

IN VITRO ANTIFUNGAL ACTION OF DIFFERENT SUBSTANCES OVER MICROWAVED-CURED ACRYLIC RESINS

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ABSTRACT

Objective: The presence of *Candida albicans* on the surfaces of denture-base acrylic resins is strongly related to the development of oral stomatitis. This study evaluated the antifungal action of different agents over microwave-cured acrylic resin without polishing specimens previously contaminated with *Candida albicans*. Material and Methods: Sixty specimens were immersed in BHI broth previously inoculated with the yeast and stored for 3 h at 37°C. They were divided into 5 experimental groups (n=10): G1: 2% chlorhexidine solution (10 min); G2: 0.5% sodium hypochlorite (10 min); G3: modified sodium hypochlorite (10 min); G4: effervescent agent (5 min); G5: hydrogen peroxide 10v (30 min). The specimens of the control group 1 (C1) were not disinfected. Ten additional specimens of the control group 2 (C2) were not infected with the yeast, aiming to check the asepsis during the experiment. The disinfection agents were neutralized and the acrylic resin specimens were immersed in BHI Broth for 24 h. Culture media turbidity was evaluated spectrophotometrically according to the transmittance degree, i.e. the higher the transmittance the stronger the antimicrobial action. Statistical analysis was performed (Kruskal-Wallis Test, p<0.05). Results: The results, represented by the medians, were: G1 = 40; G2 = 100; G3 = 100; G4 = 90; G5 = 100; C1 = 40; C2 = 100. Conclusions: This *in vitro* study suggested that sodium hypochlorite-based substances and hydrogen peroxide are more efficient disinfectants against *C. albicans* than 2% chlorhexidine solution and the effervescent agent.

Key Words: *Candida albicans*. Denture cleaners. Acrylic resin.

INTRODUCTION

The presence of bacterial plaque and fungi are the main etiological factors of denture-induced stomatitis¹⁹. According to Ramage²⁰, 11% to 67% of complete denture users present candidiasis. Denture-induced stomatitis is characterized by the alteration of tissues under dentures, such as the presence of bright red colored areas and wrinkled surfaces¹². Poor hygiene favors *Candida albicans* infection. Therefore, indication of denture cleansing is of paramount importance²³.

The inner surface of dentures acts as a *C. albicans* reservoir, which is responsible for the beginning, progression and maintenance of the infection. Measures for controlling the colonization by this fungus may be adopted by the users of dentures^{12,22}. A routine of cleaning may be instituted to

prevent and remove the accumulation of microorganisms and to remove mucine, food remains, calculus and stains.

Denture cleaning may be achieved mechanically by brushing, chemically by use of chemical agents or by the association of both methods. Mc Callum, et al.¹⁵ affirmed that it is very difficult for dentists to recommend some sort of cleanser to their patients. Hence, patients themselves choose the product they will use without information about their benefits or risks. It is also important to point out that the cleaning agents employed must have the capacity to dissolve organic deposits, must not be toxic or irritate the mucosa, must be stable for storage, preferentially bactericidal and fungicide and must be harmless for the denture^{1,11}.

Cleaning agents for dentures are classified as abrasive

or chemical cleansers. Among the chemical agents, alkaline hypochlorites, peroxides and diluted acids may be cited¹⁶. Budtz-Jorgensen⁵ described the use of chlorhexidine for disinfection of dentures and for the treatment of candidiasis. Hypochlorite agents dissolve organic material, calculus and mucine, disinfect dentures and are good for stain removal. On the other hand, these agents are corrosive to metals and may be harmful for dentures when these are immersed in it for long periods²¹.

Alkaline peroxides, when dissolved in water, become alkaline hydrogen peroxide solutions. The degradation of peroxide liberates oxygen bubbles that mechanically clean dentures when in contact with debris. Such peroxides are recommended for the removal of mucine present in saliva and food remains and can also prevent the formation of stains and calculus³. They can be used as antimicrobial agents as well. However, they present the adverse effect of bleaching plastic materials.

Chlorhexidine was developed during the 1940s as the result of a research that intended to seek an antiviral agent but rather observed a remarkable antibacterial effect⁹. Characterized as a strong base, it is commonly prepared as the digluconate salt that confers to it a greater stability and water solubility. The use of chlorhexidine seems to be beneficial to patients with denture-induced stomatitis, but its continuous use causes the discoloration of acrylic resin and, therefore, should not be indicated for daily use⁶.

Since the chemical method for disinfection of dentures is widely used by patients^{3,16} and as it is one of the processes for the treatment of candidiasis, this study was designed to evaluate the antifungal action of 5 denture cleaning chemical agents.

MATERIAL AND METHODS

Sixty specimens were fabricated with a heat-cured resin (Vipi Wave®; VIPI, Pirassununga, SP, Brazil) according to manufacturer recommendations. Matrices (10 x 10 x 5 mm) were produced with a condensation silicone impression material (Zetalabor® (Zhermack S.p.A., Rovigo, Italy). These matrices were invested in flasks, pressed and, after plaster setting, the matrices were removed and casts were obtained. The casts were coated with a thin layer of acrylic separating film (Cel-Lac®; S. S. White Artigos Dentários, Rio de Janeiro, RJ, Brazil), and were filled with plastic stage acrylic resin. The flasks were pressed slowly with a hydraulic presser until the excess material extruded from the borders, and were then opened for complete removal of the excess material. The flasks were again closed, taken to the hydraulic presser and pressed until both halves were again in contact, and then screwed. Polymerization procedures were performed, according to the cycle described by the manufacturer, which was of 20 min with at 180W power output and 5 min with a 540W power output. The specimens were removed after opening the flasks. No finishing and polishing procedures were done in order to simulate the inner surface of a complete denture. Only remaining excesses were

removed with the aid of 320-grit wet sandpaper.

The specimens were immersed in distilled water in order to promote the maximum water sorption to prevent, when in culture, the occurrence of distortion and the release of residual monomer after polymerization⁴.

Pure cultures of *C. albicans* were grown on Agar Sabouraud plates (Oxoid, Basingstoke, UK) containing 500 mMol/L of sucrose at 25°C. After 24 h, the colonies were suspended in tubes containing 5 mL of brain heart infusion (BHI) broth (Oxoid). The cell suspension in each tube was adjusted spectrophotometrically at 800 nm (O.D.800) to match the transmittance of 90 T (equivalent to 0.5 McFarland scale = 1.5×10^8 cfu). The *C. albicans* (R-68) strain was obtained from the Laboratory of Mycological research (LAPEMI) of the Department of Microbiology and Parasitology of the Federal University of Santa Maria, Brazil.

Next, the specimens were placed into the tubes containing BHI plus inoculum and remained for 11 h at 37°C in order to favor an initial colonization of the acrylic resin surfaces. The 60 specimens were distributed in 5 test groups and 2 control groups according to the disinfecting treatment to which they were subjected.

Each specimen was first washed with saline after immersion in the contaminated culture broth. Saline excess was removed with a gentle compression of sterile gauze. Then, the disinfection step was performed as follows: a) Control Group 1 (C1) - contaminated specimens, exposed to saline solution for 10 min; b) Control Group 2 (C2) - specimens without contamination, exposed to saline solution for 10 min; c) Group 1 (G1) - 2% chlorhexidine solution for 10 min; d) Group 2 (G2) - 0.5% sodium hypochlorite solution for 10 min; e) Group 3 (G3) - modified hypochlorite (50% v/v 0.5% hypochlorite solution and 96° GL alcohol) for 10 min; f) Group 4 (G4) - Corega Tabs (GlaxoSmithKline, Brentford, United Kingdom) for 5 min; g) Group 5 (G5) - 10 v hydrogen peroxide for 30 min.

Each specimen was then washed again with saline and the excess was removed with sterile gauze. It was then transferred to individual tubes containing 5 mL of BHI broth + neutralizers, in order to prevent continued action of the disinfectants tested. The neutralizer for NaOCl was 0.6% sodium thiosulfate, while Tween 80 plus 0.07% lecithin was used for chlorhexidine. After 24 h of incubation, the tubes were individually transferred to a spectrophotometer at 530 nm wavelength in order to measure the turbidity degree, through the transmittance. Aliquots of 10 iL of each tube were then collected and inoculated into Agar Sabouraud plates (Oxoid) containing 500 mMol/L of sucrose, which were incubated for 24 h at 37°C, in order to check microbial growth. The purity of the positive cultures was confirmed by Gram staining, by colony morphology on agar plates and by the use of biochemical identification kits (API 20 C Aux, BioMérieux, Marcy l'Étoile, France, for yeast identification).

The results obtained were statistically treated using the software SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA) using the Kruskal-Wallis test, with significance level set at $p < 0.05$.

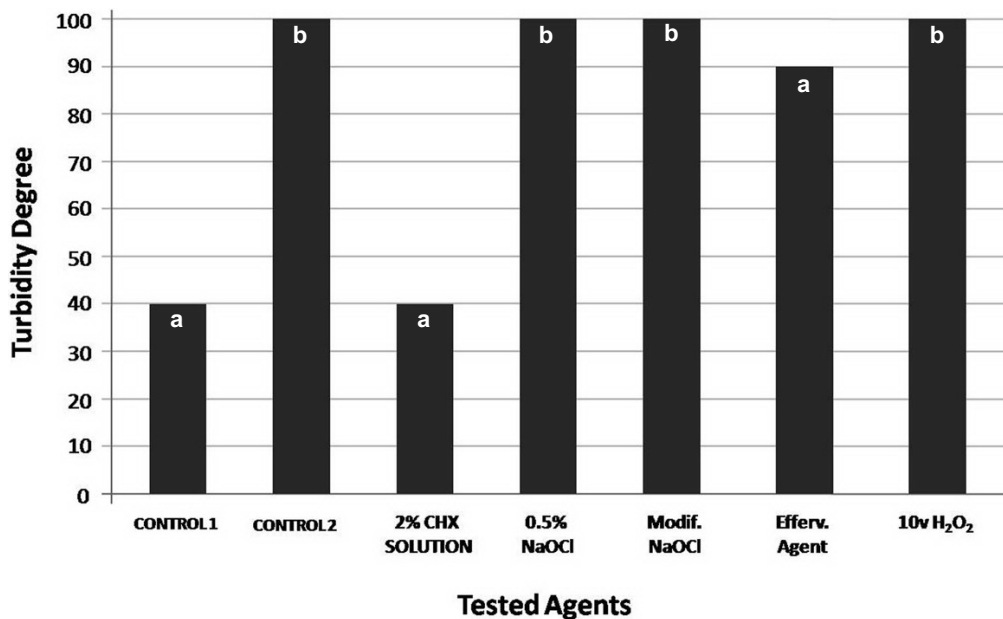


FIGURE 1- Medians of the turbidity degrees for all tested groups (n=10). Different letters mean statistically significant difference

RESULTS

Figure 1 shows the antimicrobial activity of each chemical agent. The greater the transmittance values the better the antimicrobial action. The results of the spectrophotometer measurements were represented by medians and minimum and maximum values, as follows: G1 – 2% chlorhexidine solution = 40 (minimum = 60, maximum = 20); G2 – 0.5% sodium hypochlorite = 100 (minimum = 100, maximum = 80); G3 – Modified sodium hypochlorite = 100 (minimum = 100, maximum = 90); G4 – effervescent agent = 90 (minimum = 100, maximum = 60); G5 – 10 volumes hydrogen peroxide = 100 (minimum = 100, maximum = 80); C1 – specimens without disinfecting agents = 40; C2 – specimens without contamination = 100.

Microbial growth and the purity of the growth were checked after 24 h by inoculating 10 µL of the suspension medium in agar plates to certify that the transmittance values achieved were due to the *Candida* growth and not to any potential contamination during the procedures. Chlorhexidine solution and the non-disinfected specimens did not inhibit the growth of the target microorganism, as observed by the microbial growth on the agar plates.

DISCUSSION

Oral environment temperature and the acquired pellicle formed over dentures promote *Candida albicans* adhesion to resin materials, indicating the need of an adequate plaque control for maintaining oral health¹⁸. Chandra, et al.⁷ showed that the development of this yeast on acrylic resins happens in three distinct stages: initial stage – up to 11 h from the colonization, when some micro-colonies begin to be formed; intermediate stage – from 12 to 30 h after colonization, when extracellular material begins to accumulate over colonies;

and maturation stage – from 38 to 72 h after colonization, when *Candida* colonies become totally involved by the extracellular matrix forming a biofilm. They also concluded that antifungal resistance increases during biofilm development, as the extracellular matrix acts as a barrier to the action of the antifungal.

In the present study, the 0.5% hypochlorite and the modified hypochlorite solution were able to eliminate *C. albicans* from the resin surface. Several studies showed that 0.5% sodium hypochlorite solution had excellent results regarding *C. albicans* removal^{2,6,8,14,16,21}. According to Chau, et al.⁸, a 10-min immersion in 5.25% sodium hypochlorite solution is effective for the disinfections of both the external and internal surfaces of the acrylic resin. However, hypochlorite use must be recommended only once a week as it bleaches and damages acrylic resin⁶. Further studies are necessary to elucidate the action of the modified sodium hypochlorite and to determine its effect on acrylic resins.

Hydrogen peroxide agents promote oxidation and also liberate oxygen during their degradation, with simultaneous chemical and mechanical actions. The peroxide products used in this research showed different results. The 10 V hydrogen peroxide was efficient when used during 30 min. On the other hand, Corega Tabs™, used during 5 min, as recommended by the manufacturer, did not show the same efficiency. The manufacture's information suggests that plaque remaining after the immersion period recommended is not enough for stomatitis development²¹. However, according to literature, these products are ineffective during short immersion periods¹⁷. Budtz-Jorgensen⁶ related the advantages of effervescent products to the fact that they are safe and do not damage acrylic resin even when constantly used.

Iacopino, et al.¹² reported that chlorhexidine is highly effective against denture-induced stomatitis. Its effect as mouth rinsing agent during the treatment can be observed

in the oral mucosa covered by the prosthetic device, allowing a better tissue conditioning and healing. Chlorhexidine destroys bacteria by breaking their membrane and inducing cytoplasmic precipitation^{10,13}. The chlorhexidine cationic molecule is capable of interacting with inorganic human dentine particles and also bonds to negatively charged surfaces, such as the bacterial cellular wall. In the present study, this substance was not effective against *Candida albicans*. This lack of antimicrobial action might be justified by the concentrations and immersion periods used. Pavarina, et al.¹⁹ recommend the use of 4% chlorhexidine for 10 min to disinfect complete dentures. Moreover, alike the substrates that chlorhexidine can bind, acrylic resin is an organic compound of non-ionic charged particles. Then, higher concentrations of chlorhexidine for disinfecting prosthetic devices and its topic effect as mouth rinse could act simultaneously for best treating stomatitis associated with fungi.

Chemical methods may be recommended for patients with candidiasis to complement the cleaning of acrylic resin dentures¹¹. The results of the present study suggested 0.5% sodium hypochlorite- or 10 V hydrogen peroxide-based products as disinfecting agents for dentures because they are affordable and show adequate antifungal action.

The knowledge of denture cleansing techniques is important to dental surgeons, as there are several solutions available on the market promising to clean and disinfect dentures in short periods. The dentist should be able to suggest an efficient method that does not damage the denture material and is safe for the patient²³.

CONCLUSIONS

Based on the results, it may be concluded that the tested agents containing sodium hypochlorite or 10 V hydrogen peroxide in their composition showed excellent antifungal action. The effervescent agent used according to the manufacturer's instructions was not effective in removing *C. albicans* colonies. Moreover, 2% chlorhexidine solution was not effective up to 10 min immersion.

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