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The Effect of Disinfection Methods on *C. Albicans* in Three Types of Denture Base Materials

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Abstract: *This study aimed to evaluate the efficacy of disinfectant solutions on Candida albicans (C. albicans) for different types of denture materials. A total of 144 specimens (10x10x2 mm) were obtained from three different materials: autopolymerized acrylic resin, heat-cured acrylic resin, and hard relining material (n = 6). Three disinfectant solutions were used: 100% white vinegar, 2% chlorhexidine digluconate (Saver) and denture cleaning tablets (Corega). The specimens were placed on Eliza plates and 1.5 mL of Yeast Extract Peptone (YPD) was added to each well. Then, 30µL of candida culture was added to the wells. Next, the specimens were incubated at 37°C at 80 rpm for 48 h. Disinfectants were added to the Eliza plates. For all specimens, the disinfectants were replaced with 2 mL of sterile water and kept at 100 rpm for 30 min. Then, 0.1 mL of the liquid was taken and inoculated into the pads containing YPD medium. After incubation, the candida colony growth on the pads was measured. Scanning electron microscope (SEM) images were taken from randomly selected specimens from each group. Statistically significant differences ($P < 0.05$) were found between the disinfectant method groups and the control group for the three types of denture materials. The 2% chlorhexidine gluconate (Saver) disinfectant was the most effective for C. Albicans ATCC 60193 and oral isolate of C. Albicans for all three of the tested denture materials. The effect of cleaning of tabs (Corega) and white vinegar was found to be similar.*

Keywords: *Candida albicans, vinegar, chlorhexidine digluconate, denture cleaning tabs*

1.Introduction

Denture stomatitis is one of the most common types of oral fungal infection [1]. The most common causes of stomatitis are poor oral hygiene and poorly fitting removable dentures [2]. The etiology of denture stomatitis includes local and systemic factors [3]. Despite the multifactorial etiology, the main cause is *Candida spp.*, especially *Candida albicans* (*C. albicans*) [4]. *C. albicans* is a candida species in the human oral microflora [5].

Denture stomatitis is difficult to treat [6]. To reduce the risk of this disease, oral and denture hygiene should be improved [6], antiseptic or disinfectant solutions should be applied to dentures regularly [3,6-9], and dentures should be relined or replaced [3,8]. Moreover, treatments may be required, which include topical and/or systemic antifungal drugs [3,8].

If patients who use dentures cannot practice adequate oral hygiene, it is difficult to control the infection. Therefore, disinfectant solutions reduce to need to use systemic antifungal drugs, which has been recommended to prevent denture stomatitis and to protect oral microbiota [10-12].

The present study aimed to compare three types of disinfectant solutions: easily available 100% white vinegar, 2% chlorhexidine digluconate solution (Saver), and denture cleaning tablets (Corega). The null hypothesis of this study was that the 2% chlorhexidine digluconate (Saver) solution is the most effective treatment for all types of denture materials.

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2.Materials and methods

A total of 144 specimens were obtained from three different materials: autopolymerized acrylic resin (S.C. Self Cure, Imicryl, Konya, Turkey), heat-cured acrylic resin (I.Q.-15 Heat Cure, Imicryl, Konya, Turkey), and hard relining material (Ufi Gel Hard, Voco, Cuxhaven, Germany) (n = 6). Three disinfectant solutions were used in the study: 100% white vinegar (Taris Tarpak, İzmir, Turkey), 2% chlorhexidine digluconate (Saver), and denture cleaning tablets (Corega).

The dimensions of the prepared wax specimens were 10x10x2 mm. All of the wax specimens for the heat-cured acrylic resin were placed in the muffle furnance and negative spaces were obtained. Then, the monomer and liquid were mixed in a glass container in accordance with the manufacturer's instructions. Finally, the heat-cured acrylic specimens were prepared by placing them in the muffle furnance.

Silicone molds were used for the autopolymerized acrylic resin and the hard relining material, and specimens, with dimensions of 10x10x2 mm, were prepared using liquid/powder in accordance with the manufacturer's instructions.

One specimen from each group was selected and scanning electron microscopy (SEM) images were obtained before the three disinfectant methods were applied (Figure 1).

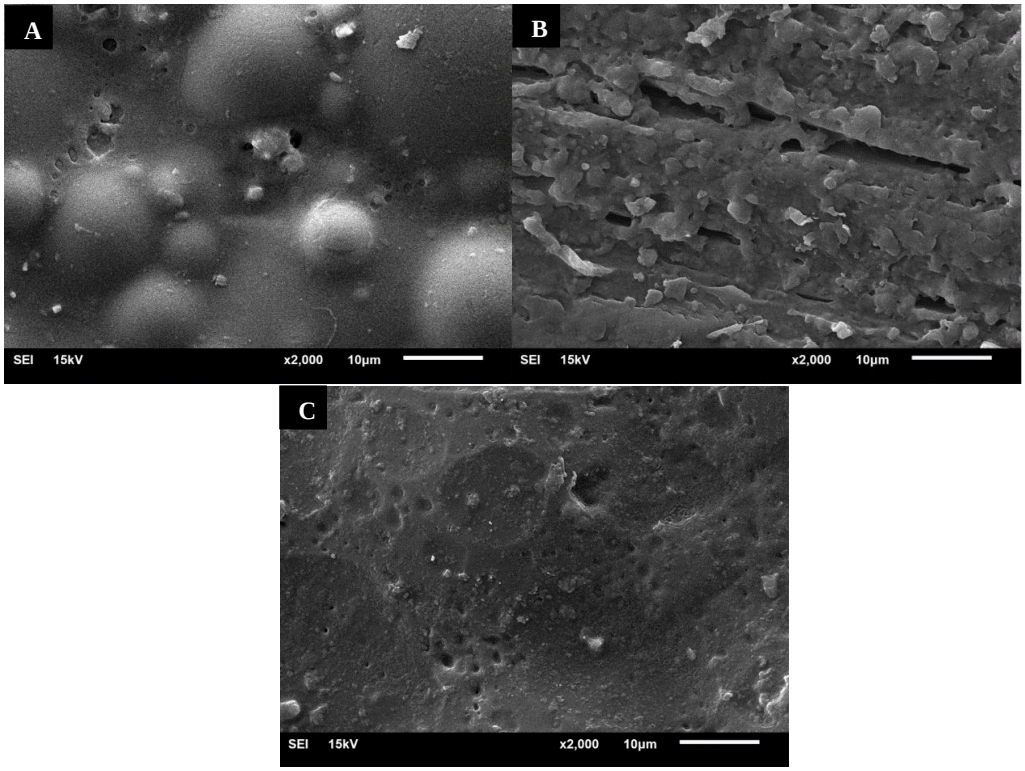

Figure 1. SEM images before the *C. albicans* strains were applied. (**A**) autopolymerized acrylic resin, (**B**) heat-cured acrylic resin, (**C**) hard relining denture material

Next, C. albicans ATCC 60193 and an oral isolate of *C. albicans* were reactivated. A suspension of Mc. Farland 0.5 / $x10^{7-8}$ cfu/mL C. albicans was prepared. The optical density (OD) of both *C. albicans* strains were taken at 600 nm and recorded as 0.10.

The specimens were placed on Eliza plates and 1.5 mL of Yeast Extract Peptone (YPD) was added to each well. Next, 30µL of candida culture was added to the wells. Then, the wells were incubated at 37°C at 80 rpm for 24 h. At the end of 24 h, 1.5 mL of YPD was drained and 1.5 mL of new YPD was added to each well. The wells were then incubated again at 37°C at 80 rpm for 24 h.

The OD 600 nm spectrum was measured from the control group without *C. albicans* (0), the oral isolate of *C. albicans* strains (2.586), and the *C. albicans* ATCC 60193 strains (2.326) after 48 h.

The wells were washed twice with 2 mL of sterile water. After washing, the OD was measured at 600 nm from the oral isolate of *C. albicans* (0.036) and the *C. albicans* ATCC 60193 (0.050) strains.

Disinfectants were added to the Eliza plates. Thus, 2.5 mL of white vinegar was added to the vinegar group and left for 10 min. For the Corega group, Corega solution was prepared according to the instructions for use, and 2.5 mL of it was added to the Eliza plates and left for 5 min. Moreover, for the Saver group, 2.5 mL of chlorhexidine digluconate was added to the Eliza plates and left for 10 min. Finally, 2.5 mL of sterile water was added to the control group and left for 10 min.

For all the groups, the disinfectants were drained and replaced with 2 mL of sterile water and kept at 100 rpm for 30 min. After vortexing, the specimens were transferred to Eppendorf tubes by making 10^{-3} dilution. Then, 0.1 mL of the liquid was taken and inoculated into pads containing YPD medium. The pads were incubated for 48 h in an oven at 37°C. After incubation, the candida colony growth was measured on the pads. SEM images were taken from each group using randomly selected specimens.

The data were analyzed by means of one-way ANOVA. The mean values and standard deviations of the groups were calculated.

3. Results and discussions

Statistically significant differences ($P < 0.05$) were found between the three disinfecting method groups and the control group for the three denture materials according to both the *Candida albicans* ATCC 60193 and oral isolate of *Candida albicans* strains (Table 1).

Table 1. Statistical analysis of antifungal effects

Materials	C. albicans strains	Methods	Mean	Std. Deviation	P
Autopolymerized acrylic resin	ATCC 60193	Control	30.33	25.48	0.0015
		White vinegar	4.83	2.85	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	5.16	5.11	
	Oral	Control	31.00	21.86	0.003
		White vinegar	6.33	4.17	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	2.33	1.63	
Heat.cured acrylic resin	ATCC 60193	Control	28.00	19.55	0.010
		White vinegar	6.33	2.94	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	8.00	4.97	
	Oral	Control	20.00	14.38	0.007
		White vinegar	4.50	3.50	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	2.83	3.54	
Hard relining denture material	*ATCC 60193*	Control	64.50	20.76	0.000
		White vinegar	19.00	7.37	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	9.50	10.78	
	Oral	Control	23.66	7.63	0.007
		White vinegar	19.16	15.94	
		Chlorhexidine digluconate	0.00	0.00	
		Corega	2.33	2.80	

Saver (2% chlorhexidine gluconate) was more effective than Corega or 100% white vinegar for *Candida albicans* ATCC 60193 and the oral isolate of *Candida albicans* for the autopolymerized acrylic resin, heat-cured acrylic resin, and hard relining material.

No statistically significant differences ($p > 0.05$) were found between the final counts *of Candida albicans* ATCC 60193 and oral isolate of *Candida albicans* after disinfection with 100% white vinegar and denture cleaning tablets (Corega). However, for the hard relining material, the amount of oral isolate *Candida albicans* was found to be statistically significant ($p < 005$) after disinfection with 100% white vinegar and denture cleaning tablets (Corega).

The SEM images shows Candida biofilm on the denture base materials (Figure 2, Figure 5).

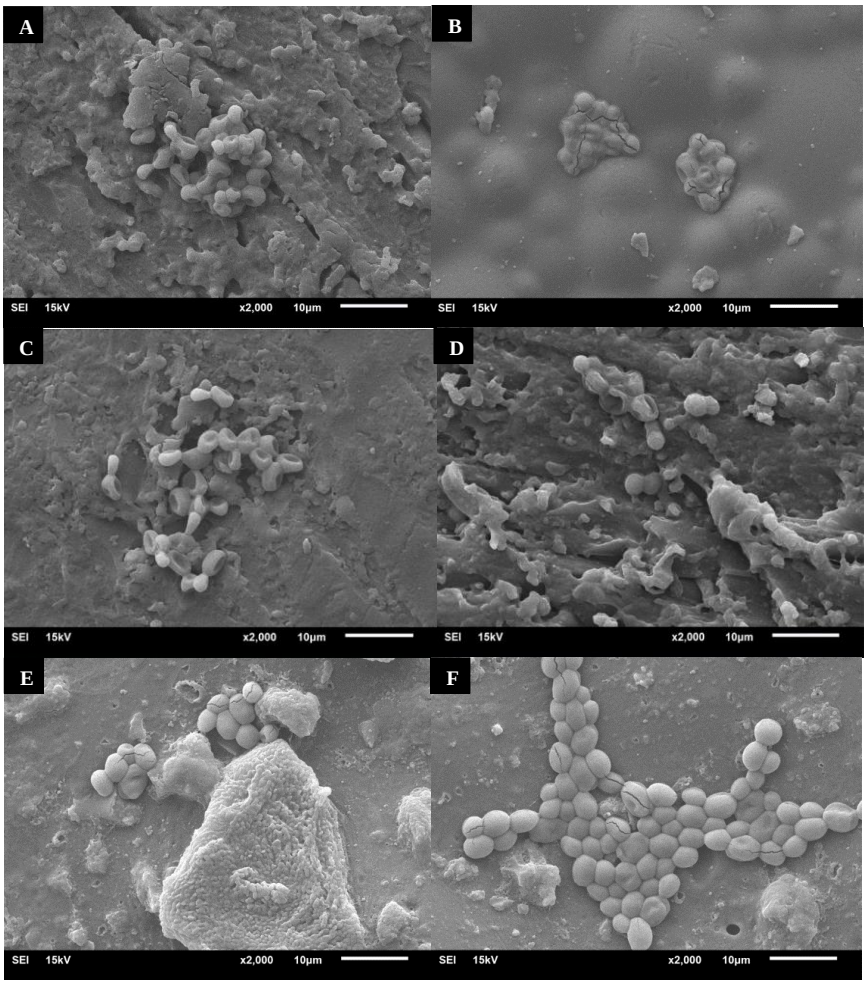

Figure 2. SEM images of the Control autopolymerized acrylic resin group (**A**) *C. albicans* ATCC 60193 and (**B**) Oral isolate of *C. albicans*. SEM images of the Control heat-cured acrylic resin group (**C**) *C. albicans* ATCC 60193 and (**D**) Oral isolate of *C. albicans*. SEM images of the Control hard relining denture material group (**E**) *C. albicans* ATCC 60193 and (**F**) Oral isolate of *C. albicans*

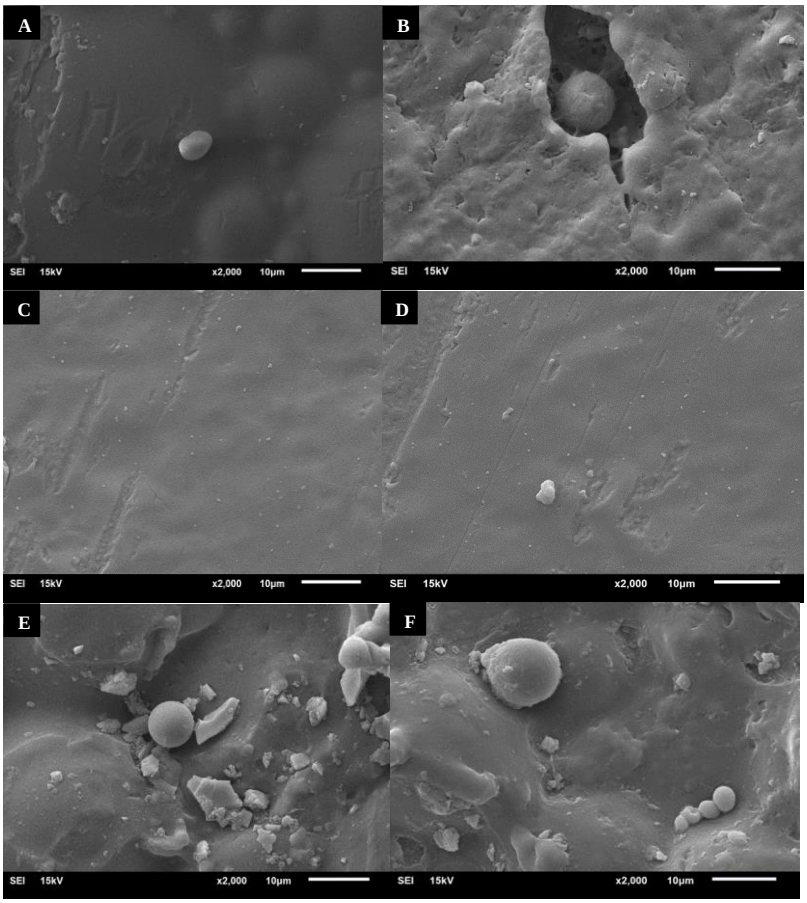

Figure 3. SEM images of the 100% white vinegar autopolymerized acrylic resin group (**A**) *C. albicans* ATCC 60193 and (**B**) Oral isolate of *C. albicans*. SEM images of the 100% white vinegar heat-cured acrylic resin group (**C**) *C. albicans* ATCC 60193 and (**D**) Oral isolate of *C. albicans*. SEM images of the 100% white vinegar hard relining denture material group (**E**) *C. albicans* ATCC 60193 and (**F**) Oral isolate of *C. albicans*

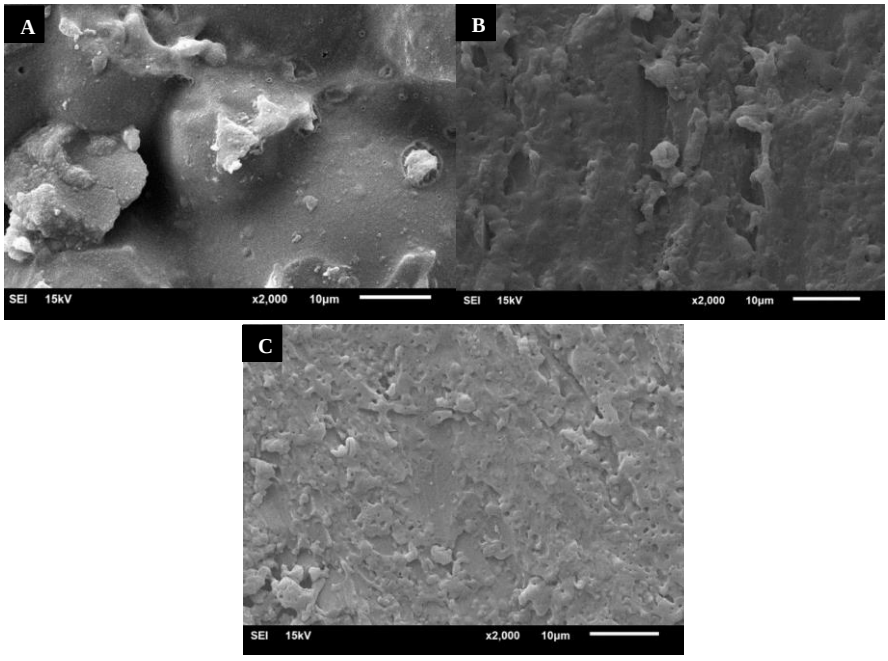

Figure 4. SEM images of the chlorhexidine digluconate (Saver) group (**A**) autopolymerized acrylic resin, (**B**) heat-cured acrylic resin, (**C**) hard relining denture material

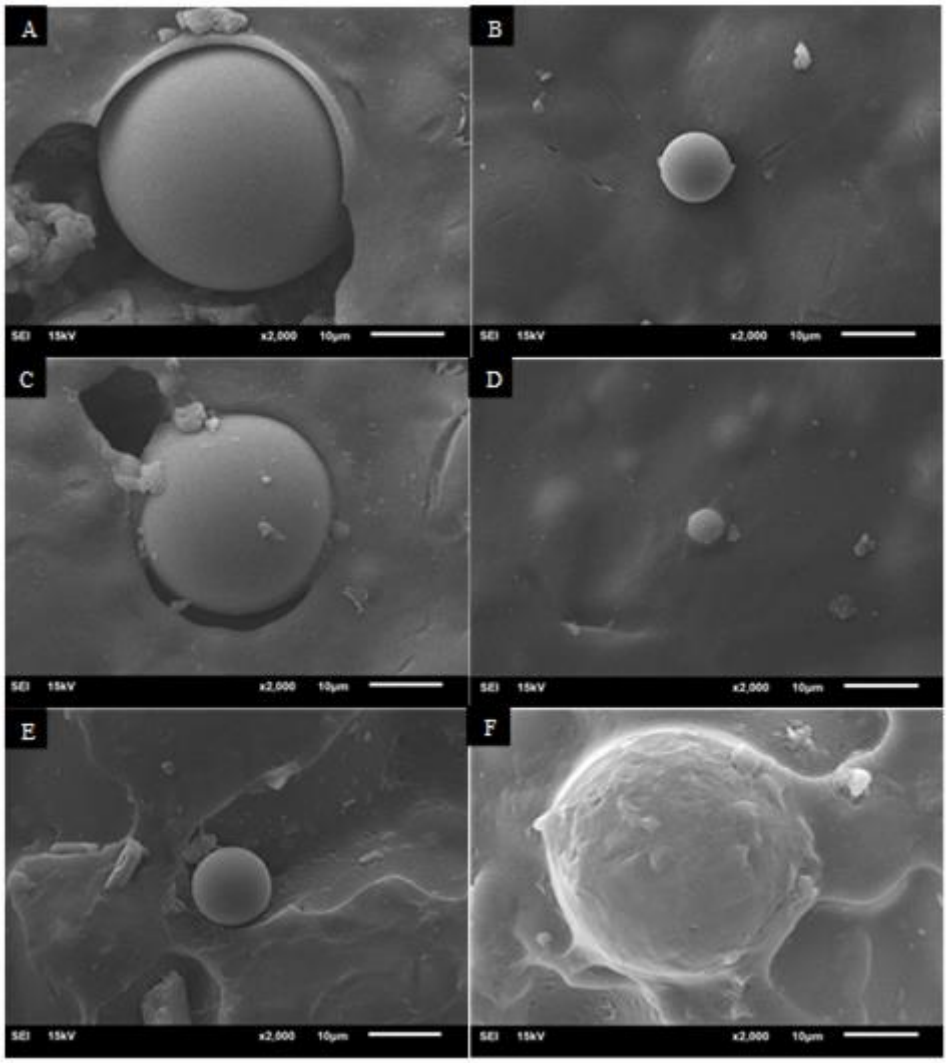

Figure 5. SEM images of the denture cleaning tablets (Corega) autopolymerized acrylic resin group (**A***) C. albicans* ATCC 60193 and (**B**) Oral isolate of *C. albicans***.** SEM images of the denture cleaning tablets (Corega) heat-cured acrylic resin group (**C**) *C. albicans* ATCC 60193 and (**D**) Oral isolate of *C. albicans.* SEM images of the denture cleaning tablets (Corega) hard relining denture material group (**E**) *C. albicans* ATCC 60193 and (**F**) Oral isolate of *C. albicans*

Routine cleaning is necessary to prevent denture stomatitis [13]. Chemical disinfectants are easier to use and more effective than mechanical cleaning [14]. The 2% chlorhexidine digluconate (Saver) solution was found to be successful, so the null hypothesis was supported.

Vinegar is nontoxic; it is also an affordable household product. Yildirim-Bicer et al. [15] reported that use of 100% vinegar for 10 min effectively reduced *C. albicans*. In vitro studies have shown that the low doses of acetic acid in vinegar induce programmed cell death in *C. albicans* [16]. In the present study, the effect of white vinegar was significant on blocking and reducing the growth of colonies of *C. albicans.*

CHX 0.2% has been used to treat Candida-associated dental illness [17]. Pavarina et al. [18] immersed complete dentures in a 4% CHX solution for 10 min, which was effective in elimination of the *C. albicans.* Da Silva et al. [19] advocated the use of chlorhexidine digluconate (Saver) and showed that is highly effective *against C. albicans, S. mutans,* and *S. aureus*. In the present study, CHX (Saver) was found to be very efficient for preventing the growth of *C. albicans*.

Denture cleaning tablets (Corega) help remove biofilm and stains [20, 21]. De Freitas Fernandes et al. [22] showed that denture cleaning tablets were effective in reducing *C. albicans* strains. In the present study, the effect of denture cleaning tablets was found to be similar to that of white vinegar.

4. Conclusions

The effect of different disinfectant methods on reducing the growth of *C. albicans* was found to be significant. The 2% chlorhexidine digluconate (Saver) solution was found to be the most effective on *C. albicans* when different disinfectant methods were evaluated. The effect of the denture cleaning tablets (Corega) and 100% white vinegar was found to be similar.

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