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In Vitro Comparisons of Minimal Inhibitory Concentrations between NaOCL, CHX, MTAD and EDTA against *Candida Albicans*

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Abstract

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX, MTAD, and EDTA against *Candida albicans (C. albicans).*

Methods and Results: Certified strain of *C. albicans* (ATCC 10231 OXOID, Hampshire, UK) was used to determine the MIC of 3% NaOCl, 2% CHX, MTAD, and EDTA in vitro. The broth dilution method was used to determine the MIC and to ensure the test was highly accurate. *C. albicans* and respective irrigants were gradually placed into the appropriate test tubes, starting from 1 mL to 0.06 mL of irrigant (getting halved each time). The test tubes were incubated at 37°C for 24 h. MIC was then recorded as the lowest concentration of irrigant that inhibited microbial growth, based on based on spectrophotometry. Our study showed that some of the tested irrigants retained an antifungal effect after dilution, which is valuable because dilution reduces toxicity. 3% NaOCl has an efficient antifungal effect against *C. albicans* both at full concentration and when diluted fivefold. The antifungal effect of 2% CHX for *C. albicans* cultures increases with its dilution. MTAD retains a good antifungal effect even when diluted fivefold.**(International Journal of Biomedicine. 2023;13(4):345-349.)**

Keywords: *Candida albicans* • minimal inhibitory concentration • MTAD • CHX • NaOCl • EDTA

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Introduction

Microorganisms can be a major cause of pulp and periapical pathosis.[1-3] To avoid the formation of periapical lesions, endodontic techniques such as cleaning, shaping root canals, and sealing the entire root canal are preventive measures for fluids entering into the root canal that serve as nutrients for the remaining microbes within the canals.[4,5]

Candida albicans (C. albicans) is an opportunistic pathogen but also a part of the human microbiota and is present in different areas, such as the oral cavity, skin, gastrointestinal tract, and vagina.[6] The root canal system is frequently subjected to microorganisms' colonization, spreading into infections and then persistent inflammation. Different studies have found numerous species to be responsible for causing apical periodontitis, but highlighted *C. albicans* as the most frequently isolated one.[7] In vulnerable individuals, when conditions are favorable, *C. albicans* prevalently behaves as a pathogen, causing oral candidal infections and complications with any other endodontic treatments.[8] *C. albicans* plays an important role in endodontic treatment failure; therefore, prompt intervention is important to eradicate the infection. Not many remedies for oral diseases caused by *C. albicans* have been discovered; hence, it is necessary to find new compounds.[9]

As established, fungi are the cause of failures of root canal treatment and are more common in secondary endodontic infections.[10] One-third of people have fungi as their normal flora, but sometimes *C. albicans* is one of the most frequent causes of oral infections.[11] Approximately 30%-45% of healthy adults carry this fungus; meanwhile, in patients with human immunodeficiency virus, this rate is at 95%, meaning

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that in patients with an impaired immune system, this species can expand.[12-14]

To date, NaOCl, EDTA, CHX, and MTAD are available as irrigant solutions for endodontic use, but each one presents advantages and disadvantages. Sodium hypochlorite (NaOCl) is an efficient disinfection organic solvent, commonly used by dentists for cleaning root canals, that causes dentinal degeneration through collagen dissolution; however, it cannot remove the smear layer.[15] Chlorhexidine (CHX) exhibits antimicrobial activity and biocompatibility but has no tissue-dissolving capabilities.[16] MTAD combines antibiotics (doxycycline), chelator (citric acid), and detergent (Tween-80). The citric acid chelator contributes to smear layer removal, allowing doxycycline to penetrate dentinal tubules with opened orifices due to the detergent effect.[17] Ethylenediaminetetraacetic acid (EDTA) is a neutral or slightly alkaline solution used as an irrigant. It is prevalently used for smear layer removal.[18] Although little is known about its antibacterial efficacy, some authors have described its strong effect on removing *C. albicans* in root canals.[19]

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX, MTAD, and EDTA against *C. albicans.*

Materials and Methods

Candida strain

In this study, *C. albicans* (ATCC 10231 OXOID, Hampshire, UK) was used to determine the antimicrobial effect of irrigants *in vitro*. *C. albicans* was grown in a culture medium prepared according to the manufacturer›s guidelines. The strain suspension was transferred into Sabouraud dextrose and incubated overnight at 30°C. The colonies were then isolated and maintained by weekly reinoculations in the same media.

Standardization of microorganisms

Brain heart infusion broth (BHI-Oxoid LTD., Hampshire, UK) was inoculated with *C. albicans* and incubated for 6–7 h at 37°C to achieve a mean optical density of 0.5 McFarland (approximately equal to 1.5×10^{8} CFU/mL). Then, 1 mL aliquots of each suspension culture were transferred to the required number of sterile tubes. All procedures were performed using sterilized instruments and reagents.

Selection of irrigants

The following irrigants were used:

- 3% NaOCl (ChlorcID, Ultradent Products, Inc. South Jordan, UT, USA)
- 2% CHX (Consepsis, Ultradent Products, Inc. South Jordan, UT, USA)
- MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA)
- 17% EDTA (CALASEPT EDTA, Nordiska Dental, Ängelholm, Sweden)

Determination of the minimal inhibitory concentration

MIC value represented the lowest concentration that inhibited 100% of the fungal growth and was determined by the minimum concentration of a respective irrigant. Therefore, the MIC was used as a reference criterion for the susceptibility of the microorganisms to the compound. The broth dilution method was used to determine the MIC and to ensure the test was highly accurate. C. albicans and respective irrigants were gradually placed into the appropriate test tubes, starting from 1 mL to 0.06 mL of irrigant (getting halved each time). The test tubes were incubated at 37°C for 24 h. MIC was then recorded as the lowest concentration of irrigant that inhibited microbial growth, based on spectrometry at the wavelength of 540 nm (Smart-CCD Spectrophotometer).

Statistical analysis was performed using *statistical software* package *SPSS version 20.0* (*SPSS* Inc, Armonk, NY: IBM Corp).

Results

Table 1 shows MICs and standard deviations (SD) of tested materials according to absorbance rate. The higher the value of the absorbance rate, the smaller the antimicrobial effectiveness of the tested substance. By diluting the substances, their antimicrobial activity was reduced.

The higher the value of the purity percentage, the greater the antimicrobial effect (Table 2). By diluting the substances, their antifungal activity was reduced. The antifungal effect of 3% NaOCl decreased with its increasing dilution: the absorbance increased from 0.16±0.01 (at 1mL dilution) to 0.58±0.005 (at 0.06 mL dilution); similarly, purity decreased from 70.1±1.223 (at 1mL dilution) to 26.45±3.13 (at 0.06 mL dilution) (Figure 1).

Table 1.

MICs of tested materials according to the absorbance rate.

Irrigants	1000 uL		500 uL		250 uL		125 uL		60 uL	
	x̄	SD	x̄	SD	x̄	SD	x̄	SD	x̄	SD
3% NaOCl	0.16	0.01	0.17	0.01	0.39	0.02	0.61	0.06	0.58	0.05
2% CHX	0.62	0.01	0.63	0.01	0.65	0.15	0.18	0.001	0.04	0.003
MTAD	0.04	0.001	0.08	0.01	0.31	0.01	0.32	0.003	0.35	0.001
17% EDTA	0.19	0.01	0.21	0.01	0.49	0.01	0.56	0.01	0.62	0.01

Table 2.

MICs of tested materials according to the purity percentage.

Irrigants	1000 uL		500 uL		250 uL		125 uL		60 uL	
	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD
3% NaOCl	70.11	1.22	63.44	2.04	41.53	1.62	24.63	3.23	26.45	3.13
2% CHX	23.68	0.86	23.32	0.77	22.95	8.35	66.89	1.59	93.33	5.44
MTAD	99.18	0.00	82.67	0.57	48.74	1.16	48.24	0.46	44.52	0.03
17% EDTA	63.87	1.75	62.46	0.12	32.19	0.60	27.81	1.04	24.21	1.00

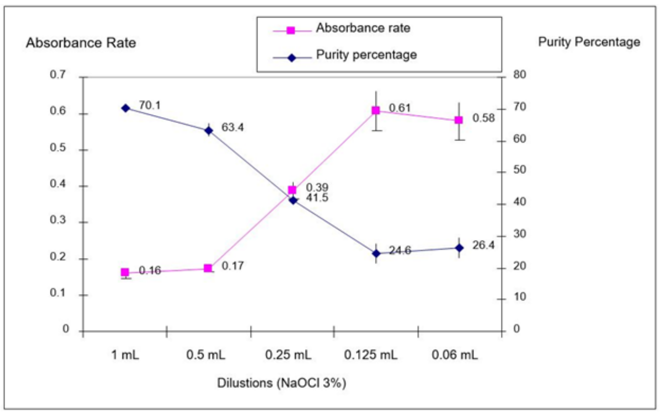

Fig. 1. *MIC of 3% NaOCl against C. albicans.*

The antifungal effect of 2% CHX for *C. albicans* cultures increased with its dilution: the absorbance decreased from 0.62±0.01 (at 1mL dilution) to 0.04±0.003 (at 0.06 mL dilution); similarly, purity increased from 23.7±0.86 (at 1mL dilution) to 93.3±5.44 (at 0.06 mL dilution) (Figure 2).

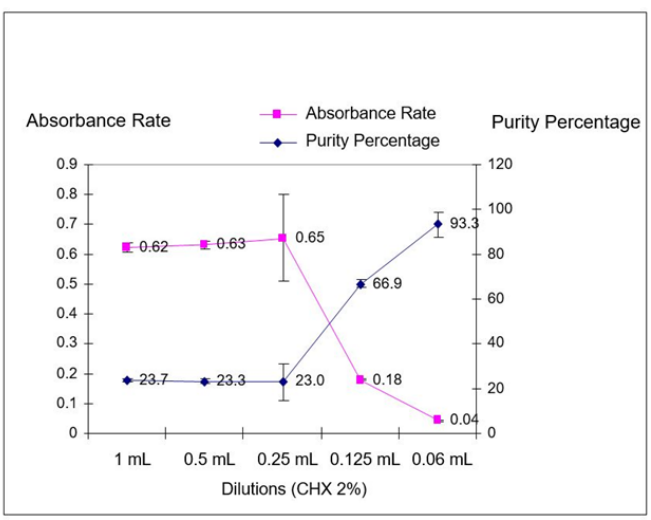

Fig. 2. *MIC of 2% CHX against C. albicans.*

The antifungal effect of MTAD exhibited variable outcomes, with a general tendency to decrease with increasing dilution: absorbance increased from 0.04±0.001 (at 1mL dilution) to 0.35±0.001 (at 0.06 mL dilution); similarly, purity decreased from 99.2±0.0 (at 1mL dilution) to 44.5±0.03 (at 0.06 mL dilution) (Figure 3). Notably, the antifungal effect of 17% EDTA decreased as it was diluted: absorbance increased from 0.19±0.01 (at 1mL dilution) to 0.62±0.001(at 0.06 mL dilution), while purity decreased from 63.9±1.75 (at 1mL dilution) to 24.21±1.0 (at 0.06 mL dilution) (Figure 4).

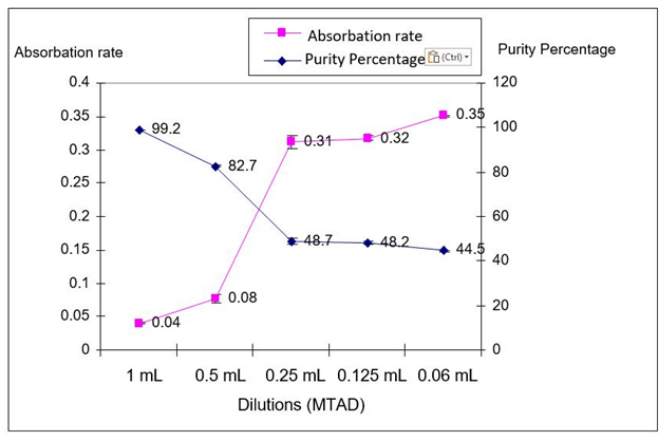

Fig. 3. *MIC of MTAD against C. albicans.*

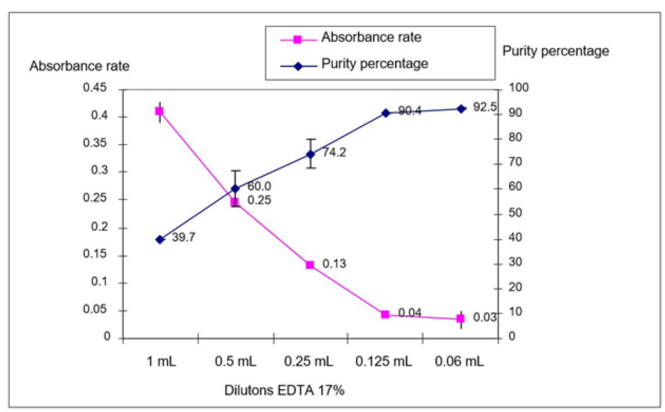

Fig. 4. *MIC of 17% EDTA against C. albicans.*

Discussion

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX, MTAD, and EDTA against *C. albicans*. *C. albicans* was selected because it is the most dominant fungal species in persistent endodontic infections. ATCC 10212 strain is a commonly used quality control for *in vitro* antifungal studies.[20]

Our study showed that some of the tested irrigants retained an antifungal effect after dilution, which is valuable because dilution reduces toxicity. Notably, a fivefold dilution of 3% NaOCl caused purity to decrease from 70.11% to 26.45%. Antifungal activity for NaOCl remained with 41% for 0.25 mL dilution. Regarding the efficacy of 3% NaOCl against *Candida*, it has been shown that its dilution reduces the antifungal effect. Until dilution of 3% NaOCl three times, the irrigant had a good antifungal effect against *C. albicans*, which meant that the MIC of NaOCl against *C. albicans* was 0.7%.

Antifungal activity for CHX increased from 23% at 1000 uL to 93% after dilution at 60 uL. Similarly, diluting CHX increased its antifungal effect. Meanwhile, the antifungal effect of MTAD was decreased from 99% to 44% after being diluted five-times. This result still showed that MTAD had quite a good effect even after diluting it five times. EDTA 17% showed lower antifungal activity after being diluted four times, from 69% to 24%. EDTA is a substance with a very good chelating effect, is biocompatible with periapical tissue, and has an antifungal effect against *C. albicans*.[21] In other studies, in which an agar diffusion method was used, EDTA 17% has shown the best antifungal effect compared to other antifungal drugs and test solutions.[22]

NaOCl is a disinfecting and oxidizing agent with bactericidal, virucidal, and fungicidal activity. Due to its antimicrobial and tissue-dissolving properties, it is commonly used by dentists for cleaning root canals.[15] Although it has a positive antimicrobial effect, the quantity could provoke NaOCl extrusion with the consequent bleeding or other severe symptoms in the patients.[23] Nevertheless, there is a lack of agreement about the exact concentration of NaOCl to use. Moreover, its efficacy is strongly associated with the volume and frequency of irrigation.[24] In relation to these factors, NaOCl is essential for the effective shaping and cleaning of root canals, for its proteolytic and dissolution capacity, and for its debridement properties. Hence, it remains a common compound on which studies regarding its exact concentration were necessary.[25]

Meanwhile, CHX is a disinfectant and antiseptic compound used for multiple reasons, including dental use. CHX acts as an antibacterial thanks to its chemical structure, for which its positively charged lipophilic/hydrophobic form interacts with phospholipids and lipopolysaccharides of the bacterial cell membrane and consequently alters their osmotic equilibrium.[16] Regarding its antifungal activity, numerous studies help to assess and encourage its use, especially against *C. albicans*. Moreover, some studies have also highlighted its use as a substitute for NaOCl for its lower cytotoxicity and its efficient clinical performance.[26]

MTAD is an aqueous solution constituted of 3% antibiotics doxycycline, 4.25% of the demineralizing agent citric acid, and another small percentage of TWEEN 80 detergent. Its composition makes possible its solubilization in water.[17] MTAD is commonly used as an endodontic irrigant with antibacterial activity and for its ability to remove the smear layer. Not many studies have been made about its antifungal activity, but it is frequently used in combination with NaOCl to increase its efficacy.[27] As for EDTA, although little is known about its antibacterial efficacy, some authors have described its strong effect on the removal of *C. albicans* in root canals.[19] EDTA acts by reducing colonization and growth of *C. albicans*, interfering with its morphogenesis and adherence capacity.[22]

Based on the best antifungal effect, the test substances are sorted according to the size of the inhibition zone: 3% NaOCl > 2% CHX > MTAD > 17% EDTA. Our data are consistent with those of other authors who have evaluated the antimicrobial effect of CHX and have compared it to the effect of NaOCl. CHX 2% gel and solution and NaOCl 0.5%-5.25% have eliminated *C. albicans*.[28,29] Despite that, our study had limitations. The main limitation of this *in vitro* study was that the findings might not be representative of *in vivo* outcomes; therefore, additional *in vitro* and *in vivo* studies are needed to confirm the apparent antibacterial activities and MICs of these irrigants against *C. albicans* to support their clinical applications.

In conclusion, the results of this study indicate that 3% NaOCl has an efficient antifungal effect against *C. albicans* both at full concentration and when diluted fivefold. The antifungal effect of 2% CHX for *C. albicans* cultures increases with its dilution. MTAD retains a good antifungal effect even when diluted fivefold.

Competing Interests

The authors declare that they have no competing interests.

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