ACP
AMERICAN COLLEGE OF PROSTHODONTISTS®

Effect of Three Methods for Cleaning Dentures on Biofilms Formed In Vitro on Acrylic Resin

Helena F. Oliveira Paranhos, DDS, PhD,[1] Cláudia H. Silva-Lovato, DDS, PhD,[1] Raphael F. de Souza, DDS, PhD,[1] Patricia C. Cruz, DDS, MS,[1] Karina M. de Freitas-Pontes, DDS, PhD,[1] Evandro Watanabe, PhC, PharmD,[2] & Izabel Y. Ito, PhC, PharmD[2]

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

Keywords
Candida; bacteria; biofilm; complete dentures; oral hygiene; denture cleansers; acrylic resins.

Correspondence
Raphael Freitas de Souza, São Paulo University, Department of Dental Materials and Prosthodontics, Avenida do Café s/n Bairro Monte Alegre, Ribeirão Preto, São Paulo 14040-904, Brazil. E-mail: raphael@forp.usp.br

Financial support provided by FAPESP (The State of São Paulo Research Foundation – 02/12724-9).

Accepted May 13, 2008

doi: 10.1111/j.1532-849X.2009.00450.x

Abstract

Purpose: The aim of this study was to evaluate the effect of three denture hygiene methods against different microbial biofilms formed on acrylic resin specimens.
Materials and methods: The set (sterile stainless steel basket and specimens) was contaminated (37°C for 48 hours) by a microbial inoculum with 10^6 colony-forming units (CFU)/ml (standard strains: *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli, Candida albicans, Pseudomonas aeruginosa*, and *Enterococcus faecalis*; field strains: *S. mutans*, *C. albicans*, *C. glabrata*, and *C. tropicalis*). After inoculation, specimens were cleansed by the following methods: (1) chemical: immersion in an alkaline peroxide solution (Bonyplus tablets) for 5 minutes; (2) mechanical: brushing with a dentifrice for removable prostheses (Dentu Creme) for 20 seconds; and (3) a combination of chemical and mechanical methods. Specimens were applied onto a Petri plate with appropriate culture medium for 10 minutes. Afterward, the specimens were removed and the plates incubated at 37°C for 48 hours.
Results: Chemical, mechanical, and combination methods showed no significant difference in the reduction of CFU for *S. aureus*, *S. mutans* (ATCC and field strain), and *P. aeruginosa*. Mechanical and combination methods were similar and more effective than the chemical method for *E. faecalis*, *C. albicans* (ATCC and field strain), and *C. glabrata*. The combination method was better than the chemical method for *E. coli* and *C. tropicalis*, and the mechanical method showed intermediate results.
Conclusion: The three denture hygiene methods showed different effects depending on the type of microbial biofilms formed on acrylic base resin specimens.

Denture biofilm is defined as a dense microbial layer comprising 10^{11} microorganisms per gram in wet weight and their metabolites.[1] The biofilm formed with *Candida* species has been shown to be a causative factor in denture stomatitis.[2] Several oral bacteria have also been found to be important in this disease process.[3] In addition, it has been pointed out that the colonization of oral surfaces, including intaglio surface of dentures, could serve as a reservoir for disseminated infections.[4] Appropriate denture hygiene is an important factor for maintaining mucosal tissue health as well as overall health, particularly in the elderly.[5]

Dentures can be cleaned mechanically, chemically, or by a combination of the two.[4] A significant feature of the chemical method is the variety of possible active agents. Previous studies have evaluated enzymes,[6-8] hypochlorite solutions,[6,9-11] and peroxide solutions.[6,11-13] Effective disinfection can be attained by 0.5% sodium hypochlorite.[6,10,11] Enzymes have shown less antimicrobial activity than sodium hypochlorite[6] and peroxides,[7] despite early reports of their potential usefulness as denture cleansers.[8] On the other hand, alkaline peroxide solutions present good antimicrobial activity against denture biofilm, comparable with that of sodium hypochlorite solutions.[11] This property, in addition to the absence of odor and aftertaste, makes peroxide solutions good choices for denture cleansing.

The efficacy of denture hygiene methods has been examined by means of an in vivo accumulated-plaque model[6,14,15] and an in vitro microbial model;[7,8,16,17] however, controversial results were attained as in vivo denture biofilm is a mixed-species community. Some in vitro assays have focused on a limited number of isolated species, such as *Candida albicans*.[7,8] A possible

source of controversy is that some species are more sensitive to certain substances than others, such as monocaprin against *C. albicans*.[15] A previous study found better antimicrobial performance of alkaline peroxides when compared to brushing.[16] However, this report was restricted to anaerobic bacteria; the same assay could yield different results with aerobes. Another important limitation of several studies is the comparison of a chemical method with only one negative control, as opposed to comparing with another treatment modality, such as brushing or another substance.[8,14,15,17]

In summary, previous comparisons among a small number of microbial species or the employment of in vivo designs has not thoroughly elucidated the antimicrobial effect of denture cleansing methods. The aim of this study was to evaluate the effect of three denture hygiene methods against microbial biofilm formed on the surface of acrylic resin specimens. The null hypothesis for each microorganism was that the colony-forming units (CFU) obtained from specimen surfaces would be the same following the three methods tested, that is, chemical, mechanical, or their combination.

Materials and methods

Specimen fabrication and sterilization

Two hundred and twenty cylindrical denture base acrylic resin specimens were obtained by means of wax patterns with the same dimensions (diameter 15 mm, width 4 mm). Hard wax (Wilson, Polidental Ind. Com Ltda., São Paulo, Brazil) was melted and poured in a stainless steel mold. Patterns were invested in a metallic flask No. 6 (Jon, São Paulo, Brazil) and type III dental stone (Herodent, Vigodent S/A Ind. Com, Rio de Janeiro, Brazil). A layer of type IV dental stone (Herostone, Vigodent S/A Ind. Com) was applied around the patterns to avoid stone fragments in the acrylic resin. After the investing material had set, the flask halves were separated, and wax was removed with boiling water and liquid detergent. Two coats of a separating medium (Al-quote, Dentsply Ind. Ltda., Petrópolis, Brazil) were applied on the stone surfaces. Heat-processed acrylic resin (Vipi, Dental Vipi Ltda., Pirassununga, Brazil) was mixed according to the manufacturer's recommendations and packed into the stone mold. A hydraulic press (PM-2000, Techno Máquinas Ltda., Vinhedo, Brazil) was used for packing the denture base resin at 1250 kgf, maintained for 30 minutes. Specimens were then polymerized by a conventional heat method with metal flasks in an automatic polymerization water tank (Ribeirão Preto Dental School, Ribeirão Preto, SP, Brazil) at 65°C for 1 hour, followed by a stage at 100°C for half an hour.

All specimens were bench cooled overnight before deflasking. They were then deflasked and immersed in distilled water at 50°C for 24 hours for residual monomer elimination. The excess resin was trimmed with a bur (Maxi-Cut, Malleifer SA, Ballaigues, Switzerland) and one of the surfaces was finished using 180-, 220-, 360-, and 400-grit wet/dry sandpapers (Norton, Saint-Gobain Abrasivos Ltd., Guarulhos, Brazil), and polished on a wet rag wheel with pumice slurry followed by calcium carbonate. Polishing was carried out for a better visualization through the specimens. This procedure intended to prevent incomplete contact between acrylic resin and solid culture media during the microbial counting stage. The other surface was employed for microbial counting and did not receive polishing. This way, it simulated the intaglio surfaces of a complete denture.

Specimens were sterilized with ethylene oxide gas. The hygiene methods' efficacies were evaluated against standard strains of American Type Culture Collection (ATCC) and field strains (Table 1). These microorganisms have been used to analyze the antimicrobial activity of diverse biocides and/or have been found in the oral cavity and dental prostheses.

Table 1 Origin and morphotype of microorganisms

Code	Microorganisms	Origin	Morphotype
St	*Staphylococcus aureus*	ATCC 6538	Gram-positive cocci
Smp	*Streptococcus mutans*	ATCC 25175	Gram-positive cocci
Sm	*Streptococcus mutans*	Field strain	Gram-positive cocci
Ef	*Enterococcus faecalis*	ATCC 10541	Gram-positive cocci
Ec	*Escherichia coli*	ATCC 10538	Gram-negative rod
Pn	*Pseudomonas aeruginosa*	ATCC 2327	Gram-negative rod
Cap	*Candida albicans*	ATCC 1023	Yeast
Ca	*Candida albicans*	Field strain	Yeast
Cg	*Candida glabrata*	Field strain	Yeast
Ct	*Candida tropicalis*	Field strain	Yeast

ATCC: American Type Culture Collection.

Contamination of the specimens

Five specimens were placed into a stainless steel basket (6.0-cm length × 3.0-cm width × 3.0-cm height) with six compartments (2.0 × 1.5 cm^2) and an appropriate cover. This basket was employed for the formation of biofilm without sedimentation of microorganisms onto the specimens' surfaces, as long as the biofilm formation occurred in static culture. The set was completely immersed in a container with culture medium broth (200.0 ml) with 1.0% microbial inoculum at 0.5 McFarland scale, which corresponds to 10^6 CFU/ml. After incubation at 37°C for 48 hours, the baskets containing the contaminated specimens were then dried by placing them in Petri plates with filter papers.

Experimental and control groups

The set containing the contaminated specimens (n = 5 for each microorganism) was randomly assigned to one of the cleansing methods evaluated, as follows:

(1) Chemical method: the contaminated specimens were transferred to a sterilized basket, which was immersed in a container with 200 ml of distilled sterilized water at 37°C and one effervescent tablet of alkaline peroxide (Bonyplus, Bonyf GAC, Vaduz, Liechtenstein) for 5 minutes. Volume, temperature, and time of immersion followed manufacturer's recommendations.
(2) Mechanical method: the contaminated specimens were brushed on all faces with a soft-bristle toothbrush (Tek, Johnson & Johnson Ltd, São José dos Campos, Brazil) for

20 seconds. A new toothbrush was used for each specimen, associated with a specific dentifrice for removable prostheses (Dentu Creme, Dentco, Inc., Jersey City, NJ). Fifty microliters of distilled sterilized water were also used for wetting the bristles. After brushing, the specimens were then transferred to a sterilized basket, which was immersed in a container with distilled sterilized water for 5 minutes.

(3) Combination method: the contaminated specimens were first submitted to the mechanical method, followed by the chemical method.

The other part of the sample was assessed as an experimental control carried out simultaneously for the treatment groups for each microorganism. It was divided in two groups:

(1) Negative control: The intent of this group was to confirm sterilization of the specimens. Previous immersion in culture broth and incubation was similar to the other groups, except for the absence of inoculum. Ten sets of two sterilized specimens were transferred to a container with distilled and sterilized water (200.0 mL) for 5 minutes.

(2) Positive control: five specimens for each microorganism were transferred to another sterilized basket, which was immersed in a container with distilled and sterilized water (200.0 mL) for 5 minutes.

Regardless of the group, each set was dried in Petri plates (20×100 mm^2) with filter papers. This procedure was similar for the three treatment groups and the two control regimens.

Microbial counting

For all specimens, the nonpolished surface was placed in contact with appropriate culture medium contained by a 20×100 mm^2 Petri plate. Specimens were discarded after 10 minutes, the Petri plates were incubated at 37°C for 48 hours, and next, the number of CFU was counted. The culture media used in Petri plates to recover/count the different microorganisms from specimens were: Ni[18] for *Streptococcus aureus*; SB20[19] for *S. mutans*; Mueller Hinton agar (Oxoid, Basingstake, Hampshire, United Kingdom) for *Enterococcus faecalis, E. coli,* and *Pseudomonas aeruginosa*; and agar Sabouraud (Difco, Detroit, MI) for *Candida* spp.

Statistical methods

The data obtained for microbial counts were expressed according to an ordinal scale. The following values were considered: (1) no microbial growth (0 CFU), (2) slight growth (1 to 20 CFU), and (3) large growth (more than 20 CFU). Values of each microorganism were grouped and analyzed according to the different hygiene methods by the Kruskal-Wallis test. Multiple comparisons were performed when indicated according to the Dunn test. Procedures were performed with $\alpha = 0.05$. Data were analyzed with GraphPad InStat 3.06 (GraphPad Software Inc., San Diego, CA).

Table 2 The number of denture base resin specimens according to an ordinal scale of microbial contamination

	Hygiene method (CFU)								
	Chemical			Mechanical			Combination		
Code	0	1–20	>20	0	1–20	>20	0	1–20	>20
St	3	2	0	3	2	0	5	0	0
Smp	0	0	5	0	0	5	0	0	5
Sm	0	0	5	0	0	5	1	1	3
Ef	0	1	4	5	0	0	5	0	0
Ec	0	1	4	0	3	2	1	4	0
Pn	0	0	5	0	3	2	0	1	4
Cap	0	0	5	2	3	0	0	5	0
Ca	0	0	5	4	1	0	2	3	0
Cg	0	0	5	1	4	0	2	3	0
Ct	0	0	5	0	2	3	0	5	0

St = *S. aureus*; Smp and Sm = *S. mutans* (ATCC and field); Ef = *E. faecalis*; Ec = *E. coli*; Pn = *P. aeruginosa*; Cap and Ca = *C. albicans* (ATCC and field); Cg = *C. glabrata*; Ct = *C. tropicalis*.

Table 3 Comparison among hygiene methods against microorganisms

	Mean ranks			Kruskal-Wallis test	
Microorganisms	I	II	III	KW	*p* value
S. aureus	9.4	8.6	6.0	2.62	0.270 ns
S. mutans	8.0	8.0	8.0	0.00	1.000 ns
S. mutans (field)	9.0	9.0	6.0	4.29	0.117 ns
E. faecalis	13.0 (A)	5.5 (B)	5.5 (B)	13.64	0.001*
E. coli	11.10 (A)	8.3 (AB)	4.6 (B)	6.75	0.034*
P. aeruginosa	10.0	5.5	8.5	4.46	0.108 ns
C. albicans	13.0 (A)	4.5 (B)	6.5 (B)	12.15	0.002*
C. albicans (field)	13.0 (A)	4.5 (B)	6.5 (B)	11.17	0.004*
C. glabrata (field)	13.0 (A)	6.0 (B)	5.0 (B)	11.08	0.004*
C. tropicalis (field)	11.5 (A)	8.5 (AB)	4.0 (B)	9.50	0.009*

I = chemical method; II = mechanical method; III = combination method; ns = nonsignificant difference, $p > 0.05$; *significant difference, $p < 0.05$. Values with same capital letter are not significantly different (Dunn test, $p < 0.05$).

Results

As presented in Tables 2 and 3, the Kruskal-Wallis test failed to find significant differences among chemical, mechanical, and combination methods for *S. aureus*, *S. mutans* (ATCC and field strain), and *P. aeruginosa*. Additionally, mechanical and combination methods were similar and more effective than the chemical method for *E. faecalis*, *C. albicans* (ATCC and field strain), and *C. glabrata*. Moreover, the combination method was better than the chemical method for *E. coli* and *C. tropicalis*, and the mechanical method attained intermediate results.

No microbial growth (score "1") was observed for the negative control group, either in culture broth or Petri plates. For the positive control, all specimens resulted in large growth (score "3"). These results confirm that the sterilization and contamination procedures were adequate.

Discussion

In this study, the data support rejection of the null hypothesis for six microorganisms. It was shown that the three denture hygiene methods presented variations in antimicrobial activities, depending on the type of microbial biofilms formed on acrylic base resin specimens.

The combination method was more effective than the chemical method for *E. faecalis*, *E. coli, C. albicans* (ATCC and field strain), *C. glabrata*, and *C. tropicalis*. This is in agreement with Lee et al,[20] who stated that the combination of brushing and chemical methods was the most effective type of denture hygiene method. A clinical trial recently pointed out that the brushing method has a better effect regarding denture hygiene than immersion in a peroxide solution;[21] however, some clinical studies have found that brushing alone is less effective than soaking in reducing microbial counts.[16,22,23] A possible reason for these different results is the set of participants' characteristics, that is, brushing can be influenced by patients' dexterity. Besides, diverse information on cleansing methods can also be provided through verbal information and/or through visual demonstration, and this can influence outcomes.[24]

E. coli is found as a transient microbiota in the oral cavity[25] and is able to promote initial adherence of yeasts on host surfaces.[26] Furthermore, *E. faecalis* is able to colonize several sites, including the oral cavity,[27] and has been associated with oral mucosal lesions in immunocompromized subjects.[28] In this study, a better reduction for both species was attained by means of the combination method; however, the combination and mechanical methods were similar against *E. faecalis*, which implies a minimal effect of the chemical method. For *E. coli*, intermediate results for the mechanical method indicate that immersion in a peroxide solution contributed to a further antimicrobial effect than shown by the combination method.

Yeast counts were high following the chemical method, but further reduction was attained by brushing. This points out that the mechanical method should be recommended for removal of *Candida* species from acrylic resin surfaces; however, this does not mean that the tested chemical method is ineffective. It was reported that peroxide solutions are able to reduce *C. albicans* from denture bases;[17] however, soaking should be combined with brushing to control candidal growth more effectively.

Specimen hygiene with chemical, mechanical, and combination methods showed no statistically significant difference in the counts for *S. aureus*, *S. mutans* (ATCC and field strain), and *P. aeruginosa*. The considerable counts found for *S. mutans* and *P. aeruginosa* could be a consequence of a higher resistance against denture cleansing methods when compared with other species.

In this study, *S. mutans* (field strain) was not affected differently by the tested methods. Some effect might be expected after the use of peroxide solution[29] or brushing;[30] however, it can be stated that it is a relatively resistant microorganism, and none of the tested cleansing protocols was able to reduce it to low levels. A possible reason for this resistance is the synthesis of extracellular polysaccharides.[31]

Both *S. aureus* and *P. aeruginosa* can lead to opportunistic infections associated with a decrease in immune function.[32] Diverse microorganisms have been used as indicators for denture disinfection effectiveness.[33] Thus, careful hygiene methods should not preclude disinfection protocols when dentures are transferred from a dental laboratory to patients.

Microbiological assessment was based on the nonpolished surface of the acrylic resin specimens. This procedure intended to simulate the intaglio surface of removable dentures, as different degrees of polishing may influence microorganism adherence.[34] There is a significant association between *C. albicans* adhesion and denture base acrylic resin roughness.[35,36]

The procedures that have been used in studies on the effect of cleansing methods on denture plaque include placing the contaminated specimen in a sterile container with sterile saline, vortex-mixing, serial dilutions of the sonicate, plating on culture medium, incubation, and counting of microorganisms (CFU/ml).[33,34] The method used in this study (applying the nonpolished surface of the specimens onto a Petri plate with appropriate culture medium for 10 minutes), although simple, has disadvantages. The resolution is lower, since no continuous quantitative variable as log CFU could be obtained. In addition, an ordinal scale, with a cut-off point of only 20 CFU, had to be used because of the distribution of results found for the Petri dishes. Two distinct microbial growth patterns were found. Some specimens resulted in slight growth, with a maximum of 20 CFU, while several specimens presented intense growth that resulted in colony aggregation, which made counting impossible.

The sample size of this study demanded a nonparametric analysis. The reduced number of specimens does not provide a descriptive procedure based on median and quartiles. Therefore, an ordinal scale was used, as data do not allow a parametric description, that is, mean and standard deviation; however, the use of specimens in a stainless steel basket was a useful and inexpensive method to simulate denture biofilm, as well as to evaluate a large variety of hygiene methods.

Another significant limitation of this study was that mixed microbial biofilms were not assessed. In the oral cavity, microorganisms exist in polymicrobial communities and different species interact in a complex manner to modulate biofilm nature.[37] It was found that some of the analyzed microorganisms displayed distinct responses against denture cleansing methods. Thus, further studies should look at the in vitro response of mixed communities. Another possibility is the molecular analysis of biofilm composition[38] as an outcome variable for clinical trials on denture hygiene.

Conclusion

The three denture hygiene methods showed different effects depending on the type of microbial biofilms formed on acrylic resin specimens. The combination method provided results similar to the mechanical method and was more effective than the chemical method for the majority of the tested species (*E. faecalis*, *E. coli*, *C. albicans* [ATTC and field strain], *C. glabrata*, and *C. tropicalis*).

References

1. Nikawa H, Hamada T, Yamamoto T: Denture plaque – past and recent concerns. J Dent 1998;6:299-304

2. Akpan A, Morgan R: Oral candidiasis. Postgrad Med J 2002;78:455-459
3. Theilade E, Budtz-Jørgensen E: Predominant cultivable microflora of plaque on removable dentures in patients with denture-induced stomatitis. Oral Microbiol Immunol 1988;3:8-13
4. 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
5. Shay K: Denture hygiene: a review and update. J Contemp Dental Pract 2000;15:28-41
6. Lima EMCX, Moura JS, Del Bel Cury AA, et al: Effect of enzymatic and NaOCl treatments on acrylic roughness and on biofilm accumulation. J Oral Rehabil 2006;33:356-362
7. Nakamoto K, Tamamoto M, Hamada T: Evaluation of denture cleansers with and without enzymes against *Candida albicans*. J Prosthet Dent 1991;66:792-795
8. Tamamoto M, Hamada T, Miyake Y, et al: Ability of enzymes to remove *Candida*. J Prosthet Dent 1985;53:214-216
9. Barnabe W, De Mendonça Neto T, Pimenta FC, et al: Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, *Streptococcus mutans* and *Candida albicans*. J Oral Rehabil 2004;31:453-459
10. Bell JA, Brockmann SL, Feil P, et al: The effectiveness of two disinfectants on denture base acrylic resin with an organic load. J Prosthet Dent 1989;61:580-583
11. Pavarina AC, Pizzolitto AC, Machado AL, et al: An infection control protocol: effectiveness of immersion solutions to reduce the microbial growth on dental prostheses. J Oral Rehabil 2003;30:532-536
12. De Paola LG, Minah GE, Elias SA: Evaluation of agents to reduce microbial growth on dental prostheses of myelosuppressed cancer patients. Clin Prev Dent 1984;6:9-12
13. Sharp EW, Verran J: Denture cleansers and in vitro plaque. J Prosthet Dent 1985;53:584-585
14. Sheen SR, Harrison A: Assessment of plaque prevention on dentures using an experimental cleanser. J Prosthet Dent 2000;84:594-601
15. Thorgeirsdottir TO, Kristmundsdottir T, Thormar H, et al: Antimicrobial activity of monocaprin: a monoglyceride with potential use as a denture disinfectant. Acta Odontol Scand 2006;64:21-26
16. Chan EC, Iugovaz I, Siboo R, et al: Comparison of two popular methods for removal and killing of bacteria from dentures. J Can Dent Assoc 1991;57:937-939
17. Lin JJ, Cameron SM, Runyan DA, et al: Disinfection of denture base acrylic resin. J Prosthet Dent 1999;81:202-206
18. Ito IY: Caracterização e Incidência de *Staphylococcus aureus* de Cepas Hospitalares. Thesis, Ribeirão Preto, São Paulo University, 1973
19. Davey AL, Rogers AH: Multiple types of the bacterium *Streptococcus mutans* in the human mouth and their intra-family transmission. Arch Oral Biol 1984;29:453-460
20. Lee HE, Wang CC, Wang JC, et al: The effect of denture cleansers and cleaning methods on the microflora of denture plaque. Gaoxiong Yi Xue Ke Xue Za Zhi 1985;1:88-94
21. 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
22. Dills SS, Olshan AM, Goldner S, et al: Comparison of the antimicrobial capability of an abrasive paste and chemical-soak denture cleaners. J Prosthet Dent 1988;60:467-470
23. Kulak Y, Arikan A, Albak S, et al: Scanning electron microscopic examination of different cleaners: surface contaminant removal from dentures. J Oral Rehabil 1997;24:209-215
24. Ambjornsen E, Rise J: The effect of verbal information and demonstration on denture hygiene in elderly people. Acta Odontol Scand 1985;43:19-24
25. Samaranayake LP, Lamb AB, Lamey PJ, et al: Oral carriage of *Candida* species and coliforms in patients with burning mouth syndrome. J Oral Pathol Med 1989;18:233-235
26. Makrides HC, MacFarlane TW: An investigation of the factors involved in increased adherence of *C. albicans* to epithelial cells mediated by *E. coli*. Microbios 1983;38:177-185
27. Smyth CJ, Matthews H, Halpenny MK, et al: Biotyping, serotyping and phage typing of *Streptococcus faecalis* isolated from dental plaque in the human mouth. J Med Microbiol 1987;23:45-54
28. Wahlin YB, Holm AK: Changes in the oral microflora in patients with acute leukemia and related disorders during the period of induction therapy. Oral Surg Oral Med Oral Pathol 1988;65:411-417
29. Drake D, Wells J, Ettinger R: Efficacy of denture cleansing agents in an in vitro bacteria-yeast colonization model. Int J Prosthodont 1992;5:214-220
30. Paranhos HFO, Lara EHG, Panzeri H, et al: Capacity of denture plaque removal and antimicrobial action of a specific paste formulated for denture cleaning. Braz Dent J 2000;11: 97-104
31. Tam A, Shemesh M, Wormser U, et al: Effect of different iodine formulations on the expression and activity of *Streptococcus mutans* glucosyltransferase and fructosyltransferase in biofilm and planktonic environments. J Antimicrob Chemother 2006;57:865-871
32. Tada A, Senpuku H, Motozawa Y, et al: Association between commensal bacteria and opportunistic pathogens in the dental plaque of elderly individuals. Clin Microbiol Infect 2006;12:776-781
33. Silva MM, Vergani CE, Giampaolo ET, et al: Effectiveness of microwave irradiation on the disinfection of complete dentures. Int J Prosthodont 2006;19:288-293
34. Moran TD, Wilson M: The effects on surface roughness and type of dentures acrylic on biofilm formation by *Streptococcus oralis* in a constant depth film fermentation. J Appl Microbiol 2001;91:47-53
35. Radford RD, Sweet SP, Challacombe SJ, et al: Adherence of *Candida albicans* to denture-base materials with different surface finishes. J Dent 1998;26:577-583
36. Taylor R, Maryan C, Verran J: Retention of oral microorganisms on cobalt-chromium alloy and dental acrylic resin with different surface finishes. J Prosthet Dent 1998;80: 592-597
37. Thein ZM, Samaranayake YH, Samaranayake LP: Effect of oral bacteria on growth and survival of *Candida albicans* biofilms. Arch Oral Biol 2006;51:672-680
38. Sakamoto M, Umeda M, Benno Y: Molecular analysis of human oral microbiota. J Periodont Res 2005;40:277-285