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CLINICAL RESEARCH

Crossover clinical trial of different methods of removing a denture adhesive and the influence on the oral microbiota

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Many wearers of complete dentures use or have used some sort of denture adhesive (DA).[1-3] Several studies have concluded that 1 of the most frequent complaints among users of these products is the difficulty of removing them from the oral tissues and the prosthesis.[4,5] In contact with saliva, these products usually have a sticky consistency, making the dentures and oral mucosa difficult to clean and limiting their acceptance by patients. Because denture wearers are mostly older people who are susceptible to a decline in general health, cognitive impairment, motor difficulties, and decreased visual acuity, greater difficulty in oral hygiene and prosthesis is predictable.[6]

ABSTRACT

Statement of problem. The difficulty of removing denture adhesive is a common problem reported by users of these products.

Purpose. The purpose of this clinical study was to investigate the effectiveness of different cleaning protocols for removing a denture adhesive (DA) and the influence on the oral microbiota.

Material and methods. Twenty participants wearing well-fitting complete dentures were instructed to use a denture adhesive 3 times a day during a 4-week trial, divided into 4 stages: (A) control—3 daily denture brushings using water at ambient temperature, (B)—3 daily denture brushings using water at ambient temperature plus coconut soap, (C)—3 daily denture brushings using water at ambient temperature plus dentifrice; (D)—3 daily denture brushings using water at ambient temperature combined with immersion in sodium perborate solution for 5 minutes before nocturnal sleep. After each 1-week stage, saliva specimens were collected. A dye was used to display and quantify the remaining DA on the internal surface of the maxillary dentures as a percentage. For microbiological analysis, the saliva was diluted and plated onto Petri dishes containing a nonselective culture medium and *Candida* spp culture media. After the incubation period, *Candida* species were identified and the number of colony forming units (CFU/mL) was calculated.

Results. A significant difference was found among the 4 cleaning methods for the quantification of remaining DA (Friedman, P=.036). Brushing the dentures with coconut soap, dentifrice, or water combined with immersion in sodium perborate solution was more effective in removing DA than brushing with only water. The cleaning methods did not influence the quantification of microorganisms in general or *Candida albicans* and other *Candida* species in particular.

Conclusions. Brushing the dentures with coconut soap, dentifrice, or water combined with immersion in sodium perborate solution was more effective for removing cream-type denture adhesive than brushing with only water. (J Prosthet Dent 2016;115:462-468)

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Clinical Implications

Cleaning dentures with coconut soap, dentifrice, or water combined with immersion in sodium perborate solution could be recommended as effective cleaning methods for users of denture adhesives.

A combination of mechanical and chemical methods for denture cleansing is recommended.[7] Brushing with dentifrices or soap is an effective method of biofilm control[8,9] and should be combined with the immersion of the dentures in alkaline peroxides, enzymes, acids, or disinfectant solutions.[10-13]

Oral care is essential among denture adhesive users. Because these products become viscous and can leave a residue that is difficult to remove, pathogenic oral bacteria and yeasts may proliferate on the denture surface.[14] However, the effect of denture adhesives on oral microbiota and their biocompatibility remains uncertain.[15] Some in vivo studies found that the use of DA did not significantly alter the oral microbiota over 14 days[16-18] or over 2 months of continuous use of these products.[19] Sampaio-Maia et al[20] observed in vitro that some denture adhesives showed microbial contamination and others had a significant inhibitory effect on the growth of *Candida albicans*. In addition, some bacteria species such as *Streptococcus oralis*, *Prevotella oralis*, *Fusobacterium nucleatum*, and *Streptococcus mutans* may play a role in halitosis, a common problem in denture wearers.[21]

Because the microorganisms present on the denture bases are known to cause oral infections and because older people tend to lose the necessary dexterity to perform oral hygiene,[10,22] a cleaning method for complete denture wearers using denture adhesives that provides effective removal of DA is needed. The literature concerning denture adhesives and oral hygiene is relatively scarce. Sato et al[23] investigated in vivo the ease of removing denture adhesives from the oral mucosa. Harada-Hada et al[14] investigated in vitro the efficiency of denture cleansers on adhesive removal and observed that some denture cleaners composed of enzymes and hydrogen peroxide could be indicated for the removal of cream-type adhesives.

The purpose of this clinical study was to investigate the effectiveness of different cleaning methods for removing DA and the influence on the oral microbiota of complete denture wearers. The null hypothesis was that the proposed cleaning methods would not influence the amount of adhesive remaining on the internal surface of maxillary dentures and the number of colony forming units (CFU/mL) obtained from saliva.

MATERIAL AND METHODS

The study was approved by the institutional ethics committee of Araraquara Dental School, Sao Paulo State University, Brazil (CAAE:17845913.50000.5416) and registered in the ensaiosclinicos.gov.br database (Identifier: RBR-8dy6c3). All participants were asked to sign a written consent form before enrollment.

A convenience sample of 50 individuals was assessed for participation in this research. The inclusion criteria were that the individuals should be mentally receptive and have been wearers for up to 3 years of well-fitting bimaxillary complete dentures that had not been rebased or relined. Participants who had dysfunctional disorders of the masticatory system, debilitating systemic diseases, diabetes mellitus, or xerostomia or who used antibiotics in the experimental period or in the 3 months preceding the study were excluded.

After the initial evaluation, 15 individuals did not meet inclusion criteria (diabetes mellitus [n=9]; recent history of antibiotic use [n=3]; physical limitations of locomotion [n=2]; cognitive or psychological problems [n=1]), and 7 refused to participate. Written consent was obtained from the participants before enrollment.

The participants used a DA (Ultra Corega cream; GlaxoSmithKline) during the 4-week trial. The investigator (N.M.O.J.) demonstrated the placement of the DA in the maxillary and mandibular dentures to the participants according to the strip method.[24] Three 1-cm strips were applied to the frontal, right, and left middle region of the posterior segments of the dentures.

The participants were instructed to use the DA 3 times a day (before and after breakfast and after lunch). After dinner, adhesive use was only required if the participant was involved in some activity and/or social interaction. These recommendations were based on a previous report.[25] The participants were also instructed to clean their dentures and mucosa 3 times a day after the main meals (breakfast, lunch, dinner), not to use any mouthwash, and to remove their dentures before sleeping,[26] keeping them in water.

The trial was divided into 4 stages of 1 week each: (A) Control. 3 daily denture brushings with water at ambient temperature, (B) 3 daily denture brushings with water at ambient temperature plus coconut soap (Mon Bijou; Bombril), (C) 3 daily denture brushings with water at ambient temperature plus dentifrice (Colgate Máxima Proteção Anticáries; Colgate-Palmolive), and (D) 3 daily denture brushings with water at ambient temperature combined with immersion in perborate sodium solution (Corega Tabs; Stafford-Miller Ind) for 5 minutes before nocturnal sleep and according to the manufacturer's recommendations. Soft bristle brushes (Colgate Extra Clean; Colgate-Palmolive) were used to perform the cleaning procedures, and these brushes were replaced every week.

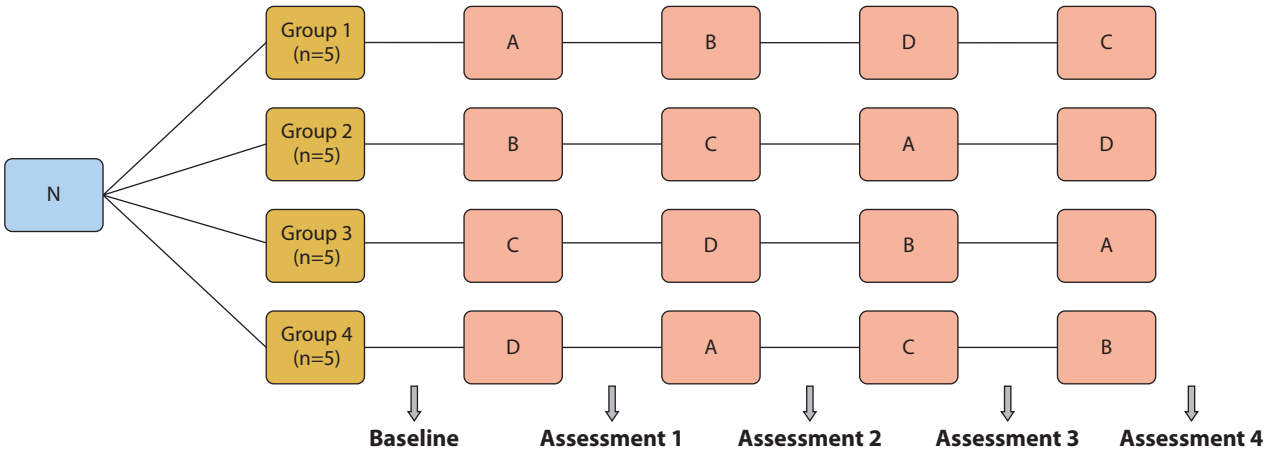

Figure 1. 4×4 Latin square experimental design. Flowchart of steps involved in study, wherein letters represent cleaning methods.

The study design followed the criteria established for a crossover study, where all treatments (cleaning methods) were applied to all participants, thereby eliminating the possibility of variation among individuals in response to these treatments. A 4×4 Latin square experimental design was used wherein the cleaning methods are represented by the random distribution of letters (Fig. 1).

The sample was divided into 4 groups according to the sequence of cleaning methods performed for 1 week each as follows: group 1 (A, B, D, C), group 2 (B, C, A, D), group 3 (C, D, B, A), group 4 (D, A, C, B). The sequence for each participant was randomly determined by sex and age by another researcher (G.G.) using computer-generated numbers (BioEstat 5.0; Federal University of Pará).

Specimens of saliva were collected at different times of the study (baseline, assessments 1, 2, 3, and 4). Participants were instructed not to swallow the saliva for 90 seconds and then expel 5 mL into a sterile Falcon tube. These procedures were performed by another researcher (E.M.N.). Each tube was vortexed for 1 minute to suspend the microorganisms from the saliva.

Serial dilutions were made from replicate aliquots of 100 μL of solution transferred to Eppendorf tubes containing 900 μL of sterile saline. An aliquot of 25 μL of the resulting suspensions was then plated onto sterile Petri dishes containing a nonselective medium (Mueller Hinton Agar) and a selective medium for *Candida* spp. (Sabouraud Dextrose Agar). These procedures were performed in duplicate. After 48 hours at 37°C incubation, the viable colonies of each Petri dish were counted with a digital colony counter (CP 600 Plus; Phoenix Ind Com Equipamentos Científicos Ltda). The estimated number of colony-forming units (CFU) per mL was calculated by multiplying the mean of the number of colonies from the 2 Petri dishes by the dilution factor and aliquot used. The microbial count data obtained were expressed as log (CFU + 1)/mL.

Candida species were identified on CHROMagar *Candida* media. An aliquot (25 μL) from this suspension was spread-plated on CHROMagar *Candida* and incubated at 37°C for 5 days, and the colonies were presumptively identified by colony color.[27] The colonies of *C. albicans* appear light green to medium green, *C. tropicalis* appear greenish-blue to metallic blue, and *C. krusei* appear light pink with a whitish edge. *Candida (Torulopsis) glabrata* colonies usually appear mauve to dark mauve on this medium.

The methodology used in this study to quantify the remaining adhesive was adapted from previous studies.[7,9,18,23,28] Initially, a control was established which corresponded to the total internal surface area of each maxillary denture. The calculation of the area was performed with software (Image Tool, Windows, v3.00; UTHSCSA).

On the days of the assessments (1, 2, 3, and 4), each participant performed the corresponding cleaning method, and then each maxillary denture was placed on a sterilized 20×100-mm Petri dish. The presence of remaining adhesive was displayed by applying 1 mL of 0.4% indigo carmine colorant (Renylab Química e Farmacêutica) with a disposable syringe over the internal surface of the maxillary denture.[23,28] The denture was rinsed in running water for 5 seconds to remove excess colorant. The denture was then positioned on a clamp, and the disclosed surfaces were photographed with a digital camera (Cyber-shot DSC-F717; Sony) fixed on a stand at a standardized distance and 45 degrees of inclination.

The photographs were processed, and the areas (total internal surface and surface stained with adhesive) were quantified by another researcher (V.B.P.). The percentage of remaining adhesive was calculated by the ratio between the adhesive area multiplied by 100 and the total area of the internal surface of the maxillary complete denture.

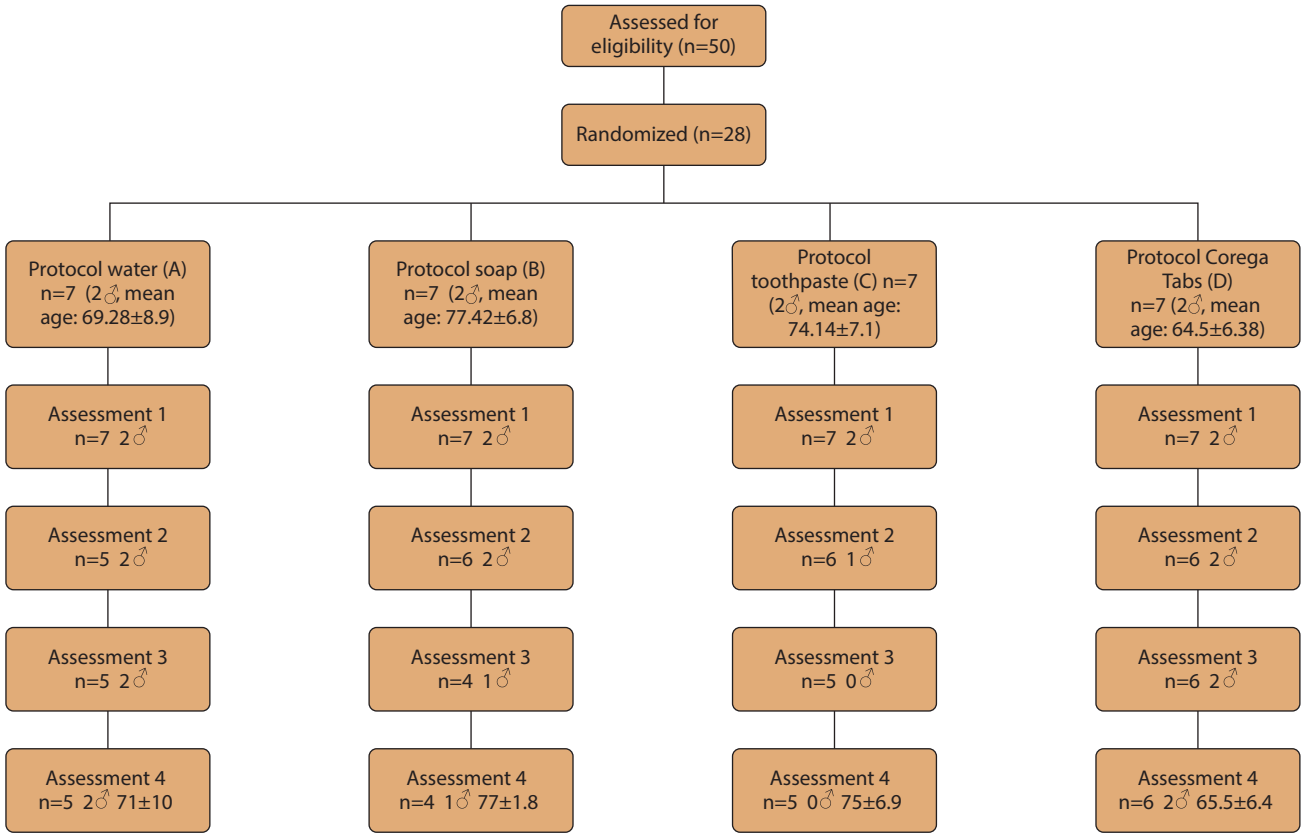

Figure 2. Flow diagram of participants. Adapted from CONSORT statement.

After these procedures, the dentures of each participant were cleaned by 1 researcher (A.R.P.L.), according to the protocol followed by the patient during the week to remove any adhesive or remaining microbiological material without interfering with the microbiological evaluation performed in the following period.[18] This procedure was carried out to quantify only the microorganisms and residual denture adhesive formed in the respective 7-day periods.

Data collection was performed by one researcher (P.M.S.). Statistical analysis was performed by another researcher (D.O.M.M.), who was masked to all procedures. The data of the adhesive-covered area (%) were compared with the Friedman nonparametric paired sample test. For microbiological analysis, the data obtained from selective media for *Candida* spp and CHROMagar *Candida* were compared with the Friedman test, and ANOVA was performed for the nonselective culture medium. All analyses were performed with α=.05 using software (PASW Statistics, v18; SPSS Inc).

RESULTS

A diagram of participants throughout the research is presented in Figure 2. It was adapted from the CONSORT statement.[29] Twenty-eight participants were recruited in this study. During the follow-up, 8 participants were lost from the study. The final sample was composed of 15 women (mean age: 70.9 ±8.4 years) and 5 men (mean age 73.6 ±7.2 years).

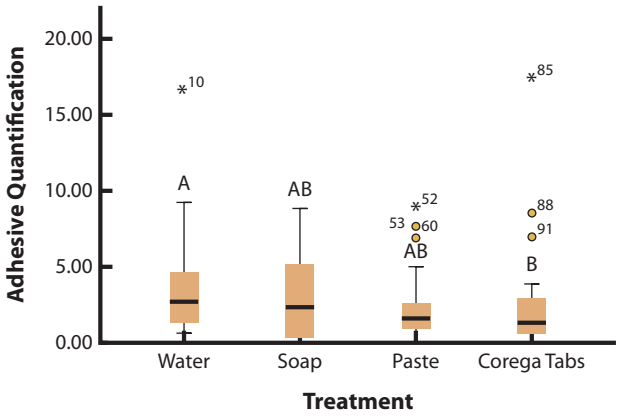

Figure 3. Box-plot graph comparing effectiveness of different cleaning protocols to remove denture adhesive (Ultra Corega Cream). Different uppercase letters signify statistical difference (Dunn test, *P*=.004).

The remaining DA was influenced by the cleaning method (Friedman test, *P*=.036). Figure 3 shows that brushing the dentures with water combined with immersion in perborate sodium solution (Corega Tabs) was

Table 1. Mean values (standard deviation) of log (CFU +1/mL) for nonselective culture medium (Mueller Hinton Agar), selective medium for *Candida* spp. (Sabouraud Dextrose Agar), and CHROMAgar *Candida* medium

	Mean (Std. Deviation)					
			CHROMAgar *Candida*			
Protocol	Nonselective Culture Medium	*Candida spp.*	*C. albicans*	*C. tropicalis*	*C. krusei*	*Candida (Torulopsis) glabrata*
Baseline	9.54 (±0.67)	6.21 (±4.13)	1.55 (±1.86)	0.57 (±1.18)	0.64 (±1.37)	1.17 (±1.49)
Water	9.60 (±0.86)	7.04 (±4.20)	1.31 (±1.70)	0.00 (±0.00)	0.40 (±0.90)	0.55 (±1.15)
Coconut soap	9.19 (±0.97)	7.29 (±3.26)	1.05 (±1.51)	0.00 (±0.00)	0.27 (±0.86)	0.95 (±1.36)
Dentifrice	9.57 (±0.64)	7.49 (±3.30)	1.44 (±1.70)	0.27 (±0.84)	0.42 (±1.30)	1.35 (±1.58)
Sodium perborate	9.26 (±1.09)	7.02 (±3.72)	1.82 (±1.79)	0.11 (±0.51)	0.26 (±0.83)	0.98 (±1.38)
P	*.34***	*.70**	*.28**	*.06**	*.97**	*.36**

*Friedman test, α=.05.
**ANOVA, α=.05.

more effective in removing DA than brushing with only water (control) (Dunn test, *P*=.004). The results also demonstrated that the remaining DA was similar when the participants cleaned their dentures with coconut soap, dentifrice, or a combination of brushing with water and immersion in perborate sodium solution. The cleaning methods using water, coconut soap, and dentifrice showed similar results.

Table 1 shows the influence of the cleaning methods on the oral microbiota of the participants. For the nonselective culture medium, no statistically significant difference was found (ANOVA, *P*>.05) on the colony counts for the cleaning methods proposed in this study. Similar colony counts were also observed for the selective culture media for *Candida* spp. and selective media for other *Candida* species (Friedman, *P*>.05), regardless of the cleaning method.

DISCUSSION

The null hypothesis of the study was partially accepted because the cleaning methods had no influence on the oral microbiota of the denture wearers using a denture adhesive during the experimental period; however, it was demonstrated that easy removal of the adhesive depended on the cleaning method. Cleaning the dentures with coconut soap, dentifrice, or water combined with immersion in a sodium perborate solution (Corega Tabs) was more effective for removing DA than brushing with only water.

These results are consistent with previous studies that have shown that soaking in commercial effervescent products or 1% sodium hypochlorite solution is effective.[8,20] According to Sato et al,[23] only brushing fails to promote adequate cleaning in all regions of the denture, which indicates the need to soak the denture in solutions of effervescent tablets. Harada-Hada et al[14] also observed that immersion in denture cleaning solutions could be an alternative method of denture adhesive removal.

Among the denture cleaning methods described in the literature, the combined use of mechanical and chemical cleaning methods is generally recommended to obtain adequate control of the biofilm on the surface of the dentures.[7] In the present study, no influence on the oral microbiota of the participants was observed, regardless of the cleaning method. The effervescent solution was used for 5 minutes according to the manufacturer's recommendations. Previous studies have stated that the period of immersion is crucial for antimicrobial efficacy and that immersion in effervescent solutions is not effective when used from 15 to 30 minutes.[10,11] Rossato et al[12] showed that 30 minutes of immersion in sodium perborate solution performed similarly to the alkaline hypochlorite in removing plaque. McCabe et al[13] compared the efficacy of 2 immersion products (experimental tablet and Steradent) with brushing using toothpaste, soap, or water at removing stains, plaque, and calculus from dentures. The authors concluded that the mechanical-chemical method (immersion in Steradent combined with brushing) was more effective in removing plaque and stains when compared with the other cleaning methods. Despite these contrasting results, in the present study, immersion in sodium perborate solution was evaluated according to the manufacturer's recommendation of 5 minutes because it would represent the habitual use of this product among users.

Some studies have suggested that the proliferation of microorganisms and subsequent biofilm formation could increase due to the viscosity of the adhesive or its accumulation on the surface of the prosthesis if the product is not replaced[16] and that adhesives could change the surface topography of the denture in contact with the mucosa, which also favors the proliferation of microorganisms.[17] Despite these considerations, in this study the amount of remaining adhesive was not decisive in the quantification of microorganisms, even in participants brushing only with water. These results are in agreement with previous studies showing that the use of adhesive does not influence biofilm formation or the increase of microorganisms in the oral cavity. Kim et al[16] and Oliveira at al[17] found no significant differences in the

absolute number of CFU of *Candida* and other yeasts between participants who used denture adhesives and those who did not use the same adhesive for 15 days. Leite et al[18] found that the number of microorganisms collected from the palatal mucosa and the intaglio of the dentures was similar after 15 days for those who used DA and those of did not. Ozcan et al[19] extended the use of adhesives in denture wearers up to 2 months and found no increase in microbiota or any adverse effects. In this study, based on previous studies regarding denture hygiene methods and microbiological analysis, a period of only 7 days was considered sufficient for the microbiological evaluation.[9,30]

In addition, it should be noted that the participants wore well-fitting dentures, with a smaller space between the denture base and denture bearing areas. This study design was adopted to avoid thicker layers of denture adhesive and to standardize this space among the participants. Thicker denture adhesive films could produce different effects on the oral microbiota of denture wearers.[16-18] To reinforce these arguments, a recently published systematic review concerning denture adhesives[15] stated that an increased thickness of adhesives may represent a risk related to the continuous wearing of ill-fitting dentures.

One of the limitations of this study was the loss of 8 participants during the follow-up period, bringing the number of participants to 20. The sample size used in this study was based on previous studies.[1,18,19] As in this study, in which most of the losses (5 participants) were due to not using the adhesive because of the onset of nausea, a considerable number of patients interviewed by Coates[2] reported that denture adhesives had an undesirable flavor and viscous texture, causing nausea and were difficult to use and to remove from the oral tissue and dentures. Another limitation was the evaluation of only 1 type of adhesive.

Some participants reported informally that the use of coconut soap and of sodium perborate solution as denture cleaning methods were well accepted. The majority of participants reported well on the use of sodium perborate solution for removing stains and whitening the surface of the denture.

This study demonstrated that the combination of a mechanical method and a chemical cleaning agent removes remaining adhesive from the denture surface better than brushing with only water and is well accepted among users.

More studies are needed on the removal of remaining adhesive on prostheses. The combination of brushing with soap together with immersion in disinfectant solutions could be suggested, which also represent effective cleaning methods,[8] to assess the possibility of different results in order to improve the quality of life of denture wearers and adhesive users.

CONCLUSIONS

Based on the results of this crossover clinical trial, it was concluded that brushing the dentures with coconut soap, dentifrice, or water combined with immersion in sodium perborate solution was more effective for removing cream-type denture adhesive than brushing with only water.

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Noteworthy Abstracts of the Current Literature

Characterization of cement particles found in peri-implantitis-affected human biopsy specimens

Burbano M, Wilson TG Jr, Valderrama P, Blansett J, Wadhwani CP, Choudhary PK, Rodriguez LC, Rodrigues DC

Int J Oral Maxillofac Implants 2015;30:1168-73

Purpose. Peri-implantitis is a disease characterized by soft tissue inflammation and continued loss of supporting bone, which can result in implant failure. Peri-implantitis is a multifactorial disease, and one of its triggering factors may be the presence of excess cement in the soft tissues surrounding an implant. This descriptive study evaluated the composition of foreign particles from 36 human biopsy specimens with 19 specimens selected for analysis. The biopsy specimens were obtained from soft tissues affected by peri-implantitis around cement-retained implant crowns and compared with the elemental composition of commercial luting cement.

Materials and Methods. Nineteen biopsy specimens were chosen for the comparison, and five test cements (TempBond, Telio, Premier Implant Cement, Intermediate Restorative Material, and Relyx) were analyzed using scanning electron microscopy equipped with energy dispersive x-ray spectroscopy. This enabled the identification of the chemical composition of foreign particles embedded in the tissue specimens and the composition of the five cements. Statistical analysis was conducted using classification trees to pair the particles present in each specimen with the known cements.

Results. The particles in each biopsy specimen could be associated with one of the commercial cements with a level of probability ranging between .79 and 1. TempBond particles were found in one biopsy specimen, Telio particles in seven, Premier Implant Cement particles in four, Relyx particles in four, and Intermediate Restorative Material particles in three.

Conclusion. Particles found in human soft tissue biopsy specimens around implants affected by peri-implant disease were associated with five commercially available dental cements.