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CLINICAL RESEARCH

Chemical hygiene protocols for complete dentures: A crossover randomized clinical trial

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ABSTRACT

Statement of problem. Clinical evidence on the best chemical protocol for the disinfection and removal of biofilm from complete dentures is lacking.

Purpose. The purpose of this crossover randomized clinical trial was to assess the effectiveness of various chemical hygiene clinical protocols in reducing the microbial viability of biofilm formed on complete dentures.

Material and methods. In this triple-blind (participants, dentist, and outcome evaluator) study, complete denture wearers without candidiasis were randomly divided into 4 groups (n=40) according to the chemical hygiene protocol: water (placebo), 0.5% sodium hypochlorite solution, 0.12% chlorhexidine gluconate solution, and 5% sodium bicarbonate solution. The biofilm formed on the palate intaglio and denture teeth was collected and assessed in each experimental phase for quantitative microbial viability at the seventh and 14th day after using the chemical protocol.

Results. Two participants were lost. Data were analyzed by MANOVA and Tukey HSD tests. Soaking dentures was not effective in decreasing *Candida albicans*, *C.* non-*albicans*, and *lactobacillus* counts. The use of sodium hypochlorite and chlorhexidine decreased total microorganisms and *Streptococcus mutans* counts for both palate and teeth compared with water and sodium bicarbonate. The intaglio of the dentures always presented higher microbial counts than did the denture teeth.

Conclusions. The use of sodium hypochlorite and chlorhexidine and mechanical cleansing with a toothbrush decreased microbial viability in healthy complete denture wearers. (J Prosthet Dent 2019;121:83-9)

Poor denture hygiene is commonly found in complete denture wearers.[1,2] Biofilm formed on the tissues and denture surface is a significant cofactor in the pathogenesis of denture stomatitis, whereas biofilm accumulation when not removed may also be responsible for multiple local and systemic diseases[3-5] including bacterial endocarditis, gastrointestinal infection, aspiration pneumonia, and respiratory infections.[2,5] This occurs because dentures serve as a reservoir for distant infections, compromising health and resulting in higher susceptibility to infection.[4]

Although brushing alone has been recommended to decrease biofilm formation,[6,7] effective biofilm removal requires a degree of manual dexterity often lacking among elderly individuals.[1,7] A combination of mechanical and chemical hygiene protocols might be required to reduce denture biofilm accumulation to effectively maintain a disease-free oral environment.[1,2,5,8,9]

Alkaline peroxides,[8,10,11] sodium bicarbonate,[12,13] chlorhexidine,[10,14-16] and hypochlorite solutions[17,18] are effective in reducing biofilm formation on complete dentures. However, most in vitro studies may not accurately reflect the clinical situation,[19] because not only the substrate but also the antimicrobial properties of saliva

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Clinical Implications

Weekly use of 0.5% sodium hypochlorite or 0.12% chlorhexidine gluconate is effective in controlling biofilm in denture wearers.

may contribute to the tissue and/or patient factors that influence biofilm formation.[20]

Because clinical evidence on the best chemical protocol for disinfecting and removing biofilm from complete dentures is limited,[9,21,22] this clinical trial assessed which chemical hygiene protocol would lower microbial viability on maxillary complete dentures. The hypothesis assumed differences among treatments and time points.

MATERIAL AND METHODS

This randomized clinical trial had a crossover and triple-blind (participant, dentist, and outcome evaluator) design with 4 phases of 14 days each. Complete denture wearers were randomized into 4 groups depending on the chemical hygiene protocol to be used to soak dentures once a week (water [placebo], sodium hypochlorite, chlorhexidine, or sodium bicarbonate). Biofilm that formed on the intaglio of the palate and buccal posterior denture teeth was collected after 7 and 14 days of use of the protocol. The study was registered at ClinicalTrials.gov (NCT01906242), approved by the local Research and Ethics Committee (no. 140/2009), and reported according to the CONSORT statement.[23]

The trial was conducted from April to October 2014. One examiner (F.V-M.) carried out an intraoral examination of the soft tissues and complete dentures of all participants from April to June 2014. The oral health of the participants was assessed, and all participants signed written informed consent before being accepted into the study. One hundred forty-three (N=143) complete denture wearers who had their dentures provided at the School of Dentistry and had been wearing the maxillary complete denture for at least 1 year and a maximum of 2 years were selected and invited to participate in the study. Inclusion criteria were individuals who could comply with the experimental protocol, wear a maxillary complete denture, and had good general and oral health. Participants were screened for *Candida* species without, however, having candidiasis. For the screening, swabs from the palate were cultured in CHROMagar *Candida* (Difco; Sparks) at 37ºC for 48 hours. After the study was explained, 128 individuals agreed to participate. However, the exclusion criteria eliminated those taking antibiotics or antifungal agents during the 3 months prior to the study (n=3) and those with clinically diagnosed denture stomatitis or systemic diseases (n=10). Also, 45 participants could not comply with the experimental protocol because of the study's timeframe, and 30 participants were not eligible because they were enrolled in another study. Forty participants (31 women and 9 men) between 58 and 67 years old were selected (Fig. 1).

The primary study outcome was to reduce viable microorganism counts after using different chemical hygiene protocols on the dentures. Sample size calculation was based on this outcome, presuming a superiority trial with statistical tests performed with 80% power and α=.05. Considering the data from a previous study,[24] 24 participants were required for the experimental and control group. Taking possible losses into account due to the crossover nature of the design (estimated around 50%), the final sample size was n=40.

Before the study began, the original dentures of all participants received a standardized mechanical polishing to obtain the same smooth surface, as previously described.[19] Every time a phase ended, this procedure was redone to standardize the denture surfaces. The 40 participants were randomized and assigned to each group using a computer-generated list (Excel 2010; Microsoft Corp) of random numbers. As a result, neither the investigators nor the participants knew which treatment was being used. The solutions were placed in plain white receptacles of the same size and shape by a researcher not involved in the study and identified by Greek letters according to the treatment previously randomized. An identifying number was assigned to each participant, who received 1 receptacle (enough for 2 weeks of use) for each test phase. Each participant received a bowl with a marked line on the outside where the liquid should be placed and the denture soaked. The amount was enough to cover the prosthesis. After immersion, the solution was discarded, and a new solution was used for the next immersion phase.

The participants were asked to return the receptacle at every visit. Before the experiment began, all participants had an initial run-in week with water to get acquainted with the experiment. All participants were asked to perform the chemical treatment protocol on Saturdays and return the next Tuesday for the biofilm analyses. Participants were instructed to wear the dentures at all times[24,25] and did not receive any instructions regarding daily diet. Likewise, written instructions were provided on how to use the product, along with the treatment protocol.

At each experimental phase, participants were randomly assigned into 1 of the 4 subgroups, according to the chemical treatments: water (placebo control), 0.5% sodium hypochlorite solution,[26,27] 0.12% chlorhexidine gluconate solution,[10,27] and 5% sodium bicarbonate solution.[28] When a treatment ended, the participants started another treatment until all participants had used all 4 treatments (Fig. 2).

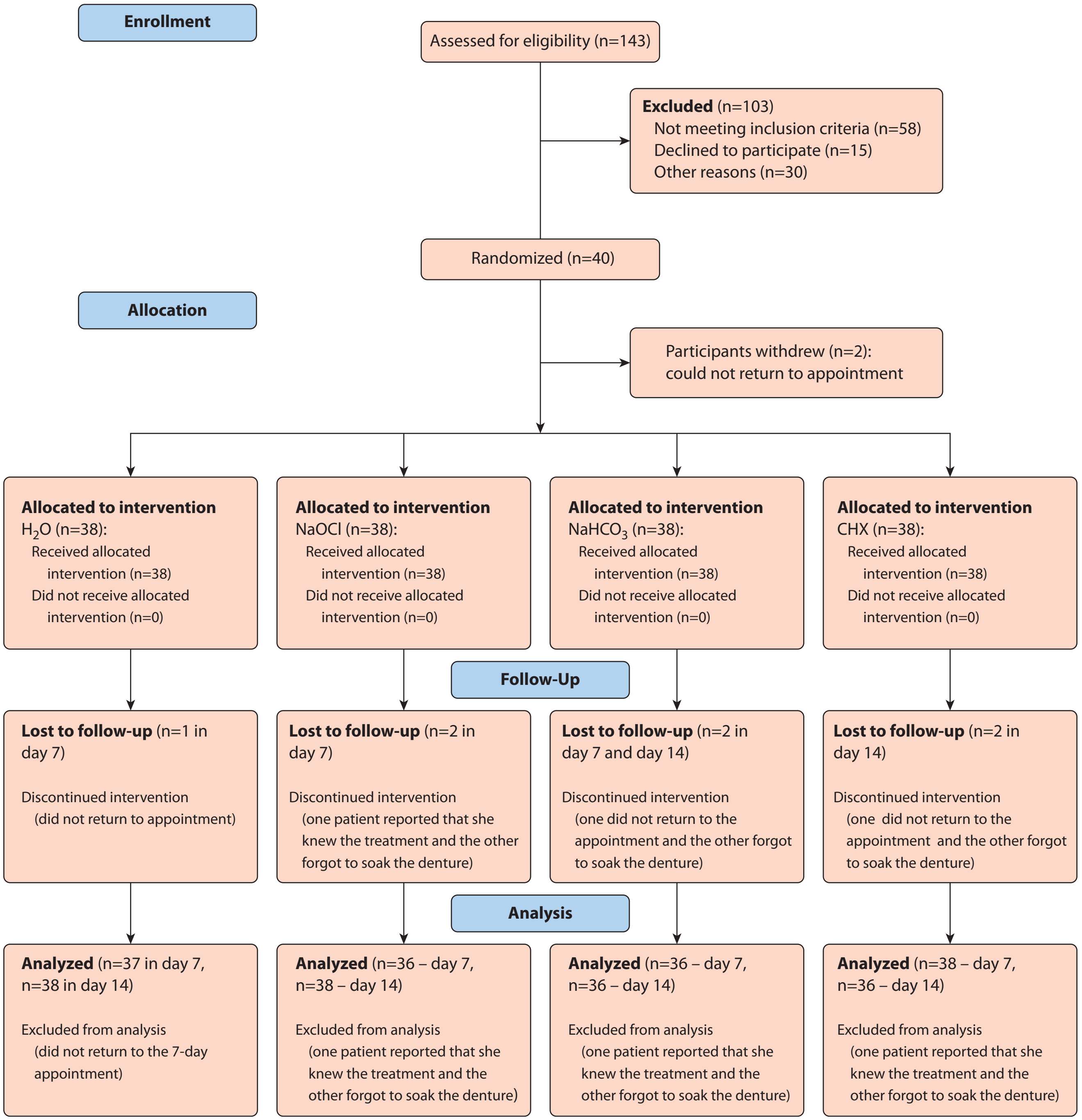

Figure 1. Trial phases.

The same company manufactured all solutions to International Organization for Standardization (ISO) certified standards. All cleansing protocols were the same, soaking the denture for 10 minutes once a week for 2 weeks.[10,26] After completing each phase, the participants returned to change the product for another. In between phases, the participants used running water (washout) for 7 days for each experimental phase to eliminate possible residual effects from the chemical hygiene protocol. They were instructed to brush their dentures 3 times a day for 1 minute with a toothbrush and toothpaste (Total 12; Colgate-Palmolive) provided by the researchers and to rinse with tap water after mechanical cleansing with a toothbrush during experimental periods and washouts.[28] The total study period was therefore 77 days (4 phases of 14 days each for each participant and 3 washout periods of 7 days between each phase).

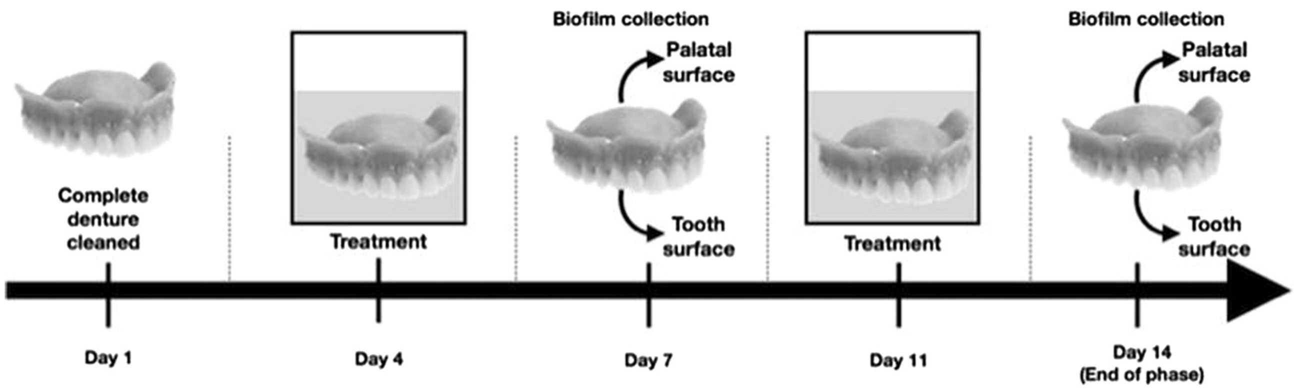

Figure 2. Timeframe of experiment.

Table 1. Microbiological results for microorganisms in biofilm according to experimental conditions (CFU/mm^2, mean ±standard deviation)

Treatment	Area	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days
		***Candida albicans* (10^3)**		***Candida* non-*albicans*(10^2)**		**Total Microorganisms (10^7)**	
H_2O (n=37/38)	Palate	2.0 ±6.5^a	1.0 ±3.3^a	11 ±62	0.1 ±1		
	Teeth	0.3 ±1.0^a	0.2 ±0.8^a	10 ±4	1 ±0.3		
CHX (n=38/36)	Palate	0.4 ±2.2^b	1.2 ±4.3^a	1.0 ±3.0	13 ±53		
	Teeth	0.7 ±2.7^a	0.4 ±1.4^a	0.2 ±0.1	2 ±10		
NaOCl (n=36/38)	Palate	0.01 ±0.1^b	0.2 ±1.0^a	0.01 ±0.1	0.01 ±0.3		
	Teeth	0.4 ±2.2^a	0.02 ±0.1^a	0 ±0	0.01 ±0.1		
$NaHCO_3$ (n=36/36)	Palate	1.7 ±6.3^a	1.4 ±7.4^a	74 ±440	220 ±1312		
	Teeth	1.9 ±7.0^a	1.0 ±5.0^a	6 ±24	2 ±9		
		***Streptococcus mutans* (10^5)**		**Lactobacilli (10^6)**			
H_2O (n=37/38)	Palate	1.3 ±5.7^a	4.3 ±18.8^a	7 ±54	7 ±18	6 ±10^a	8 ±20^a
	Teeth	0.2 ±0.6^a	1.0 ±5.4^a	2 ±6	10 ±3	7 ±12^a	5 ±7^a
CHX (n=38/36)	Palate	0.0001 ±0.3^b	0.1 ±0.8^a	1 ±2	4 ±18	1 ±2^a	3 ±2^b
	Teeth	0.4 ±1.6^b	0.1 ±0.4^a	8 ±23	4 ±8	2 ±6^a	1 ±4^b
NaOCl (n=36/38)	Palate	0.3 ±1.5^b	3.8 ±23.3^a	1 ±4	2 ±4	3 ±9^a	1 ±3^b
	Teeth	0.1 ±0.4^b	0.2 ±0.5^a	1 ±4	2 ±8	3 ±6^a	1 ±3^b
$NaHCO_3$ (n=36/36)	Palate	1.6 ±6.4^a	1.5 ±6.9^a	5 ±12	4 ±8	3 ±6^a	6 ±9^a
	Teeth	8.0 ±3.2^a	0.7 ±3.9^a	2 ±5	6 ±26	9 ±17^a	4 ±7^a

Lowercase superscript letters represent statistically significant differences among treatments for each group of microorganisms at time points in each area collected (P<.05, MANOVA).

The biofilm that formed on 1 randomly selected (using a computer-generated list, Excel 2010; Microsoft Corp) side of the palate and the posterior teeth of the denture was collected with a sterile microbrush according to a 2×2-cm template of the area to be swabbed after 7 and 14 days of treatment. The microbrush was inserted into microcentrifuge tubes containing sterile saline solution (1 mL) and vortexed for 30 seconds followed by sonication (20 W with 3 pulses of 10 seconds each). The suspensions were serially diluted, inoculated on specific media, and incubated at 37°C (anaerobiosis: blood agar, rogosa agar, and mitis salivarius agar supplemented with 0.2 U/mL of bacitracin; aerobiosis − CHROMagar *Candida*) for 24 to 96 hours.[19] The colony-forming units (CFUs) were counted with a stereomicroscope and the results expressed in CFU/mm^2.

The data were analyzed with statistical software (IBM SPSS Statistics, v20; IBM Corp) (α=.05). To compare the different treatment groups, the investigator in charge of the statistical analysis (T.T.M.) was informed of the tube letters corresponding to each tested product and those corresponding to the active treatment product. For microbiological analysis, data that violated the assumptions of equality of variances and normal distribution of errors were ranked and analyzed by MANOVA and the Tukey HSD test. Time was considered as within the participant factor and treatment as between participant factors.

RESULTS

Two participants withdrew from the experiment, both for medical reasons. Data from another patient were only considered in 2 of the 4 treatments, because on the third treatment she reported smelling a different odor from the solution; the researchers decided to remove her from the experiment because she could not adhere to the given protocol, compromising the blindness of the trial. During the experiment, 6 cases of data loss at some phases occurred, and the data for the phase was considered as "missing data" in the analysis (Fig. 1).

No statistically significant differences were found in *C.* non-*albicans* and lactobacilli counts for all treatments, areas, and time points assessed (P>.05, Table 1). Regarding total microorganisms on denture teeth, statistically significant differences were found among treatments after 14 days, with sodium hypochlorite performing better when compared with water (P<.001) and sodium bicarbonate (P=.008). Chlorhexidine was also better than water (P=.001) and sodium bicarbonate (P=.048) and similar to sodium hypochlorite (P>.05). For the palate, the same occurred after 14 days: sodium hypochlorite was better than sodium bicarbonate (P=.002) and water (P=.030), and the use of chlorhexidine resulted in lower counts compared with water (P=.019) and sodium bicarbonate (P=.001).

For *Streptococcus mutans* collected from the denture teeth, sodium hypochlorite led to lower counts after 7 (P=.046) and 14 (P=.047) days when compared with water. After 14 days, statistically significant differences were also seen for chlorhexidine (P=.016). The same effect occurred in the biofilm collected from the palate after 7 days: sodium hypochlorite decreased microbial viability compared with water (P=.005) and sodium bicarbonate (P=.044). Chlorhexidine and sodium hypochlorite performed similarly (P=1.00).

Candida albicans (P=.027) and *lactobacilli* (P=.001) presented higher counts after 14 days and *Streptococcus mutans* (P=.043) and total microorganisms (P=.001) after 7 days. *Candida albicans* counts also decreased on the palate on day 7 with the use of sodium hypochlorite compared with water (P=.0014) and sodium bicarbonate (P=.034), but these results were not sustained at 14 days, with no statistically significant differences among treatments (P>.05).

DISCUSSION

This clinical trial showed that sodium hypochlorite and chlorhexidine can both effectively decrease microbial viability on denture surfaces and could be used together with mechanical toothbrushing to prevent microbial colonization. In general, sodium hypochlorite and chlorhexidine led to lower *Streptococcus mutans* and total microorganism counts and similar performances for the palate and denture teeth. Therefore, the research hypothesis was rejected because the use of sodium hypochlorite and chlorhexidine solution resulted in lower microbial viability.

In this study, the participants used all treatments once a week for 2 weeks.[4] These time points were chosen because they represent the initial development and maturing (14 days) of biofilms on complete dentures. Also, a time-dependent effect or a possible recolonization could be observed at these time points. The main effect occurred after 14 days, except for total microorganism counts, where no differences were found among the tested cleaning protocols after 7 days. In vitro studies have shown that chemical hygiene is effective in reducing several microorganisms.[10,13,14,17] However, those results are not sustained in vivo, as other factors are involved in microorganism colonization.

In fact, it seems that the same *Candida* species show increased resistance to commonly used antifungal therapies.[19] One possible hypothesis for the lack of effect of denture cleansers on *Candida albicans* might be the lower concentrations of the chemical treatments (0.5% sodium hypochlorite solution, 0.12% chlorhexidine gluconate solution, and 5% sodium bicarbonate solution), the short immersion period used (10 minutes once a week), or even the short follow-up (14 days). Studies have shown that at high concentrations the chemical hygiene clinical protocols were more effective at killing microorganisms,[14] but with the disadvantage of roughening denture surfaces, possibly resulting in more biofilm accumulation.

Although reports have related denture base infections to a single species of *Candida*,[20] denture stomatitis is a polymicrobial disease in which bacterial and fungal interactions form a symbiotic biofilm.[4,20,29] For example, the *Lactobacillus* species has an antagonistic relationship with *Candida,* and investigations suggest that these bacteria can modulate the host response, upregulating cytokines when co-cultured with *C. albicans*; this could be associated with the clearance of fungal infection.[20]

Lower *Streptococcus mutans* counts were found when sodium hypochlorite and chlorhexidine were used. These results are important because, although the ability of *Candida albicans* to form biofilms on denture surfaces is a significant cofactor in the pathogenesis of denture stomatitis, other bacterial species are also important components of denture biofilm.[29] This occurs because during the stage of denture biofilm formation, the rate of adherence increases with anaerobic bacteria such as *Streptococcus mutans;* these appear in the initial phases of biofilm development and seem to have synergism with the *Candida* species.[15,29] Also, *Streptococcus mutans* may be located in more superficial layers of the biofilm and consequently are more exposed to antimicrobial agents. Yet, these bacteria also increase acidity, which generates environmental conditions for yeast growth.[26] In a multispecies biofilm, *Candida* spp. would be protected from denture cleanser action by layers of extracellular matrix and bacterial cells.[28] As shown in this study, decreasing the viability of mutans streptococci may have a positive influence, inhibiting growth of the *Candida* species. Total microorganism viability was reduced with sodium hypochlorite and chlorhexidine after 14 days. The lower the number of microorganisms in the oral cavity, the lower the rate of microbial adherence and biofilm formation will be. It is expected that these cleansing protocols will indeed help maintain oral health.

Healthy individuals wearing new complete dentures (without clinically diagnosed denture stomatitis) were selected, which may explain the present results. Although this study showed no differences in *C. albicans* counts, *C. albicans* is recognized as contributing to denture stomatitis. These fungi can proliferate in healthy hosts by surviving immune factors, demonstrating increased resistance to commonly used antifungal drug therapies. This is a limitation of the present study, as participants diagnosed with denture stomatitis may present different results; this remains to be tested.

From a microbiological point of view, whether to use sodium hypochlorite or chlorhexidine gluconate solution makes no difference. This is further reinforced by the findings that both sodium hypochlorite[17,21,22] and chlorhexidine gluconate solution[10,21,22] significantly reduce biofilm formation. Still, other properties should be considered when choosing one or the other. A chemical disinfectant should be chosen because of its efficiency in eliminating microorganisms without adversely affecting the denture materials. Chlorhexidine gluconate solution presents substantivity, which results in a desirable residual antimicrobial effects for days to weeks when used.[15] In contrast, its prolonged use also presents some unwanted effects, including mild discomfort or burning sensation, epithelium exfoliation, tooth staining, enhancement of supragingival calculus, and discoloration of artificial teeth and denture basis.[14]

The current gold standard solution (sodium hypochlorite) acts directly on the organic matrix,[17] has fungicidal and bactericidal properties, and can remove plaque stains[8] resulting in the dissolution of the polymer structure probably because of its alkaline pH. Such alterations could be attributed to the loss of cell membrane integrity and release of cytoplasmic content caused by this alkaline solution. It also has some unwanted effects, including soft-tissue dissolution, bad taste, objectionable smell, corrosive effect on metals, and increased roughness on the denture surfaces when used in concentrations above 0.5%, and with a soaking time greater than 10 minutes.[9]

Removal of the denture at night and good hygiene with denture cleaning solutions are effective in the prevention and treatment of denture stomatitis.[4,20] In this study, the participants were instructed not to remove the prosthesis at night because all participants reported not removing their dentures as their established habit. This is a personal and cultural issue also reported in other studies.[24,25] Nevertheless, the results should be interpreted with caution. This study was carried out in healthy individuals, the hygiene clinical protocols were standardized, a short period was evaluated (14 days), and all participants received oral hygiene instruction and motivation at every appointment. Also, recolonization occurs naturally at all times, emphasizing why brushing with a toothbrush and toothpaste combined with a daily hygiene routine is most important for the maintenance of clean complete dentures.

The results of this clinical study indicate that strict denture disinfection procedures should be recommended to prevent denture stomatitis. Within this context, both sodium hypochlorite and chlorhexidine gluconate have proved to be safe, straightforward, effective, and inexpensive chemical hygiene protocols that can be used to disinfect dentures.

CONCLUSIONS

From the findings of this clinical study, the following conclusion was drawn:

1. Soaking complete dentures in 0.5% sodium hypochlorite or 0.12% chlorhexidine gluconate solution for 10 minutes once a week and mechanical cleansing 3 times a day with a toothbrush and toothpaste effectively reduced microbial viability on complete dentures.

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