Purpose: To evaluate and compare the effects of 0.2% sodium hypochlorite, Efferdent, and 6.25% Ricinus communis on biofilm removal and antimicrobial action on dentures and brushes using nonimmersion and immersion protocols for the brushes. Materials and Methods: A total of 45 denture wearers were randomly assigned to a denture immersion protocol for 7 days: 0.85% saline solution for 20 minutes (control); 0.2% sodium hypochlorite for 20 minutes (0.2% SH); Efferdent for 3 minutes; or 6.25% R. communis for 20 minutes (6.25% RC). The participants were also randomized to immersion (n = 23) or no immersion (n = 22) of their brushes with their dentures in the same solutions. For biofilm evaluation, the dentures were stained and photographed, and the area of the biofilm was measured using Image Tool 3.0 (University of Texas Health Science Center). To evaluate microbial load on dentures and brushes, the biofilm was collected, and the Candida spp and Streptococcus mutans colonies were counted. Results: The 0.2% SH, Efferdent, and 6.25% RC groups showed reduced biofilm and Candida spp on dentures regardless of the immersion protocol for the brushes. However, no difference was found in the Candida spp counts that were collected from the immersed brushes compared to the nonimmersed brushes. The 0.2% SH and Efferdent groups showed reduced S. mutans on both dentures and brushes, except for in the nonimmersion subgroups. Conclusion: All solutions reduced denture biofilm and microbial load. However, immersion of brushes in the solutions did not contribute to reducing the microbial load. Int J Prosthodont 2021;34:291–299. doi: 10.11607/ijp.6665

When biofilm forms on a denture, it can act as a reservoir for pathogenic microorganisms, which can recolonize not only the denture but also the oral cavity.1 Furthermore, the presence of an extracellular matrix can protect microorganisms against the effects of antimicrobials2 and lead to the development of local diseases, such as denture stomatitis,3 and systemic diseases, such as bacterial endocarditis, aspiration pneumonia, gastrointestinal infection, and chronic obstructive pulmonary disease, especially in individuals with compromised immunity.4,5 The presence of Candida spp and Streptococcus mutans is of great importance in biofilm formation and stabilization.6–8 These microorganisms should be controlled and reduced to avoid transfer from dentures to the oral mucosa and other parts of the body.

Appropriate denture biofilm removal is essential to control the presence of microorganisms. Brushing is effective in controlling the biofilm and is the method commonly used by denture wearers,9–12 but the application of auxiliary solutions has been recommended, as they enhance effective biofilm control.13–15 Studies have suggested that microbial adhesion occurs not only on the oral mucosa8 but also on the surface of prostheses.16 Therefore, it is also important to control the growth and proliferation of microorganisms in the bristles of brushes to avoid inflammatory reactions in the
oral tissues that may result in adverse clinical implications. However, the literature regarding the benefit of appropriate hygiene for the denture brush is scarce, and most of the studies refer to the hygiene provided by toothbrushes of dentate individuals.

Diluted (0.5% and 0.25%) concentrations of sodium hypochlorite have been shown to be clinically effective for removing biofilm and reducing microorganism colonies. Lower concentrations, such as 0.2%, have also been reported to be clinically effective for biofilm removal and against Candida spp when used for short immersions of 20 minutes. In addition, this lower concentration minimized damage to the denture resin after a simulated period of 5 years of use. These results suggest that 0.2% sodium hypochlorite is suitable as a safe and effective denture cleanser.

Alkaline peroxide solutions are also widely used by denture users, although the antimicrobial action of these products is unclear. Their efficacy has been reported for denture hygiene, although other studies have reported ineffective antimicrobial action. Clinical trials have demonstrated that alkaline peroxide solutions can reduce the mass of denture biofilm and the Candida spp count when compared to storing the denture dry or in water. However, when compared to 0.5% sodium hypochlorite, they were less effective for biofilm removal.

The effectiveness of these denture cleansers has been shown to increase with increased periods of immersion. However, extended immersion in peroxide alkaline can discolor a removable prosthesis. Efferdent (Prestige Consumer Healthcare) has been reported to be effective against Candida glabrata, S mutans, Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa; however, these findings were based on an in vitro evaluation with a simple biofilm. The effectiveness of this denture cleaner should be verified on an in vivo standardization.

Ricinus communis is a plant produced in several countries with a potent protein toxin that can break down the sugar molecules of the cellular membrane of pathogenic yeasts. It does not have an unpleasant color or odor. This solution has been studied as a denture cleanser and was reported to reduce not only the growth of microorganisms such as Candida spp or S mutans, and are thus necessary for evaluating such a concentration.

The importance of brushing with chemical solutions has been stressed for denture hygiene. However, these solutions must be evaluated by clinical randomized trials to determine their effectiveness in removing biofilm and providing antimicrobial action against important microorganisms in the biofilm formation and stabilization process. In addition, microorganisms are retained in the bristles of brushes, so contamination of the brush bristles should be evaluated. Randomized clinical studies involving these solutions are necessary to determine a safe hygiene protocol that protects the prosthesis and maintains the general health of the patient.

The aim of this clinical trial was to evaluate the effect of 0.2% sodium hypochlorite, Efferdent, and 6.25% R communis on biofilm removal and antimicrobial action (dentures and brushes) using nonimmersion or immersion protocols for the brushes (α = .05). The null hypothesis was that immersion in the different denture cleansers would be similar to the control solution in both protocols.

MATERIALS AND METHODS

Forty-five participants from the Complete Denture Clinic of the Ribeirão Preto Dentistry School, University of São Paulo, Brazil were included in the clinical trial after approval from the Institutional Review Board (CAAE: 48725015.6.0000.5419). The participants were screened and enrolled if they presented without denture stomatitis or immunosuppressive systemic diseases. The participants were wearers of maxillary complete dentures with an acrylic resin denture base and teeth, without fracture, repair, or reline. Those using dentures for less than 1 year were excluded. The presence of biofilm was evaluated using the Additive Index. Age or sex was not considered an inclusion or exclusion factor.

An a priori sample size estimation used data from a previous clinical study, identifying a difference between the two means of 8.14 for biofilm removal. Therefore, a total of 42 participants (power of 80%; α = .05) were required to form two groups (brush immersion or nonimmersion), with at least 21 participants. In order to standardize the initial condition of the dentures, the biofilm was completely removed at baseline (first visit) and before the use of each solution. The intaglio surfaces of the dentures were disclosed with 1% neutral red and brushed by a researcher (M.M.S.) until they were properly cleaned.

The participants brushed their dentures with a specific brush (Denture, Condor) and neutral liquid soap (JOB Quimica, Hygiene Products) three times per day, according to the researcher’s instructions (H.F.O.P.). The
participants were randomized for denture immersion in solutions (200 mL) after the last tooth brushing: control = 20 minutes in 0.85% saline solution; 0.2% SH = 20 minutes in 0.2% sodium hypochlorite (Inject Center); Efferdent = 3 minutes in Efferdent; and 6.25% RC = 20 minutes in 6.25% R communis oil solution (Institute of Chemistry, University of São Paulo, Brazil). Regarding brushes, participants were randomized to immerse (n = 23) or not immerse (n = 22) their specific brushes with their dentures in the same solutions. They received one brush for each solution. Participants were instructed to continue with their routine brushing of the oral mucosa, to immerse their dentures in 200 mL of filtered water while sleeping (8 to 10 hours), and to rinse them in abundant tap water (20 seconds) before inserting them.

The present study followed the Latin square design for participant randomization. Participants used each solution for 7 days, and a 7-day washout period was established to prevent carry-over effects. In addition, in the wash-out period, participants were instructed to brush their dentures with neutral soap. Data on biofilm removal and the microbial load of the dentures and brushes were collected before (baseline) and after each period of use.

Denture biofilm removal was evaluated as described by Arruda et al.21 Briefly, dentures were stained (1% neutral red) and photographed (Canon EOS Digital Rebel EF-S 18-55) at 45 degrees, and the biofilm area (%) was quantified using software Image Tool 3.0 (University of Texas Health Science Center).32 For antimicrobial action analysis, all procedures were performed in an aseptic zone. Dentures were brushed with 10 mL of saline by the same researcher for 2 minutes, and a biofilm suspension was obtained and diluted from 100 to 10^-3 in a selective culture medium of Candida spp (CHROMagar, Difco Laboratories) and S mutans (SB-20, HiMedia Laboratories).33

For analysis of the brushes, the participants returned the brushes to be analyzed for the presence of Candida spp and S mutans after the hygiene protocol. All brushes were cut at the same point to remove the handle and deposited in Falcon tubes containing 20 mL of Letheen Broth to neutralize the remnants of the antimicrobial agents. The Falcon tubes were placed in an ultrasonic bath (Altsonic Clean 9CA) for 20 minutes, followed by vortex agitation to ensure the release of microorganisms resistant to the hygiene procedure. The tubes were then centrifuged at 6,000 rpm for 7 minutes, and the supernatant was discarded. One milliliter of phosphate-buffered saline (PBS) was added to the pellet to elute the microorganisms. Serial dilutions (100 to 10^-3) were performed as for the dentures.

For analysis of the dentures and brushes, the Petri dishes were incubated (37°C/48 hours), and Candida spp and S mutans colonies were counted to up to 300 colonies using the formula: CFU/mL = number of colonies x 10^n/q (n: dilution absolute value of 0, 1, 2, or 3; q: 0.05 mL).

To control for bias, different researchers performed different parts of the study as follows: random lists for brushes and solutions (A.P.M.); distributed solutions into similar and unlabeled bottles (C.H.L.S.); photographed dentures for biofilm (M.M.S.); and collected antimicrobials (V.C.O.). The other researchers were responsible for scheduling the participants, providing the solutions to the participants, and explaining the hygiene protocol (H.F.O.P.); collecting and preparing the variables (C.N.F.A.); and performing the statistical analysis (A.P.M.). Thus, the study was blinded for the participants and most of the researchers.

Kolmogorov-Smirnov test did not show normal distribution for biofilm removal and antimicrobial action (dentures and brushes). As a result, Friedman test was used for the analysis of these variables, followed by Wilcoxon test (α = .05). Mann-Whitney test (α = .05) was used to analyze the difference between the brush immersion and nonimmersion protocols.

RESULTS

Figure 1 shows the participant flowchart. A total of 64 participants were invited after screening. Nine individuals did not wear maxillary complete dentures and were excluded, and 3 declined to participate. Of the 52 remaining individuals, 1 did not attend scheduled appointments because of health problems, 2 abandoned the study, and 4 were withdrawn from the study because of inadequate use of the solutions. Thus, 45 individuals completed the study (29 women, 16 men; average age: 72 years).

Friedman test showed significant differences for biofilm removal regarding immersion of the brushes (Friedman rank sum tests = 65.89/P < .001) and nonimmersion (Friedman rank sum tests = 53.75/P < .001). For both protocols, multiple comparisons showed that 0.2% SH (immersion: mean rank [MR] = 1.41; nonimmersion: MR = 1.48), Efferdent (immersion: MR = 2.41; nonimmersion: MR = 2.25), and 6.25% RC (immersion: MR = 2.48; nonimmersion: MR = 2.77) reduced denture biofilm when compared to control (immersion: MR = 3.91; nonimmersion: MR = 4.18) and baseline (immersion: MR = 4.78; nonimmersion: MR = 4.32). 0.2% SH (P = .011) and Efferdent (P = .043) showed higher effectiveness for the nonimmersion protocol. For baseline, control, and RC, no significant difference was found in the biofilm percent reduction for immersion or nonimmersion protocols (Figs 2 and 3).

Regarding the denture cleanser effect on dentures, statistical analyses for Candida spp and S mutans reduction showed significant differences between the solutions for immersion and nonimmersion protocols. Table 1
**Fig 1** Participant flowchart (adapted from CONSORT statement).

- **Screening**: Assessed for eligibility (n = 64)
  - Excluded (n = 12)
    - Not meeting inclusion criteria (n = 9)
    - Declined to participate (n = 3)

- **Randomized**
  - Allocated to intervention (n = 52)
    - Received allocated intervention (n = 52)

- **Follow-up**
  - Lost to follow-up (n = 7)
    - Did not show up to appointments because of health problems (n = 1)
    - Stopped treatment (n = 2)
    - Did not use products correctly (n = 4)

- **Analysis**
  - Statistically analyzed (n = 45)

**Fig 2** Biofilm-covered area after use of each product for nonimmersion and immersion protocols. Different uppercase letters indicate significant differences between treatments in the same protocol, and different lowercase letters indicate significant differences between nonimmersion and immersion protocols ($P < .05$). SH = sodium hypochlorite; RC = *Ricinus communis*. 

- **Nonimmersion**
  - Baseline
  - Control
  - 0.2% SH
  - Efferdent
  - 6.25% RC

- **Immersion**
  - Baseline
  - Control
  - 0.2% SH
  - Efferdent
  - 6.25% RC
Fig 3  Denture biofilm after the use of each product. Nonimmersion: (a) control, (b) 0.2% sodium hypochlorite (SH), (c) Efferdent, (d) 6.25% Ricinus communis (RC). Immersion: (e) control, (f) 0.2% SH (g) Efferdent, (h) 6.25% RC.
shows the mean ranks of CFU/mL values transformed into log10 (CFU + 1). All of the solutions were effective against Candida spp in both protocols. The control group showed no significant difference for Candida spp from baseline for the immersion protocol (P = .153), and the nonimmersion protocol showed intermediate values compared to baseline and other solutions.

For immersion or nonimmersion protocols, 0.2% SH and Efferdent were more effective against S mutans, while 6.25% RC showed intermediate values. The control solution was similar to 6.25% RC and baseline.

Table 1 also shows the mean ranks of Candida spp and S mutans for brushes. No difference was found in the Candida spp CFU/mL count between solutions and control for immersion (P = .467) and nonimmersion (P = .108) protocols. The nonimmersion protocol did not show a significant difference among tested solutions (P = .288) for S mutans. The immersion protocol showed a difference among solutions (P = .001); control showed higher S mutans counts for brushes than 0.2% SH (P = .008) and Efferdent (P = .018), while 6.25% RC showed intermediate values (P = .406). Table 2 shows means and differences of CFU/mL log for Candida spp and S mutans for each solution compared to baseline.

**DISCUSSION**

Inadequate oral hygiene and biofilm accumulation have been correlated with oral and systemic diseases. Thus, the efficacy of denture hygiene products in biofilm elimination should be evaluated to preserve microbial adhesion to oral tissues and prevent oral and systemic pathology. This study evaluated the effect of 0.2% sodium hypochlorite, Efferdent, and 6.25% R communis on dentures and brushes against biofilm removal and antimicrobial action in a randomized clinical trial. The null hypothesis was rejected for denture analysis, since all test solutions reduced the presence of biofilm and the microbial count. However, it was only partially rejected for brushes, as microbial reduction occurred only for S mutans.

Studies with sodium hypochlorite and other hygiene solutions are plentiful, but there is a large variation in the concentrations used. After immersion and nonimmersion of the brushes, the 0.2% sodium hypochlorite was effective and similar to other solutions in reducing denture biofilm. Sodium hypochlorite for biofilm removal has been reported to have better efficacy compared to alkaline peroxide and 2% R communis if used in a concentration of 1% with 20-minute immersions. This difference in sodium hypochlorite performance may be related to the different concentrations used, as the higher the concentration, the greater the effectiveness. Concentrations of 0.5% and 0.25% of sodium hypochlorite showed similar results and better performance in biofilm removal than 10% R communis. However, it seems that the hydrophilic property of R communis can improve its effectiveness, as the concentration of 6.25% was also similar to Efferdent and, in addition, showed better results than 8% R communis when compared to 0.2% sodium hypochlorite.

In addition, 0.2% sodium hypochlorite was effective for immersion and nonimmersion of the brushes for Candida spp and S mutans on dentures, showing higher microorganism reduction for nonimmersion protocols. Diluted concentrations of sodium hypochlorite have been shown in clinical studies to decrease long-term effects on denture materials. The efficacy of 0.2% and 0.1% to control biofilm without changes in acrylic resin color, surface roughness, or flexural strength promotes greater denture longevity. The results presented in this study confirmed the possible use of lower concentrations of sodium hypochlorite in short immersion times to prevent damage to the acrylic resin.

Controlled clinical studies with peroxides are sparse. Additionally, a wide variety of results have been reported, and their effectiveness has not been conclusively demonstrated. Regarding biofilm removal, Efferdent was similar to 0.2% sodium hypochlorite and 6.25% R communis in the present study. Another study reported that the use of an alkaline peroxide for 20 minutes was less effective than 1% sodium hypochlorite; however, the concentration of the sodium hypochlorite was higher than that used in the present study. In addition, some studies only compared alkaline peroxide to water or dry storage, so the lack of an alkaline hypochlorite solution should result in the greater efficacy of this solution in those studies.

Concerning antimicrobial action, alkaline peroxide solution was not effective in reducing multispecies biofilm (C albicans, C glabrata, and S mutans) in 10 seconds or the microbial total count at 5 and 30 minutes, differing from Efferdent in the present study, which was effective at 3 minutes. Similar results for the efficacy of alkaline peroxides were reported by Lucena-Ferreira et al, who evaluated multispecies biofilm using immersions of 3 minutes in alkaline peroxides in an in vitro evaluation. However, the present study did not evaluate the total microbial reduction, which should present different results. Coimbra et al also showed a great reduction in C glabrata and S mutans counts with Efferdent. This effectiveness was confirmed in the present in vivo biofilm study, demonstrating that Efferdent was effective against Candida spp and S mutans.

The diversity of results in relation to alkaline peroxides can be related not only to the different methodologies applied but also to the different formulations of those solutions, which can interfere with efficacy and lead to changes in the properties of acrylic resin. Although the alkaline peroxides of different commercial brands present...
some common ingredients such as sodium carbonate, sodium perborate, and sodium bicarbonate in their compositions, they are present in different concentrations, perhaps explaining the difference in effectiveness among products. The main active ingredients of Efferdent are sodium perborate and ethylenediaminetetraacetic acid (EDTA) tetrasodium. The sodium perborate consists of a stable salt that, when in contact with water, decomposes into sodium metaborate, oxygen, and hydrogen peroxide. Hydrogen peroxide is responsible for the release of active oxygen, which promotes the antimicrobial and stain removal effect. The action of EDTA tetrasodium is related to structural damage caused in the cell membrane, causing changes in cell permeability and facilitating the action of antimicrobial agents.

Table 1 Mean Ranks and Pairwise Comparisons of Log (CFU/mL + 1) Values of Candida spp and S mutans Counts at Baseline and After Use of Solutions for Dentures and Brushes

<table>
<thead>
<tr>
<th></th>
<th>Baseline Control</th>
<th>0.2% SH</th>
<th>Efferdent</th>
<th>6.25% RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida spp Dentures</td>
<td>Immersion 57.53 (&lt; .001)</td>
<td>4.74A</td>
<td>3.61A</td>
<td>2.22B</td>
</tr>
<tr>
<td></td>
<td>Nonimmersion 29.92 (&lt; .001)</td>
<td>4.34A</td>
<td>3.25AB</td>
<td>2.09B</td>
</tr>
<tr>
<td>S mutans Dentures</td>
<td>Immersion 46.32 (&lt; .001)</td>
<td>4.54A</td>
<td>3.67AB</td>
<td>2.13C</td>
</tr>
<tr>
<td></td>
<td>Nonimmersion 53.95 (&lt; .001)</td>
<td>4.52A</td>
<td>3.68AB</td>
<td>1.96C</td>
</tr>
<tr>
<td>Candida spp Brushes</td>
<td>Immersion 2.543 (.467)</td>
<td>2.57A</td>
<td>2.50A</td>
<td>2.24A</td>
</tr>
<tr>
<td></td>
<td>Nonimmersion 6.54 (.108)</td>
<td>2.86A</td>
<td>2.09A</td>
<td>2.36A</td>
</tr>
<tr>
<td>S mutans Brushes</td>
<td>Immersion 17.33 (&lt; .001)</td>
<td>3.26Ab</td>
<td>2.04B</td>
<td>2.13B</td>
</tr>
<tr>
<td></td>
<td>Nonimmersion 3.762 (.288)</td>
<td>2.84Aa</td>
<td>2.30A</td>
<td>2.41A</td>
</tr>
</tbody>
</table>

Different uppercase letters indicate significant differences between treatments in the same protocol, and different lowercase letters indicate significant differences between nonimmersion and immersion protocols (P < .05). CFU = colony-forming units; SH = sodium hypochlorite; RC = Ricinis communis.

*Friedman test.

Table 2 Mean and Log Differences (CFU/mL) of Candida spp and S mutans for Each Solution Compared to Baseline

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Control</th>
<th>0.2% SH</th>
<th>Efferdent</th>
<th>6.25% RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida spp</td>
<td>Immersion</td>
<td>3.65</td>
<td>2.44</td>
<td>0.71</td>
<td>1.49</td>
</tr>
<tr>
<td>Nonimmersion (Baseline – solution)</td>
<td>1.21</td>
<td>2.94</td>
<td>2.16</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>Immersion</td>
<td>3.87</td>
<td>2.67</td>
<td>1.11</td>
<td>1.17</td>
<td>1.60</td>
</tr>
<tr>
<td>(Baseline – solution)</td>
<td>1.20</td>
<td>2.76</td>
<td>2.70</td>
<td>2.27</td>
<td></td>
</tr>
<tr>
<td>S mutans</td>
<td>Immersion</td>
<td>4.65</td>
<td>3.51</td>
<td>0.37</td>
<td>0.88</td>
</tr>
<tr>
<td>Nonimmersion (Baseline – solution)</td>
<td>1.14</td>
<td>4.28</td>
<td>3.77</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>Immersion</td>
<td>4.78</td>
<td>3.91</td>
<td>1.45</td>
<td>1.51</td>
<td>2.22</td>
</tr>
<tr>
<td>(Baseline – solution)</td>
<td>0.87</td>
<td>3.33</td>
<td>3.27</td>
<td>2.56</td>
<td></td>
</tr>
</tbody>
</table>

CFU = colony-forming units; SH = sodium hypochlorite; RC = Ricinis communis.
In addition, the type of biofilm (in vitro or in vivo) is important to consider, as it may interfere in the effectiveness of these solutions. Clinical randomized studies evaluating the biofilm removal of these solutions are sparse, as most of the research on alkaline peroxide solutions involves in vitro biofilm. Thus, the effectiveness of Efferdent found in the present study was relevant, as it was evaluated by a randomized clinical study.

*R communis* (6.25%) was effective for biofilm removal and against *Candida* spp and showed moderate action against *S. mutans*. Studies of the effect of *R communis* as a denture cleanser on biofilm removal showed similar results to 2% alkaline peroxide, but less effective than 1% sodium hypochlorite. Regarding antimicrobial action, 10% *R communis* has been reported to be effective against *S. mutans* and biofilm removal, but it was less effective than a 0.5% sodium hypochlorite solution. The 8% *R communis* was not as effective as 6.25% *R communis* and was similar to saline solution. The findings of the present study were the most promising for this solution so far, showing that this concentration may be used as a denture cleaner.

The effect of hygiene on specific denture brushes is unclear because most studies of antimicrobial action on brushes are related to conventional toothbrushes. Although data for the sodium hypochlorite and alkaline peroxide solutions did not show any statistical difference, the log reduction of these solutions was higher for all evaluations than for the *R communis* solutions. Log reduction is an important factor that can imply a better clinical performance of these solutions. Furthermore, sodium hypochlorite showed lower CFU/mL means for the nonimmersion group for both microorganisms tested, while higher Efferdent and *R communis* CFU/mL reduction occurred in the immersion group. This may be related to the deleterious effects of the hypochlorite solution on the nylon bristles of the brushes, which may have contained a greater number of grooves and favored the deposition of microorganisms.

The present study found that immersing brushes in the same solutions used for denture hygiene did not appear to provide additional health benefits. More studies are needed to find the best hygiene protocol for brushes using different methodologies such as immersing brushes separately from the dentures, thereby increasing the effect of the solutions. However, brush bristles should be evaluated for damage depending on the solution and concentration applied. The immersion time can affect the physical integrity of the bristles.

Limitations of the present study included the inability to prevent participants from identifying Efferdent. It was stored without identification, but its shape (powder) and protocol of use were different from those of the other solutions, which were stored in unlabeled bottles. Although they were clearly different solutions, participants did not report any differentiation between sodium hypochlorite and *R communis* solutions. The authors believe that the low concentration of sodium hypochlorite used in the study allowed the blinding of participants regarding those solutions.

An additional limitation was the nonevaluation of the mandibular prostheses, since these may have higher biofilm levels than the maxillary ones. This study was performed with a 20-minute immersion period, but these solutions should be clinically evaluated in 8-hour immersions, since such a period has also been recommended and used by denture wearers.

**CONCLUSIONS**

Sodium hypochlorite (0.2%), Efferdent, and 6.25% *R communis* were effective for the control of biofilm and may be indicated as denture cleansers. The immersion of brushes did not show better efficacy in microbial reduction.

**ACKNOWLEDGMENTS**

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The peri-implant soft tissue phenotype (PSP) encompasses the keratinized mucosa width (KMW), mucosal thickness (MT), and supracrestal tissue height (STH). Numerous approaches to augmenting soft tissue volume around endosseous dental implants have been investigated. To what extent PSP modification is beneficial for peri-implant health has been the subject of debate in the field of implant dentistry. The aim of this systematic review was to analyze the evidence regarding the efficacy of soft tissue augmentation procedures aimed at modifying the PSP and their impact on peri-implant health. A comprehensive search was performed to identify clinical studies that involved soft tissue augmentation around dental implants and reported findings on KMW, MT, and/or STH changes. The effect of the intervention on peri-implant health was also assessed. Selected articles were classified based on the general type of surgical approach to increase PSP, either bilaminar or an apically positioned flap (APF) technique. A network meta-analysis including only randomized controlled trials (RCTs) reporting on PSP outcomes was conducted to assess and compare different techniques. A total of 52 articles were included in the qualitative analysis, and 23 RCTs were included as part of the network meta-analysis. Sixteen RCTs reported the outcomes of PSP modification therapy with bilaminar techniques, whereas 7 involved the use of APF. The analysis showed that bilaminar techniques in combination with soft tissue grafts (connective tissue graft [CTG], collagen matrix [CM]), and acellular dermal matrix (ADM) resulted in a significant increase in MT compared to nonaugmented sites. In particular, CTG and ADM were associated with higher MT gain compared to CM and nonaugmented sites. However, no significant differences in KMW were observed across different bilaminar techniques. PSP modification via a bilaminar approach utilizing either CTG or CM showed beneficial effects on marginal bone level stability. APF-based approaches in combination with free gingival graft (FGG), CTG, CM, or ADM showed a significant KMW gain compared to nonaugmented sites. However, compared to APF alone, only FGG exhibited a significantly higher KMW gain. APF with any evaluated soft tissue graft was associated with reduction of probing depth, soft tissue dehiscence, and plaque index compared to nonaugmented sites. The evidence regarding the effect of PSP modification via APF-based approaches on peri-implant marginal bone loss or preservation is inconclusive. Bilaminar approach involving CTG or ADM obtained the highest amount of MT gain, whereas APF in combination with FGG was the most effective technique for increasing KMW. KMW augmentation via APF was associated with a significant reduction in probing depth, soft tissue dehiscence, and plaque index, regardless of the soft tissue grafting material employed, whereas bilaminar techniques with CTG or CM showed beneficial effects on marginal bone level stability.