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ORIGINAL ARTICLE

Effect of Alkaline Peroxide-type Denture Cleaners to the Microbial Profile in Maxillary Complete Denture

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ABSTRACT

Objective: To investigate the effect of alkaline peroxide-type denture cleanser agents on the microbial profile, namely *Streptococcus*, *Staphylococcus*, and *Candida* species, in maxillary complete dentures. **Methods:** A total of 20 patients with maxillary complete dentures were recruited. Three denture cleanser agents were selected ($n = 5$): Polident[®] (P); Steradent[®] (S); and Pearlie White[®] (PW), with distilled water (DW) as control. Dentures were brushed before immersion with denture cleansers. Denture biofilm was collected from the palatal impression surface of the denture before and after immersion. The collected microorganisms were cultured and colony-forming units (CFU) were counted. **Results:** Significant reduction in the numbers of *Streptococcus* and *Staphylococcus* was observed after immersion with all denture cleanser agents ($p < 0.05$). On the other hand, no denture cleanser was significantly effective against *Candida* species ($p > 0.05$). Furthermore, S was more effective among other cleansers compared to DW ($p < 0.05$). **Conclusion:** Immersion of dentures in denture cleansers can reduce the amount of microorganisms on denture surfaces; thus, leading to effective denture hygiene. Steradent[®] denture cleanser showed the highest percentage reduction of polymicrobial organisms.

Keywords: complete denture, denture cleanser, denture hygiene, microbial profile

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INTRODUCTION

According to the National Oral Health Survey of Adults 2010 (NOHSA, 2010) performed by the Oral Health Division in the Ministry of Health Malaysia, 7.3% of adults 15 years and older are affected with complete edentulism, defined as the loss of all permanent teeth.¹ The survey also reported that 6.0% of adults had maxillary and mandibular edentulism that led them to have complete dentures for functioning.¹ Maintenance of complete dentures is crucial to prevent further problems, such as denture stomatitis, which has been reported to be related to good cleaning habits.^{2,3} Failure to clean dentures properly could lead to growth of microbials on the denture surface, which could further harm the soft tissues due to biofilm developing on the surface of the dentures.³ Denture biofilm has been identified as a dense microbial layer comprising 10^{11} gm⁻¹ microorganisms in liquid measure that could lead to local and systemic infection.^{4,5} Previous study

has shown that denture biofilm can be present on fitting and polished surfaces of the denture, more so in undercut areas.^{2,4}

One common infection in complete denture wearers is denture stomatitis. Denture stomatitis is always present on the palatal mucosa beneath the fitting surface of the maxillary complete denture.⁶ In most cases, denture stomatitis usually is asymptomatic, but can be linked to a mucosal burning sensation, bleeding, an unpleasant taste, and halitosis. Few microorganisms have been identified to contribute to the infection, namely *Streptococcus*, *Staphylococcus*, *Candida*, *Neisseria*, *Lactobacillus*, and *Actinomyces*.^{3,5,7} Recent studies have reported coadhesion between *Candida* and *Streptococcus* species, which promotes oral colonization by yeast cells that leads to denture stomatitis.^{3,6}

Denture hygiene is important in reducing the amount of denture biofilm, as well as the incidence of

Table 1. Ingredients for all cleansing agents used

Cleansing Agents	Ingredients	Form	Immersion Time (mins)	Manufacturer
Polident® (P)	Sodium bicarbonate, citric acid, sodium carbonate, potassium monopersulfate, sodium perborate, sodium polyphosphate, sodium benzoate, PEG-8000, sodium lauryl sulfoacetate, PVP/VA S630, flavor, sodium stearate, CI 73015, CI42090, CI19140	Tablet	3	Stafford-Miller (Ireland), Ltd. Dungarvan, Co. Waterfrond, Ireland
Steradent® (S)	Sodium bicarbonate, sodium sulfate, citric acid, sodium carbonate peroxide, sodium carbonate, potassium caroate, malic acid, PEG-150, PEG-90, sodium cocoyl isethionate, aroma, glucose, sodium chloride, CI73015	Tablet	10	Reckitt Benckiser (Deutschland) GmbH, Theodor-Heuss Anlage 12, 68165 Mannheim, Germany
Pearlie White® (PW)	Sodium bicarbonate, citric acid, corn syrup solids, sodium carbonate, potassium persulfate compound, sodium perborate, sodium tripolyphosphate, sodium lauryl sulfoacetate, PEG 8000, mint flavor, FD&C Yellow #5 Dye, FD&C Blue #2 Dye, FD&C Blue #1 Lake, FD&C Yellow #5 Lake, magnesium stearate, tetrasodium EDTA dihydrate	Tablet	3	Corlison Pte Ltd, 7030 Ang Mo Kio Avenue 5, 569880 Singapore
Distilled water (DW)	-	-	10	-

denture stomatitis. Denture hygiene procedures can be performed either mechanically or chemically. A combination of both methods has shown effective results in reducing the amount of microorganisms present.^{2-4,7,8} Chemical methods may be classified into four groups, based on the mechanism of action or main ingredients of cleansers, namely sodium hypochlorites, alkaline peroxides, mouth rinses and enzymes.^{3,4} Alkaline peroxide solutions are widely indicated for controlling biofilm; however, the efficacy of such agents remains inconclusive. Some studies have reported their ineffectiveness on biofilm removal while others have demonstrated otherwise.⁹ Alkaline peroxides usually are the choice due to their good antimicrobial activity against denture biofilms in the absence of odor and aftertaste.¹⁰ However, they are quite costly compared with other agents.

Limited studies have evaluated the effectiveness of available denture cleanser agents. In the Malaysia market, few alkaline peroxides are available and their price varies. Therefore, we investigated the effect of these alkaline peroxide-type denture cleanser agents on the microbial profile in maxillary complete dentures.

METHODS

A total of 20 patients who complied with the inclusion criteria of wearing maxillary complete dentures and agreed to participate in the study were recruited.

Exclusion criteria included those who were taking any antifungal agents or medications known to predispose them to oral candidiasis, as well as patients who currently were using a denture cleanser. Ethical approval was obtained from the Research Ethics Committee, the Medical Research and Industry Secretariat, Universiti Kebangsaan Malaysia Medical Committee and informed consent was obtained from all patients.

All patients were divided into four groups ($n = 5$) according to the cleansing agents used in this study, which were: (1) Polident® (P; GlaxoSmithKline, Cork, Ireland); (2) Steradent® (S; Reckitt Benckiser GmbH, Heidelberg, Germany); (3) Pearlie White® (PW; Pearlie White, Singapore); and (4) distilled water (DW) as the negative control. The ingredients of all cleansing agents are shown in Table 1.

The methods in this study have been modified from those of previous studies by Nishi et al.,¹¹ Nishi et al.,¹² and Uludamar et al.¹³ All maxillary denture-bearing areas in each patient were examined at chairside and the maxillary denture was rinsed with one cup of distilled water (DW). Denture biofilm of the left lateral half of the denture fitting surface was collected using a sterile swab (Figure. 1a). The cotton swab then was placed into a universal bottle that contained phosphate buffered saline (PBS; Oxoid, Altringham, England) solution and was sealed with a parafilm strip. The denture then was brushed with a soft bristle toothbrush and one cup of DW for 10 s.

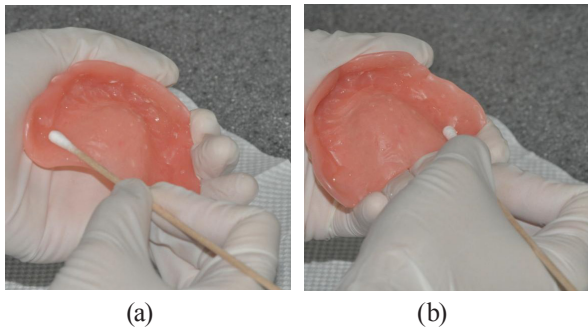


Figure 1. (a) Left lateral half of maxillary complete denture fitting surface sample was collected. (b) Right lateral half of maxillary complete denture fitting surface sample was collected after immersion in denture cleanser.



Figure 2. Specimens transferred to the agar plates using a micropipette.



Figure 3. Agar plate was tilted to allow specimens to flow vertically.

Next, a denture cleanser tablet was placed in a kidney dish filled with warm water at 55 °C and the maxillary denture was immersed according to the manufacturer's instructions. For the DW group, dentures were immersed for 10 min. After immersion, the denture was brushed again with a soft bristle toothbrush with one cup of DW for 10 s. Denture biofilm on the right lateral half of the denture fitting surface was collected using a new sterile swab (Figure. 1b). The cotton swab then was placed in a universal bottle that contained PBS solution and sealed with a parafilm strip. The denture was rinsed under running tap water and returned to the patient.

A 10-fold serial dilution was performed for all specimens (before and after immersion). All universal bottles were well mixed for 10 s using a vibrating

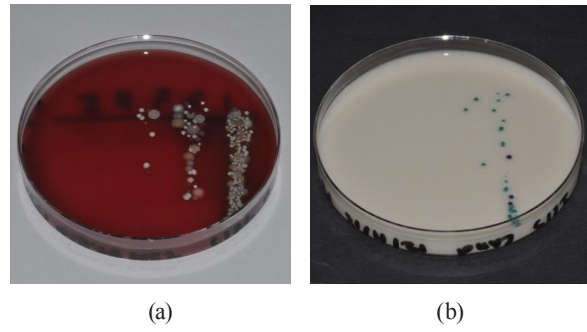


Figure 4. (a) Colonies formed on TSA-SBA. (b) Colonies formed on BCA

shaker before transferring. A different micropipette tip was used for each transfer. The specimens were transferred to trypticase soy agar with 5% sheep blood agar (TSA-SBA; Fisher Scientific, Selangor Malaysia) and Brilliance candida agar (BCA; Fisher Scientific) plates from lowest to highest concentration. Each plate was labeled with numbers at the base of the agar plate, which indicated the bottles of diluted specimens.

Then, 10µL of specimens from each bottle was transferred to the agar in the form of droplets using a micropipette (Figure. 2). The agar plates then were tilted to let the droplets flow vertically (Figure. 3). Once all moisture of the droplets was gone, the plates were covered and inverted.

TSA-SBA and BCA were incubated aerobically at 37 °C for 48h. However, only TSA-SBA was enriched with 5% carbon dioxide. After 48 h, all agar plates were taken out and colony-forming units (CFU) were counted (Figure. 4a, 4b). The lane with the number of colonies <100 but nearest to 100 was chosen. CFUs for the lane number chosen were calculated using the formula: $\text{Flask's CFU/mL} = (\text{colonies on the plate}) \times 10^{2+\text{number of lane}}$.

Calculation was repeated twice for every specimen and the average value was taken. Data were analyzed using SPSS Version 21. One-way analysis of variance was used to determine any statistically significant difference among the four groups of cleansing methods. A paired sample *t*-test also was used in this study to determine any statistically significant differences before and after the use of each cleansing method. Tukey's test was used to determine which specific group of cleansing method was statistically significant from others.

RESULTS

Demographically, the majority of the subjects involved in this study were male (mean age, 68.2 ± 7.6 years).

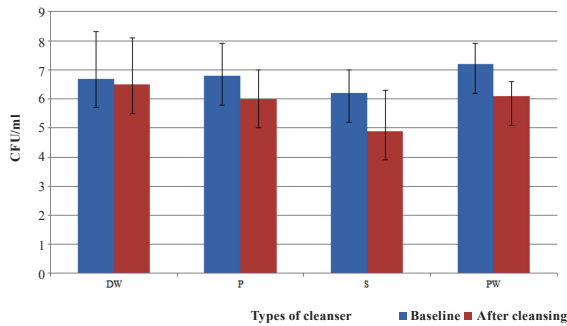


Figure 5. CFU of polymicrobial on TSA-SBA at baseline and after cleansing

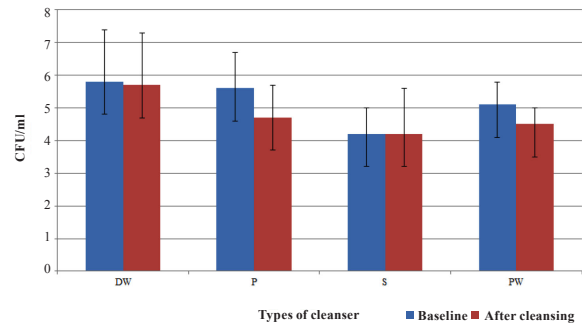


Figure 6. CFU of *Candida* species on BCA at baseline and after cleansing

The ethnicity of the subjects was mainly Chinese (60%) with an equal number of Malay and Indian (20%) subjects.

The quantities of polymicrobials on TSA-SBA and *Candida* species on BCA on the dentures before and after each cleansing method are shown in Figs. 5 and 6. There were no significant differences in the amount of polymicrobials and *Candida* species ($p > 0.05$) on the dentures at baseline for each cleansing method. All cleansers were significantly effective against polymicrobials on TSA-SBA ($p < 0.05$). However, they were not effective against *Candida* species on BCA ($p > 0.05$).

When compared with Group DW, Group S shows the highest reduction of polymicrobial by comparison with Groups P and PW. Since all cleansing methods were significantly effective only against polymicrobials on dentures, Tukey's test was performed further to compare the effectiveness of reducing polymicrobials using various cleansing methods. This test demonstrated that only Group S showed significant value ($P < 0.05$) in reduction of polymicrobials compared with Group DW.

DISCUSSION

Polident[®], Steradent[®] and Pearlie White[®] were chosen to be tested, as these three brands of alkaline peroxide-type denture cleansers are widely available in Malaysia. Alkaline peroxides were shown to be effective on newly-formed plaque and stains.⁹ With the current rising number of the elderly, the demand for dentures has been increasing dramatically over the years. Thus, a denture hygiene procedure is of paramount importance to denture wearers.

Several articles have proven that a combination of mechanical and chemical method is more efficient in removing denture plaque than a mechanical or chemical method alone.^{2-4,9} Therefore, a soft bristle toothbrush was used to remove the debris and deposit it

mechanically from the denture, followed by immersion of the denture in alkaline peroxide-typed denture cleansers. However, the intention of this study was to test the efficacy of various brands of peroxide-type denture cleansers on microbials on dentures.

At baseline, the insignificant CFUs of polymicrobials and *Candida* species demonstrated that the similar initial plaque that formed would not affect the apparent efficacy of various cleansing methods. Previous studies have shown that denture cleansers are effective only against polymicrobials, but are not effective against *Candida* species, which coincides with the result of this study.^{3,14,15} The different response achieved for different microorganisms may be due to the location of the microorganism itself. Polymicrobials may be located in more superficial layers of the biofilm and consequently are more exposed to denture cleanser agents compared with *Candida*. Thus, in a multispecies biofilm, *Candida* would be protected from denture cleanser action by layers of extracellular matrix and bacterial cells.⁶ However, chlorhexidine has been shown to remove *Candida* biofilm effectively when used together with denture cleanser agents.¹⁰

When compared with distilled water, Steradent[®] showed a higher percentage reduction of polymicrobials than Polident[®] and Pearlie White[®]. It is postulated that this might be due to the longer immersion time of Steradent[®], which is 10 min, compared to that of Polident[®] and Pearlie White[®], which both have 3-min immersion times. Longer immersion time may have an effect on reduction of the microbes as suggested in a previous study.¹⁶ Denture cleansers should be introduced to all denture wearers, together with brushing techniques. These acts would improve denture hygiene by minimizing the presence of microbes on the denture, and thus would prevent further problems, such as infection of the soft tissues.

There are a few limitations of this study, which include the facts that the presence of microbes was evaluated from only one lateral side of the denture and the specific

types of polymicrobial and *Candida* species were not evaluated. Furthermore, due to time constraints, this study only evaluated alkaline peroxide-type cleansers, and the manufacturer's instruction was followed throughout the research.

Future studies can be suggested to overcome these limitations. Denture biofilm can be collected from the whole fitting surface of the denture. The types of polymicrobial and *Candida* species can be identified using special techniques, such as Gram staining. Different types of denture cleansers can be compared to evaluate their effectiveness in removing denture biofilm. Different immersion times of denture cleansers also could be evaluated for the effectiveness of removing microbes on the denture surface.

CONCLUSION

All denture cleansers can remove polymicrobials effectively, but not *Candida* species. Steradent® denture cleanser shows the highest percentage reduction of polymicrobials. Immersion of denture in denture cleansers can reduce the microorganisms on denture surfaces, thus leading to effective denture hygiene, and should be recommended to all denture patients.

CONFLICT OF INTEREST

All authors declare no conflict of interest throughout the research.

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