**Denture Disinfection by Brushing and 6% Hydrogen Peroxide Immersion on Denture Base Resin after Biofilm Formation: An In vitro Study**

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**Abstract**

**Background:** Biofilm formation on acrylic dentures is often seen in denture wearers for which wide variety of denture cleansers are available. This study uses the commonly available 6% British Pharmacopoeia grade 20 volume hydrogen peroxide as denture cleanser. **Aim:** The aim of this study was to evaluate colonization and population dynamics of oral pathogenic microbial biofilm formation after assigning to one of the cleansing methods, manual brushing, or compound method (manual brushing with soft bristle wetted in distilled water and rinsed then immersion in 6% hydrogen peroxide solution) for the heat-cure acrylic denture base resin. **Materials and Methods:** Specimens of heat-cure acrylic resins discs were fabricated \( n = 120 \). Specimens \( n = 60 \) were placed in bacterial culture (Group I) and \( n = 60 \) were placed in fungal culture (Group II) until the formation of a biofilm which then was subjected to denture cleansing. They were then immersed in respective broth. The specimens were discarded, and Petri plate was incubated, and colony-forming units (CFUs) were counted. Values of microorganisms were grouped and analyzed according to the different hygiene methods by the Kruskal–Wallis test. Pairwise comparison was done by Mann–Whitney U-test. **Results:** The CFU in Group I and Group II after brushing was 60 CFU/ml and 50 CFU/ml, respectively. The CFU in Group I and Group II after subjecting to compound method was 1 CFU/ml. **Conclusion:** The compound method of this study was better when compared to only brushing method. Therefore, for the better maintenance of oral hygiene in denture wearers, the dentures need to be cleaned by manual brushing followed by immersion in hydrogen peroxide solution.

**Keywords:** Biofilm, denture cleanser, denture stomatitis, denture wearer, hydrogen peroxide

**Introduction**

“Biofilm” accumulates as a consequence of poor denture hygiene, which in turn leads to onset of several systemic and oral infections.[1] The continuous presence of biofilm formed by yeasts and bacteria in such denture wearer causes an inflammatory condition called as denture stomatitis. Cultures and smears of denture plaque in such denture wearers validate higher concentration of *Candida* species, especially *Candida albicans*.[2,3]

The flora of denture wearers with healthy palatal mucosa primarily has microbial bacteria such as Streptococci, Staphylococci, *Actinomyces*, Lactobacilli, and Gram-negative Cocci, but very few Gram-negative rods and yeast.[4] Denture cleansing is necessary for removal of biofilm from dentures. Denture cleansing can be achieved mechanically by manual brushing, chemically involving wide varieties of chemical agents, and by combination of both.[1,2] Studies have been done to evaluate the effect of cleansing dentures on individual microorganisms that form the biofilm on acrylic dentures, but oral cavity habitats various species behaving in a complex manner.[5] Hence, the present study was done to evaluate colonization and population dynamics of mixed fungal and mixed bacterial microbial biofilms formation after subjecting to different denture cleansing methods for the heat-cure acrylic denture base resin.

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MATERIALS AND METHODS

A total of 120 heat-cure acrylic resin discs of 15-mm diameter and 4-mm thick were incorporated in the study. Polishing was done on one surface of the disc, and the other surface was left unpolished simulating the intaglio surface of a complete denture. These specimens were sterilized with ethylene oxide gas and were evaluated against standard strains of the American Type Culture Collection and field strains to ensure no cross-contamination. Of which, 60 specimens for mixed bacterial (Group I) and other 60 specimens for mixed fungal (Group II) contamination were separately placed in a stainless steel basket with respective culture. The brain heart infusion (BHI) culture media was used in Petri plates to recover/count *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and sabouraud dextrose agar (SDA) for *Candida* spp. form specimens. They were placed as long as the biofilm occurred in those static cultures. Then, they were immersed in petri plate with the unpolished surface in contact with appropriate culture medium broth (200 ml) with 1.0% microbial inoculums on 0.5 McFarland scale (which corresponds to $10^6$ CFU/ml).

Specimens from broth were discarded after 10 min, and those Petri plates were incubated at 37°C for 48 h, and colony-forming units (CFUs/ml) were counted after assigning to one of the cleansing methods [Figures 1 and 2]. Group I and Group II were subdivided into A and B for different cleansing methods. In “Method A”, specimens (Group IA and Group IIA) were just brushed after wetting the bristles in distilled water and rinsed, in “Method B”, specimens (Group IB and Group IIB) were brushed after wetting the bristles in distilled water then rinsed in a container with 200 ml of 6% British Pharmacopoeia (BP) 20 volume hydrogen peroxide at 37°C for 5 min.

RESULTS

The data obtained for microbial counts were expressed according to an ordinal scale. The following values were considered as follows: (1) no microbial growth (0 CFU), (2) slight growth (1–20 CFU), and (3) large growth (more than 20 CFU).

Values of microorganisms were grouped and analyzed according to the different hygiene methods by the Kruskal–Wallis test. Pairwise comparison was performed according to Mann–Whitney U-test. Procedures were performed with $\alpha = 0.05$. Data were analyzed with IBM SPSS Statistics 20 software package. The comparative analysis between Groups for CFU in BHI and in SDA was assessed. In Group IA and Group IIA, the maximum CFU was 60 CFU/ml and 50 CFU/ml, respectively. The microbial count in Group IB and Group IIB was 1 CFU/ml for both [Figures 3 and 4]. There is statistical difference in mean score in Group IA, Group IB, Group IIA, and Group IIB at 5% significance level. Pairwise comparison showed statistically significant difference between Group IA and Group IB, Group IA and Group IIB, Group IB and Group IIA, and between Group IIA and Group IIB at 5% significance level [Table 1]. The significant decline in microbial count in “Method B” suggests that 6% BP 20 volume hydrogen peroxide can be adjutant for denture cleansing.
Denture disinfection by brushing and 6% hydrogen peroxide immersion

**DISCUSSION**

Oral cavity of humans harbors various species of microorganisms.[6] These microorganisms are introduced into the mouth from birth, and among them few species establish. In an adult edentulous mouth, yeasts predominate compared to few bacteroïds or spirochetes. In a denture wearer, various factors are involved in the pathogenesis of denture stomatitis.[2,7] Drake et al. also found that denture plaque containing *C. albicans* plays a major role.[8] The microflora in mouth also varies at different surfaces as a result of respective physical and biological properties.[9] Surface roughness in denture aid in colonization of oral micro organisms by adhering to it and then mature plaque forms.[10] Microbial communities so formed have a complex structure but does not exist as a single living cells.[6] Infection of the oral cavity in geriatric patient is the main drawback. A strict denture hygiene protocol, especially in elders, should be followed for better mucosal tissue health as well as overall health. It has been shown that several denture hygiene methods present variation in antimicrobial activities depending on the type of microbial biofilm formed on acrylic base resin specimens.[10,11,12]

Denture hygiene can be performed mechanically, chemical, or by a combination of both. Mechanical brushing is simple method, effective, and economical, but for patients with low dexterity; it becomes difficult to perform and may be subjected to superficial wear. The chemical denture cleansers also have implemented cleansing the surfaces effectively.[1,2]

Gornitsky recommended chemical denture cleansers as a better option for the elderly. These can be hypochlorites, peroxides, enzymes, acids, crude drugs, and disinfectants.[1] Montagner *et al.*[13] used 10v H2O2 and found effective against *C. albicans*, but not according to Hashizume *et al.*[14] Surface roughness in denture aid in colonization of oral micro organisms by adhering to it and then mature plaque forms.[10] Buergers *et al.*[3] stated that effective denture cleansing of soft denture relining material by soaking dentures in 1% sodium hypochlorite for 10 mins, microwave irradiation immersed in water at 800W for 6mins and application of effervescent cleansing tabs were effective against *C.albicans* colonization da Silva *et al.*[15] proved that 1% sodium hypochlorite, 2% chlortohexidine digluconate, 2% glutaralddehyde, 100% vinegar, tabs of sodium perborate-based denture cleaner, and 3.8% sodium perborate solutions are the various disinfectant solution which can be used alternatively and are effective against analyzed organism, but Paranhos *et al.*[16] concluded that brushing alone was less effective than the chemical method employed.

de Andrade *et al.*[11] suggested that the effects of ultrasonic cleaning could be associated with the chemical immersion for better efficiency. Nishi *et al.*[12] also stated that the use of a denture brush and the daily use of a denture cleanser should be recommended to complete denture wearers to effectively reduce the quantity of microorganisms adhering to dentures. Barroeta *et al.*[18] proved that 2.0% sodium hypochlorite, 5.0% acetic acid, peroxides, 0.12% chlortohexidine disinfectants were effective in eliminating *C. albicans* after 20 minutes of immersion in different immersion times. Ingrid Machado *et al.*[19] claimed that nonthermal effects of microwave energy resulted in reduction of microorganisms. Lee *et al.*[18] stated that the combination of brushing and chemical immersion was the most effective type of denture hygiene method. In the present *in vitro* study, the heat-cure resin discs with either colony of mixed bacterial flora or mixed fungal flora were cleansed by compound method, involving manual brushing with soft-bristle toothbrush wetted with distilled water and rinsing followed by immersion in 200 ml of 6% BP 20 hydrogen peroxide for 5 min. This method of cleansing could be used as one of the combination methods for efficiently disinfecting the heat-cure acrylic denture bases as no clinically, and statistically significant microbial CFU existed in both the groups.

**CONCLUSION**

Within the limitation of the study, the following conclusions were drawn. Colonization and population dynamics of oral
pathogenic microbes’ biofilm formation varies drastically for different denture cleansing methods. In the present study, biofilm formed from mixed bacterial flora and mixed fungal flora microbes on acrylic discs were assessed separately, and no emphasis was done on individual microorganism or entirely mixed bacterial and fungal biofilm forming microbes. Compound method of this study has been proved to be more effective disinfection method on mixed microbial biofilms formed on heat-cure acrylic resin specimens.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. de Andrade IM, Cruz PC, Silva-Lovato CH, de Souza RF, Souza-Gugelmin MC, Paranhos Hde F, et al. Effect of chlorhexidine on denture biofilm accumulation. J Prosthodont 2012;21:2-6.
2. Dhamande MM, Pakhan AJ, Thombare RU, Ghodpage SL. Evaluation of efficacy of commercial denture cleansing agents to reduce the fungal biofilm activity from heat polymerized denture acrylic resin: An in vitro study. Contemp Clin Dent 2012;3:168-72.
3. Buergers R, Rosentritt M, Schneider-Brachert W, Behr M, Handel G, Hahnel S, et al. Efficacy of denture disinfection methods in controlling Candida albicans colonization in vitro. Acta Odontol Scand 2008;66:174-80.
4. 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.
5. Paranhos HF, Silva-Lovato CH, de Souza RF, Cruz PC, de Freitas-Pontes KM, Watanabe E, et al. Effect of three methods for cleaning dentures on biofilms formed in vitro on acrylic resin. J Prosthodont 2009;18:427-31.
6. Prakash B, Shekar M, Maiti B, Karunasagar I, Padiyath S. Prevalence of Candida spp. Among healthy denture and nondenture wearers with respect to hygiene and age. J Indian Prosthodont Soc 2015;15:29-32.
7. Pereira CA, Toledo BC, Santos CT, Pereira Costa AC, Back-Brito GN, Kaminagakura E, et al. Opportunistic microorganisms in individuals with lesions of denture stomatitis. Diagn Microbiol Infect Dis 2013;76:419-24.
8. Drake D, Wells J, Ettinger R. Efficacy of denture cleansing agents in an in vitro bacteria-yeast colonization model. Int J Prosthodont 1992;5:214-20.
9. Marsh PD, Percival RS, Challacombe SJ. The influence of denture-wearing and age on the oral microbiota. J Dent Res 1992;71:1374-81.
10. Pesci-Bardon C, Fosse T, Serre D, Madinier I. In vitro antifungal properties of an ammonium compound combined with denture base acrylic resin. Gerodontology 2006;23:111-6.
11. de Andrade IM, Cruz PC, da Silva CH, de Souza RF, Paranhos Hde F, Candido RC, et al. Effervescent tablets and ultrasonic devices against Candida and Mutans streptococci in denture biofilm. Gerodontology 2011;28:264-70.
12. Nishi Y, Seto K, Kamashita Y, Kaji A, Kurono A, Nagaoka E, et al. Survival of microorganisms on complete dentures following ultrasonic cleaning with immersion in peroxide-based cleanser solution. Gerodontology 2014;31:202-9.
13. Montagner H, Montagner F, Braun KO, Peres PE, Gomes BP. In vitro antifungal action of different substances over microwaved-cured acrylic resins. J Appl Oral Sci 2009;17:432-5.
14. Hashizume LN, Hoscharuk MF, Moreira MJ. Effect of affordable disinfectant solutions on Candida albicans adhered to acrylic resin for dental prosthesis. Rev Gaúcha Odontol 2015;63:309-14.
15. da Silva FC, Kimpara ET, Mancini MN, Balducci I, Jorge AO, Koga-Ito CY, et al. Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of acrylic resin. J Prosthodont 2008;17:627-33.
16. Barroeta AU, Mendez GR, Leis AB. Accion de agentes químicos en la eliminacion de Candida albicans sobre protesis dentales. Acta Odontol Venez 2007;45:172-7.
17. Machado AL, Breeding LC, Puckett AD. Effect of microwave disinfection procedures on torsional bond strengths of two hard chairside denture reliner materials. J Prosthodont 2006;15:337-44.
18. Lee HE, Wang CC, Wang JC, Chen CP. The effect of denture cleaners and cleansing methods on the microflora of denture plaque. Gaoxiong Yi Xue Ke Xue Za Zhi 1985;1:88-94.