



ANTIMICROBIAL ACTIVITY OF ROOT CANAL FILLING MATERIALS FOR ENDODONTIC TREATMENT IN PRIMARY DENTITION

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ABSTRACT

Aim. To evaluate in vitro the antimicrobial activity of materials used as root canal filling pastes for endodontic treatment in primary dentition.

Material and methods. The antimicrobial effectiveness of six root canal filling materials with different chemical compositions was tested against eight microorganisms commonly found in infected root canals. The agar diffusion test was used. Eight wells per plate were created at equidistant points and filled with the test and control materials, and the plates were incubated at 37°C in the incubator. Following 24, 48, and 72 hours, a digital caliper was used to measure the diameter of the growth inhibition zones for each material in millimeters.

Results. The difference between the mean diameter of growth inhibition zones of root canal filling materials tested (11.5 mm) and that of the control template (Vaseline) is statistically significant ($p < 0.001$). The antimicrobial effect is found to be large (Cohen's $d = -2.46$). Formalin-resorcin-zinc oxide paste exhibited the highest antimicrobial activity among the investigated materials. The mean diameter of the growth inhibition zone concerning all microorganisms was 30.27 mm. The combination of Calcium hydroxide + Iodofom paste had the lowest antimicrobial activity. The mean diameter of the growth inhibition zone was 6.73 mm.

Conclusions. The antimicrobial activity of root canal filling materials can be summarized as follows: Formalin-Resorcin-Zinc Oxide>Zinc oxide eugenol>Zinc oxide eugenol+Chlorhexidine dihydrochloride>Calcium hydroxide+Chlorhexidine dihydrochloride>Calcium hydroxide>Calcium hydroxide+Iodoform>Vaseline.

Keywords: antimicrobial activity, root canal filling pastes, primary dentition,

INTRODUCTION

Endodontic infections in the primary dentition are a serious problem for paediatric dentistry. Structural differences of dentin, as well as anatomical variations in root canal systems of primary teeth, significantly influence the complexity of treatment planning. Vidi et al. found primary roots were longer, thinner, curved, and divergent [1]. Other authors reported that primary maxillary first and second molars, predominantly three-rooted with 3 canals, exhibited a high prevalence of fused roots, accessory canals, and thinner dentin walls. Primary mandibular first and second molars were described as two-rooted with 4 canals, showing high variability in canal configurations [2, 3].

There is a suitable environment for microbial species and their growth in the root canal system. Many studies confirm the polymicrobial nature and mixed non-specific microflora of the infection in the root canals of primary teeth [4, 5]. With the development of modern microbiological diagnostic technologies, it has been established that the majority of species are represented by obligate anaerobic species, followed by facultative anaerobes. The most common microorganisms in endoinfections of primary teeth are *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Candida albicans* [4, 5]. *Enterococcus faecalis* is one of the most resistant microorganisms of the root canal flora. In prolonged endodontic infection, this microorganism can enter the canal system, resist antibacterial treatment, and remain there even after root canal filling. Once it enters the microtu-

bular system of dentin, it becomes difficult to treat [5].

Microbial species can be isolated from the entire root canal system – dentinal tubules, lateral root canals, accessory canals, apical foramen, the cementum surface of the apical foramen, as well as from the outer surface of apical root areas where root resorption has begun [6].

The success of endodontic treatment of primary teeth is determined by the elimination or reduction of microorganisms from the root canal system and includes three mandatory stages [7]:

1. Adequate mechanical preparation of the root canal system

2. Use of antimicrobial irrigants with a short-term effect on the root canal system

3. Use of root canal filling agents with antimicrobial activity

A significant number of agents have been studied in search of an ideal root canal filling agent for endodontic treatment of primary teeth. However, the antimicrobial activity is essential for the success of endodontic treatment – to eliminate the residual microflora after mechanical treatment, to neutralize microbial toxic products, to prevent reinfection, and to create a favorable environment for the healing process until the physiological shedding occurs [8]. The modern scientific literature presents a significant number of studies on the antimicrobial activity

of root canal filling pastes in primary teeth, but the results are contradictory [9, 10].

The most commonly used root canal filling materials are based on calcium hydroxide, iodoform, zinc oxide, and eugenol [11, 12]. They could be used alone or in combination with other active substances (formocresol, chlorhexidine, tetracycline, chloramphenicol, zinc acetate, metronidazole, etc.), as well as being available in pre-mixed, ready-to-use paste or extemporaneous preparation.

The purpose of this study was to evaluate in vitro the antimicrobial activity of materials used as root canal filling agents for endodontic treatment in primary dentition against the most common microbial agents in endodontic infections of primary teeth. To realize this aim, two tasks were assigned:

- to study the antimicrobial activity of different commonly used root canal filling materials

- to optimize the antimicrobial activity of recently used root canal filling materials by mixing with additional agents

MATERIALS AND METHODS

The antimicrobial effectiveness of six root canal filling materials with different chemical compositions was tested against eight microorganisms commonly found in infected root canals (Table 1).

Table 1. Tested root canal filling agents.

| Main ingredients | Brand name/Chemical composition | Form |
|---|--|----------------------------|
| Calcium hydroxide, Ca(OH) ₂ [5, 12] | Calcipast, Cerkamed | pre-mixed and ready-to-use |
| Calcium hydroxide+Iodoform [7, 11, 12, 13] | Calcipast+I, Cerkamed | pre-mixed and ready-to-use |
| Zinc oxide-eugenol paste, ZOE [8, 14] | Zinc oxide dispersed in eugenol in a ratio of 0.2:0.07 (g: ml) | extemporaneous preparation |
| Zinc oxide – eugenol - chlorhexidine paste, ZOE-ChX | Zinc oxide dispersed in eugenol in a ratio of 0.2:0.07 (g: ml) with added chlorhexidine hydrochloride in a concentration of 0.1% w/v. | extemporaneous preparation |
| Calcium hydroxide - chlorhexidine paste, Ca(OH) ₂ -ChX | Chlorhexidine hydrochloride in a concentration of 0.1% w/v, dispersed in a semi-solid base of calcium hydroxide 38% w/v (Calcipast, Cerkamed). | extemporaneous preparation |
| Formalin-resorcin and zinc oxide paste, HCHO-Res-ZnO | A base of 40% formalin with dissolved resorcinol crystals in a ratio of 1:4, in which zinc oxide is dispersed to obtain a pasty consistency. | extemporaneous preparation |
| Negative control agent | Vaseline | ready-to-use |

The agar diffusion test has been widely used for this purpose [2, 3, 10, 11, 12]. As an in vitro method, the investigated material diffuses into the agar. The inhibitory effects are a combination of diffusion potential and antimicrobial activity using the agar-well diffusion method. Eight wells

per plate were made at equidistant points and filled with the test and control materials, and the plates were incubated at 37 °C in the incubator. Following 24, 48, and 72 hours, a digital caliper was employed to measure the diameter of the growth inhibition zones for each material in millimeters.

Statistical methods: One-way analysis of variance (One-Way ANOVA) and non-parametric Kruskal-Wallis analysis for independent samples were used to compare the mean values of the antimicrobial activity of the investigated root canal filling pastes. Statistical analysis and data visualization were conducted using the statistical software product SPSS v.21.0 and Microsoft Excel 2010 (SPSS Inc., Chicago, IL, USA). The significance level was $p < 0.05$. $p = 0.05$. The differences between the groups were analyzed using non-parametric tests because the normality assumption could not be met (Shapiro-Wilk test, $p < 0.05$).

RESULTS:

Intervention vs Control

The average diameter of the growth inhibition zone for the investigated root canal agents was 16.44 mm (95% CI, 15.32 -17.56), which is a 2.7 times wider zone of inhibition compared to the control sample (Vaseline). The difference between the average diameter of growth inhibition zones (11.5 mm) and of the control template (Vaseline) is statistically significant ($p < 0.001$). The difference between the mean diameter of inhibition (10.44 mm) of each investigated agent was statistically significant ($p < 0.001$) (Table 2, Figure 1).

Table 2. Difference in the size of the growth zone (intervention vs control).

| | | Difference | L95%CI | U95%CI | SE | df | t | p |
|---------|--------------|------------|---------|--------|-------|---------|---------|-------|
| control | intervention | -10.411 | -12.183 | -8.639 | 0.902 | 613.003 | -11.539 | <0.01 |

The antimicrobial effect is found to be large (Cohen's $d = -2.46$, 95% CI [-2.68, -2.24]).

No statistically significant dynamics in the antimicrobial activity of the agents investigated were observed between 12, 48, and 72 hours (Table 3).

Pre-mixed ready-to-use vs extemporaneous preparation

Comparing the two types of products – extemporaneous preparation and ready-to-use root canal agents - a statistically significant difference (8.69, 95% CI, 7.47 - 9.75) was found in the mean diameter of growth inhibition, considered averaged over all microorganisms (Figure 2). The extemporaneous root canal agents (mean diameter, 19.9 mm; 95% CI, 18.96-20.83) had an average of 1.76 times wider antimicrobial zone compared to the used ready-to-use pre-mixed pastes (mean diameter, 11.32 mm; 95% CI, 10.33-12.31) (Table 4).

Fig. 1. Comparison of the growth inhibition zones: intervention vs control.

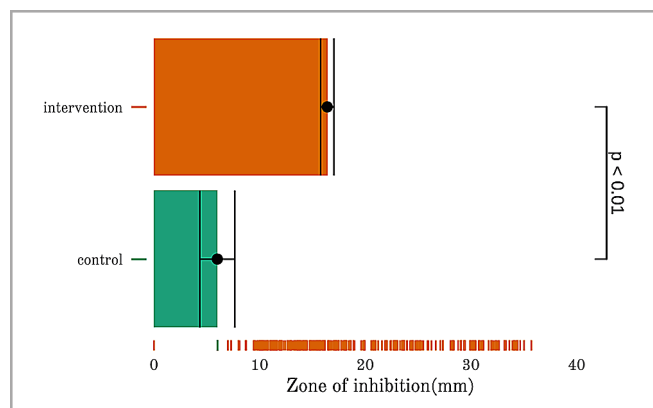


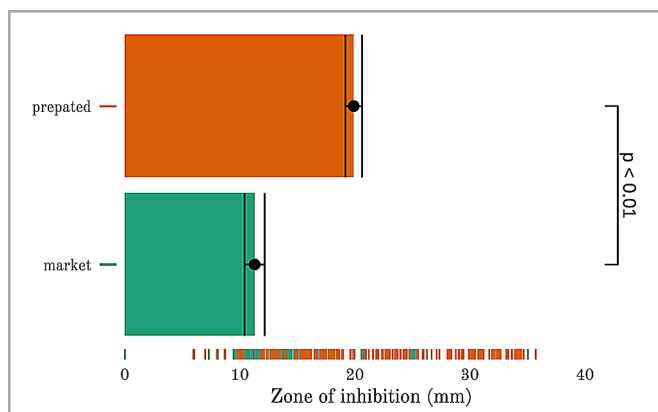
Table 3. Difference in the size of the growth inhibition zone over time.

| | | Difference (mm) | L95%CI | U95%CI | SE | df | t | p |
|------|------|-----------------|--------|--------|-------|---------|-------|-------|
| 24 h | 48 h | 0 | -1.779 | 1.779 | 0.741 | 613 | 0 | 1 |
| 24 h | 72 h | 0.176 | -1.604 | 1.955 | 0.741 | 613.029 | 0.237 | 0.969 |
| 48 h | 72 h | 0.176 | -1.604 | 1.955 | 0.741 | 613.029 | 0.237 | 0.969 |

Table 4. Difference in the size of the growth inhibition zone according to the preparation type of the paste – pre-mixed ready-to-use versus extemporaneous root canal filling pastes.

| | | Difference (mm) | L95% CI | U95% CI | SE | df | t | p |
|--|--|-----------------|---------|---------|-------|---------|---------|-------|
| pre-mixed ready-to-use root canal filling pastes | extemporaneous root canal filling pastes | -8.609 | -9.747 | -7.471 | 0.579 | 524.032 | -14.862 | <0.01 |

Fig. 2. Comparison of the growth inhibition zones: extemporaneous preparation vs pre-mixed ready-to-use pastes.



Comparing the antimicrobial activity of pre-mixed, ready-to-use root canal filling pastes with extemporaneous pastes, considering the microorganisms investigated, a statistically significant difference was not found, except for *Candida albicans* ($p = 0.103$). The greatest antimicrobial effect was observed for *E. coli*, where the growth inhibition zone of the extemporaneous pastes was 14.65 mm larger than the pre-mixed, ready-to-use pastes (Figure 3).

Type of paste

Formalin-resorcin-ZnO paste had the highest antimicrobial activity among the investigated materials. The mean diameter of the growth inhibition zone concerning all microorganisms was 30.27 mm (95% CI, 29.72 -30.81) (Table 5). The average diameter of the inhibition zone of this extemporaneous paste, considered for all microorganisms, is 30.27. Calcium hydroxide + Iodoform paste (Calcipast+I) had the lowest antimicrobial activity. The

mean diameter of the growth inhibition zone was 6.73 mm (95% CI, 6.18-7.27) (Figure 4).

DISCUSSION:

Antimicrobial activity assessment helps assess the clinical adequacy of the most commonly used root canal filling pastes in clinical practice for treatment of endodontic infections in the primary dentition [3, 8, 14].

Based on the results obtained, our study determines the application of formalin-resorcin-zinc oxide paste as a method with significant effectiveness on the infection in the primary dentition. Our results are in line with a few studies that investigated the effectiveness of mortal pulpotomy using the formalin-resorcin method [14,15]. Given the debate on the negative consequences of its use and certain limitations in accessibility, this agent should be the subject of subsequent studies along with other agents in terms of biotolerance and cytotoxicity [5, 6, 15].

Fig. 3. Comparison of antimicrobial activity between the pre-mixed ready-to-use paste and extemporaneous pastes, considering the microorganisms investigated.

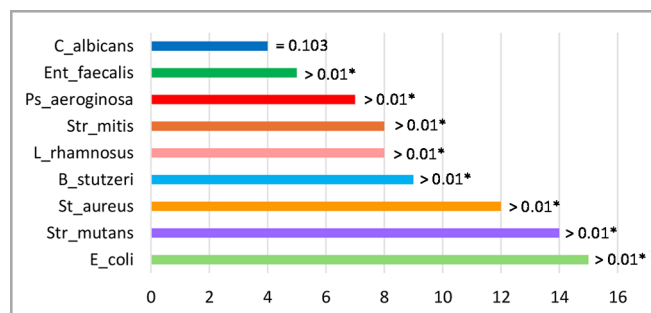
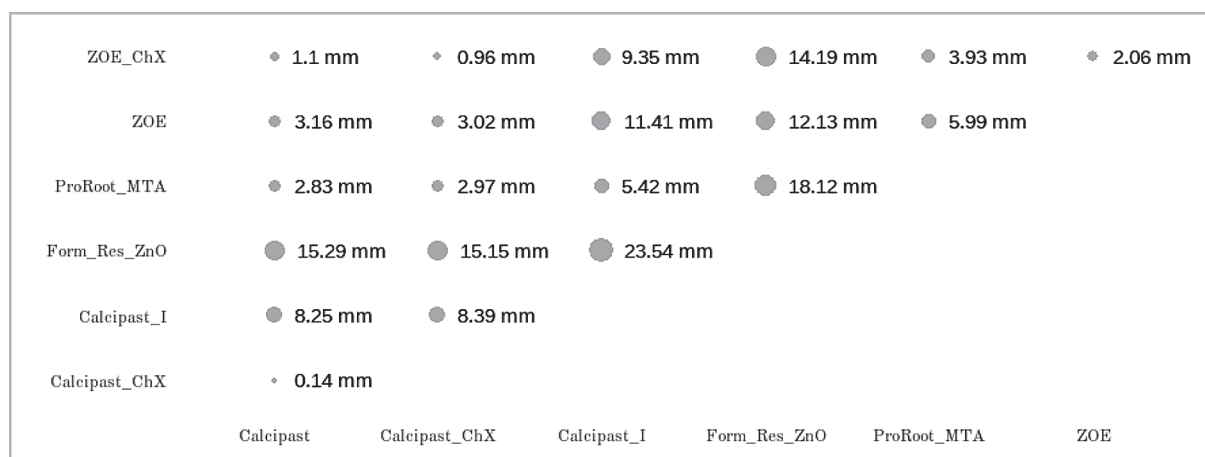


Table 5. Mean inhibition zone according to the type of paste.

| Root canal filling paste | zone (mm) | L95%CI CI | U95% CI | SE | df |
|--------------------------|-----------|-----------|---------|------|-------|
| Calcipast | 14.978 | 14.42 | 15.536 | 0.28 | 74.25 |
| Calcipast_ChX | 15.113 | 14.555 | 15.671 | 0.28 | 74.25 |
| Calcipast_I | 6.727 | 6.169 | 7.285 | 0.28 | 74.25 |
| Form_Res_ZnO | 30.265 | 29.707 | 30.823 | 0.28 | 74.25 |
| ZOE | 18.136 | 17.578 | 18.694 | 0.28 | 74.25 |
| ZOE_ChX | 16.075 | 15.517 | 16.633 | 0.28 | 74.25 |

The extemporaneous preparation exhibited twice as strong activity against the tested microorganisms in comparison with the pre-mixed, ready-to-use pastes.

Fig. 4. Difference in the inhibition zone between extemporaneous and pre-mixed ready-to-use pastes.



Numerous authors investigated Zinc Oxide Eugenol (ZOE) as a root canal filling material of primary teeth [1, 3, 6, 9, 12, 16]. Previous studies emphasize the good antibacterial properties of the agent in comparison with widely used root canal pastes in practice [4, 5, 6, 7, 8]. Our results also confirm that ZOE remains the Gold standard root canal filling material in primary dentition [4, 5, 6, 7, 8, 9, 10]. Pedrotti et al., Tchaou et al., and Queiroz et al. reported greater antibacterial effectiveness of ZOE than that of other most commonly used calcium hydroxide-based and iodoform-calcium hydroxide-based agents [5, 6, 13, 14]. However, our results differ from those obtained by the studies by other authors, which showed a higher chance of failure for ZOE compared to other pastes [15, 16, 17]. The different design of studies is considered to be the possible reason.

Several authors compared the antimicrobial activity of ZOE with an Iodoform paste (IP), a calcium hydroxide cement (Ca(OH)₂), and IP + (Ca(OH)₂) and concluded there was no convincing evidence to support the superiority of any material over ZOE [5, 18]. However, the modern concept points out that ZOE and Iodoform+Calcium hydroxide paste are suitable as root canal fillings for deciduous teeth [5, 18].

The trend reported by other authors for a better effectiveness of the Iodoform paste in combination with calcium hydroxide (IP + Ca) than ZOE was not observed in our study [18]. One research monitored the long-term antimicrobial activity of root canal filling pastes for primary dentition, and it was concluded that an Iodoform paste with calcium hydroxide (IP + Ca) had no antimicrobial activity [9]. Similar to our study's analysis, the recommended and widely used root canal filling pastes, which are based on a combination of Calcium hydroxide and iodoform, are the least effective in terms of de-

stroying the residual microflora in the root canal.

Indeed, among the materials tested in the present study, calcium hydroxide is the most studied in relation to its antimicrobial action [4, 6, 9, 10]. In contrast to the results obtained in the present research, Khoramian et al., Nadkarni et al. researched the clinical, radiographical, and microbiological effectiveness of root canal pastes for primary teeth and reported that calcium hydroxide root canal filling paste had better antibacterial activity than ZOE [19,20]. Asgari et al. and Lilian et al. demonstrated significantly high antimicrobial activity after 24, 48, and 72 hrs of calcium hydroxide [27, 28]. Our results are consistent with those of other authors [11, 24]. The Ca(OH)₂ pastes presented the highest probability of being the worst option, and the success rate was significantly lower when compared to ZOE and IP + Ca [19, 20].

The current experimental study is of great significance due to the use of chlorhexidine as an additional agent with antimicrobial activity and demonstrated potential action of the ex tempore prepared root canal filling pastes. Compared to the most commonly used root canal filling materials, in the present study, two ChX-based pastes exhibited similar performance in terms of consistency, manipulation time, flow, thickness, and superior antimicrobial activity. ZOE-ChX paste, as well as Ca(OH)₂-ChX paste, could be a promising root filling material for primary teeth and may be a potential alternative to existing materials.

In an in vitro study, Ercan et al. found high efficacy of the combination of 2% ChX gel and Ca(OH)₂ against *E. faecalis* inside dentinal tubules [21]. In another in vivo study using primary teeth, 1% ChX gluconate gel in combination with chitosan demonstrated sufficient effectiveness against *E. faecalis* within 48 hours [22]. In another study, 0.5% and 1%

chlorhexidine (CHX)-loaded calcium hydroxide (CH) pastes were used as intracanal medications (ICMs). Exposure to 0.5% and 1% CHX-loaded ICMs decreased the growth inhibiting an *Enterococcus faecalis* biofilm. Results indicated that the root canal may serve as a reservoir for periodontal drug delivery [23].

However, studies suggest that chlorhexidine in high concentrations poses a potential systemic risk and is a cytotoxic agent with carcinogenic potential in humans [23, 24]. Our study demonstrated additive antimicrobial activity of the extemporaneous root canal pastes prepared by adding 0.1% ChX compared with the same agents used as a one-component root canal filling paste. In contrast to the results obtained by the numerous studies, Haenni et al. found no additive antibacterial effect by mixing chitosan powder with 0.5% CHX. [24]

LIMITATIONS:

A limitation of the present study is the inability to perfectly replicate the complex anatomy of teeth and the dynamic physiological factors in the oral cavity. High variability in the antibacterial activity of root canal filling materials limits current evidence, emphasizing

the need for future clinical trials to investigate the long-term effects of endodontic treatment in primary teeth and to test new experimental materials and determine a suitable alternative root canal filling material. Consensus among paediatric dentists regarding the best available treatment option will play a crucial role in improving and optimizing clinical outcomes.

CONCLUSION:

In conclusion, it was found that the root canal filling agents have significantly better antimicrobial activity than the control used. The antimicrobial activity of root canal filling materials according to results obtained from the present study can be summarized as follows: Formalin-Resorcin-Zinc Oxide>Zinc oxide eugenol >Zinc oxide eugenol+Chlorhexidine dihydrochloride >Calcium hydroxide+Chlorhexidine dihydrochloride >Calcium hydroxide > Calcium hydroxide+Iodoform >Vaseline.

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