

Antifungal properties of sodium peroxide and sodium hypochlorite as a denture cleanser for full acrylic denture in vitro

Gantini Subrata

Department of Prosthodontics Faculty of Dentistry Universitas Padjadjaran

ABSTRACT

Widely used materials are reported as denture cleansers are peroxide and hypochlorite. Many contradictions on the effectiveness of the commercial peroxide base solution against *Candida albicans* (*C. albicans*). Low concentration sodium hypochlorite (0.5%) is used as household sanitizer. But it is still unknown whether it has antifungal effect, what is the optimum concentration and contact time to destroy the yeast. The purpose of this study is to examine the antifungal efficacy of commercial peroxide-based soaking solution and low concentration sodium hypochlorite against *C. albicans*, to determine the optimum concentration and contact time, and at the end, to obtain an effective denture soaking solution which is safe to use, easy to get, affordable and could be use to destroy *C. albicans* on dentures. The research conducted was an in vitro practical test for surface disinfectant. Sixty plates of acrylic which were already incubated with *C. albicans* are immersed in peroxide and hypochlorite base soaking solution in different concentration and contact time. The result showed that peroxide base was not effective to *C. albicans* and hypochlorite base solution can destroy *C. albicans* in 10 minute at concentration of 0,125%. Thus, the use of low concentration sodium hypochlorite as a denture soaking solution can be suggested.

Key words: *Candida albicans*, sodium peroxide, sodium hypochlorite, denture soaking solution

INTRODUCTION

Studies show that many patients who wear acrylic-based full dentures suffer from denture stomatitis or denture-induced stomatitis (DIS), denture sore mouth or prosthetic stomatopathy. This abnormality is an inflammation on mucous membrane that directly touches the denture and is often found on the palate right underneath the denture on the upper jaw. According to the early researchers, one of etiology of this abnormality was *Candida albicans* (*C. albicans*).¹⁻¹³

Local predisposing factors such as dirty dentures and other traumatic factors can alter this fungus to pathogen. Based on earlier studies, *C. albicans* is attached to the denture fitting surface more than it is to oral tissues. This would make the treatment more difficult, since *C. albicans* uses the surface of the denture as its reservoir.^{3,4,6,14,15} Even though DIS is a minor disorder for a healthy person, the prevention of *C. albicans* infections should not be taken for granted. This is because people who wear dentures are mostly elderly, who are prone to oral and systemic candidiasis, thus

DIS will become a serious threat for them.⁴

The cleanliness of the denture needs to be regularly maintained to avoid plaque build up and growth of microorganism, especially *C. albicans*.^{4,16} The simplest and the most effective way to maintain the cleanliness of denture is by soaking it in a cleaning solution. A soaking/immersion technique in a solution which can remove plaque and destroy microorganism is the simplest and the most effective way to maintain a clean denture.^{17,18}

This soaking technique is intended for infection prevention, but not for treatment, as dentures need to be free from plaque and microorganism before each usage. Widely used substances or bases to soak dentures are peroxide and hypochlorite.^{16,18,19-21} A clean denture must be free of plaque and *C. albicans* to prevent DIS infection.

Peroxide based disinfectant has been sold as denture soaking solution in a form of powder or fast-dissolving (effervescent) tablets with alkaline peroxide as the active material/compound. This commercial solution is intended to remove plaque and stain on dentures, but not to destroy *C. albicans* yeast. There have been many researches done on the effectiveness of this solution against *C. albicans* and reported contradicting results.^{10,16,18,22}

Sodium hypochlorite, on the other hand, is a disinfectant, bactericide, as well as fungicide, which can be also used as a soaking solution for denture, to remove stains, dissolve organic materials, even if it has not been known as such.^{9,20,22-26} Substance that contains 0.2-2.0 ppm chloride is categorized as bactericide and viricide; whereas a minimum 100 ppm is needed to obtain a fungicide effect.^{23,27}

Sodium hypochlorite in a low concentration, about 0.5%, is usually used as a household sanitizer. It is affordable, easy to use, available almost everywhere, odourless, tasteless and no side effect on skin and other goods, thus can be used as a potential soaking solution for dentures.^{23,27} Based on preliminary research on sodium hypochlorite antifungal efficacy against *C. albicans*, it is shown that sodium hypochlorite 0.5% can destroy *C. albicans* in only 5 minutes. Therefore, low concentration of sodium hypochlorite could be a potential active ingredient of soaking solution

for cleaning the dentures. Because one of the etiology of DIS is *C. albicans*, it is expected that the soaking solution for dentures also have positive effect to it. It is still in contradiction whether commercial peroxide soaking solution has antifungal action and it is still unknown what is the optimum concentration and contact time of sodium hypochlorite that still has antifungal effect.

From the explanation above, some questions arise. It has to be examined whether commercial sodium peroxide solution for dentures has antifungal efficacy on *C. albicans*. It has to be proven also whether low concentration sodium hypochlorite (0.5%) have antifungal efficacy on *C. albicans*. If it really has an antifungal effect on *C. albicans*, what are the optimum concentration and the optimum soaking time for denture in sodium hypochlorite solution, which still has an antifungal efficacy.

The purpose of this study is to examine the antifungal efficacy of sodium peroxide denture cleanser that are available on the market and to learn antifungal efficacy of low concentration sodium hypochlorite against *C. albicans*, to determine the optimum concentration of sodium hypochlorite, and the optimum soaking time needed by sodium hypochlorite to destroy *C. albicans*, and at the end, to obtain an effective denture soaking solution which is safe to use, easy to get, affordable and could be used to destroy *C. albicans* on dentures.

This study was done at Prosthodontic Laboratory Faculty of Dentistry Universitas Padjadjaran and Metallurgy Laboratory of Mechanical Department of Institute Technology Bandung for making the acrylic specimens, Biochemistry Laboratory at School of Medicine of Universitas Padjadjaran for making the artificial saliva, and Laboratorium Diagnostik Klinik of PT. Bio Farma (Persero) for research on antifungal efficacy.

MATERIALS AND METHODS

This research is a laboratory-experimental research to test antifungal efficacy of sodium peroxide and hypochlorite as denture soaking solutions, against *C. albicans* which is attached to the fitting surface of acrylic denture base.

Materials used as denture soaking solutions

Material A: randomly chosen denture soaking solutions from the available commercial brands that are marketed by distributors of dental materials and appliances in Bandung. Active ingredient of materials A is sodium peroxide (7,24%). Concentration of material A: one tablet is dissolved in 200 ml of sterile distilled water that is warmed in 40°C water bath in accordance to the usage instruction.²⁸

Material B: sodium hypochlorite 0.5% that is obtained from dilution of 5 ml sodium hypochlorite solution 11.4%, in 109 ml sterile distilled water, with a total of 114 ml sodium hypochlorite 0.5% solution (solution B1), then further diluted to a concentration of B2, 0.25%; B3, 0.125%; B4, 0.062%; and B5, 0.031%.

Contact time

Immersion time of acrylic plates in soaking solutions are 10, 20, and 30 minutes. Contact time is determined based on time listed on usage instruction of commercially denture soaking solutions.^{10,16,28-30} and the closest it is to the real-life usage.

Microorganism test

The strain of *C. albicans* used is ATCC 10231, obtained from the Laboratorium Diagnostik Klinik of PT. Bio Farma (Persero). The microorganism test is identified and tested by germ tube production and by sugar assimilation and fermentation techniques.³¹⁻³⁵ A stock culture is maintained in Sabouraud Dextrose Agar (SDA) slopes at 4°C.

Then a subculture in SDA plate is made. This subculture is then stored in incubator for 48 hours at 37°C to get the pure culture of *C. albicans* (Fig. 1). To prepare a test yeast suspension, some of the pure *C. albicans* colonies is suspended in sterile PBS until it reaches saturation point which contains $1,22 \times 10^7$ cells/ml.^{15,36}

Testing specimens

Sixty one pieces of acrylic plates (polymethylmethacrylate: Stellon 100 De Trey usually use in Laboratory of Prosthodontics Faculty of Dentistry Universitas Padjadjaran), with the size of 12x12x5 mm are then made according to manufacturer's manual. One side of the plate surface is wet ground using 320 grid silicon carbon

sand papers until smooth³⁷ (Fig. 2A). Grinding the test specimens made the testing surface of acrylic plates all in a same roughness stage.

The surfaces which are not ground, are marked for the ease of the testing (Fig. 2B). These plates are thoroughly washed before use, with distilled water under ultrasound agitation for 1 minute. These plates were maintained in sterile distilled water for 48 hours in room temperature prior to adherence assay to let leach excess monomer into the water. Distilled water is changed every 24 hours.^{15,37-39}

Testing procedures

According to Russel et al.⁴⁰ the yeast adherence assay on acrylic surfaces which will be conducted here is practical test for surface disinfectant. First, acrylic plates are sterilized in autoclave for 15 minutes at 121°C under pressure 5.1 kg/cm².⁴¹ For negative control: one sterile acrylic plate is placed into sterile container filled with 5 ml Sabouraud broth and incubated for 48 hours at 37°C to make sure there is no contamination. Sabouraud broth needs to remain clear to indicate no yeast/bacterial growth on the plates. On six Petri dishes (diameter 9 cm), put in 10 acrylic plates subsequently to be soaked with artificial saliva. The artificial saliva are made according to Arends et al.⁴² Fifty ml sterile saliva is then poured on top of each Petri dish, let soaked for 30 minutes in room temperature to get layers of salivary coat on acrylic surfaces (Fig. 3).³⁶ Then, each acrylic plate are removed, the remaining/excess saliva is absorbed in a sterile absorbent paper on another petri dishes and is then moved to other sterile Petri dishes.

Fifty ml of test yeast suspension is poured on to each Petri dish to soak these acrylic plates. Incubate for 60 minutes at 37°C for the yeast cells to adhere to the acrylic surface.^{36,39,43} After incubation, washing is done by taking these acrylic plates out and dipping them one by one into tubes filled with 20 ml PBS sterile. This tube is then closed and shaken for ten times (Fig. 4). This is done to dislodge all the loosely adherent yeast from the surface of acrylic plates.^{38,39,44} These plates are then removed and the remaining PBS is absorbed with sterile absorbent paper and after that the plates are moved to the new sterile Petri dishes.



Figure 1. Pure colony of *C. albicans*.



Figure 2. Testing specimens.
Upper: Surface grinding on one side of acrylic plates.
Lower: Marking on non-ground side of acrylic plates.

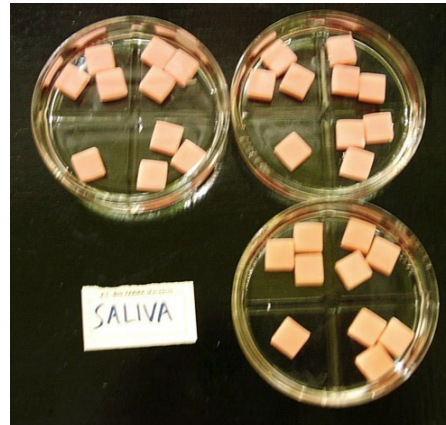


Figure 3. Soaking in saliva.

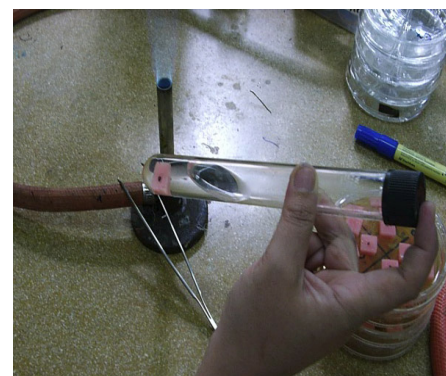


Figure 4. Acrylic plate washing.



Figure 5. Positive control result.

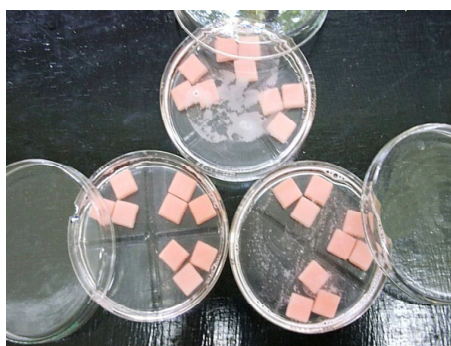


Figure 6. The immersion of acrylic plates in soaking solution.

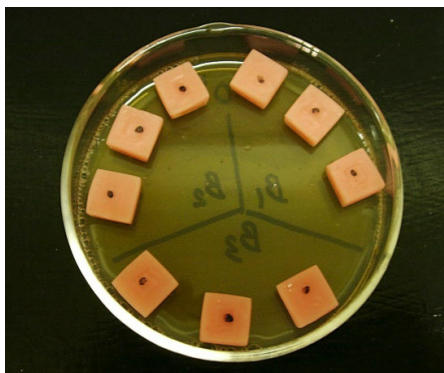


Figure 7. The acrylic plates placement in SDA.

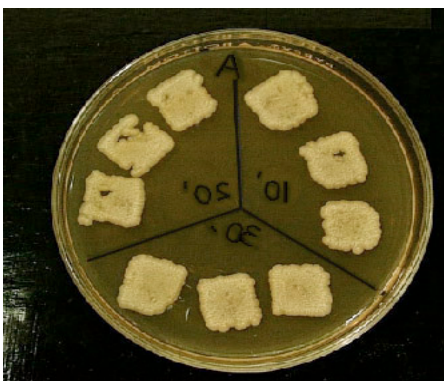


Figure 8. The growth of colony after soaking in solution A in different contact time.



Figure 9A. Solution B1, B2, B3 in 10, 20, and 30 minutes + positive control.

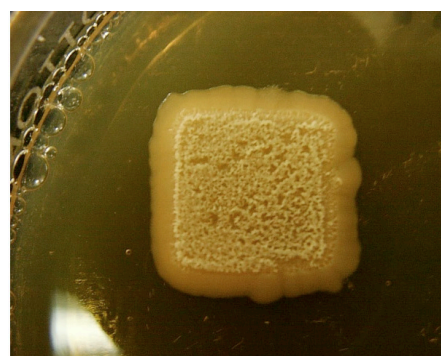


Figure 9B. Positive control: uncountable colony.



Figure 9C. Solution B4 in 10, 20, and 30 minutes.

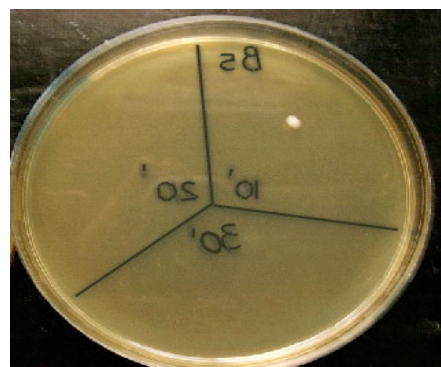


Figure 9D. Solution B5 in 10, 20, and 30 minutes.

Figure 10. Working procedures.

For positive control, a piece of acrylic plate is taken from each dish. The ground sides of the plate are attached to SDA for 30 minutes, then removed and the SDA is incubated for 48 hour at 37° C.⁴⁴ This positive control plate must always produce *C. albicans* colonies on SDA which indicates that the yeast incubation process on acrylic plates is successful. Otherwise, the process needs to be redone (Fig. 5).

Other nine acrylic plates are ready to be given treatments under soaking solution. These nine acrylic plates are immersed in two types

of denture cleaning solutions in with different concentrations (Fig. 6). After immersion, take out the acrylic plates in group of three at 10, 20, and 30 minutes interval. Rinse each group of acrylic plates with 20 ml of sterilized PBS in a test tube. Capped the test tube and turn it over 10 times to remove excess of the denture cleaning solutions. Remaining excess is then absorbed with sterilized absorbent paper in a Petri dish.^{38,39,44} To start the incubation process, divides an SDA in a Petri dish into 3 quadrants and place 3 acrylic plates from different concentrations in each quadrant in

reference of the time when each group is picked up (10, 20, and 30 minutes) (Fig. 7). After 30 minutes, removes the acrylic plates and incubates the agar for 48 hour at 37° C. Then observe the presence of the yeast on the agar.

Analysis method

The adhesion of *C. albicans* to acrylic plates is observed in the form of present (+) or not present (-) of yeast in SDA agar. These are data with nominal scale, so to compare statistically whether there is influence or not from the solutions used, the concentration of the solutions, and the contact time on the yeast colonies formation, statistic analysis of non parametric with Fisher Exact Probability test and Q or Cochran test are used.^{45-47.}

RESULT

Exact Fisher Test used to determined whether there is differences between treatment with material A and B to the growth of *C.albicans*. The conclusion derived from the statistically calculation shows that solution A and B has significant different influence on the formation of *C. albicans* colony. This shows that solution A and B has different antifungal action. In the colony proportions on Table 1, it is clear that solution A has no effect on inhibiting the growth of the yeast in different contact time: 9 the incident of colony present (+) and 0 the colony was absent

(-) (Fig. 8). While in Table 3 for solution B, there are 43 incident (-) and only 2 incident (+). This is to identify that on all solution A replications there are colony growth, while in solution B there are only 2 replications that showed the growth of colony (Fig. 9 C-D). This proved that solution B has greater influence to destroy *C. albicans* compare to solution A.

Cochran or Q test is used to determine whether there is influence from concentration of the materials and contact time to the growth of *C. albicans*. The conclusion derives from Table 2 shows that besides the effect of types of materials (A and B), there are also effects from concentration and contact time differences on the formation of colony. Table 2 shows that the lower the concentration of solution B and the shorter it's soaking time, the less effect of solution B to destroy *C. albicans*.

The immersion in solution B1 (sodium hypochlorite 0.5%) for 10 minutes is enough to kill *C. albicans*. Similar with B3 solution in small concentration (0,125%), can kill *C. albicans* in 10 minutes (Fig. 9A). While for more less concentration that is B4 and B5 the time needed is longer, which is 20 minutes.

DISCUSSION

Material B (sodium hypochlorite) was found to have antifungal efficacy (fungicide) on *C. albican*, while material A (sodium peroxide) did

Table 1. Growth of *C. albicans* colonies on SDA after being treated with solution A

Material contact time	Material A 1:200 (3 replications)
10 minutes	TT, TT, TT
20 minutes	TT, TT, TT
30 minutes	TT, TT, TT

Note: Positive control: TT, TT,TT. (TT: uncountable)

Table 3. The influence of solution A and B on the growth of *C. albicans* colonies

Material	<i>C. albicans</i> colony		Total
	Absent (-)	Present (+)	
A	0	9	9
B	43	2	45
Total	43	11	54

Table 2. Growth of *C. albicans* colonies on SDA after being treated with solution B

Material B contact time	NaOCL 0,5% B1	NaOCL 0,25% B2	NaOCL 0,125% B3	NaOCL 0,062% B4	NaOCL 0,031% B5
10 minutes	0, 0, 0	0, 0, 0	0, 0, 0	0, 1, 0	0, 1, 0
20 minutes	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
30 minutes	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

not have any. This can be explained by the ability of hydrogen peroxide which in low concentration (3-6%) has the character of bactericide and at higher concentration (10-25%) has a fungicide/ sporocide function and is used for sterilization.^{23,48} Peroxide content in the solution used in this study is 7.34%, which explains why in this concentration solution A has no fungicide character yet. While for solution B (sodium hypochlorite), the solution must have a minimal active chloride as much as 100 ppm to have fungicide effects and for shorter contact time, the higher concentration is needed.²³ In this study, the lowest concentration of B solution that can kill the yeast is 0.125% or equivalent to 1250 ppm chlorine active. Table 2 shows that the lower the concentration of B solution and the shorter it's soaking time, the less action of B solution to destroy *C. albicans*. This is in accordance with the previous data indicating that the lower concentration of the cleanser and the shorter the contact time, the weaker its antibacterial effect.⁴¹

The test done here is a practical test for a certain surface disinfectant with the result of concentrations that can be used practically (use dilution), although this test is not a test for determining MIC of a drug. Because the test done is a practical test, that's why the way of applying it for practical usage possibly can be suggested here. If 2 until 8 ml sodium hypochlorite 11.4% is added to 200 ml water, it will make solution B mixture become 0.125% up to 0.5%.

Although low concentration of sodium hypochlorite can be used as a denture soaking solution, but it is strictly advised to clean the denture carefully after immersion. This is important because the disinfectant used for unliving things can be dangerous if it get into contact with mucosal tissue.^{18,25,49}

In addition to this, hypochlorite acid, which is an active ingredient from this group, besides having a germicide/fungicide property can also dissolve the necrotic tissues. So it is hoped that this solution is able to prevent the new plaque formation on the denture. By looking at the advantage of sodium hypochlorite, which is in low concentration can inhibit the growth of *C. albicans* and it probably can also dissolve organic tissue, the use of this solution as the soaking material to keep the cleanliness of denture is

really recommended.

Nevertheless, alkaline peroxide cleansers are reported not having deleterious side effect and has a pleasant odour that makes them popular. While sodium hypochlorite is reported having a bleaching effect on denture material, severe tarnish on metal, bad odour and aftertaste in full strength concentration.¹⁹ In small concentration this sodium hypochlorite has no odour, no aftertaste, and no bleaching effect.

CONCLUSION

The sodium peroxide solution as a commercial product for denture cleanser does not have antifungal effect on *C. albicans*. While sodium hypochlorite in low concentration (0.5%) has the antifungal effect on *C. albicans*. The lowest concentration of sodium hypochlorite that can inhibit the growth of *C. albicans* in 10 minutes is 0.125%. Due to several reasons such as: the low concentration of sodium hypochlorite is safe for the tissue and denture, reasonable in price, the ease of use, and can give an antifungal efficacy, the use of this solution as a soaking denture cleanser can be suggested to patients who wear dentures. It is also suggested to do more research on the influence of low concentration of sodium hypochlorite towards acrylic usage in the long term period.

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