canal pastes prepared in different ratios for *E. faecalis*

E. faecalis					
Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values	Amount of OCT (g)	Amount of CaOH (g)	Inhibition Zone Measurement Values
1 gr	0.5 gr	2.6 cm	0.5 gr	1 gr	1.2 cm
1 gr	1 gr	2 cm	1 gr	1 gr	2 cm
1 gr	1.5 gr	1.8 cm	1.5 gr	1 gr	2.3 cm
1 gr	2 gr	1.3 cm	2 gr	1 gr	2.5 cm

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The Tukey multiple comparison test revealed a statistically significant difference in the antibacterial effect between the OCT–CaOH (except with a 1:2 ratio) and iodoform root canal sealers, with the OCT–CaOH sealers having significantly greater antibacterial effects than the iodoform sealer. No statistically significant difference in antibacterial effect was found between the iodoform and eugenol canal sealers, which are routinely used in clinical practice (Table 4).

DISCUSSION

Developmental, anatomical and physiological differences between the primary and permanent teeth determine the properties of the root canal filling material to be used.[17] A wide variety of bacteria are involved in primary tooth root canal infections.[18] The success of canal treatment in primary teeth depends on the elimination or reduction of bacteria in the root canals. Bacterial elimination is achieved

Table 4. Tukey multiple comparison test (OCT: Octenidine dihydrochloride)

(I) VAR00001	(J) VAR00001	Interlameller difference (I-J)	Significance
OCT/CaOH=1/2	OCT/CaOH=2/3	-.63333	.315
	OCT/CaOH=1/1	-.80000	.125
	OCT/CaOH=3/2	-1.00000*	.035
	OCT/CaOH=2/1	-1.60000*	.001
	Iodoform	.83333	.102
	Eugenol	-.03333	1.000
OCT/CaOH=2/3	OCT/CaOH=1/2	.63333	.315
	OCT/CaOH=1/1	-.16667	.996
	OCT/CaOH=3/2	-.36667	.831
	OCT/CaOH=2/1	-.96667*	.044
	Iodoform	1.46667*	.002
	Eugenol	.60000	.370
OCT/CaOH=1/1	OCT/CaOH=1/2	.80000	.125
	OCT/CaOH=2/3	.16667	.996
	OCT/CaOH=3/2	-.20000	.989
	OCT/CaOH=2/1	-.80000	.125
	Iodoform	1.63333*	.001
	Eugenol	.76667	.152
OCT/CaOH=3/2	OCT/CaOH=1/2	1.00000*	.035
	OCT/CaOH=2/3	.36667	.831
	OCT/CaOH=1/1	.20000	.989
	OCT/CaOH=2/1	-.60000	.370
	Iodoform	1.83333*	.000
	Eugenol	.96667*	.044
OCT/CaOH=2/1	OCT/CaOH=1/2	1.60000*	.001
	OCT/CaOH=2/3	.96667*	.044
	OCT/CaOH=1/1	.80000	.125
	OCT/CaOH=3/2	.60000	.370
	Iodoform	2.43333*	.000
	Eugenol	1.56667*	.001
Iodoform	OCT/CaOH=1/2	-.83333	.102
	OCT/CaOH=2/3	-1.46667*	.002
	OCT/CaOH=1/1	-1.63333*	.001
	OCT/CaOH=3/2	-1.83333*	.000
	OCT/CaOH=2/1	-2.43333*	.000
	Eugenol	-.86667	.083
Eugenol	OCT/CaOH=1/2	.03333	1.000
	OCT/CaOH=2/3	-.60000	.370
	OCT/CaOH=1/1	-.76667	.152
	OCT/CaOH=3/2	-.96667*	.044
	OCT/CaOH=2/1	-1.56667*	.001
	Iodoform	.86667	.083
Confidence level 0.05			

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by mechanical debridement and the use of antibacterial irrigation agents and root canal filling materials.[19] Bacterial elimination in primary teeth is not fully achieved by mechanical debridement.[20] The search for material that can be the ideal root canal material for primary teeth is still ongoing. For this reason, in this study, the antibacterial effect of an experimental primary pulp canal sealer, which is an alternative to the primary tooth root canal sealer used in the clinic, was investigated.

The filling materials most commonly used for primary pulp canals are ZOE paste, IP, CaOH and CaOH and in combination.[21] When the studies were examined, it was seen that the most used primary tooth root canal sealers are the pastes containing iodoform.[22] For this reason, for comparison with the experimental canal sealer prepared in our study in terms of antibacterial effect, pastes containing ZOE and iodoform were also used.

Various bacteria cause primary pulp canal infections.[23] In the study carried out by Fabris et al.,[14] the root canal microflora of the primary teeth with necrotic pulp was examined, and it was observed that the active bacteria species were gram positive cocci. Moreover, Sassone et al.[24] investigated the root canal microflora of primary teeth with necrotic pulp and found that the most prevalent bacterial species was *E. faecalis*. In this study, the antibacterial properties of the OCT–CaOH-containing, IP-containing, and ZOE-containing primary tooth canal sealers on gram-positive aerobic cocci *S. mutans*, *S. aureus,* and *E. faecalis* were compared.

Octenidine hydrochloride by Sterling Winthrop Research Institute is an improved derivative of bispyridine It has been put into clinical use as an alternative to chlorhexidine, povidone iodine, and triclosan. Biocompatibility of OCT to mucosa has a high antibacterial effect and does not have any stated side effects. Therefore, it has many uses such as acne treatment, treatment of nail infections, wound antisepsis, and biofilm elimination.[11]

OCT exhibits properties of positively charged (cation-active) chemical compounds, high antimicrobial efficacy, and specific ability to form and adhere to complexes with the chemical components of cells and whole cells.[25] The antibacterial potential of OCT has been well documented and compared with other disinfectants used in endodontics. Tirali et al.[13] reported that OCT may be an alternative irrigation solution to sodium hypochloride for the elimination of *S. aureus*. Similarly, Şahinkesen et al.[26] proposed OCT as an alternative irrigation solution to hypochlorite and chlorhexidine for the elimination of *S. aureus*. In this study, the OCT and calcium hydroxide mixture was found to be an alternative material for eradicating *S. aureus* bacteria. However, no previous study has evaluated the antibacterial effect of the octenidine dihydrochloride–calcium hydroxide mixture against *S. aureus*. Similarly, no studies have compared the antibacterial effect of the OCT–CaOH mixture against *S. aureus* with that of primary tooth canal sealers containing eugenol and iodoform. Tirali et al.[13] reported that higher concentrations of OCT had greater antibacterial effects on *S. aureus*. In our study, the antibacterial effect on *S. aureus* increased with the increase in OCT ratio in the experimental primary canal sealer containing OCT. Şahinkesen et al.[26] found that OCT was more effective than chlorhexidine digluconateagainst *S. mutans*. *S. mutans* has been reported to have comparable susceptibility to OCT and chlorhexidine digluconate.[27] Similarly, in our study, the OCT–CaOH mixture was found to be more effective than other materials against *S. mutans*. However, no previous studies have compared the antibacterial effects of OCT–CaOH mixtures against *S. mutans* with those of primary teeth canal pastes containing eugenol and iodoform.

Studies have associated the failure of endodontic treatment in adults with *E. faecalis* infection. This microorganism shows high resistance to calcium hydroxide and the irrigating solutions commonly used in endodontic treatments. Studies reported that 77% of failed root canal treatment cases suffered reinfection due to *E. faecalis* resistance. The high resistance of *E. faecalis* is because of various virulence factors, including its ability to compete with other microorganisms in invading dentinal tubules and to survive in high temperatures and in a broad pH range.[28] In addition, this bacterial resistance may also occur in the treatment of primary teeth, leading to persistent infection.[29] Guneser et al.[30] reported that the OCT–CaOH mixture used in their study was effective in eliminating *E. faecalis* from root canals in permanent teeth. Tandjung et al.[31] demonstrated the efficacy of OCT against *E. faecalis* in an infected root canal dentin model. Similarly, in the present study, our OCT–CaOH mixture was effective in eliminating *E. faecalis*. In addition, Tirali et al.[32] reported that the OCT–CaOH mixture used in their study showed a better antibacterial effect on *E. faecalis* than MTAD–calcium hydroxide and chlorhexidine–calcium hydroxide mixtures. In another study, octenidine used as intracanal medicament showed more effective antimicrobial effect against *E. faecalis* than CaOH.[33] Similarly, our study demonstrates that the OCT–CaOH mixture was more effective than other materials in eliminating *E. faecalis*.

The results of this study show that the antibacterial effects of the experimental primary tooth root canal sealer prepared with an OCT–CaOH mixture against *S. mutans*, *S. aureus*, and *E. faecalis* were significant compared with those of eugenol- and iodoform-containing sealers. On the basis of the study findings demonstrating the antibacterial efficacy of the experimental primary tooth canal paste prepared by mixing OCT and CaOH, it is reasonable to suggest its use as an alternative to the primary tooth canal pastes containing eugenol and iodoform, which are routinely used by dentists in clinical practice. However, further clinical trials are needed to recommend it for routine use in the treatment of primary tooth root canal infections.

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