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Full Length Research Paper

Effect of chemical denture cleansers on microorganisms over heat-polymerized acrylic resin

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The aim of this study was to measure the anti-microbiological effect of the denture cleansers both on polished and non-polished surface six hundred disc shaped polished and non-polished resin specimens, were used. Six different oral microorganisms were selected. Each specimen was then inoculated into the specific medium for cultivation and than removed. Subsequently the media were incubated 24 h at 37°C. After colony counting, contaminated specimens were subgrouped and cleaned with Correga, Protefix, 2% sodium hypochlorite, 2% glutaraldehyde and distilled water. Each specimen was then inoculated into new cultivation medium, after incubation, colonies were recounted in each group. Data were analyzed with Wilcoxon and Mann Whitney U tests. Cleaning efficiency of sodium hypochlorite and glutaraldehyde were better than Correga and Protefix. The latter two agents cleaned polished surfaces better than non-polished. *S. aureus* was the most adherent microorganism.All the agents except distilled water cleaned polished surfaces effectively but 2% hypochlorite and 2% glutaraldehyde cleaned non-polished surfaces also. Despite of the corrosion and bleaching effects, in need of intense cleaning of resin materials, hypochlorite or glutaraldehyde may be the first choice.

Key words: Denture cleaners, sodium hypochloride, dentures, denture polish, oral microorganisms.

INTRODUCTİON

Oral care is important for the prevention of caries, periodontal disease and many systemic diseases. Also denture care is indispensable for general health of not only elderly, fragile and immunocompromised patients but also for healthy patients (Orsi et al., 2010). Surface roughness of dentures influence the adherence of microorganisms (Radford et al., 1998; Taylor et al., 1998). Willams and Lewis have also reported that surface roughness favors the microbial colonization (Williams et al. 2000). Bacteria on the surface of the denture can cause fatal infections such as pneumonia and endocarditis due to poor hygiene. Yoneyama et al. reported that oral care can decrease the risk of pneumonia(Yoneyama et al., 1999). Hence, denture care especially in the elderly becomes vital.

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Dentures can be cleaned mechanically, chemically or by their combination. Denture cleaning pastes with their active ingredients and/or tooth pastes are commonly used in the mechanical method. The abrasive, detergent, humectant and flavoring properties of the pastes provide potential effects for removing the debris from the denture surface. Chemical cleansers contain a variety of active agents. Effective disinfection can be attained by enzymes, hypochlorite solutions, acids, mouth washes and peroxide solutions (Nikawa et al., 1999). The sodium hypochlorite-based denture cleansers are fungicidal and are known to be effective by dissolving mucin and other organic substances (Harrison et al., 2004). Sodium hypochlorite does not change the roughness, but can deteriorate the base material by bleaching and corrosion (Harrison et al., 1997).

Alkaline peroxides are the most commonly used denture cleaners (McCabe et al., 1995; Kulak et al., 1997). Besides their chemical effects, they can remove stains mechanically by releasing oxygen. Alkaline peroxides

present good antimicrobial activity against denture biofilms in the absense of odor and after taste (Paranhos et al., 2009).

Glutaraldehyde based solutions have also proved to be potent antimicrobial agents, and are often used in dentistry. They are not inactivated when in contact with organic materials, are not corrosive, and do not degrade rubber or plastic materials(da Silva et al., 2008). However the researchers are still ambigious about the toxicity of glutaraldehyde thus, its usage is considered to be limited.

Several disinfectants have been suggested for denture cleaning. The current expectation for cleaning agents is to clean simply and effectively, with no risk to human health, and with no adverse effects on the properties of the denture material.

In light of these observations, this study investigated the efficiency of different denture cleaning agents against microorganisms formed on polished and non-polished surfaces of denture base materials.

MATERİALS AND METHODS

Specimen fabrication

Six hundred acrylic denture base resin specimens were obtained from wax patterns with the same dimensions (diameter 15 mm, width 4 mm). Patterns were invested in metallic flask and type III dental stone (Herodont Soli Rock, Rio de Janeiro, Brasil). One flask contained 10 disk-shaped wax patterns. After the setting of dental stone, the flasks were opened, the wax was removed. Heat-polymerized acrylic resin (QC 20, Densply Ind. Com. Ltd, Petropolis, RJ, Brasil) was mixed for 60s with a ratio of 23g/10ml powder/liquid according to manufacturer's recommendations, and was packed into the mold at the dough stage 12-15 minutes after mixing using conventional technique. The metal flasks were then closed. Polymerization process was accomplished according to manufacturer's recommendations.

All flasks were allowed to cool for 3 hours at room temperature and then opened. The excess resin was smooted with a hard bur (KG Sorensen, Barueri, SP, Brasil). 300 specimens were progressively smoothed with aluminum oxide papers of 320, 400 and 600 grid and polished with a paste (Composite Polish; Ultradent Products Inc, South Jordan, Utah). The remaining specimens were left non-polished. All specimens were ultrasonically cleaned and immersed in distilled water at 37ºC for 48±2 hours for elimination of the residual monomer.

Contamination of the Specimens

After sterilization with ethylene oxide, each polished [P(+)] and non-polished [P(-)] specimens were divided into 6 groups (n=50). In each group, the specimens were inoculated with one of the following microorganisms: *Staphylococcus aureus* (ATCC 6538), *Streptococcus mutans* (ATCC 35688), *Enterococcus faecalis* (ATCC 10541), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 2327) or *Candida albicans* (ATCC 18804) which were adjusted to 0.5 McFarland standard (10^8 cfu/ml bacteria) and were incubated for 1 hour at 37°C. Specimens were rinsed for 15s with distilled water and inoculated face down into the specific cultivation media (sheep blood agar, Eosin-Methylene-Blue (EMB) agar, Sabouraud dextrose agar) for each microorganism for 30 minutes. After removing the contamined specimen, the media were incubated for 24 h at 37°C.

The number of colonies were counted with BioImaging Systems (UVP, Upland, CA) in each group and expressed as groups of "1-9" and "≥10" colonies (Table 1).

Experimental and Control Groups

Five groups, each containing contamined specimens of 5 P(+) and 5 P(-) (n=10) were randomly assigned to one of cleaning method as follows, to assay one microorganism with one disinfectant (table 2):

1) Correga (Dungarvan, Co. Waterford, Ireland): specimens were immersed in a cap containing Correga alkaline peroxide efervescent tablet for 5 minutes and then cleaned with tap water.

2) Protefix (Queisser, Flensburg, Germany): specimens were immersed in a cap containing this alkaline peroxide efervescent tablet for 5 minutes and then cleaned with tap water.

3) Sodium hypochlorite: specimens were immersed in a cap containing 2% hypochlorite for 5 minutes and then cleaned with tap water.

4) Glutaraldehyde: specimens were immersed in a cap containing 2% glutaraldehyde for 5 minutes and then cleaned with tap water.

5) Distilled water (control group): specimens were immersed in distilled water for 5 minutes.

Each specimen was then dried with filter papers and inoculated into a new medium (sheep blood agar, EMB, Saboraud dextrose agar). After 30 minutes the specimens were removed, and the media were incubated for 24 h at 37°C.

The number of colonies were re-counted in each group after the procedure and expressed as groups of "1-9" and "≥10" colonies.

Statistical Methods

Statistical analysis was performed with "PASW 18.0 Statistics" (IBM Company, New York, USA) statistical software. The data were analyzed with Wilcoxon

Table 1. Number of specimens of each group and effect of cleaners over microorganisms.

Microorganims	No. of colonies	Specimen(n)	Before Correga		After Correga		Before Protefix		After Protefix		Before Distiled Water		After Distiled Water		Before 2% Glutarald.		After 2% Glutarald.		Before 2% Hypochlorite		After 2% Hypochlorite	
			P(+)	P(-))	P(+)	P(-))	P(+)	P(-))	P(+)	P(-)	P(+)	P(-)	P(+)	P(-)	P(+)	P(-)	P(+)	P(-)	P(+)	P(-)	P(+)	P(-)
P. aeruginosa	<10	50	5	5	0	1	5	5	0	1	5	5	1	3	5	5	0	1	5	5	0	0
	≥10	50	5	5	0	0	5	5	0	0	5	5	1	2	5	5	0	0	5	5	0	0
E. coli	<10	50	5	5	0	1	5	5	0	1	5	5	2	2	5	5	0	0	5	5	0	0
	≥10	50	5	5	0	0	5	5	0	1	5	5	2	2	5	5	0	0	5	5	0	0
S. mutans	<10	50	5	5	0	1	5	5	0	2	5	5	1	2	5	5	0	0	5	5	0	0
	≥10	50	5	5	0	1	5	5	0	1	5	5	1	2	5	5	0	0	5	5	0	0
E. faecalis	<10	50	5	5	1	2	5	5	1	2	5	5	2	3	5	5	1	1	5	5	0	0
	≥10	50	5	5	1	2	5	5	1	1	5	5	2	2	5	5	0	1	5	5	0	0
S. aureus	<10	50	5	5	1	2	5	5	1	2	5	5	2	3	5	5	1	2	5	5	0	0
	≥10	50	5	5	1	1	5	5	1	2	5	5	3	3	5	5	1	1	5	5	0	0
C. albicans	<10	50	5	5	0	2	5	5	0	2	5	5	1	2	5	5	0	1	5	5	0	0
	≥10	50	5	5	0	0	5	5	1	1	5	5	1	2	5	5	0	0	5	5	0	0

(Glutarald: glutaraldehyde).

test to determine the difference between cleaning methods before and after contamination. Differences between polished and non-polished surfaces were compared statistically with Mann Whitney U test. Results were considered statistically significant at $p<0.05$.

RESULTS

The results of the effects of denture cleaning methods are presented in Table 1. Significant differences were observed between "before" and "after" groups ($p<0.05$). Among the disinfectants, 2% sodium hypochlorite solution showed the highest effect of cleaning followed by 2% glutaraldehyde solution, Correga tabs and Protefix tabs. As expected, the least effective cleaner against all microorganisms was distilled water ($p<0.05$).

Efficiency of cleaners on polished and non polished surfaces were significantly different for correga tabs, protefix tabs and distilled water ($p<0.05$). The cleaning effects of Correga tabs, Protefix tabs and distilled water on polished surfaces were better than their effects on nonpolished surfaces ($p<0.05$). Sodium hypochlorite and glutaraldehyde solutions were able to clean both polished and non-polished surfaces effectively.

Mean cleaning efficiencies of distilled water, Correga, Protefix and 2% glutaraldehyde were 68%, 92%, 90% and 95% respectively at polished surfaces. These rates decreases at nonpolished surfaces to 52%, 78%, 72% and 88% respectively.

Correga was more effective against *P. aeruginosa, E. coli, S. mutans* and *C. albicans* than *E. faecalis* and *S. aureus* at both polished and nonpolished surfaces. Protefix disinfected specimens less effectively than correga when mean efficiencies were evaluated, but cleaned *E. faecalis* significantly better than Correga. There was no statistical differences among the efficiencies of cleaners when the number of colonies are "1-9" or "≥10".

S. aureus was the most adherent microorganism on the acrylic resin surface followed by *E. faecalis, C. albicans, S. mutans, P. aeruginosa* and *E. coli* but this data was statistically insignificant.

DİSCUSSİON

With the limitations, this study compared the efficacy of denture cleaners on contaminated specimens. Among all the agents evaluated against selected microorganisms, 2% sodium hypochlorite solution demonstrated the best cleaning effect on denture base material. Owing to the antimicrobial strength of hypochlorite, no colonization was found in any of the specimens.

Poor oral hygiene is not only associated with periodontitis, but also with systemic diseases such as aspiration pneumonia, cardiovascular diseases and diabetes (Orsi, Junior et al., 2010). Especially in the elderly, existence of oral microorganism may become a potential indicator for high risks of certain diseases. Mechanical cleaning, like brushing, is inexpensive and effective. However, some patients have restricted hand con-

Table 2. Denture cleansers used in the study. *:Experimental group, **:Control group.

Groups	n P(+)	n P(-)	n(total)	Immersion
Corega Tabs (GlaxoSmithKline, Brentford, UK)*	10	10	20	5 min
Protefix (Queisser Pharma GmbH&Co., Flensburg, Germany)*	10	10	20	5 min
Sodium hypochlorite (%2)*	10	10	20	5 min
Glutaraldehyde (%2)*	10	10	20	5 min
Distilled water **	10	10	20	5 min
Total	50	50	100	

trol, especially because of elderness. Chemical method merely requires soaking the denture into the solution only. Some researchers have found that mechanical cleaning is better than chemical cleaning(Nikawa, Hamada et al. 1999; Marchini et al. 2004; Hashiguchi et al. 2009). However, chemical disinfectants have some advantages over mechanical cleaning such as effectivity and ease of use especially for elderly(Paranhos Hde et al., 2000). This was the primary reason of choosing commonly used chemical cleaners.

In this study heat polymerized acrylic resin was chosen. Besides its low porosity incidence and ease of manipulation, acrylic resin is an appropriate material for evaluating the effect of disinfectants on the surface. Microbial assessment was based on both polished and nonpolished surfaces of heat polymerized denture base resin. Polished specimens simulated the outer surface, while non-polished specimens simulated the inner surface of the acrylic base resin. This study evaluated the association between microorganism adhesion and surface polishing. The adherence and colonization of microorganism may change by ongoing usage and cleaning cycle. Since continous application of disinfectants may alter the surface characteristics(Kulak, Arikan et al., 1997), only one cycle of usage and cleaning was assessed in this study.

Six different microorganisms were tested both on polished and non-polished surfaces so as to compare the efficiency of chemical cleaners in this study. Many studies have investigated the effect of chemical cleaners on microorganisms, but most of them have only focused on the adhesion and cleaning methods of *C. albicans*(Radford, Sweet et al., 1998; Harrison, Johnson et al., 2004; Moura et al., 2006; Paranhos, Silva-Lovato et al., 2009; Redding et al., 2009; Uludamar et al., 2010; Salerno et al., 2011). In our study we have found that *S. aureus* was the most adherent microorganism followed by *E. faecalis, C. albicans, S, mutans, P. aeruginosa* and *E. coli* to the acrylic resin, but this data was statistically insignificant. As these microorganisms have high adhesion potential to the acrylic resin surfaces and have resistance to disinfectants the activity of cleaners may have been decreased.

Microbial growth patterns of this study were classified in two colony groups. Some specimens resulted in slight growing in colonization and were classified as “1-9”, while the rest presented high growth, and were classified as “≥10 “. Some of the specimens showed intense growth and resulted in agregation of colonies. These uncountable specimens were discarded from the study. According to the present results, the disinfectant solutions reduced microbial contamination independently from colony numbers (1-9 or ≥10) on both polished and non-polished heat polymerized acrylic resin, but none of them were able to destroy all the microorganisms except 2% sodium hypochlorite solution. This result was similar to Chau et al. (1995), although they performed cleaning procedure with 0.5% sodium hypochlorite for 10 minutes. The contamination incidence may vary according to materials and methods used. In the present study the data shows that 2% sodium hypochlorite solution is very active at disinfecting microorganisms. Although glutaraldehyde is not a commonly used disinfectant because of its toxicity, we found that it was the second active cleaner. It is known that 2% sodium hypochlorite and 2% glutaraldehyde penetrate into the resin surfaces, dissolute the surface materials and inhibit the enzymatic activities of microorganisms (Maillard, 2002). This may explain the success over the other cleaners.

Correga and Protefix are mostly used denture cleaners. In our study they were found to be significantly insufficient at cleaning non-polished surfaces ($p<0.05$).

This feature may be due to their insufficiant surface active characteristics compared with chemical agents such as enzymatic or acidic reactions. Correga seem to be more effective than Protefix at cleaning the microorganisms except *E. faecalis* on both polished and non-polished surfaces of the acrylic resin. On polished surfaces they were both statistically effective against *C. albicans, S. mutans and E. coli* ($p<0.05$) but were less effective at cleaning *S. aureus, P. aeruginosa and E. faecalis*.

CONCLUSİON

Within the limitations of this study;

1) 2% sodium hypochlorite showed the best cleaning effect on all microorganisms, followed by 2% glutaraldehyde solution, Correga tabs, Protefix tabs. and distilled water.

2) Cleaning effects of Correga and Protefix tabs on polished surfaces were better than on non-polished surfaces. Sodium hypochlorite and glutaraldehyde solutions were able to clean both polished and non-polished surfaces effectively.

3) *S. aureus* was the most adherent microorganism to the acrylic resin surface followed by *E. faecalis, C. albicans, S, mutans, P. aeruginosa* and *E. coli*.

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