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# Vinegar as a Removing Agent of *Candida albicans* From Acrylic Resin Plates

Abbas Ali Jafari [1], Abbas Falah-Tafti [2*], Mohammad Hossein Lotfi-Kamran [2], Arezzo Zahraeii [3], Abdolhassan Kazemi [4]

[1] Department of Medical Parasitology & Mycology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, IR Iran

[2] Department of Prostodontics, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, IR Iran

[3] Shahid Sadoughi University of Medical Sciences, Yazd, IR Iran

[4] Infections and Tropical Disease Research Center of Immunology, Tabriz University of Medical Sciences, Tabriz, IR Iran

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**ABSTRACT**

***Background:*** Adherence of *Candida* species, mainly *C. albicans* to denture surfaces, forms a biofilm which causes denture stomatitis in denture users. Removal of *Candida* plaque on dentures is essential to control the colonization of this yeast and to prevent infections related to *C. albicans*.

***Objectives:*** The aim of this study was to compare the effectiveness of sodium hypochlorite with white vinegar for the disinfection of *C. albicans* from acrylic resin.

***Materials and Methods:*** 82, 10×10×1 mm acrylic resin plates were inoculated with $1\times10^3$ *C. albicans* suspension for 24 hours to prepare experimental *Candida* biofilm. The total number of *Candida* cells which adhered to 10 acryl resin plates was determined and the remaining 72 plates were randomly divided into four groups. The test plates were immersed in a solution of 1% sodium hypochlorite, 5% or 10%white vinegar for a period of 8 hours and distilled water was used as the negative control group. The *Candida* removing ability of the 3 disinfectants and the negative control group was assessed by comparing the number of colony forming units per 1 mL of the plates washing solution before and after the removing protocol. Data was analyzed using Kruskal-Wallis and Mann-Whitney tests.

***Results:*** Sodium hypochlorite (1%) and white vinegar (10%) removed 100% of the *C. albicans* cells, followed by white vinegar (5%), which removed 99% of the adhered *C. albicans* from the acrylic resin plates. There wasn't any significant statistical difference found between the 5% and 10% white vinegar in removing *Candida* from the acryl resin plates ($P = 0.161$).

***Conclusions:*** Overnight immersion of complete removable dentures in 10% or even a 5% white vinegar solution effectively removed *C. albicans* cells that had adhered to the denture surface and their removal properties the same as 1% sodium hypochlorite.



*Implication for health policy/practice/research/medical education:*
The results of this study indicate that vinegar is useful for removing of *Candida* biofilm from complete removable denture.

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## 1. Background

Denture stomatitis, formerly known as denture sore mouth, relates to an inflammatory lesion of the mucosa followinthe use of complete or partial removable dental prostheses in about 60% of denture wearers (1). *Candida* species, which are a part of the human oral microbial flora in particular *C. albicans*, are the main etiologic agents responsible for the development of this opportunistic infec-

* *Corresponding author:* Abbas Fallah-Tafti, Department of Prostodontics, School of Dentistry, DahaFajr Boulvard, Yazd, IR Iran. Tel: +98-3518228717, Fax: +98-3518367636, *E-mail:* Jaabno@gmail.com

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tion (2). Poor oral hygiene, badly fitting dentures and using denture liners are the most common causes of denture stomatitis (3). The ability of *C. albicans* to adhere to host mucosal tissues (4) as well as acrylic denture surfaces (5), the production of proteolytic enzymes that prepare penetration into tissues, phenotype switching of yeast to the hyphal form (6) and several immunomodulatory activities are known to be virulent factors for this fungus (7). Salivary flow is also reduced in patients with dentures, which decreases the physiological cleaning properties of the tongue and prepares a suitable environment for microbial survival and colonization in the oral cavity of denture users (8). Attachment and colonization of *Candida* on the surface of the denture, produces biofilm that is implicated as a possible source for oral and disseminated candidiasis in immunocompromisedpatients and denture wearers with poor oral hygiene (9, 10), cleaning and removal of this biofilm is necessary for the control of denture stomatitis in edentulous patients (11, 12). There are many studies in the literature suggesting the use of mechanical, chemical and even a combination of both methods for denture hygiene (13-16). Immersion of dentures in a commercial or a household solution is a commonly used chemical for the disinfection of denture biofilm. Several cleaning agents that are relatively expensive and to damage the acrylic resin and metal alloys of the dentures were also reported (17, 18).

Vinegar is a sour liquid comprised mainly of acetic acid, which is prepared in households by the fermentation of fruit such as grapes and apples in Iran. This solution is also commercially available, it is cheap and easily found in Iranian markets. There are many studies which support the antimicrobial effects of vinegar (19-22). Vinegar has also been shown to be effective in the prevention and control of microbial contamination in intra-canal treatment of apical periodontitis in dog teeth (23).

## 2. Objectives

The general purpose of the present study was to evaluate the effectiveness of household and commercial vinegar for disinfecting *C. albicans* from acrylic resin.

## 3. Materials and Methods

### *3.1. Preparation of Acrylic Resin Plates*

82 square shaped, 10×10×1mm, acrylic resin plates (Acropars, Iran) were prepared using thermally activated acrylic resin according to the manufacturer's instructions (*Figure 1*). The specimens were kept in a flask containing physiological serum (NaCl 0.85%), sterilized in an autoclave (Labtron, Iran) at 121°C/15 min and incubated at 4°C for adherence testing.

### *3.2. Preparation of Fungal Suspension and Experimental Biofilm*

A standard strain of *C. albicans* (ATCC 10231) was cultured on Sabouraud dextrose agar plates (Merck, Germany) and incubated at 37°C for 24 hours. A suspension containing

**Figure 1.** Standard Acrylic Resin Plates (10×10×1 mm)

$1\times10^3$ viable cells per milliliter was prepared in a sterile saline solution (NaCl 0.85%) using a haemocytometer slide. Experimental biofilm was created using 24 well cell culture plates by adding 1.5 mL sterile Sabouraud Broth, one acrylic resin plate and 100µL of *C. albicans* suspension were added to each well. The plates were sealed and incubated at 37°C in a shaker (100 rpm) for 24 hours to prepare the experimental *C. albicans* plaque. The acrylic resin plates were then washed 3 times with 1 mL of sterile distilled water and used for the removing test. Ten plates were transferred to 10 falcon tubes containing1ml of sterile physiological solution (NaCl 0.85%) and glass pearls, then agitated in a Sonicator (Elma, Germany) for 5 minutes (45 KH/ 5 min) to remove the adhered cells. 10 µL of each suspension was added to 90 µL of sterile physiological solution and plated in duplicate on Sabouraud dextrose agar plates. All plates were incubated

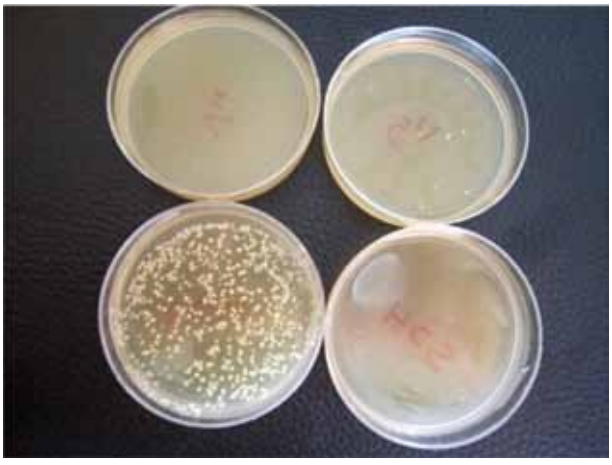

**Figure 2.** Isolated *C. albicans* Colonies From Culture of 10 µL of Washing Solution

for 48 hours at 37°C and the number of colony-forming units per 1 mL of the plates' washing solution (cfu/mL) was determined by the number of attached *Candida* cells before entering the removing protocol (*Figure 2*).

### 3.3. Removal of Candida

Acrylic resin plates covered in plaque were then randomly divided into 4 groups of 18. Group 1 plates were immersed in 50 mL 10% white vinegar (Yek & Yek, Iran), group 2 in 5% white vinegar (grape), group 3 in 1% sodium hypochlorite, and group 4 in distilled water as a negative control, then incubated at 37°C for 8 hours in a shaker (100 rpm). After incubation, the plates were washed 5 times with 1 mL of sterile distilled water and transferred to sterile falcon tubes for examination of the number of viable *Candida* cells that had adhered to the plates as described previously. The *Candida* removing ability of the three disinfectants and the negative control group were assessed by comparing the number of colonyforming units per 1 mL of plate washing solution before and after the removing procedure.

### 3.4. Statistical Tests

A Kruskal-Wallis test was used to compare the average viable number of *Candida* cells in the four groups. A Mann-Whitney test was also used to compare the results in the 5% and 10% vinegar groups. Differences in the *Candida* removing ability of the tested chemicals were considered significant if $P < 0.05$. All statistical calculations were performed using SPSS15 software.

## 4. Results

The mean value of viable *C. albicans* cells which adhered to the acrylic resin plates before and after disinfection, as well as the percentage that were removed are illustrated in *Table 1*. *Candida* growth was not present in the culture of group 1 and group 3 plates, meanwhile the mean value of CFU/mL ± SD is shown in *Table 1*. The 10% vinegar and 1% sodium hypochlorite groups showed the highest rate of *C. albicans* removal from the acrylic resins (100%) followed by 5% vinegar (99%) when compared to the number of viable *Candida* cells before the removing procedure in the control group. There was no statistically significant difference between the vinegar at 5% and 10% ($P = 0.161$) or sodium hypochlorite in removing *Candida* from the resin plates. In fact all three disinfectants presented statistically significant differences when compared with the before and after removing protocol ($P < 0.01$).

## 5. Discussion

Denture stomatitis is a prevalent disorder in patients using dentures caused mainly by the adherence and colonization of *C. albicans* on the surface of the denture. This results in direct cytotoxicity and produces an inflammatory effect on the mucosal epithelium that covers the denture bearing tissues (8, 24). There are several previous studies which have shown that disinfection of complete dentures with sodium hypochlorite is an effective treatment (25, 26), however there is controversy over the effectiveness of white vinegar in the prevention and treatment of denture stomatitis and its ability to completely remove *C. albicans* adhered to the surface of the acrylic resin (27, 28).

In the present study, disinfecting *C. albicans* from the surface of acrylic resin plates using 1% sodium hypochlorite, 5% and 10% white vinegar solutions were compared for their removing abilities. There are several methods which have been used for evaluating the plaque removing efficacy of denture disinfectants. Experimental *in vitro* formation of *Candida* biofilm on the surface of acrylic resin plates and quantitative assessment of the remaining viable *Candida* adheredto the plates is more accurate than other methods such as weight (29) and staining methods (30) suggested by some investigators. White vinegar is a readily available, inexpensive household solution usually found in Iranian homes, which was used in the current study for the removal of *C. albicans* biofilm on acryl resins. 10% white vinegar and 1% sodium hypochlorite solutions were shown to be equally effective as both removed 100% of the *C. albicans* yeast adhered to the resin plates. The 5% white vinegar solution also showed effective removing properties eliminating 99% of the attached *C. albicans* cells from the surface of the resin plates ($P = 0.161$).

These results were comparable with a study by Basson *et al.* who also reported the effectiveness of undiluted vinegar for killing adherent microorganisms when used as a disinfection agent for denture cleansing (27), conversely Pinto *et al.* in a recent study reported that 10% vinegar wasn't able to eliminate *C. albicans* counts on the dentures and saliva of denture users (28). However the removing property of 10% vinegar was a little higher than 5% white vinegar for eradicating *Candida* from acryl plates in the current study, but this wasn't statistically significant ($P = 0.161$), representing the same level of effectiveness. *In vitro* experimental studies have already shown that low doses of vinegar can induce apoptosis

**Table 1:** Disinfectant Effectiveness in Removing *C. albicans* From Acryl Resins

| Disinfectants | Initial Culture, Mean ± SD | *C. albicans* After the Protocol, Mean ± SD | Removing Ability, % | *P* value |
|---|---|---|---|---|
| 10 % vinegar | $1.32 \times 10^3 \pm 1.1 \times 10^2$ | - | 100% | 0.0001 |
| 5 % vinegar | $1.32 \times 10^3 \pm 1.1 \times 10^2$ | $1.3 \pm 2.7$ | 99% | 0.001 |
| 1 % sodium hypochlorite | $1.32 \times 10^3 \pm 1.1 \times 10^2$ | - | 100% | 0.0001 |
| D.W. (negative control) | $1.32 \times 10^3 \pm 1.1 \times 10^2$ | $1.25 \times 10^3 \pm 1.06 \times 10^2$ | 5.3% | 0.18 |

and programmed cell death of *C. albicans* (31). The acetic acid present in vinegar is also used to control other pathogenic fungi such as dermatophytes, the agent of tineapedis (32). Komayama *et al.* (33) in their study reported that 50% vinegar was not effective for disinfecting *C. albicans* on toothbrushes; however it was useful for several bacteria such as *Streptococcus mutans, Streptococcus pyogenes* and *Staphylococcus aureus* (33).

Immersion of acryl plates in 1% sodium hypochloritewas also effective for removing *Candida* from acryl resin plates, removing 100% of the attached *Candida* in the present study. The presence of undissociatedhypochlorous acid (HCOl), the concentration of which is dependent on pH, oxidizes the sulfhydryl groups (-SH) of amino acids and proteins to produce disulphide forms (S-S) (34, 35). There are many studies which have proven the effectiveness of 1% sodium hypochlorite in removing *Candida* species from the surface of dentures as shown in this current study. However it produces an unpleasant smell and taste, which must be properly washed to remove the product when used for disinfecting dentures. Continuoususe of sodium hypochlorite can also produce stains on the surface of the dentures and damage them (14, 16), which did not occur with the use of 10% vinegar (28).

Soaking dentures overnight in 10% or even 5% vinegar solution effectively removed *C. albicans* cells adhered to the denture surface. The removing effect of both 5% and 10% vinegar solutions is comparable with 1% sodium hypochlorite.

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