

# Comparison of Antifungal Activity of 2% Chlorhexidine, Calcium Hydroxide, and Nanosilver gels against *Candida Albicans*

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## Abstract

**Objectives:** Residual microorganisms in the root canal system (RCS) after endodontic therapy such as *Candida albicans* are a major cause of endodontic failure. Calcium hydroxide (CH) and chlorhexidine (CHX) have suitable antimicrobial activity against bacteria and can be used as intracanal medicaments. Nanosilver has also shown antimicrobial activity against microorganisms. This study aimed to compare the antifungal effect of calcium hydroxide, 2% chlorhexidine and nanosilver gels on *Candida albicans*.

**Materials and Methods:** Eighty-one single-rooted teeth were selected. After root canal preparation, the teeth were contaminated. After culture, the teeth were randomly divided into 4 groups. In experimental groups, 24 teeth were selected and completely filled with CH, 2% CHX and nanosilver gels in each group. Nine teeth were selected in the control group and filled with saline solution. After 1, 3, and 7 days, samples were obtained by #30 sterile paper points, and #2 and #4 Gates Glidden drills and cultured on solid Sabouraud agar.

**Results:** The results demonstrated that CH and 2% CHX had equal antifungal effects on samples taken by paper point and #2 Gates Glidden drill at all time points. Both CH and 2% CHX were more effective than nanosilver at all time periods. There was no statistically significant difference between medicaments in samples taken by #4 Gates Glidden drill.

**Conclusion:** CH and 2% CHX gels have significantly higher antifungal activity than nanosilver gel. Also, CH and 2% CHX gels are equally effective against *Candida albicans*.

**Keywords:** Calcium hydroxide; Chlorhexidine; *Candida albicans*

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## INTRODUCTION

The primary goal of endodontic treatment is elimination of bacteria and their products from the root canal system [1]. In routine endodontic therapy, most bacteria are removed by instrumentation and irrigation [2]. However, it has been confirmed that instrumentation and

irrigation of root canal system remove only 50-70% of intracanal microorganisms [3]. Residual microorganisms in the RCS are a major cause of endodontic failure [4]. Application of intracanal medicaments in-between endodontic treatment sessions may be effective against resistant microorganisms [4,5].

CH is the most commonly used material in endodontic therapy with significant antibacterial effects on intracanal microorganisms [5]. In order to have optimal efficacy in the RCS, CH should be spread all over the canal walls to be in close contact with them. The efficacy of this material depends on the penetration of hydroxyl ions into the dental tubules and accessory canals, where bacteria and their products accumulate. Furthermore, as a physical barrier, CH can prevent reinfection of the canal and impair nutritional supply of the residual microorganisms in the RCS [6-8]. Nevertheless, CH does not affect all bacterial strains in the same manner and therefore, cannot be used as a general intracanal medicament in all situations. Many studies have shown its failure in elimination of some types of microorganisms such as Enterococci species and *Candida albicans* [4,5]. Furthermore, CH is toxic and can have destructive effects on peri-radicular tissues, which in turn leads to chronic inflammation and cell necrosis [8].

CHX is widely used as an antimicrobial agent and medicament in periodontal and endodontic treatments. This material is also effective against gram-positive and gram-negative microorganisms [1,4]. Although CHX has a cationic component that binds to the negatively charged sites of the cell membrane causing cell lysis, it is incapable of solving debris and pulp tissues [9].

Today, by the advancements made in nanotechnology, nanoparticles are used for medical and industrial purposes. The antimicrobial effect of silver has long been understood. However, demand for it decreased after the advent of antibiotics. However, production of silver in a nano-crystalline structure has significantly enhanced its biological and antimicrobial properties [10]. Silver nanoparticles provide greater contact surface in comparison with silver particles in an aggregate form; thus, a small volume of silver nanoparticles has an antimicrobial property similar to that of much larger amount of aggregated silver.

Therefore, it may be effective against different root canal microorganisms [11-14]. At present, special attention is given to the role of different fungal species in failure of endodontic treatments. Almost all the fungi isolated from the RCS belong to the *Candida* clade, predominantly *Candida albicans*; which, is the culprit responsible for development of pulpal and periapical infections [15]. Several studies have investigated and compared the efficacy of CH and CHX against *Candida albicans* [7,16-18] indicating that 2% CHX gel was more effective against *Candida albicans* and *Enterococcus faecalis* compared to CH or the combination of both medicaments [7,17]. Also, one study showed marked antifungal activity of nanosilver particles against *Candida albicans* [19]. The aim of the present in-vitro experimental study was to determine and compare the antifungal efficacy of CH, 2% CHX and nanosilver gels against *Candida albicans*.

## MATERIALS AND METHODS

This study was carried out on 81 newly extracted single-rooted teeth with straight roots without calcification, supplementary canals or root decay. After extraction, the teeth were cleaned and debrided immediately. All the tissue appendages were also removed carefully using a scaler. For surface disinfection, the samples were immersed in 5.25% sodium hypochlorite for 24 hours. Then, the teeth were stored in 0.9% sterile saline solution at room temperature. All teeth were cut 2-3mm below the cemento-enamel junction perpendicular to the long axis of the tooth using a hand piece with rotary diamond disc at 700 rpm under abundant irrigation in a way that all samples had approximately the same length (14-16mm). Afterwards, root canals were prepared using the following mechanical and chemical instrumentation techniques:

Pulpal remains and debris were removed from the canal. Based on canal diameter, a #20 or #25 hand file (Dentsply-Maillefer, Ballaigues, Switzerland) was inserted into the root canal

until its tip appeared at the apical foramen. Working length was determined 0.5mm shorter than the measured length.

ProTaper system sequence was used up to F3 for cleaning and shaping of the canals. In all phases of instrumentation, canals were irrigated with copious 5.25% sodium hypochlorite. Afterwards, the root canals were irrigated with 17% EDTA solution for one minute for efficient smear layer removal. The samples were immersed in an ultrasonic bath for 5 minutes. Finally, the root canals were rinsed with 10ml of saline solution to wash off all the remaining medicaments. The teeth were then transferred to a microbiology laboratory. For this purpose, 2cc of the rain-heart infusion (BHI) culture medium was poured into sealed micro-tubes. The samples were then sterilized twice in an autoclave (121 °C, 15 PSI, 30 minutes). Afterwards, the teeth were incubated at 37 °C for 24 hours. The teeth were then contaminated. BHI medium was extracted from the micro-tubes by an insulin syringe in a way that the level of solution was adjusted 1ml below the coronal region of the root canal. Microbial suspension was prepared at 0.5 McFarland standard ( $1.5 \times 10^8$ ), and 0.1 ml of the inoculum was injected into the canals using an insulin syringe. The tubes were sealed again and incubated at 37 °C for 24 hours. Three micro-tubes were evaluated to determine the growth of *Candida albicans* species. All micro-tubes were subsequently incubated at 37 °C for 21 days to allow the proliferation of microorganisms and their further penetration into the dentinal tubules. In order to avoid the desiccation of teeth, BHI medium was added to the micro-tubes every 3 days.

Preparation of the materials: 2% methylcellulose was used as a neutral carrier to prepare the medicaments in gel form with the same standard concentration. The medicaments were added to the neutral gel as follows:

1.CH gel: One g calcium hydroxide powder (Merck, Darmstadt, Germany)+ 1g methylcellulose gel.

2.CHX gel: One g of 4% CHX solution+ 1g of 2% methylcellulose

3.Nanosilver gel: One g of 50ppm nanosilver solution (Lotus Nanochemistry Pasargad, Tehran, Iran) + 1g methylcellulose gel.

4.Neutral gel: Mixture of normal saline and methylcellulose gel (for the control group)

A few drops of viscose polyethylene glycol (PEG 400) were added to achieve a gelatin form. Then, the medicaments were injected into the root canals by a syringe with a 25-gauge needle.

After the completion of 21-day period of *Candida albicans* culture in dentinal tubules of root canals, samples were obtained from the root canals by #30 paper points to estimate the average (standard) number of the microbial colonies before the intervention. The paper points were placed in Sabouraud agar culture medium. Next, 24 teeth were randomly (Random allocation software, Isfahan, Iran, version 1.0) selected from each experimental group and the assigned medicaments were inserted into the root canals to completely fill the root canal from the apex to the coronal region. Nine teeth were selected for the control group. After this procedure, the tubes were sealed and the teeth were incubated at 37 °C. For sample size determination, 2-level factorial was used in Minitab software (with  $\alpha=0.05$ ,  $\beta=0.2$ , the mean standard deviation of 5.102, and the mean change of 1.104). According to this calculation, the minimum sample size was calculated to be 8 samples for each experimental group.

#### **Sampling:**

Eight teeth from each experimental group were selected for sampling. First, each tooth was taken out from its micro-tube, and the intracanal medicament was completely washed off via irrigation with normal saline solution. Then, a sample was obtained from the canal contents using #30 sterile paper points and cultured on solid Sabouraud agar, as mentioned earlier. Another sample was taken from the apical  $\frac{1}{3}$  of the root canals, using

#2 and #4 Gates Glidden drills. The obtained dentinal powder (#2 Gates=200  $\mu\text{m}$  and #4 Gates= 400  $\mu\text{m}$  of dentinal tubules) was transferred from the drills to the BHI culture media using a paper point. In order to facilitate the procedure and reduce the number of plates, each plate was divided into 4 equal sections and 4 paper points (1 paper point prior to application of the antimicrobial medicaments and 3 paper points for sampling after the completion of culture period) were placed in each section. The collected data regarding the colony forming units (CFU) was analyzed using SPSS. Number of CFUs in each group was classified as low (<1000), low-medium (1000-10,000), medium (10,000-100,000), and high (>100,000). The Kruskal-Wallis test was used for the comparison of CFUs among groups, and the Mann Whitney U test with Bonferroni adjustment was used for pairwise comparisons.

## RESULTS

Qualitative evaluation of CFUs in samples taken by paper points and #2 and #4 Gates Glidden drills after the application of intracanal medicaments at one, 3, and 7 days is demonstrated in Tables 1 to 3, respectively.

### One day:

The Kruskal Wallis test demonstrated statistically significant differences between medicaments in sampling by paper point ( $P<0.006$ ) and #2 Gates drills ( $P<0.003$ ) at one day post-intervention. However, no statistically significant differences were observed in sampling by #4 Gates Glidden drills. Based on the obtained results, the gels employed in this study had different antifungal effects after one-day period.

The Mann-Whitney U test found no statistically significant differences between CH and 2% CHX in sampling by paper point and #2, and #4 Gates Glidden drills in number of *Candida albicans* CFUs. Thus, the antifungal effects of CH and CHX were similar after one-day use.

In the same period, CH gel demonstrated superior antifungal effects in comparison with nanosilver gel in sampling by paper point ( $P<0.007$ ) and #2 Gates Glidden drills ( $P<0.005$ ). Nevertheless, the difference between these gels was not statistically significant in sampling by #4 Gates Glidden drills. Also, 2% CHX gel had higher antifungal effects compared to nanosilver gel (in sampling by paper point with  $P<0.01$  and #2 Gates with  $P<0.007$ ). However, no statistically significant difference was noted in sampling by #4 Gates. Number of *Candida albicans* CFUs after the application of 2% CHX gel was less than that for nanosilver gel, indicating the greater efficiency of CHX gel.

### Three days:

At 3 days post-intervention, statistically significant differences were detected between sampling by paper point ( $P<0.0001$ ), #2 Gates ( $P<0.001$ ) and #4 Gates Glidden drills ( $P<0.004$ ). Pairwise comparison of CH and 2% CHX in this time period revealed no statistically significant differences in sampling by paper point, #2 Gates, and #4 Gates Glidden drills. Therefore, the antifungal efficacy of CH and 2% CHX gels was similar at 3 days post-intervention. Also, the antifungal effect of CH was significantly higher than that of nanosilver in sampling by paper point ( $P<0.0001$ ), #2 Gates ( $P<0.01$ ), and #4 Gates Glidden drills ( $P<0.04$ ) at 3 days. In the same time period, the efficacy of 2% CHX was significantly higher than that of nanosilver gel in sampling by paper point ( $P<0.0001$ ), #2 Gates ( $P<0.002$ ) and #4 Gates Glidden drills ( $P<0.01$ ). Furthermore, lower level of *Candida albicans* CFUs following the use of 2% CHX gel in comparison with nanosilver revealed greater antifungal efficacy of the former agent.

### Seven days:

At 7 days, the medicaments showed different antifungal efficacies in sampling by paper point ( $P<0.0001$ ), #2 Gates ( $P<0.0001$ ) and #4 Gates Glidden drills ( $P<0.009$ ).

**Table 1.** CFUs in samples taken by paper point following the application of medicaments

Time Point	Medicaments	Low	Low to Moderate	Moderate	High
1 day	CH (n=8)	1(12.5%)	6(75.0%)	0	1(12.5%)
	2% CHX (n=8)	1(12.5%)	4(50.0%)	2(25%)	1(12.5%)
	Nanosilver (n=8)	0	1(12.5%)	0	7(87.5%)
	Control (n=3)	0	0	0	3(100%)
3 days	CH (n=8)	4(50.0%)	3(37.5%)	1(12.5%)	0
	2% CHX (n=8)	8(100%)	0	0	0
	Nanosilver (n=8)	0	0	0	8(100%)
	Control (n=3)	0	0	0	3(100%)
7 days	CH (n=8)	8(100%)	0	0	0
	2% CHX (n=8)	8(100%)	0	0	0
	Nanosilver (n=8)	0	0	5(62.5%)	3(37.5%)
	Control (n=3)	0	0	0	3(100%)

**Table 2.** CFUs in samples taken by #2 Gates Glidden drill following the application of medicaments

Time Point	Medicaments	Low	Low to Moderate	Moderate	High
1 day	CH (n=8)	6(75.0%)	2(25.0%)	0	0
	2% CHX (n=8)	5(62.5%)	3(37.5%)	0	0
	Nanosilver (n=8)	1(12.5%)	1(12.5%)	3(37.5%)	3(37.5%)
	Control (n=3)	0	0	0	3(100%)
3 days	CH (n=8)	5 (62.5%)	2(25.0%)	1(12.5%)	0
	2% CHX (n=8)	8 (100%)	0	0	0
	Nanosilver (n=8)	1 (12.5%)	1(12.5%)	2(25.0%)	4(50.0%)
	Control (n=3)	0	0	1(33.3%)	2(67.7%)
7 days	CH (n=8)	8(100%)	0	0	0
	2% CHX (n=8)	8(100%)	0	0	0
	Nanosilver (n=8)	0	0	7(87.5%)	1(12.5%)
	Control (n=3)	0	0	0	3(100%)

**Table 3.** CFUs in samples taken by #4 Gates Glidden drill following the application of medicaments

Time Point	Medicaments	Low	Low to Moderate	Moderate	High
1 day	CH (n=8)	7(87.5%)	1(12.5%)	0	0
	2% CHX (n=8)	5(62.5%)	3(37.5%)	0	0
	Nanosilver (n=8)	3(37.5%)	2(25.0%)	2(25.0%)	1(12.5%)
	Control (n=3)	0	0	0	3(100%)
3 days	CH (n=8)	4(50.0%)	4(50.0%)	0	0
	2% CHX (n=8)	8(100%)	0	0	0
	Nanosilver (n=8)	2(25.0%)	0	3(37.5%)	3(37.5%)
	Control (n=3)	0	0	0	3(100%)
7 days	CH (n=8)	8(100%)	0	0	0
	2% CHX (n=8)	7(87.5%)	1(12.5%)	0	0
	Nanosilver (n=8)	3(37.5%)	2(25.0%)	3(37.5%)	0
	Control (n=3)	0	0	0	3(100%)

Pairwise comparison of intracanal medicaments at 7 days showed no statistically significant differences between CH and 2% CHX gels in number of CFUs in sampling by paper point, #2 Gates, and #4 Gates Glidden drills. Thus, CH and 2% CHX gels had relatively similar antifungal effects after 7 days.

In this time period, the efficacy of 2% CHX and CH gels was significantly greater than that of nanosilver in sampling by paper point and #2 Gates ( $P < 0.0001$  and  $P < 0.0001$  for CH, and  $P < 0.0001$  and  $P < 0.0001$  for CHX, respectively). In samples taken with #4 Gates, CH was significantly more effective than nanosilver ( $P < 0.04$ ). However, there was no significant difference between CHX and nanosilver in samples taken with #4 Gates Glidden drills ( $P = 0.07$ ). Number of *Candida albicans* CFUs following the use of 2% CHX gel was lower than that after the use of nanosilver in all samples.

## DISCUSSION

The present study compared the antifungal activity of CHX, CH and nanosilver gel. According to our obtained results, antifungal activities of CH and CHX gels were significantly higher than that of nanosilver gel (in all samples and time points). This finding did not match our hypothesis. The majority of previous studies have evaluated the antibacterial efficacy of nanosilver gels and no study was found on the antifungal efficacy of nanosilver in endodontics. Nanosilver is a newly manufactured product by the use of nanotechnology. Its mechanism of action is through catalytic and ionic reactions. The catalytic reactions produce hydroxyl ions by hydrolysis, and oxygen ions by oxidizing  $O_2$ , both of which are among the most powerful antimicrobial agents. In the ionic reactions,  $-SH$  bonds in the microorganisms' cell walls are converted to  $-SAg$  bonds through a substitution reaction leading to consequent degradation of microorganism. The antimicrobial efficacy of nanosilver has been proven in previous studies

[10,20,21]; however, the nanosilver gel used in this study was not similar to the product used in other studies [22,23]. It may be due to the reaction between the carrier and nanoparticles. Future studies are required to further evaluate the antifungal activity of nanosilver. Sadeghi et al. evaluated the effect of nanosilver solution on *Actinomyces viscosus* and *Streptococcus sanguinis* and showed that nanosilver solution had suitable antimicrobial properties against these species. This effect was achieved in lower concentration, in comparison to CHX [24].

In another study, Hiraishi et al. reported adequate antimicrobial effect of 3.8% silver diamine fluoride on *Enterococcus faecalis* species [25]. Furthermore, Sotiriou and Pratsinis evaluated the antimicrobial effects of silver ions and nanosilver particles and concluded that the antimicrobial activity of both was similar [26].

In the current study, we found no statistically significant difference between %2 CHX and CH gels in samples at different time points. Both medicaments were equally effective on *Candida albicans* and had suitable antifungal effects.

CHX is a cationic molecule soluble in water and lipid. Thus, it reacts with cell membrane lipopolysaccharides and phospholipids causing their degradation [27]. Many studies have demonstrated the superior antimicrobial activity of CHX against microorganisms in comparison to other medicaments [7,17,27-29]. Haffajee et al. showed greater efficacy of CHX against 40 oral microbial strains [29]. Ercan et al. reported that CHX had the greatest efficacy for elimination of *Enterococcus faecalis* and *Candida albicans* from the RCS [7]. Waltimo et al. obtained similar results as well. They investigated the antimicrobial efficacy of sodium hypochlorite (0.5% and 5%), CHX acetate (0.5%), iodine (4%), potassium iodide (2%) (IKI) and CH against *Enterococcus faecalis* and *Candida albicans* and revealed that sodium hypochlorite (both 0.5% and 5% con-

centrations), iodine (2%) and potassium iodine (4%) eliminated all yeasts within 30 seconds, but chlorhexidine acetate had the greatest efficacy after 5 minutes. Furthermore, they demonstrated that *Candida albicans* was resistant to CH [18].

In our study, CH was effective against *Candida albicans* at all tested time points. This finding further confirms the efficient antimicrobial properties of CH. Antimicrobial properties of CH are attributed to its alkaline pH and the ability of this medicament to degrade cytoplasmic membrane [23]. Despite the limitations of CH, it has suitable biological properties. This medicament neutralizes the bacterial lipopolysaccharides and their resorption activity, and also induces the hard tissue formation [30]. Previous studies confirmed the antimicrobial effects of CH [4,7,15,31]. Nevertheless, some studies have reported that *E. faecalis* and *Candida albicans* are resistant to CH [32,33]. Ballal et al. compared the antimicrobial activity of CH paste, 2% CHX gel, and their combination against *E. faecalis* and *Candida albicans*. They stated that 2% CHX gel was more effective than CH or mixture of both medicaments at 72 hours. However, CH showed higher efficacy at the first 24 hours against *Candida albicans* and its effect was reduced after 72 hours on both microorganisms [17]. In a study by Delgado et al, the number of *E. faecalis* CFUs was markedly reduced in CH and CHX groups in comparison to the control group. However, CHX was more potent than CH [4]. Nonetheless, in the current study, no statistically significant differences were detected in antifungal efficacy of CH and CHX at all time periods.

Some studies have suggested the combination of CH and CHX [7,17,27,34,35]. Gomes et al. reported that the combination of CH and CHX yielded higher antimicrobial efficacy compared to the combination of CH and sterile water [35]. But, the antimicrobial activity of the mixture of CH and CHX was weaker than that of CHX gel alone.

This result was achieved in many other studies as well [7,17].

A shortcoming of the current study is its experimental nature and that the results cannot be generalized to the clinical setting. Additionally, this study was performed on one fungal strain and did not investigate other fungal species.

## CONCLUSION

Within the limitations of the current study, the antifungal efficacy of CH and 2% CHX gels was significantly higher than that of nanosilver gel in all samples at the tested time points. Furthermore, no significant differences were noted between CH and 2% CHX gel in CFUs at different time points. Accordingly, these gels are equally effective against *Candida albicans* and have suitable antifungal properties.

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