ACP AMERICAN COLLEGE OF PROSTHODONTISTS
Your smile. Our specialty.

Effect of Chlorhexidine on Denture Biofilm Accumulation

Ingrid Machado de Andrade, DDS, PhD,[1] Patricia C. Cruz, DDS, MS,[1] Cláudia H. Silva-Lovato, DDS, PhD[1] Raphael F. de Souza, DDS, PhD,[1] Maria Cristina Monteiro Souza-Gugelmin, DDS, PhD,[2] & Helena de Freitas Oliveira Paranhos, DDS, PhD[1]

[1]Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, Brazil
[2]Department of Clinical, Toxicological and Bromatological Analysis, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Keywords
Complete denture; biofilm; denture cleansers; chlorhexidine; oral hygiene.

Correspondence
Helena de Freitas Oliveira Paranhos, Ribeirão Preto Dental School – Dental Materials and Prosthodontics, Av. café, s/n Ribeirão Preto 14040–904, Brazil. E-mail: helenpar@forp.usp.br

Financial support from the São Paulo Research Foundation (FAPESP; grant no. 2005/55705–2).

Accepted February 14, 2011

doi: 10.1111/j.1532-849X.2011.00774.x

Abstract

Purpose: Adequate denture hygiene can prevent and treat infection in edentulous patients, who are frequently elderly and have difficulty brushing their teeth. This study evaluated the efficacy of complete denture biofilm removal using a chlorhexidine solution in two concentrations: 0.12% and 2.0%.

Materials and Methods: Sixty complete denture wearers participated in a trial for 21 days after receiving brushing instructions. They were distributed into three groups, according to the tested solution and regimen (n = 20): (G1) Control (daily overnight soaking in water); (G2) daily immersion at home in 0.12% chlorhexidine for 20 minutes after dinner; and (G3) a single immersion in 2.0% chlorhexidine for 5 minutes at the end of the experimental period, performed by a professional. Biofilm coverage area (%) was quantified on the internal surface of maxillary dentures at baseline and after 21 days. Afterward, the differences between initial and posttreatment results were compared by means of the Kruskal-Wallis test ($\alpha = 0.05$).

Results: Median values for biofilm coverage area after treatment were: (G1) 36.0%; (G2) 5.3%; and (G3) 1.4%. Differences were significant (KW = 35.25; $p < 0.001$), although G2 and G3 presented similar efficacy in terms of biofilm removal.

Conclusions: Both chlorhexidine-based treatments had a similar ability to remove denture biofilm. Immersion in 0.12% or 2.0% chlorhexidine solutions can be used as an auxiliary method for cleaning complete dentures.

Increasing life expectancy has led to a rising number of elderly people worldwide,[1] resulting in a high prevalence of edentulism and complete denture wearing.[2,3] Several studies have shown that the oral health of denture wearers is precarious.[4-7] Poor hygiene is associated with lack of guidance, the intrinsic characteristics of the denture, and reduced manual dexterity as a consequence of aging.[8]

Poor denture hygiene allows biofilm accumulation, an important stage for the development of several oral and systemic infections.[9,10] The continuous presence of a biofilm formed by bacteria and yeasts is the main etiological factor of denture stomatitis.[11,12] Thus, the indication of denture cleansing is of paramount importance to prevent or treat infections in the edentulous mouth.[13,14]

Denture care products should be able to remove inorganic/organic deposits and stains, be easy to handle, have bactericidal and fungicidal properties, present no toxicity to patients, be compatible with the denture materials, and have a low cost.[15] However, these requirements are difficult to achieve in a clinical setting. Denture hygiene methods can be classified as mechanical or chemical.[8,16] Brushing is the most widespread mechanical method,[13] with the advantage of being simple, inexpensive, and effective.[8,16] However, patients with low dexterity may find it difficult to perform, and there is a possibility of acrylic resin wear and superficial damage to relining materials. Chemical methods can overcome some of these disadvantages. Chemical denture cleansers are able to dislodge food debris, biofilm, and tobacco stains from prosthodontic surfaces effectively. According to Gornitsky,[17] chemical denture cleansers could be a good choice for the elderly, who require adjunctive measures to clean their dentures. These cleansers are classified according to their composition and mechanism, namely, hypochlorites, peroxides, enzymes, acids, crude drugs, and disinfectants.

Several disinfectants have been suggested for denture disinfection. These products are readily accepted by denture wearers because they are easy to handle, accessible, and have a

pleasant odor; however, alterations to prosthetic materials may be a concern when disinfecting agents are used.[11]

Chlorhexidine is one of the most widely used agents in dentistry and has been used as an adjunct in the treatment of oral candidiasis since the 1970s. It is an antiseptic agent with a broad spectrum of antimicrobial activity including *Candida albicans* and other common non-*albican* yeast species.[18,19] The most common preparation for oral use is chlorhexidine gluconate, a water-soluble compound, which has physiological pH and is dissociable, allowing the release of positively charged chlorhexidine[20] to be attracted by negative charges of bacteria.

In a 0.2% concentration, chlorhexidine gluconate has been successfully used as an antiseptic oral rinse in the treatment of denture stomatitis.[18] In a 0.12% concentration, it has been used as an antiseptic mouthwash in periodontal management. The gel in the 2.0% concentration has demonstrated an ability to clean dentinal walls when used during endodontic treatment,[21] while the 2.0% suspension is used as an overnight denture disinfectant.[18]

Given the antimicrobial potential shown by chlorhexidine in several areas of dentistry, the aim of this study was to evaluate the efficacy of 0.12% and 2.0% chlorhexidine solutions as denture cleansers, by conducting a clinical trial to evaluate their biofilm removal capability.

Materials and methods

Patient selection

After approval by the Institutional Ethics Committee and signature of the informed consent form by the potential participants, 60 patients were selected (17 men and 43 women; age range: 45 to 80 years). They presented good overall health and healthy denture-supporting tissues. The inclusion criteria were that participants should wear maxillary and mandibular complete dentures made of heat-polymerized acrylic resin; the wearing time of the present dentures should range from 5 to 10 years. In addition, an initial biofilm score of 1 or higher should be observed on the internal surface of maxillary dentures, according to an additive index.[22]

Hygiene methods and experimental design

The experimental period lasted 21 days. Before the use of each method, biofilm was eliminated by brushing with a specific brush for complete dentures (Denture, Condor S.A., Santa Catarina, Brazil) and liquid soap (JOB Química, Produtos para limpeza Ltda., Monte Alto, Brazil). All participants were instructed to brush their dentures three times a day, after each meal for 2 minutes with tap water, using a specific brush for complete dentures (Bitufo, Itupeva, Brazil). They were also instructed to rinse the oral cavity with tap water after brushing. They were randomly assigned to one of the following hygiene methods (n = 20):

1. Control: Participants were instructed to keep their dentures immersed in water overnight.
2. 0.12% chlorhexidine: Dentures were immersed daily at home in 0.12% chlorhexidine (Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil). Immersion was to be carried out for 20 minutes after dinner. Afterward, dentures were rinsed before insertion in the oral cavity. Participants were instructed to keep their dentures immersed in water overnight.
3. 2.0% chlorhexidine: At the end of the experimental period (21 days), a researcher immersed the dentures in 2.0% chlorhexidine (Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo) for 5 minutes. Participants were instructed to keep their dentures immersed in water overnight.

Denture biofilm coverage area

The internal surfaces of the maxillary dentures were disclosed with 1% neutral red solution. The surfaces were then photographed with a digital camera and flash (Canon EOS Digital Rebel EF-S 18–55, Canon MR-14 EX, Canon Inc., Tokyo, Japan) with standard film-object distance and exposure time, and the camera fixed on a stand (CS-4 Copy Stand, Testrite Inst. Co., Inc., Newark, NJ). Total surface area and areas corresponding to the stained region were measured using image processing software (Image Tool 3.0). The biofilm percentage was calculated using the ratio between biofilm area multiplied by 100 and total surface area of the internal denture base.[8,23,24] This procedure was performed by a researcher who gave no instructions, delivered no products to patients, and did not handle the dentures. After the use of each method and quantification, the biofilm was eliminated by brushing with a specific brush for complete dentures (Denture) and liquid soap (JOB Química).

Data analysis

The outcome variable for this trial, biofilm coverage area (%), did not show distribution close to normality and had no homogeneous variations. Thus, a nonparametric analysis was used. The Kruskal-Wallis test was used for comparison among the three groups followed by the Dunn multiple comparison test. Analysis was performed at $\alpha = 0.05$ using a software package SPSS 15.0.0 (SPSS Inc., Chicago, IL).

Results

Figure 1 shows a box-plot graph with the biofilm coverage areas after the trial. The control treatment seemed to remove less biofilm than the other methods. The experimental methods presented nearly similar biofilm removal results.

The Kruskal-Wallis test found significant difference among the treatments ($KW = 35.25$; $p < 0.001$). The experimental methods were similar, whereas the control group differed significantly (Table 1). This implies that denture hygiene by means of brushing was improved with the addition of the tested chlorhexidine-based treatments; however, the two experimental regimens (0.12% and 2.0% chlorhexidine) attained similar outcomes.

No adverse effect or stains were observed after the use of any of the chlorhexidine-based treatments, as disclosed by clinical examination. Participants described no complication after using the two experimental regimens, and none complained about the aftertaste associated with the chlorhexidine-based treatments.

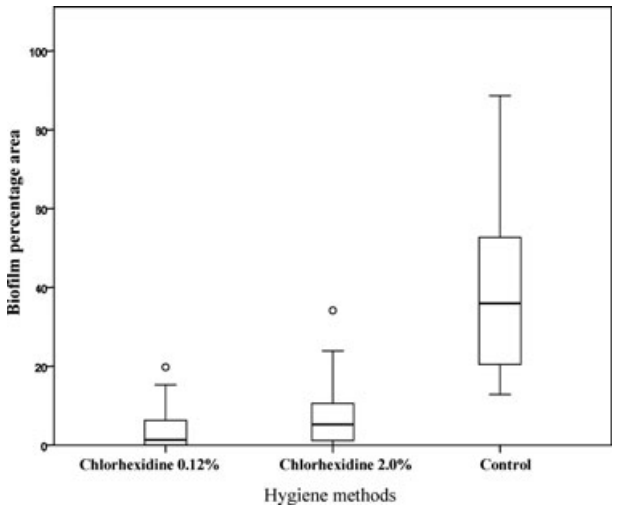

Figure 1 Biofilm coverage area for each group following treatment.

Discussion

An important feature of a complete denture cleanser is its capacity to remove biofilm, and this property should be assessed by laboratory and clinical tests.[25] This study evaluated the efficacy of complete biofilm removal from dentures using chlorhexidine solutions of two concentrations: 0.12% and 2.0%. The experimental methods presented similar results, whereas the control group (soaking in water) was significantly different. Immersion in 0.12% or 2.0% chlorhexidine solutions can improve denture hygiene when used as an auxiliary method for cleaning complete dentures.

Chlorhexidine destroys bacteria by breaking their membranes and inducing cytoplasmatic precipitation.[26] It is a cationic molecule capable of interacting with inorganic human dentine particles and also bonds to negatively charged surfaces, such as the bacterial cell wall.[12,20] Although the antimicrobial analysis was not the focus of this study, several studies that have performed antimicrobial analyses are in agreement with these results as regards the effectiveness of chlorhexidine as a denture cleanser.

Lamfon et al,[27] for example, assessed the resistance of *C. albicans* biofilms to both antifungal and antimicrobial agents in vitro. The minimal inhibitory concentration (MIC) of fluconazole, miconazole, and chlorhexidine for *C. albicans* was first determined. *C. albicans* biofilms were found to be highly resistant to fluconazole and miconazole compared with the same cells grown in suspension ($\geq$1024 $\times$ MIC). In contrast, chlorhexidine inhibited the growth of *C. albicans* biofilms at a concentration of up to 8 $\times$ MIC. When the susceptibility of biofilms over time was investigated, higher reductions were observed for chlorhexidine and miconazole than fluconazole for biofilms of 2 and 6 hours.

Similarly, Lamfon et al[28] investigated the in vitro composition of denture biofilms and the susceptibility of *Candida* spp. within these biofilms to antifungal agents. It was observed that exposure to single agents, for example, miconazole, fluconazole, or chlorhexidine did not inhibit the growth of *Candida* spp. when used in clinically relevant doses. Combinations of miconazole and chlorhexidine, to mimic patient use, did reduce bacterial and candidal growth for several days. Hence, the use of dual therapy appeared to be useful in reducing the number of viable organisms within denture plaque grown in vitro, although resistance to these agents was also evident.

Similarly, Silva-Lovato and Paranhos[23] evaluated the effectiveness of disinfectant solutions (1.0% sodium hypochlorite, 2.0% chlorhexidine digluconate, 2.0% glutaraldehyde, 100% vinegar, sodium perborate-based tabs, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by *C. albicans, Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli,* or *Bacillus subtilis* as measured by residual colony-forming units. The acrylic resin specimens were immersed in the disinfectants for 10 minutes. It was concluded that 1.0% sodium hypochlorite, 2.0% glutaraldehyde, 2.0% chlorhexidine, 100% vinegar, and 3.8% sodium perborate are valid alternatives for the disinfection of acrylic resin.

Montagner et al[12] evaluated the antifungal action of different agents on microwavable acrylic resin specimens, which were previously contaminated with *C. albicans*. They observed that sodium hypochlorite-based solutions and hydrogen peroxide are more efficacious disinfectants against *C. albicans* than 2.0% chlorhexidine solution and an effervescent agent. This lack of antimicrobial action of chlorhexidine 2.0% might be justified by the immersion periods used. The authors immersed the specimens in chlorhexidine for 10 minutes. In this study, the dentures were immersed in 2.0% chlorhexidine for 5 minutes, and the results showed that the solution was effective for biofilm removal. The in vivo design of this study and other features could be a reason for this discrepancy.

Pavarina et al[11] also noted the effectiveness of chlorhexidine as a denture cleanser, though they used the chlorhexidine in a different concentration from that adopted in this trial. In their study, the effectiveness of chemical agents (4.0% chlorhexidine gluconate, 1.0% sodium hypochlorite, iodophors, and alkaline peroxide) for cleansing and disinfecting removable dental prostheses was evaluated, and it was concluded that the 4.0% chlorhexidine gluconate, 1.0% sodium hypochlorite, and alkaline peroxide solutions were effective in reducing the growth of the microorganisms in the 10-minute immersion period.

These results with 0.12% chlorhexidine also are in agreement with Barroeta et al's study,[29] which evaluated the efficacy of four chemical agents (2.0% sodium hypochlorite, 5.0% acetic acid, peroxides, 0.12% chlorhexidine) in different immersion

Table 1 Mean ranks for the treatments and results of the Dunn multiple comparison test

Treatment	Mean rank	Grouping*
Control	39.3	A
0.12% chlorhexidine	4.1	B
2.0% chlorhexidine	7.8	B

*Identical upper case letters denote no significant differences between the treatments.

times (5, 10, 15, 20 minutes, and 8 hours) and concluded that all disinfectants were effective in eliminating *C. albicans* after 20 minutes of immersion.

Redding et al[30] determined the in vitro ability of several thin-film polymer formulations, with and without incorporated antifungal agents, to inhibit *C. albicans* biofilm growth on denture material. The fungicides incorporated were: (1) 1.0% chlorhexidine diacetate; (2) 1.0% nystatin; or (3) 1.0% amphotericin B. It was concluded that biofilm reduction with chlorhexidine (up to 98%) was significantly greater than all the other formulations tested.

Future studies should compare the antimicrobial effect of the regimens tested in this study, to better understand their effect on denture biofilm. An immediate application of 2.0% chlorhexidine may be much more efficacious than a single application of lower concentrations, but continuous use may result in biofilms with different microbial compositions. Another recommendation is the evaluation of outcome variables such as the health of supporting tissues and presence of denture stomatitis, as well as adverse effects, that is, stains on denture bases and teeth. The latter approach may lead to a definitive clinical guideline; however, it can be inferred that both tested regimens are clinically efficacious, due to their strong effect on the denture biofilm coverage area. Proprietary solutions of 0.12% chlorhexidine can be easily found by patients and are inexpensive; however, the great advantage of 2.0% chlorhexidine is the need of a single application to achieve important biofilm reduction. Based on these results, a discussion with patients about their preferences may be a reasonable approach to controlling denture biofilm.

Conclusion

The tested denture cleansing regimens based on 0.12% and 2.0% chlorhexidine solutions were equally efficacious in removing biofilm and were superior to the control method (soaking in water). Both 0.12% and 2.0% chlorhexidine solutions can be used as auxiliary methods of hygiene, contributing to the maintenance of the oral health care of complete denture wearers.

References

1. United Nations Population Division. World Population Prospects: The 2002 Revision. New York: United Nations, 2003
2. Colussi CF, Freitas SF, Calvo MCM: Perfil epidemiológico da cárie e do uso e necessidade de prótese na população idosa de Biguaçu, Santa Catarina. Rev Bras Epidemiol 2004;7:88-97
3. Moreira RS, Nico LS, Tomita NE, et al: Oral health of Brazilian elderly: a systematic review of epidemiologic status and dental care access. Cad Saúde Pública 2005;21:1665-1675
4. Coelho CM, Souza YT, Dare AM: Denture-related oral mucosal lesions in a Brazilian school of dentistry. J Oral Rehabil 2004;31:135-139
5. Kulak-Ozkan Y, Kazazoglu E, Arikan A: Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil 2002;29:300-304
6. Marchini L, Tamashiro E, Nascimento DFF, et al: Self-reported denture hygiene of a sample of edentulous attendees at a University dental clinic and the relationship to the condition of the oral tissues. Gerodontology 2004;21:226-228
7. Marchini L, Vieira PC, Bossan TP, et al: Self-reported oral hygiene habits among institutionalized elderly and their relationship to the condition of oral tissues in Taubate, Brazil. Gerodontology 2006;23:33-37
8. Paranhos HFO, Silva-Lovato CH, Souza RF, et al: Effects of mechanical and chemical methods on denture biofilm accumulation. J Oral Rehabil 2007;34:606-612
9. Donlan RM: Biofilm formation: a clinically relevant microbiological process. Clin Infect Dis 2001;33:1387-1392
10. Kuhn DM, Chandra J, Mukherjee PK, et al: Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infect Immun 2002; 70:878-888
11. Pavarina AC, Machado AL, Giampaolo ET, et al: Effects of chemical disinfectants on the transverse strength of denture base acrylics resins. J Oral Rehabil 2003;30:1085-1089
12. Montagner H, Montagner F, Braun KO, et al: In vitro antifungal action of different substances over microwave-cured acrylic resins. J Appl Oral Sci 2009;17:432-435
13. Shay K: Denture hygiene: a review and update. J Contemp Dent Pract 2000;1:1-8
14. Pires FR, Santo EB, Bonan PR, et al: Denture stomatitis and salivary *Candida* in Brazilian edentulous patients. J Oral Rehabil 2002;29:1115-1119
15. Jagger DC, Harrison A: Denture cleansing-the best approach. Br Dent J 1995;178:413-417
16. Paranhos HFO, Silva-Lovato CH, Venezian GC, et al: Distribution of biofilm on internal and external surfaces of upper complete dentures: the effect of hygiene instruction. Gerodontology 2007;24:162-168
17. Gornitsky M, Paradis I, Landaverde G, et al: A clinical and microbiological evaluation of denture cleansers for geriatric patients in long-term care institutions. J Can Dent Assoc 2002;68:39-45
18. Ellepola ANB, Samaranayake LP: Oral candidal infections and antimycotics. Crit Rev Oral Biol Med 2000;11:172-198
19. Ellepola ANB, Saramanayake LP: Adjunctive use of chlorhexidine in oral candidoses: a review. Oral Dis 2001;7:11-17
20. Mohammadi Z, Abbott PV: The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42:288-302
21. Ferraz CC: In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod 2001;27:452-455
22. Ambjørgensen E, Valderhaug J, Norheim PW, et al: Assessment of an additive index for plaque accumulation on complete maxillary dentures. Acta Odontol Scand 1982;40:203-208
23. Silva-Lovato CH, Paranhos HFO: Efficacy of biofilm disclosing agent and of three brushes in the control of compete denture cleansing. J Appl Oral Sci 2006;14:454-459
24. Salles AES, Macedo LD, Fernandes RAG, et al: Comparative analysis of biofilm levels in complete upper and lower dentures after brushing associated with specific denture paste and neutral soap. Gerodontology 2007;24:217-223
25. Nikawa H, Hamada T, Yamashiro H, et al: A review of in vitro and in vivo methods to evaluate the efficacy of denture cleansers. Int J Prosthodont 1999;12:153-159
26. Gomes BPFA, Vianna ME, Matsumoto CU, et al: Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:512-517
27. Lamfon H, Porter SR, McCullough M, et al: Susceptibility of Candida albicans biofilms grown in a constant depth film

fermentor to chlorhexidine, fluconazole and miconazole: a longitudinal study. J Antimicrob Chemother 2004;53: 383-385
28. Lamfon H, Al-Karaawi, McCullough M, et al: Composition of in vitro denture plaque biofilms and susceptibility to antifungals. FEMS Microbiol Lett 2005;242:345-351
29. Barroeta AU, Méndez GR, Lelis AB: Acción de agentes químicos en la eliminación de Cándida albicans sobre prótesis dentales. Acta Odontol Venez 2007;45:172-177
30. Redding S, Bhatt B, Rawls R, et al: Inhibition of Candida albicans biofilm formation on denture material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:669-672