Evaluation of efficacy of commercial denture cleansing agents to reduce the fungal biofilm activity from heat polymerized denture acrylic resin: An in vitro study

MITHILESH M. DHAMANDE, ASHOK J. PAKHAN, RAM U. THOMBARE, SHYAM L. GHODPAGE1

Abstract

Aims: To compare and evaluate Candida removing effects of three most commonly available varieties of commercial denture cleansers from heat polymerized acrylic resins. To compare and evaluate Candida lytic effects of denture cleansers. To assess the effect of time on ability of denture cleansers in reducing Candidal biofilm. Materials and Methods: A specially designed metal mold was fabricated to obtain wax plates of uniform dimensions which were used to fabricate heat cure acrylic resin plates. A square-shaped window of dimension 15 mm and thickness of 1.5 mm was provided in metal mould to simulate thickness of denture base. All samples used in this study were prepared using this mould. Candida albicans colonies were then cultured on this acrylic resin plates by colonization assay. Yeast removing test for samples was performed using microscope and yeast lytic test was performed using photo colorimeter. Results: Denture cleanser D2 showed the highest Candida removing activity when compared with cleansers D1, D3, and control solution. Denture cleansers D2 showed increased yeast lytic ability when compared with denture cleansers D1, D3, and control solution. More time span shared a definite influence on yeast lytic ability of denture cleansers. Conclusions: The effect of cleansing agents on removal of colonized yeasts particularly fungal biofilm from acrylic resins was assessed for clinical implications. The observation indicated superior performance of cleanser D2 when compared with D1 and D3 even though they all belong to same chemical group of alkaline peroxide. The increased effectiveness may be due to presence of sodium lauryl sulphate in formula of D2.

Keywords: Acrylic resins, Candida albicans, denture cleanser

Introduction

Denture stomatitis is a most common inflammatory condition that affects denture wearers. Microbial plaque on tissue surface of dentures is a significant co-factor in pathogenesis of denture stomatitis. Cultures and smears have demonstrated significantly higher concentration of Candida species in denture plaque from patients with denture stomatitis. Among the two strains, Candida albicans remains the most commonly implicated yeast in denture stomatitis. In oral cavity, most colonizing and infecting microorganisms are found not as single living cells but rather as complex, structural microbial communities often encapsulated within a matrix of exopolymeric material and attached to biotic and abiotic surfaces. These communities are referred to as “biofilms.”

Denture hygiene is compromised both due to the limitations of the denture material as well as the lack of manual dexterity of denture wearers. Denture cleansing that includes removal of Candida from its surface is necessary to prevent denture stomatitis, other than that caused by denture trauma. This study evaluated the effect of cleansing agents on the quality of removal of colonized yeasts, particularly fungal biofilm.

Materials and Methods

Commercial names, mode of supply, chemical composition, and manufacturers of materials used in the study are listed in Table 1.

The study protocol was divided into three parts:

• Preparation of acrylic samples
• Colonization assay
• Yeast removing test and yeast lytic test

Preparation of acrylic samples

A specially designed metal mold was fabricated and was used for obtaining wax plates of uniform dimensions of 15 mm × 15 mm and thickness of 1.5 mm which later were processed into heat cure acrylic resin samples. It had square-shaped window of dimension 15 mm × 15 mm with a thickness of 1.5 mm to simulate thickness of conventional dentures.
The metal mold was coated with thin film of petroleum jelly and was placed on glass slab already coated with petroleum jelly. Molten modeling wax was then poured into the window of the metal mould. The second glass slab also coated with petroleum jelly was then immediately placed on top of metal mold. This molten wax was then allowed to solidify undisturbed. After complete hardening, top glass slab was removed from the top of metal mold in sliding motion. Then the metal mold was separated from first glass slab in sliding motion and wax sample removed from mold. Wax sample was then processed into heat cured acrylic resin using conventional procedures, namely

i) Flasking of wax sample
ii) Wax elimination
iii) Packing
iv) Acrylization
v) Deflasking

In all, 120 samples were prepared following same procedure. These samples were then divided into four groups (30 samples each). One group was used as control group and other three were used with three denture cleanser solutions, viz D1, D2, and D3. The control group was treated with plain distill water.

**Colonization assay**

The acrylic resin samples were first coated with human serum by placing them in wells of Multiwell tissue culture plates. Now 500 µl of protein solution per well was dispensed and samples were incubated for 1 h at 37°C. After incubation, protein solution was aspirated, 50 µl of yeast suspension was inoculated in each well, and entire assembly was incubated at 37°C for 2 h to promote yeast adherence and colonization on acrylic plates. Two milliliters of sabouraud broth was then dispensed into each well and incubate for 144 h (6 days). After 6 days of incubation, samples were ready to be subjected for yeast removing test [Figure 1].

**Yeast removing test**

Fungal cells adhering to acrylic resin surfaces of samples (Group D1) were fixed with formaldehyde. Samples along with yeast cells adhered to them were examined and the total number of colonies formed over sample was counted by microscopy [Figure 2]. Now the samples were immersed in freshly prepared denture cleanser solution, D1 for 8 h. After 8 h, again samples were examined and total number of colonies remaining on sample was counted. The same procedure was performed using denture cleansers D2 and D3. For the control group, samples were immersed in distilled water for 8 h.

**Yeast lytic test**

Denture cleaner solutions were prepared according to the manufacturer’s instructions.

### Preparation of Fungal Suspension

*Candida albicans* was precultured in sabouraud glucose broth at 37°C. The cultures were centrifuged and fungal cells were washed three times with 10 ml phosphate-buffered saline (PBS) and then resuspended in PBS. The cell suspension was diluted to $3 \times 10^7$ cells per ml.

| Table 1: Commercial names, mode of supply, chemical composition, and manufacturers of materials used in the study |
| --- |
| Commercial name | Mode of supply | Chemical composition (principal component) | Manufacturer |
| Viclean Powder | Sodium Hypochlorite | Vishal Dentocare Pvt Ltd, Ahmedabad, India |
| Clinsodent Powder | Sodium Laural Sulphate | I.C.P.A Health Products Ltd, Ankleshwar, India |
| Fittydent Tablets | Sodium Hypochlorite | Group Pharmaceuticals, Thane, India |

**Figure 1:** Inoculation of yeast suspension

**Figure 2:** Microscopic picture of CFU
Dhamande, et al.: Efficacy of commercial denture cleansing agents

**Assay**

A 5-ml suspension of this fungal suspension was added to a 5-ml denture cleanser solution in a test tube. Control was added to 5 ml of sterile distill water. The tube was incubated at 37°C for 5, 30, 60, 90, and 120 min. The optical density (OD) of the tubes was surveyed at 460 nm by a photo colorimeter. Percentage OD was expressed by following equation:

\[
\% = \frac{\text{O.D of sample} - \text{O.D of control}}{\text{Initial O.D of sample} - \text{Initial O.D. of control}} \times 100
\]

The results were expressed in terms of the fall in OD of the solution as surveyed by photo colorimeter at 460 nm.

**Results**

The readings for the colonization *Candida* grown on acrylic surface with and without the use of denture cleansers were tabulated, and the *Candida* lytic results were assembled in the table as given below:

Table 2 shows the comparative analysis of reduction of CFU from heat-cured acrylic resin samples after immersion in denture cleanser solutions D1, D2, D3, and control by applying paired t-test.

Figure 3 represents mean values of all three study groups and control before and after treatment with denture cleanser solution. Control solution showed almost no change in number of count pre- and post-treatment. Denture cleanser solution D2 on the other hand showed highest reduction in the number of count.

Table 3 shows the values of OD of *C. albicans* cell suspension at different intervals of time. Initial OD of all three denture cleansers D1, D2, D3, and control (distill water) was measured. After mixing all denture cleanser solutions separately with *Candida* cell suspension in equal amounts, OD of each solution was measured separately at 5, 30, 60, 90, and 120 min. Control sample (distilled water) was also mixed with equal quantity of *Candida* cell suspension and OD measured at same intervals.

Percentage OD of cell suspension at each interval was calculated using the following formula:

\[
\% = \frac{\text{O.D of sample} - \text{O.D of control}}{\text{Initial O.D of sample} - \text{Initial O.D. of control}} \times 100
\]

Table 4 depicts the percentage decrease in OD of *Candida* cell suspension over period of time which signifies the yeast lytic activity of denture cleansers.

Figure 4 represents the percentage decrease in OD of cell suspension in the form of bar diagram. Among three denture cleanser solution, D1 and D2 showed a greater decrease in percent OD when compared with D3.

---

### Table 2: Comparison of efficiency of sample solution before and after treatment: A: Descriptive Statistic

| Group | Treatment With denture cleanser | Mean* | n  | SD  | SEM |
|-------|---------------------------------|-------|----|-----|-----|
| Control | Before treatment | 18.80 | 30 | 6.45 | 1.17 |
|         | After treatment  | 18.70 | 30 | 6.50 | 1.18 |
| D1     | Before treatment | 25.40 | 30 | 8.41 | 1.53 |
|         | After treatment  | 29.13 | 30 | 8.17 | 1.49 |
| D2     | Before treatment | 27.50 | 30 | 8.47 | 1.54 |
|         | After treatment  | 22.40 | 30 | 8.76 | 1.60 |

*Values indicating colony-forming units

### Table 3: OD of sample solutions and control

| Time interval (min) | Control | D1 | Control | D2 | Control | D3 |
|---------------------|---------|----|---------|----|---------|----|
| Initial             | -0.08   | 0.26 | -0.08   | 0.24 | -0.08   | 0.11 |
| 5                   | 1.17    | 1.43 | 1.00    | 1.25 | 1.17    | 1.33 |
| 30                  | 1.09    | 1.33 | 0.70    | 0.90 | 1.13    | 1.28 |
| 60                  | 0.90    | 1.11 | 0.64    | 0.80 | 1.12    | 1.25 |
| 90                  | 0.72    | 0.90 | 0.60    | 0.75 | 1.11    | 1.25 |
| 120                 | 0.53    | 0.69 | 0.56    | 0.70 | 0.98    | 1.10 |

### Table 4: Percentage OD of cell suspension over a period of time

| Time interval (min) | D1 | D2 | D3 |
|---------------------|----|----|----|
| 5                   | 76.4 | 78.1 | 84.2 |
| 30                  | 70.5 | 62.5 | 78.9 |
| 60                  | 58.9 | 50  | 68.4 |
| 90                  | 52.9 | 46.8 | 73.6 |
| 120                 | 47  | 43.7 | 63.1 |
Prosthesis is provided to replace the lost part and also to restore the lost or impaired functions due to missing part or organ of the body. To make it more meaningful, the care and maintenance of the prosthesis is of paramount importance including maintaining hygienic condition. Inadequate home care can seriously compromise the clinical results of even the most meticulous denture prosthesis fabricated utilizing excellent material and techniques. When denture has been stained or having tartar deposits, that resort to plain chemical or physical methods of cleaning with proprietary cleaners. Denture cleansers are either chemical or abrasive in their mode of action. Chemical cleansers may be defined as alkaline hypochlorites, alkaline peroxydes, and dilute acids. Hypochlorite is used because of their ability to dissolve organic matrix upon which tartar forms. Alkaline peroxide group comprises powder and tablets which when dissolved in water become alkaline solution of hydrogen peroxide. These products usually include oxygen liberating agent such as sodium per borate or sodium per carbonate and an alkaline detergent such as trisodium phosphate; the liberation of bubbles of oxygen from this solution exerts a mechanical cleaning effect on lightly held contaminates.

Immersion cleaners are recommended to be used for minimum 20 min or even over night, when possible. Immersion was considered ideal method of cleaning for those patients who leave their denture out overnight.[4] It was therefore decided to use an 8-h period of immersion for testing these products. The adhesion of microorganism to a surface is prerequisite for the colonization of that surface. There have been many studies on adhesion of C. albicans to denture acrylic resin caused by association of commensal opportunistic pathogenic yeast with denture-induced stomatitis. The denture may function as a reservoir of infection and surface irregularities would increase the likelihood of microorganisms remaining on the surface after the prosthesis has been cleaned.[5] Although many studies have evaluated the effect of denture cleansers and disinfectant solutions on initial Candida adherence to denture base materials, little attention has been paid to the effect of these denture cleansing agents on Candida-associated mature biofilm, the cells of which are known to be more resistant to antimicrobial compounds and chemical cleansing.[6] The effect of antiseptic solutions and denture cleaners on the adhesion of yeast cells to acrylic surfaces has been investigated in previous studies.[7]

This study was carried out to evaluate and compare particularly the yeast removing ability and yeast lytic ability of commercial denture cleansers from heat-cured denture acrylic resin. The study was conducted in two parts.

First, to simulate the condition of fungal biofilm, C. albicans strain was grown on acrylic plates. These acrylic plates were immersed in denture cleanser solution for 8 h when compared with control group of acrylic plates which were immersed in distilled water for same period. The C. albicans removing test was done to determine the efficacy of denture cleansers in preventing adherence of C. albicans onto heat-cured denture base. The results indicated that C. albicans cells adhere to acrylic resin plates even after subjecting the acrylic plates to cleanser solution. Among three cleansers tested, D2 demonstrated noticeable reduction in number of colony-forming units when compared with D1, D3, and control group. This was due the presence of sodium laural sulfate in denture cleanser D2.

The second part of the study, the yeast lytic test or turbidimetric method of photo colorimeter, was done to evaluate efficacy of denture cleansers against C. albicans cell suspension. Turbidimetry is the measurement of reduction in light transmission caused by particle formation. Light transmitted in forward direction is detected. The amount of light absorbed by a suspension of particles depends on the specimen concentration and particle size. Many clinical applications exist for turbidimetry. Various microbiology analyzers measure turbidity of samples to detect bacterial growth in broth cultures.[8] In this study, each denture cleanser solution was mixed with equal quantity of C. albicans cell suspension.

The photo colorimeter was used in the study to survey the turbidity and interpret the effect of denture cleansers on C. albicans cell suspension. The results were expressed in terms of fall in OD of solution as surveyed by photo colorimeter at 460 nm. This fall in OD of solution was due to reduction in number of C. albicans cells in solution which showed the yeast lytic ability of denture cleansers.

Conclusion

This in vitro study was conducted to compare and evaluate the efficacy of three denture cleansers. Their activity against C. albicans, one of the most opportunistic and chief causative
organism of denture stomatitis, was studied. The evaluation was based on Candida removing activity as studied directly under microscope and results of turbidimetric monitoring by photo colorimeter. Within limitations of this study, following conclusions were drawn.

Denture cleanser with sodium laural sulfate showed the highest Candida removing activity when compared with cleansers with sodium hypochlorite and control solution.

Denture cleansers with sodium laural sulfate showed increased yeast lytic ability when compared with denture cleansers with sodium hypochlorite and control solution.

Time has a definite influence on yeast lytic ability of denture cleansers, i.e., lytic activity of denture cleansers increased over passage of time.

Acknowledgment

Based on dissertation submitted to Datta Meghe, Institute of Medical Sciences University, Nagpur, in partial fulfilment of the requirements for the degree of Master of Dental Surgery in Prosthetic Dentistry including Crown and Bridge and Implantology.

References

1. Jorgensen EB, Odont. Materials and methods for cleaning dentures. J Prosthet Dent 1979;42:619-23.
2. Tamamoto M, Hamada T, Miyake Y, Suginaka H. Ability of enzymes to remove Candida. J Prosthet Dent 1985;53:214-5.
3. Ramage G, Tomsett K, Wickes BL, Lopez-Ribot JL, Redding SW. Denture stomatitis: A role for Candida biofilm. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:53-9.
4. Neill DJ. A study of materials and methods employed in cleaning dentures. Br Dent J 1968;124:107-15.
5. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77:535-9.
6. Nikawa H, Hamada T, Yamashiro H, Kumagai H. A review of In Vitro and In Vivo methods to evaluate the efficacy of denture cleansers. Int J Prosthodont 1999;12:153-9.
7. Spiechowicz E, Santarpia RP 3rd, Pollock JJ, Renner RP. In vitro study on the inhibiting effect of different agents on the growth of Candida albicans on acrylic resin surfaces. Quintessence Int 1990;21:35-40.
8. Henry JB. Clinical diagnosis and management by laboratory methods. 19th ed. New York: W.B. Saunders Company; 1996.