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RESEARCH ARTICLE

Comparative In Vitro Study of the Effect of Chlorhexidine and Sodium Hypochlorite on the Colonization of Delayed-Setting Resin by Candida Albicans

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ABSTRACT

Objectives: **The aim of this study was to test the efficacy of aseptic solutions based on chlorhexidine, a combination of chlorhexidine and cetylpyridinium chloride, and sodium hypochlorite on the colonization of delayed-setting resins by Candida albicans, and to establish a prosthetic hygiene protocol.**

Materials and Methods: **100 samples were made from acrylic resin, standardized to 40 mm × 12 mm × 3 mm, and relined with Kerr® FITT late-cure resin. These were contaminated with a solution containing Candida albicans ATCC 10231, then divided into four groups: (group 1 control), (group 2 Chlorhexidine 0.2%), (group 3 Chlorhexidine 0.12% + cetylpyridinium chloride 0.5%) and (group 4 NaOCl 0.5%). After soaking in the aseptic solutions, the number of CFU/mm^2 was determined.**

Results: **The results of this experiment demonstrated the sensitivity of Candida albicans to 0.5% sodium hypochlorite, 0.2% chlorhexidine and 0.12% chlorhexidine in combination with cetylpyridinium chloride, as well as to 0.008 CFU/mm^2, 0.0226 CFU/mm^2 and 0.0372 CFU/mm^2, respectively.**

Conclusion: **The 0.12% chlorhexidine formulation combined with 0.05% cetylpyridinium chloride provides the clinical efficacy of chlorhexidine while avoiding adverse effects, and therefore appears to be the best compromise between efficacy and absence of toxicity.**

Keywords: Candida Albicans, chlorhexidine, colonization, resin.

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1. Introduction

The oral environment is a highly favorable environment for the multiplication of micro-organisms (available nutrients, neutral pH, temperature around 37°C, humidity, and etc.). Tissue conditioners are known to be easily colonized and infected by microorganisms, in particular the yeast Candida albicans. The presence of this yeast on the prosthetic surface is considered to be one of the major causes of stomatitis. Candida albicans is a yeast that belongs to the commensal flora of healthy individuals, but which, when the delicate balance between parasite and host is disrupted, becomes opportunistic and colonizes mucocutaneous surfaces and oral and gastrointestinal cavities. Candida albicans is the pathogen most frequently implicated in the development of pathologies in patients with removable prostheses, and is isolated in increased numbers from affected sites. Tissue conditioning is an essential prerequisite for the production of a complete prosthesis.

Among other things, it uses a delayed-setting resin to fill the intrados of a used or transitional prosthesis. The aim of this stage of prosthetic treatment is to place the patient in a situation conducive to the development of a new prosthesis, by improving the histological, anatomical and physiological structures of the tissues in contact with the prosthesis. Delayed-setting resins have poor mechanical properties and high surface porosity caused by the progressive loss of certain components and the incorporation of air during preparation.

Their porous texture poses two interrelated problems:

1. Their porous surface condition, due to material ageing, facilitates greater microbial invasion than that of polymerized acrylic resins.

2. Mechanical cleaning is difficult and inefficient [1]. These two problems encourage microbial proliferation and expose the material and mucous membranes to the risk of fungal outbreaks.

This is the basis of this work, which aims to develop a prosthetic hygiene protocol based on the application of aseptic solutions. The aim of the present in vitro study is to test the action of Chlorhexidine in comparison with Chlorhexidine-based soaking solutions in association with Cetylpyridinium Chloride and sodium hypochlorite-based solutions on the invasion of Candida albicans-type yeasts on the delayed-setting resin.

2. Materials and Methods

The in vitro experimental study was carried out in the oral biology laboratory of the Faculty of Dentistry, Casablanca.

2.1. Materials

2.1.1. Biological Material

A reference strain of Candida albicans ATCC 10231 was used for the experiment. The culture medium comprises Sabouraud's Chloramphenicol Gelose (BIOKAR®) and Sabouraud broth (BIOKAR®). Sabouraud agar is a medium for the growth and isolation of a wide variety of yeasts and moulds. The addition of chloramphenicol inhibits the growth of Gram-positive and Gram-negative bacteria.

The agar consists of 1 liter of medium: Meat peptone: 10.0 g, Glucose: 20.0 g, Chloramphenicol 0.5 g, Bacteriological agar: 15.0 g. The pH of the medium at 25°C is 5.7 ± 0.2.

Sabouraud Dextrose broth is a recommended medium for the detection of Candida albicans in non-sterile products and is also used as a nutrient medium for yeast and mould growth.

The broth is composed of 1 liter of medium: Tryptone: 5.0 g, Meat peptone: 5.0 g, Glucose: 20.0 g. The pH of the medium at 25°C is 5.7 ± 0.2.

2.1.2. Resins

Acrylic resin (TRIPLEX® Ivoclar Vivadent) and Delayed-setting resin, Kerr F.I.T.T. (Functional Impression Tissue Toner) were employed for specimens.

2.1.3. Aseptic Agents

Aseptic agents used are Chlorhexidine (Curasept® 0.2% chlorhexidine), Chlorhexidine combined with cetylpyridinium chloride (Perio-Aid® mouthwash: 0.12% chlorhexidine + 0.05% cetylpyridinium chloride) and Sodium hypochlorite at a concentration of 0.5% obtained according to the following formula:100 ml × C = V ×12° with C = 0.5; V = 100 × C/12 = 100 × 0.5/12; = 4.16 ml.

2.2. Methods

2.2.1. Preparation of Specimens

100 acrylic resin specimens standardized to 40 mm × 12 mm × 3 mm representing the prosthesis base, which will be relined with Kerr® FITT late-cure resin (Fig. 1).

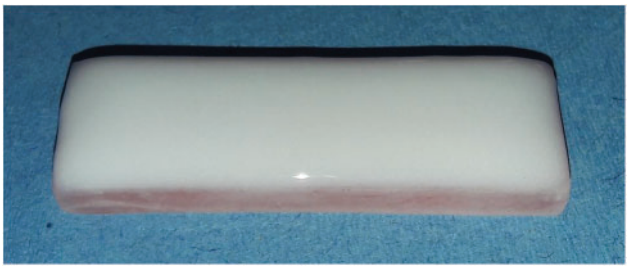

Fig. 1. Acrylic resin sample relined with delayed-setting resin.

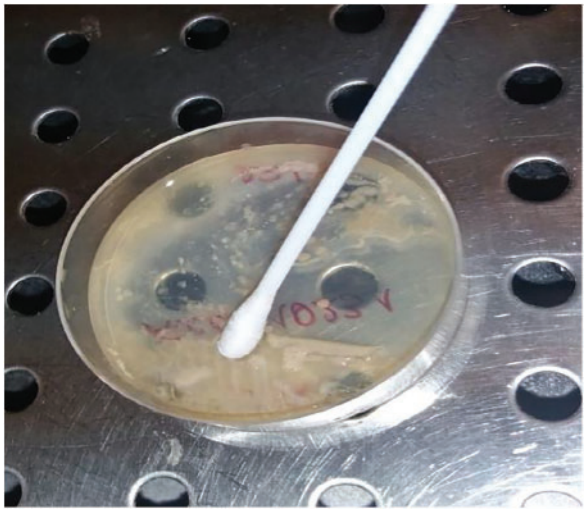

Fig. 2. Candida albicans inoculation.

2.2.2. Preparation of Culture Media

2.2.2.1. Preparation of Sabouraud Broth

30 grams of dehydrated medium is suspended in 1 litre of distilled water. The mixture is stirred slowly until completely dissolved. The resulting broth is divided into vials, autoclaved at 120°C for 15 minutes, and stored at 4°C until use.

2.2.2.2. Preparation of Agar Medium

45.5 grams of dehydrated medium are mixed with 1 litre of distilled water, and the resulting medium is slowly brought to a boil with constant stirring until the powder is completely dissolved.

2.2.3. Preparation of C. albicans

2.2.3.1. Cultivation of C. albicans

A C. albicans colony was picked from a Petri dish containing C. albicans reference strain ATCC 10231 using a sterile swab (Fig. 2).

The cells were then seeded in a tube containing Sabouraud broth, and incubated in an oven at 37°C for 48 hours (Fig. 3).

Growth is demonstrated by turbidity in the medium. The purity of the C. albicans strain is then checked by GRAM staining and optical microscopy. C. albicans growth is monitored by measuring the optical density (O.D.) of cultures at 600 nm using a spectrophotometer. A correspondence between absorbance unit and cell concentration has been determined for C. albicans. C. albicans strain ATCC 10231 is 107UFC/ml 0.257 at 600 nm.

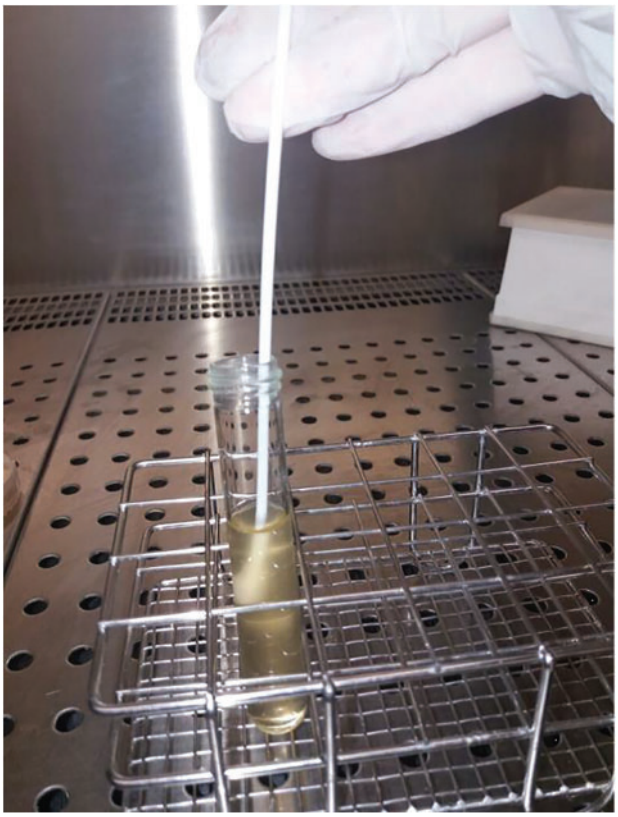

Fig. 3. Inoculate Candida albicans into a tube containing Sabouraud broth.

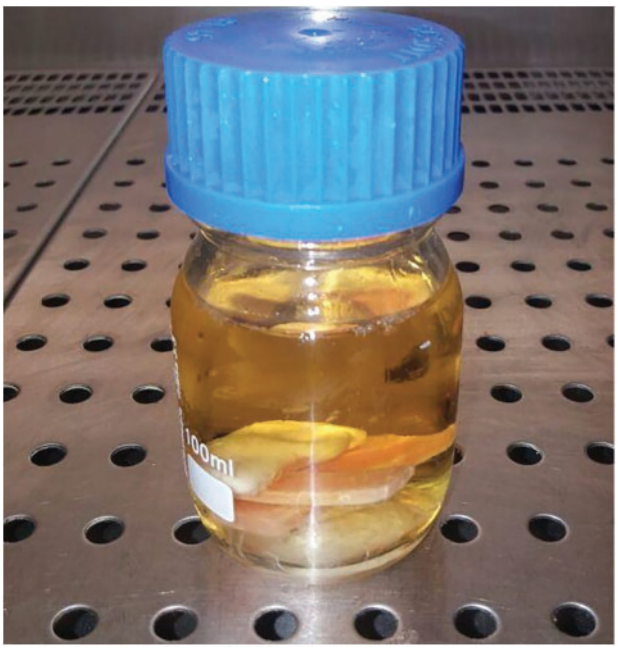

Fig. 4. Incubation of samples in Candida albicans suspension.

2.2.4. *Contamination of Specimens*

The 100 specimens are disinfected with 70°C alcohol and then immersed in a vial containing the prepared C. albicans suspension.

After incubation in an oven at 37°C for 2 hours, the samples are removed using a sterile precelle and rinsed with 10 ml sterile distilled water (Fig. 4).

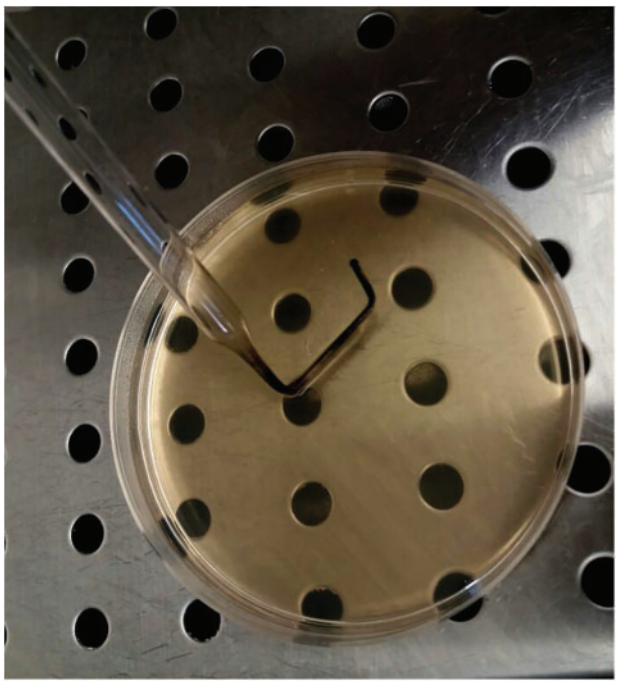

Fig. 5. Inoculate 1 ml of Sabouraud agar in a Petri dish.

2.2.5. *Application of Hygiene Measures*

Contaminated samples were randomly assigned to one of the following disinfection solutions. For the control group, after incubation with C. albicans and rinsing with distilled water, swab the surface of the samples coated with the delayed-setting resin using a sterile swab.

For test groups, after incubation with C. albicans and rinsing with distilled water, samples are soaked in vials containing disinfection solutions for 15 minutes. Each specimen is then rinsed with 10 ml distilled water. 20 ml distilled water is added to Petri dishes.

The surface of the specimens is then scraped with a sterile swab, and the sample is diluted in 20 ml.

Using a pipette, take 1 ml and inoculate into a petri dish containing Sabouraud chloramphenicol agar.

The sample is spread onto the solidified agar using a glass spreader held in contact with the agar, and the Petri dish is rotated to homogenize the surface (Fig. 5).

Incubation of seeded petri dishes in the oven at 37°C for 48 hours. The colonies in each dish were then counted to determine the number of CFU/ml, and from this, the number of CFU/mm^2 in the sample. The surface area of the sample used was 480 mm^2 (Fig. 6).

2.2.6. *Statistical Analysis*

For data processing and analysis, the various variables were cross-tabulated to obtain means with standard deviations, from which we calculated significance levels using SPSS software.

3. Results

For the control group, the mean colony-forming unit value was 634.56 CFU/ml. The ratio per unit area of the sample was 26.44 CFU/mm^2 (Table I).

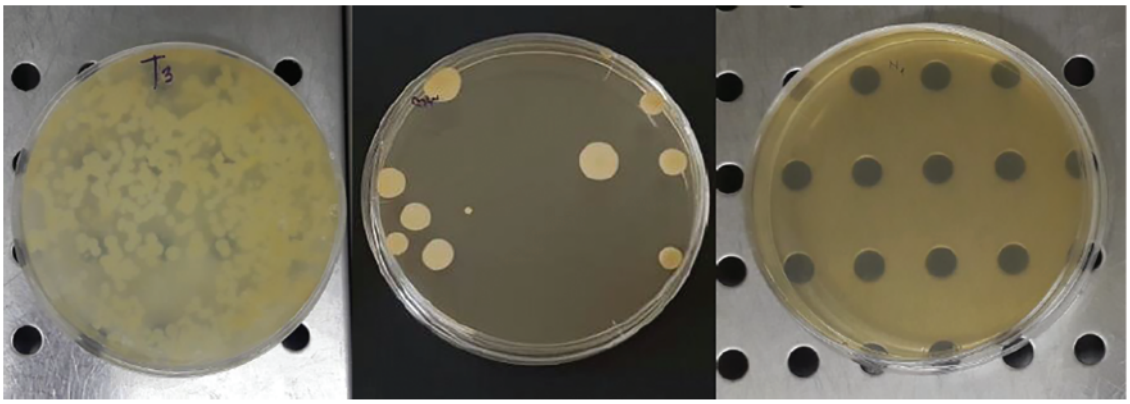

Fig. 6. Petri dishes showing CFUs from the control group, group 3 and group 4.

TABLE I: The Average CFU Value of the Control Group

	UFC/ml	UFC/30 ml	UFC/mm^2	Standard deviation
UFC/30 ml	634.56	12691.2	26.44	±2.29

TABLE II: The Average CFU Value of Group 2

	UFC/ml	UFC/30 ml	UFC/mm^2	Standard deviation
Group 2	0.54	10.848	0.0226	±0.035

TABLE III: The Average CFU Value of Group 3

	UFC/ml	UFC/30 ml	UFC/mm^2	Standard deviation
Group 3	0.89	17.856	0.0372	±0.040

TABLE IV: The Average CFU Value of the Group 4

	UFC/ml	UFC/30 ml	UFC/mm^2	Standard deviation
Group 4	0.192	3.84	0.008	±0.02

For group 2 (CHLORHEXIDINE), the mean colony-forming unit value was 0.54 CFU/ml, and 0.0226 CFU/mm^2 (Table II).

For group 3 (CHX+CCP), the mean colony-forming unit value was 0.89 CFU/ml, corresponding to 0.0372 CFU/mm^2 (Table III).

For group 4 (NaOCl), mean colony-forming unit values were 0.192 CFU/ml, and 0.008 CFU/mm^2 (Table IV).

3.1. Comparison of Mean CFU Values between Groups

Between the control group and the other groups, the p-value is less than 0.05, the difference is statistically significant between the control group and the other groups. The reduction in UFC/mm^2 is statistically significant.

Between the control group and the chlorhexidine group, there was a reduction of 1169.91 times, (99.91%).

Between the control and CHX+CCP groups, there was a 710.75-fold reduction (99.86%). Between the control group and the 0.5% NaOCl group, there was a 3305-fold reduction (99.97%).

Between Group 2 and Groups 3 and 4, the p-value is greater than 0.05, and the difference between the groups is statistically insignificant.

Between group 3 and group 4, the p-value is less than 0.05, and the difference is statistically significant between groups 3 and 4 (Fig. 7).

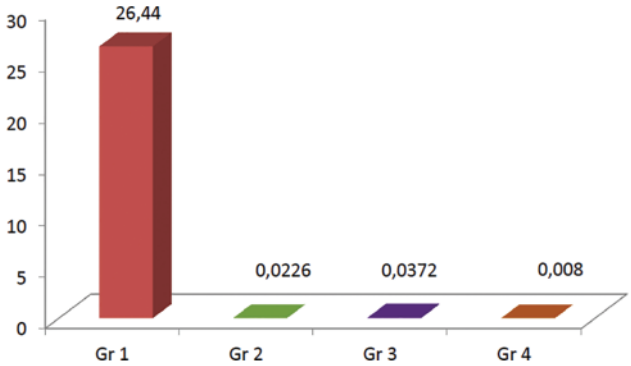

Fig. 7. Graph showing CFU/mm^2 after application of the three aseptic solutions compared with the negative control G1: Control, G2: CHX 0.2%, G3: CHX/CCP, G4: NaOCl.

4. Discussion

The oral cavity provides favourable physicochemical and nutritional conditions for the establishment and growth of an abundant, highly polymorphic and heterogeneous microbial flora. Under normal physiological conditions, there is an ecological balance between the flora and the host. They live in harmony, co-evolving in a mutually beneficial way. However, any disorder in the general or local state of health can lead to an imbalance, associated with quantitative and qualitative variations in this flora, with

the possible appearance of inflammatory and/or infectious phenomena.

Candida albicans is a unicellular fungus of the yeast category, a saprophyte of the oral mucosa, of which 40% of individuals are healthy carriers in the oral cavity [2], [3]. Candida albicans has been incriminated in several studies as the main pathogen responsible for denture-related stomatitis, the most common infection in wearers of removable dentures [4], [5].

According to a study by Daniluk *et al*. Candida albicans are isolated from the oral cavity in 74.4% of patients with prosthetic stomatitis [6]. This justifies the selection of Candida albicans as the test strain in our study. Delayed-setting resins are mainly used in tissue conditioning applications, where they are particularly well-suited. They have very interesting qualities, provided they are used wisely. Delayed-setting resins have poor mechanical properties and high surface porosity caused by the progressive loss of certain components (alcohol, phthalate, etc.) and the incorporation of air during preparation.

The average Candida albicans count per sample was 634.56 CFU/ml ± 2.29 for the control group, in agreement with the results of the study by Yilmaz *et al*. who found 620.32 CFU/ml [7]. It has often been admitted that delayed-setting resins are more susceptible to microbial accumulation on their surface than acrylic resins [8].

The results of this experiment attest to the sensitivity of Candida albicans to 0.2% and 0.12% chlorhexidine in combination with cetylpyridinium chloride, and to 0.5% sodium hypochlorite; with a reduction of 99.91%, 99.86% and 99.97%, respectively, compared to the control group. Chlorhexidine is an effective therapeutic agent, and the disinfectant and antifungal effect of chlorhexidine gluconate has been demonstrated in previous studies [9]. Chlorhexidine's effect on Candida's adhesion capacity is one of the main factors neutralizing its virulence.

A study by Pinto *et al*. [9] on the effect of repeated cycles of chemical disinfection on the roughness and hardness of relining materials showed that all five disinfection solutions studied (1%, 2%, 5.25% sodium hypochlorite; 2% glutaraldehyde; 4% chlorhexidine gluconate) produced a statistically significant decrease in hardness and an increase in surface roughness of the materials.

According to the results of our study, there was no significant difference in efficacy between 0.2% chlorhexidine and sodium hypochlorite. However, there was a statistically significant difference between 0.5% sodium hypochlorite and the 0.12% chlorhexidine formulation combined with 0.05% cetylpyridium chloride.

Sodium hypochlorite is an alkaline disinfectant that has been described as effective in reducing Candida albicans adhesion both in vitro and in vivo [10].

In the study by Yılmaz *et al*., 5.25% sodium hypochlorite was found to be the most effective concentration for prosthetic disinfection, compared with a concentration of 2%. The use of 5.25% NaOCl is not recommended, as it affects the physical properties of the materials [7].

Another study by Hahnel *et al*. [11] underlines the effectiveness of immersion for 15 min at 2% NaOCl, especially on mature biofilm, with results showing a 97% reduction in adherent Candida albicans cells on the material surface.

Several studies have indicated that the use of sodium hypochlorite at high concentrations causes discolouration and surface degradation of retarded-setting resins (8) (20) (62), which justifies our choice to test the efficacy of sodium hypochlorite at a concentration of 0.5% [12], [13].

The results of our study demonstrated the sensitivity of Candida albicans to NaOCl at 0.5%, and the efficacy of the latter with the best reduction rate (99.97%). These results corroborate those of Ferreira *et al*. [14] and are similar to those of Boscato and Gedik [15], [16].

All the above studies were carried out in vitro. However, in vitro studies have the limitations of not reproducing complex oral conditions and not involving biofilm assessment. In addition, clinical examination combined with cytological analysis confirms remission of infection, healing of palatal lesions and absence of C. albicans hyphae under the microscope [17].

5. Conclusion

The results of this study demonstrated the sensitivity of Candida albicans to disinfection solutions (NaOCl 0.5%, chlorhexidine 0.2%, chlorhexidine 0.12% combined with 0.05% cetylpyridinium chloride). The best Candida albicans reduction rate was obtained with 0.5% sodium hypochlorite. Immersion of samples for 15 min in 0.2% chlorhexidine gluconate significantly reduced Candida albicans colonization of delayed-setting resins. The 0.12% chlorhexidine formulation combined with 0.05% cetylpyridinium chloride enables us to benefit from the clinical efficacy of chlorhexidine while avoiding undesirable effects and, therefore, appears to be the best compromise between efficacy and absence of toxicity.

However, further studies are needed to assess the long-term effect of chlorhexidine use on the properties of delayed-setting resins, through more in-depth in vivo studies. Further research is also needed to test the effect of incorporating chlorhexidine gluconates into delayed-setting resins.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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