Effectiveness of a 2% chlorhexidine solution mixed with calcium hydroxide against Candida albicans

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Abstract
The purpose of this investigation was to examine the in vitro susceptibility of Candida albicans to a mixture of chlorhexidine and calcium hydroxide using a tube dilution test. A suspension containing C. albicans was exposed to 2% chlorhexidine solution, calcium hydroxide paste and 2% chlorhexidine mixed with calcium hydroxide in plastic tissue culture wells. The tested agents were incubated with C. albicans for 1, 24, or 72 h. All agents were effective antifungals at all experimental time periods tested, with the exception of exposure to calcium hydroxide for 1 h. A mixture of 2% chlorhexidine and calcium hydroxide therefore is a very effective antifungal agent against C. albicans.

Introduction
The presence of Candida albicans in the infected pulp and periradicular areas has been demonstrated through the use of light and electron microscopy, as well as using culture techniques (1–4). The incidence of C. albicans in infected root canals has been shown to vary between 7% and 55% (5,6) and occurs in approximately 11.36% of teeth with pulp lesions (7). It has also been reported that C. albicans can use dentin as a nutrient source (8).

Several studies have tested the effect of intracanal irrigants and antiseptic agents on C. albicans, including sodium hypochlorite, chlorhexidine solution and calcium hydroxide paste (9–11). These antiseptic agents have been shown to be very effective against C. albicans. Waltimo et al. (4) evaluated different oral Candida spp., including C. albicans, in the presence calcium hydroxide in vitro. They reported that Candida spp. were resistant to calcium hydroxide and concluded that there is a need for supplementary agents to effectively treat persistent apical periodontitis.

Different vehicles have been added to calcium hydroxide in an attempt to enhance its antimicrobial activity. Estrela et al. (12) tested the anti-fungal effectiveness of different mixtures of calcium hydroxide using saline, polyethylene glycol and camphorated paramonomchlorophenol. They reported complete antifungal effectiveness after 1 h of exposure, irrespective of the vehicle associated with the calcium hydroxide paste.

Chlorhexidine has been suggested to be a useful endodontic irrigant and intracanal antiseptic due to its low toxicity and excellent anti-bacterial and anti-fungal activities (11,13). A combination of chlorhexidine and calcium hydroxide was tested against bacterial species only, and was reported to be effective against Enterococcus faecalis (14,15). It would therefore be of value to determine the effectiveness of this combination of agents against C. albicans.

The purpose of the current study was to examine the in vitro susceptibility of C. albicans to a mixture of chlorhexidine and calcium hydroxide.

Materials and methods
The anti-fungal effect of 2% chlorhexidine solution, calcium hydroxide paste and 2% chlorhexidine mixed with calcium hydroxide was evaluated against C. albicans.

Stock cultures of clinical isolates of C. albicans were provided by the Microbiology Laboratory of King Khalid University Hospital (Riyadh, Saudi Arabia) and maintained in Sabouraud’s dextrose agar plates.

A suspension was prepared by transferring three colonies from a Sabouraud’s dextrose agar plate, using a sterile 4 mm diameter platinum loop, to a 10 mm
Sabouraud infusion broth in a sterilised 10 mL screw capped test tube, followed by incubation for 1 week at 37°C. Two such test tubes were prepared.

**Experimental procedure**

The experiments were performed in plastic tissue culture clusters containing 24 wells, each with an inner diameter of 16 mm. One mL of test material was placed at the bottom of each culture well, to which 1 mL of a Candida suspension was added. At the same time, 1 mL of Sabouraud infusion broth media was mixed with 1 mL of a Candida suspension in a culture well to serve as a positive control. For the negative control, 2 mL of Sabouraud infusion broth was placed in a culture well. Six wells were thus used per test. The culture clusters plates were then incubated at 37°C and evaluated after 1, 24 and 72 h.

At the end of the incubation period, aliquots of 0.1 mL were withdrawn from each well and transferred to tubes containing 5 mL of fresh Sabouraud infusion broth. The tubes were then vortexed, incubated at 37°C, and observed for 7 days. Growth of the fungi was observed daily, as indicated by the presence of turbidity in the tubes. The presence of turbidity was determined, and the purity of the cultures was checked by the morphology of colonies onto blood agar plates. The results were statistically analyzed using the Kruskal–Wallis test.

**Results**

The positive controls, in which there was no putative anti-fungal agent, exhibited growth of C. albicans at all times tested; as expected, no growth occurred in the negative control group. Calcium hydroxide was ineffective at inhibiting the growth of C. albicans after 1 h of exposure. Further, there was no significant difference (P > 0.05) in growth between calcium hydroxide treatment for 1 h and the growth in the positive control. The exposure of C. albicans to calcium hydroxide at 24 and 72 h, however, resulted in complete inhibition of growth. The growth of C. albicans was completely inhibited when exposed to 2% chlorhexidine alone or when mixed with calcium hydroxide at all times tested. Statistical analysis showed no significant difference between growth of C. albicans exposed to 2% chlorhexidine alone or mixed with calcium hydroxide and the negative control (P > 0.05).

**Discussion**

The method used to evaluate inhibition of fungal growth in this study was the dilution tube susceptibility test, which is an effective method to evaluate anti-fungal as well as anti-bacterial properties of any filling material or solution (16,17). This method allows direct contact in the solution between C. albicans and the agent to be tested. The growth of C. albicans observed in the positive controls confirmed the efficiency of the methodology. Two agents, chlorhexidine and calcium hydroxide, were chosen for this study. Chlorhexidine is a broad-spectrum antimicrobial agent that has been reported to be an effective antiseptic in endodontic therapy owing to its unique ability to bind to dentin and its substantivity in the root canal system. It is also effective against strains resistant to calcium hydroxide (18,19).

In a study comparing common endodontic disinfectants, 0.5% chlorhexidine was also significantly more effective at killing C. albicans than calcium hydroxide, 5% and 0.5% sodium hypochlorite and 2% iodine potassium iodide (4). In the present study, a high concentration of chlorhexidine (2%) was used and was found to be effective against C. albicans at all experimental times tested. Similar findings were reported by Sen et al. (11), who found that a 1 h exposure to a concentration of 1200 μg of chlorhexidine/mL was sufficient to eliminate C. albicans from dentinal surfaces with or without the presence of a smear layer.

Recent studies have questioned the efficacy of calcium hydroxide in reducing microbial numbers, even after prolonged contact with the root canal (20). Waltimo et al. (4) reported that C. albicans survived incubation in calcium hydroxide solution for 1 h and was killed after 6 h of incubation. Similar findings were obtained in the current study. Supplementing calcium hydroxide with chlorhexidine may therefore be one way to improve the efficacy of its anti-fungal activity, while also maximising microbial eradication when used as an intracanal dressing. Chlorhexidine, in different concentrations and in combination with calcium hydroxide, maintain its physicochemical properties (i.e. pH, radio-opacity and working time) that allow it to be used as an intracanal antiseptic agent (21,22). In the present investigation, mixing calcium hydroxide with chlorhexidine was found to be very effective against C. albicans at all experimental times tested. The anti-bacterial property of calcium hydroxide depends on its long-lasting alkalinity and has been reported not to be affected by the addition of chlorhexidine (15). Such a combination will result in better diffusion into the dentinal tubules of prepared root canal systems with the potential to be used as a long-term intracanal agent and therefore complete anti-microbial, as well as anti-fungal, effectiveness can be improved.

In conclusion, the results of the present study show the additive benefits achieved by combining calcium hydroxide with chlorhexidine in the treatment of C. albicans.
References