Effects of disinfectants on resilient denture-lining materials contaminated with *Staphylococcus aureus, Streptococcus sobrinus,* and *Candida albicans*

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Objectives: Resilient denture-lining materials are more susceptible to microbial adhesion than are denture base acrylic resin. As denture hygiene is essential to maintain the serviceability of the denture, disinfectant solutions have been suggested as a method for effectively cleaning resilient liners. The aim of this study was to determine the effectiveness of four different disinfectant solutions on four different resilient lining materials contaminated with *Staphylococcus aureus*, *Streptococcus sobrinus*, and *Candida albicans*. **Method and materials:** Three microorganisms (*Sta aureus*, *Str sobrinus*, and *C albicans*), four resilient lining materials (Tempo, Immediate, Flexacryl soft, Ufi Gel P), and four disinfectant solutions (5.25% sodium hypochlorite, 2% sodium hypochlorite, 5% Deconex, and 3.5% Savlex solutions) were investigated. The four resilient lining materials were contaminated with each of the microorganisms, then the disinfection tests were applied to detect the efficacy of disinfectant solutions on resilient denture base materials in an in vitro test. The specimens (n = 5, total = 240) were soaked for 5 minutes in each solution to disinfect the microorganisms. The control specimens (one of each material; n = 5, total = 60) were soaked in distilled water for 5 minutes. As a result, a reduction in microorganism counts was determined. The Kruskal-Wallis and one-way analysis of variance tests were used to analyze for significant differences among test groups at the level of significance. **Results:** For all microorganisms, soaking in 5.25% sodium hypochlorite reduced the number of viable adherent microorganisms significantly compared to soaking in 2% sodium hypochlorite, which led to greater reduction than soaking either 5% Deconex or 3.5% Savlex. The use of 5.25% sodium hypochlorite in all groups is statistically significant. **Conclusion:** Disinfection of four different resilient denture-lining materials with 5.25% sodium hypochlorite solution was the most effective immersion technique. To achieve an efficient disinfection, the immersion time of the other disinfectant solutions could be increased. *(Quintessence Int 2005;36:373–381)*

Key words: disinfection, microbiology, microorganism, resilient soft-lining materials

The success of complete or removable partial dentures depends on esthetics, comfort, and function. Unfortunately, the health of the supporting tissues may be adversely affected by the pressure of the prosthesis during use.[1] Resilient denture-lining materials are widely used to restore health of inflamed and distorted denture-supporting tissues.[1] These materials are used to make dynamic impressions,[1,2]

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and as tissue conditioners to restore traumatized oral mucosa to a healty state.[2,3] They are also used as a provisional liner to maintain the proper fit of the denture, to prevent trauma, and for trial evaluation of border extension.[1,4,5] Additional uses of resilient liners have emerged in the past few years, such as for oral cancer patients with postoperative defects requiring obturation,[6,7] and to modify transitional prostheses after stage I and stage II implant surgery.[1,8]

Resilient denture-lining materials have some disadvantages related to their physical properties and their response to microorganisms.[8] The adherence of *Candida albicans* and related microorganisms to solid surfaces, such as acrylic resin or resilient denture base lining materials, has been thought to be the first step in successful colonization, subsequent plaque formation, growth of adherent organisms, and the development of pathogenesis.[9] Resilient lining materials have been found to be more prone to microbial adhesion than acrylic resin denture base materials and have been demonstrated to interact with oral microorganisms because of their surface texture and the physical and chemical affinity between the materials.[10,11] Okita et al[10] demonstrated that resilient liners are more susceptible to microbial adhesion in vitro (*Streptococcus mutans* and *C albicans*) than the acrylic resin. In a study involving assays with multiple variables,[12] it was shown there was more adhesion to a silicone than an acrylic resin on heat-polymerized materials.

Dental prostheses are exposed to normal oral microbial flora, which include bacteria, viruses, and fungi. Oral and nonoral pathogenic organisms associated with local and systemic diseases have been cultured from contaminated prostheses such as these (ie, bacteria, viruses, and fungi).[13,14] Bacterial plaque and yeast are major factors in the etiology of denture stomatitis.[10] "Denture stomatitis" or "denture sore mouth" are terms used to describe a group of pathogenic changes found on denture-bearing tissues.[15] Surveys indicate that about 60% to 65% of denture wearers suffer some form of stomatitis.[16,17] Numerous studies have investigated the etiological agents of this inflammatory disease and have found that yeasts, particularly *C albicans*, play a major role in its pathogenesis.[18–20] Various oral bacteria also have been found to be important in this disease process.[20] *Staphylococcus aureus*, a pathogen causing respiratory infections, has been isolated from dentures and the oral cavity.[21,22] Dentures have recently been reported to be carriers of this pathogen.[23] Powell et al[24] surveyed dental laboratories in four cities and found that 67% of all materials transported from dental offices to the dental laboratories were contaminated with potentially pathogenic bacteria, including *Enterobacter cloacae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, α-hemolytic *Streptococcus* species, *Mycobacterium tuberculosis*, *Escherichia coli*, and others.

Efficient cleaning of the denture surfaces is a key factor in the maintenance of a healthy edentulous mucosa.[25] Denture hygiene is essential to maintain the serviceability of the denture because of esthetic concerns, as well as for prevention of denture-related stomatitis. The most effective preventive and curative treatment for pathogens is believed to be adequate denture hygiene; therefore, denture cleansers have been studied to identify ideal solutions.[26] During the assessment of an agent that prevents the *Candida* species from adhering to resilient lining materials, in vitro and in vivo studies have shown the effects of antifungal agents incorporated in the materials.[27,28]

Chemical disinfectants, when used after removal and before insertion of a prosthesis into the mouth, are a recommended method to prevent cross-contamination.[29] Chemical disinfectant solutions include 2% glutaraldehyde, dilute sodium hypochlorite, 5.25% sodium hypochlorite, chlorine dioxide, iodophors, alcohols, and chlorhexidine.[30–34]

Immersing dentures infected with *C albicans* in a chlorhexidine oral rinse overnight for a period of 24 days has been shown to effectively eliminate the organism from the acrylic resin surfaces.[35] Brace and Plummer[34] reported that 4% chlorhexidine scrub for 15 seconds, followed by 3-minute contact time with chlorine dioxide, was effective in disinfecting contaminated dentures.

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Table 1 The resilient denture lining materials and disinfectants tested

Brand	Type	Batch no.	Disinfectants	Type
Tempo (Lang Dental)	Liquid-powder Methacrylate polymer (auto-polymerized)	1023PNK	Deconex (Borer Chemie)	21%N,N-bis (3-aminopropyl)-dodecylamine didecyldimetylammoniumchloride 14% (5% diluted form)
Immediate (Lang Dental)	Liquid-powder Polyethyl methacrylate polymer (auto-polymerized)	3723PNK	Savlex (Drogsan)	15% Cetrimide, 1.5% chlorhexidine gluconate, Tartrazine, alcohol (3.5% diluted form)
Flexacryl Soft (Lang Dental)	Liquid-powder plasticized methacrylate polymer (auto-polymerized)	1123PNK	2% sodium hypochlorite	2% sodium hypochlorite
Ufi Gel P (Voco)	Paste-catalyst, A-silicone (auto-polymerized)	2070	5.25% sodium hypochlorite	5.25% sodium hypochlorite

Sodium hypochlorite is used as a traditional disinfectant because of its effectiveness in killing a wide range of organisms,[36] but it has some limitations.[29] The corrosive effect on metals can limit the use of this popular cleanser.[25] Other germicidal solutions are available that have been shown to be effective for use on prostheses.[37,38] Examples of such solutions are benzoic acid detergents and alkaline hypochlorite cleansers.[7] A simple, safe, and inexpensive method of disinfecting the prosthesis and resilient lining materials is needed.

The purpose of this study was to evaluate the effect of four immersion disinfection procedures on four resilient denture-lining materials contaminated with *Sta aureus*, *Str sobrinus*, and *C albicans*.

METHOD AND MATERIALS

Four commercial brands of resilient denture-lining materials—Tempo, Lang Dental; Immediate, Lang Dental; Flexacryl Soft, Lang Dental; and Ufi Gel P, Voco—were used in the present study and processed according to the manufacturers' directions. More information on the materials can be found in Table 1. Ufi Gel P contains a base and a catalyst, and the other lining materials contain liquid and polymer. Deconex, Savlex, 2% sodium hypochlorite, and 5.25% sodium hypochlorite are commonly used disinfectant solutions in dental clinics. In this study, these disinfectant solutions were used and their effects on the four resilient lining materials were observed.

The materials were prepared by using metal molds of a uniform size (10-mm diameter, 1-mm thickness) with smooth surfaces by placing glass slides over them as described by Nikawa et al.[39] Five specimens were prepared for each test group (n = 5, total = 300).

The purpose of the experiment was to contaminate sterile resilient lining materials with the microorganisms and to determine any reduction in the count of viable cells after four disinfection tests. The reduction in viable cells in phosphate-buffered saline (PBS, 20 mM potassium-buffered saline) was calculated by comparing it with appropriate control specimens (n = 5, total = 60), which were processed in the same way as test specimens, without using any disinfectant solution.

All of the resilient lining material specimens were sterilized with ethylene oxide.[40] *Staphylococcus aureus* ATCC 25923, *Str sobrinus* 6715, and *C albicans* ATCC 10231 were provided from Karadeniz Technical University, Faculty of Medicine Department of Microbiology, Turkey.

Mueller Hinton Broth (Merck, 21 g/L, infusion from meat 2.0 g; casein hydrolysate 17.5 g; starch 1.5 g) and Mueller Hinton Agar (Difco, 38 g/L, beef infusion 300 g; bacto casamino acids 17.5 g; starch 1.5 g; bacto agar 17 g) were used for *Sta aureus*. Todd Hewitt Broth (THB, LabM, 34.4 g/L, minced meat 10 g; tryptone 20 g; dextrose 2 g; sodium bicarbonate 2 g; sodium chloride 2 g; disodium phosphate anhydrous 0.4 g), supple-

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mented with agar (pH 6, 16 g/L; Merck, agar-agar ultrapure) and 5% sheep blood were used for *Str sobrinus*. Sabouraud Liquid Medium (Oxoid, 30 g/L, pancreatic digest of casein 5 g; peptic digest of fresh meat 5 g; glucose 20 g) and Sabouraud Dextrose Agar (Oxoid, 65 g/L, mycological peptone 10 g; dextrose 40 g; agar 15 g) were used for *C albicans*. All media were autoclaved at 121°C for 15 minutes. They were incubated at 37°C overnight to ensure purity. Ten μL of each culture was then inoculated onto the agar plates to form a confluent growth. After incubation at 37°C for 20 hours, the microorganism suspensions used for inoculation were prepared from the density of 3×10^8 cells/mL with broth medium.[41] A total of 300 specimens were used in this test. Each of the specimens was put into the culture with a sterile forceps.

The microorganisms that were incubated for 48 hours with sterile specimens were taken from the liquid medium and put into PBS (20 mM potassium-buffered saline, 0.15 M sodium chloride, pH 7.0). At the end of this period, test specimens were immersed in 5.25% sodium hypochlorite, 2% sodium hypochlorite, 5% Deconex, and 3.5% Savlex at 22°C for 5 minutes (n = 5, total = 240), and control specimens of each material (n = 5, total = 60) were soaked in distilled water for 5 minutes.

Liquid medium was not used while testing the effect of disinfection to eliminate new microbial growth. After 5 minutes, the materials were vigorously mixed in a vortex mixer (Agitateur, Firlabo.sa; 0 rpm, 230/50 volts/Hz, 35 watts) to pass the microorganisms into PBS.[41] Microorganisms in PBS that were still alive after the disinfection were diluted to decimal dilution (10^{-1},.....10^{-4}) by dispensing into PBS in sterile glass tubes. Twenty μL of each dilution from the tubes were inoculated onto the dry surface of agar plates with a drigalsky glass (an instrument used for homogen microorganism inoculation, Ildam A,Ş). The dilutions were aerobically incubated at 37°C for 24 hours. Then the colonies were counted macroscopically and calculated according to the formula:

$$N = X \times 1/V \times D$$

Where N is the number of microorganisms in the original suspension, X is the applied microorganism number, V is the quantity of inoculum inoculated on the dry surface of agar plate, and D is the reverse logarithm of dilution.[42] The reduction in the number of viable cells in PBS was calculated by comparison with control specimens that were contaminated with each test material and processed in the same way as the test specimens (ie, without using any disinfection solution).

All experiments were performed with five replicates. The values obtained were averaged to provide the colony-forming units (cfu), with standard deviations.

Statistical analysis

A two-way analysis of variance (ANOVA) was used for each microorganism to detect the effect of interactions between the disinfectant solutions in the lining materials on cell counts of the microorganisms. Two-way ANOVA showed an interaction between the disinfectant solution and the lining material, thus, one-way ANOVA was performed by nonparametric Kruskal-Wallis comparisons on the level of disinfectant solutions for each resilient lining material. If $P < .05$ ($\alpha = .05$), the statistical differences should be considered significant.

RESULTS

A two-way ANOVA was used for each microorganism and two-way interactions between disinfectant solutions and resilient denture liners for each microorganism were detected. For *Sta aureus* ($F = 3.952$, $P < .001$), *Str sobrinus* ($F = 16.721$, $P < .001$), and *C albicans* ($F = 36.455$, $P < .001$), two-way interactions were found to be statistically significant. Therefore, one-way ANOVA was used by nonparametric Kruskal-Wallis test to detect the effect of disinfectant solutions in each resilient denture-lining material. Application of a one-way ANOVA and nonparametric Kruskal-Wallis comparison showed a significant difference among the levels of disinfectant solutions for each lining material ($P < .001$). The results of a one-way

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Table 2 One-way ANOVA and pairwise comparisons of disinfectants by nonparametric analysis in resilient denture lining materials of each microorganism

	Sta aureus				*Str sobrinus*				*C albicans*			
	D4	D3	D1	D2	D4	D3	D1	D2	D4	D3	D1	D2
Tempo	9.80	49.00	63.80	78.40	13.00	68.20	78.00	116.00	4.20	25.20	29.40	33.60
	H = 16.597 *P* = .001 A = 3.069				H = 16.519 *P* = .001 A = 3.118				H = 13.711 *P* = .003 A = 4.553			
Immediate	10.20	46.80	53.40	63.20	13.80	55.20	69.00	96.00	6.60	30.60	33.00	31.80
	H = 16.519 *P* = .001 A = 3.337				H = 17.516 *P* = .001 A = 2.412				H = 11.472 *P* = .009 A = 5.432			
Flexacryl soft	7.00	35.00	49.40	45.20	14.00	63.20	84.00	119.00	6.80	39.60	42.40	45.20
	H = 14.632 *P* = .002 A = 4.138				H = 17.556 *P* = .001 A = 2.379				H = 12.554 *P* = .006 A = 5.027			
Ufi Gel P	9.40	58.40	65.80	80.00	22.80	114.00	149.00	218.00	11.80	82.80	88.80	100.80
	H = 16.003 *P* = .001 A = 3.427				H = 17.567 *P* = .001 A = 2.419				H = 16.532 *P* = .001 A = 3.110			

D1 = 5% Deconex; D2 = 3.5% Savlex; D3 = 2% sodium hypochlorite; D4 = 5.25% sodium hypochlorite.
Using the Kruskal Wallis test.

ANOVA with Kruskal-Wallis comparison are presented in Table 2.

Staphylococcus aureus test group

The mean cell counts of cfu/mL for each test and the control groups are shown in Fig 1. Application of a one-way ANOVA revealed a significant difference ($P < .001$) among the levels of disinfection factor. With the nonparametric Kruskal-Wallis analysis, a statistically significant ($P < .001$) difference was observed between all of the resilient lining materials—soaking in 5.25% sodium hypochlorite solution was more effective than soaking in 2% sodium hypochlorite solution for 5 minutes, which, in turn, was more effective than soaking in 5% Deconex or 3.5% Savlex. For the four resilient lining materials, soaking the lining material in 5.25% sodium hypochlorite solution was found to be extremely effective in destroying *Sta aureus*.

Streptococcus sobrinus test group

The mean cell counts of cfu/mL for each test and the control groups are presented in Fig 2. Application of a one-way ANOVA and nonparametric Kruskal-Wallis test showed differences among all the groups, with soaking in 5.25% sodium hypochlorite solution, being more effective than soaking in 2% sodium hypochlorite solution, which, in turn, was more effective than soaking in 5% Deconex or 3.5% Savlex ($P < .001$). For four resilient lining materials, soaking the the lining in 5.25% sodium hypochlorite solution for 5 minutes was found to be extremely effective in destroying *Str sobrinus*.

Candida albicans test group

Results were obtained using the same principles as with *Sta aureus* and *Str sobrinus*. The mean cell counts of cfu/mL for each group and the control groups are shown in Fig 3. Application of a one-way ANOVA and nonparametric Kruskal-Wallis showed a significant difference between all the groups ($P < .001$). Soaking in 5.25% sodium hypochlorite solution was also significantly different from the test group of soaking in 2% sodium hypochlorite solution, 5% Deconex, or 3.5% Savlex ($P < .001$) for *C albicans.*

DISCUSSION

The aim of this study was to determine the effectiveness of four different disinfection

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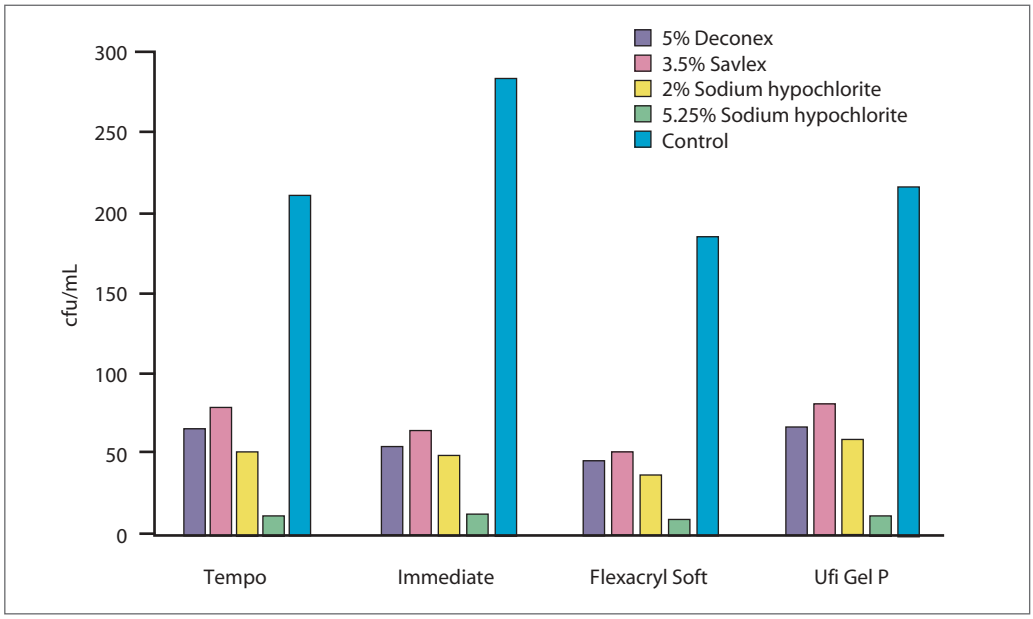


Fig 1 Comparison of cfu/mL for disinfection test groups and control groups for *Staphylococcus aureus.*

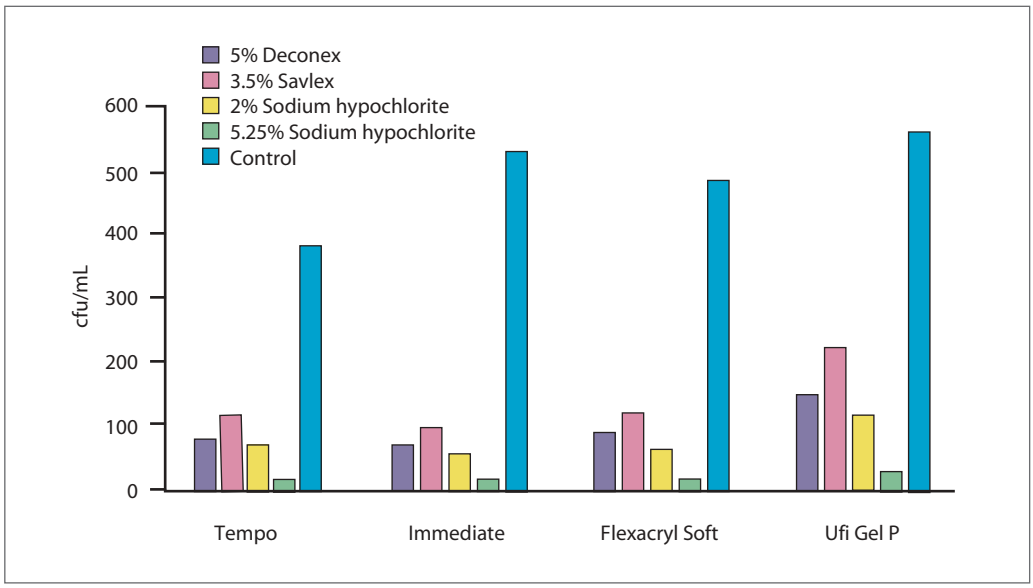


Fig 2 Comparison of cfu/mL for disinfection test groups and control groups for *Streptococcus sobrinus.*

solutions on four different resilient lining materials contaminated with *Sta aureus*, *Str sobrinus*, and *C albicans*. Results of this study showed that a 5-min contact time with four different disinfectant solutions was effective in reducing the cell counts of microorganisms. However, there was a difference in the effectiveness of all disinfection solutions to destroy the microorganisms.

According to these results, the most effective disinfection method for each of the four resilient lining materials was the application of 5.25% sodium hypochlorite solution. The order of effectiveness of the other disinfectant solutions was (in decreasing order): 2% sodium hypochlorite solution, 5% Deconex, and 3.5% Savlex. The use of 5.25% sodium hypochlorite solution for 5 minutes provided

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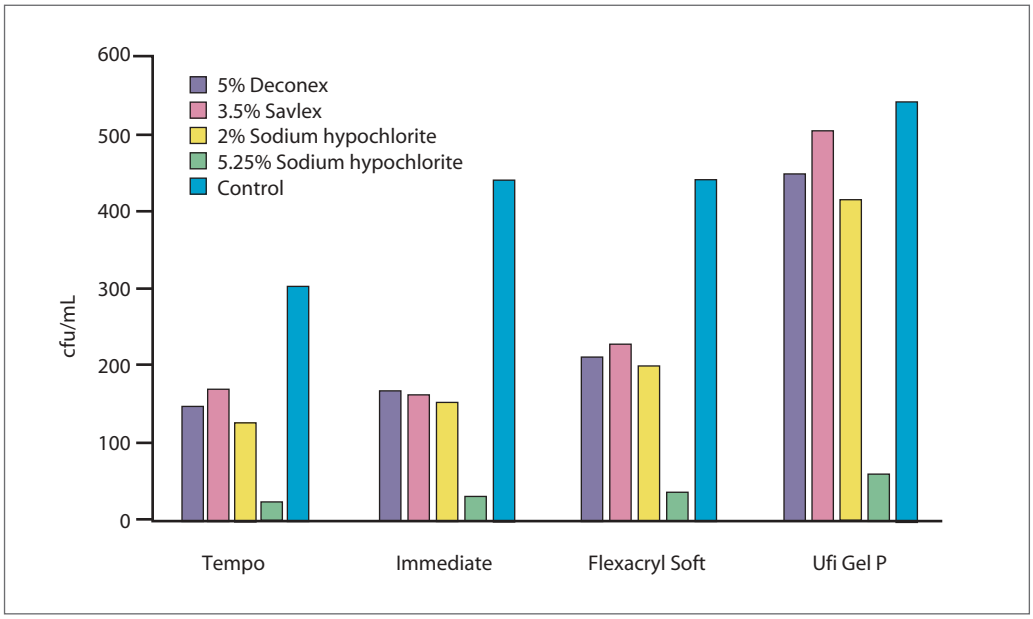


Fig 3 Comparison of cfu/mL for disinfection test groups and control groups for *Candida albicans*.

a significant reduction of all viable organism counts.

In a study by Bell et al,[33] the biocidal effectiveness of chlorine dioxide and 5.25% sodium hypochlorite (1:10 dilution) was compared using acrylic resin strips inoculated with *Sta aureus*, *C albicans*, and *Escherichia coli* in the presense of an organic load. The results revealed that chlorine dioxide achieved complete disinfection within 2 minutes for the three organisms and that sodium hypochlorite achieved complete disinfection within 4 minutes for the three organisms.

Rudd et al[31] demonstrated the sterilizing effect of 5.25% sodium hypochlorite as a denture soak. Chau et al[30] found that 10-minute immersion in 5.2% sodium hypochlorite disinfects dental acrylic resin more effectively than either Biocide or Alcide CD disinfectant solutions. In this study, the results revealed that soaking the specimens in 5.25% sodium hypochlorite for 5 minutes reduced the growth of microorganisms significantly in comparison to the other disinfectants.

Soaking dentures in sodium hypochlorite has not been recommended because of the possibility of a discoloring and corrosive effect on the denture base.[25,43] However, other studies claim that no deterioration of resilient lining materials occur when hypochlorite denture cleansers are used.[31,44] Rudd et al[31] suggested that full-strength sodium hypochlorite can kill organisms when used for only 5 minutes, but the effect of this treatment on the dentures was examined only visually to determine any deterioration in color and surface details.

In another study,[43] no detrimental effect on the denture base material was observed either at 0.02% or 0.0125% sodium hypochlorite solution, but adverse effects could manifest themselves in the long term (more than 7 years).

Baysan et al[45] determined the effectiveness of microwave energy, in the disinfection of long-term resilient lining material contaminated with *C albicans* and *Sta aureus*. They suggested that for both organisms, soaking in 2% sodium hypochlorite reduced the number of viable adherent microorganisms recovered significantly more than exposure to microwave energy, which led to a greater reduction than leaving the lining material dry overnight ($P < .001$).

Dixon et al[46] examined the efficacy of microwave irradiation against *C albicans* colonized on three soft liner materials and one heat-polymerized denture base resin. They showed that irradiating the materials for 5 minutes while the materials were immersed in water killed all *C albicans* present.

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Furukuwa et al[47] evaluated the effectiveness of 3 minutes of chlorine dioxide spray and immersion disinfection procedures on two denture liners and stainless-steel specimens used as controls. They demonstrated that chlorine dioxide was effective against nonporous stainless-steel specimens, but was inadequate for denture liners at the recommended 3-minute disinfection time. The results showed that the immersion technique was more effective than the spray technique, but the difference was not significant.

The disinfectant solutions used in this study were applied to the specimens for 5 minutes using an immersion technique. For each of the resilient lining materials and the three microorganisms, the most effective disinfectant solution was the 5.25% sodium hypochlorite solution, as it significantly reduced the microorganism number in comparison to the other disinfectant solutions.

The immersion time that we applied (5 minutes) can be increased to improve disinfection. Further studies are needed to investigate whether or not the material is physically and mechanically affected when the disinfection time is increased.

CONCLUSIONS

1. A 5-minute contact time with four different disinfectant solutions was effective in reducing the cell counts of each microorganism tested. However, there was a difference in the reduction of viable microorganisms between the disinfectant solutions.
2. Disinfection of four different resilient lining materials with a 5.25% sodium hypochlorite solution was the most effective immersion technique, and the difference from the other disinfectant solutions in all groups was statistically significant.
3. In all groups, the disinfectant solutions reduced the viable microorganisms significantly, but it is important to establish that the immersion time of the disinfectant solutions should be increased to inhibit the growth of all microorganisms.

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