Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.05.297 2019;26(8):1556-64

Original Article

Evaluation of the time-dependent effect of an enzymatic denture cleanser tablet against six microbial species

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Abstract

Aim: In elderly individuals, infection control is, essential, especially in the presence of severe bacteria such as oral environment. Prevention of biofilm formation in removable dental prosthesis, which is often used in elderly patients might help infection control. This study aimed to evaluate the time-dependent effect of an enzymatic tablet on six microorganisms cultured in two different denture base resins.

Material and Methods: Polymethylmethacrylate (PMMA) and polyamide-resin were used in this study. 480 samples were prepared for each resin, and denture cleanser tablet was tested against C.albicans, S.mutans, S.gordonii, A.actinomycetemcomitans, S.aureus, and E.coli for 3rd, 5th, 10th, 20th, 40th, 80th, 160th, and 200th minutes. Cell viability (CV) was evaluated by MTT. ANOVA was used for statistics.

Results: C.albicans exhibited higher CV in polyamide-resin compared to PMMA-resin in all tested durations. S.gordonii and A.actinomycetemcomitans exhibited higher CV in PMMA-resin except for 80th minute-S.gordonii, which had similar values. S.mutans had a higher CV in PMMA-resin than polyamide-resin at 3rd, 5th, 10th, 160th, and 200th minutes, and other values were similar to polyamide-resin. The tested concentration killed more than 75% of microorganisms except for 3rd and 5th minute-C.albicans on resins. S.aureus and E.coli had similar. CV of microorganisms on PMMA-resin was higher than on polyamide-resin. All microorganisms exhibited different adherence on the resins.

Conclusions: Tablet cleanser was effective in all microorganisms and durations and over 10-minute durations provided over 76% cell death. Patients with a compromised immune system can safely use restorations composed of polyamide-resin or PMMA-resin with 40 min-treatment of tablet cleanser.

Keywords: Bacteria; candida albicans; cell survival; denture bases; denture cleansers.

INTRODUCTION

The removable dental prosthesis is prosthetic restorations, which compensate missing teeth and soft tissues, especially in elderly patients who often have a lack of muscle tone and lip and cheek support (1). Elderly patients might have compromised immune defense and be prone to infection or superinfection, which might jeopardize the patient's overall health (2,3). Bacteremia result from oral infections or oral bacterial/fungal infection itself result from microorganisms such as *Candida albicans (C.albicans), Streptococci* or *Aggregatibacter actinomycetemcomitans (A.actinomycetemcomitans)* have a particular importance since the diseases caused by these microorganisms lead severe even lethal conditions such as infective endocarditis, nephritis, arthritis, cardiac problems or pneumonia (3-8). Apart from oral pathogens, non-oral pathogens such as *Staphylococcus aureus (S.aureus)* and *Escherichia coli* (*E.coli*) might also contribute oral biofilms and cause further infections with contaminations of a dental prosthesis in elderly patients who cannot achieve adequate oral hygiene, and the treatment would be challenging (9). Therefore strict precautions against infection control should be considered especially in the environments with a severe bacterial load such as oral cavity (3).

Infection control requires prevention of biofilm or disruption of formed biofilm on prone surfaces such as denture materials like polymethylmethacrylate (PMMA) or polyamide which are the most common denture base resins for removable prostheses (10-13). Denture base resins provide a suitable area for the growth of biofilm (14-16). Furthermore, the biofilm formed on the surface

Received: 28.05.2019 **Accepted:** 25.06.2019 **Available online:** 31.07.2019
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of the prosthesis is a structure formed by the interaction of many microorganisms, rather than a single bacterial biofilm (17-19). Such a biofilm structure in which many microorganisms are involved is quite complex, difficult to eliminate, and require potent disinfectants (20-25).

The most common inhabitants of oral biofilms are *C.albicans*, the primary cause of stomatitis (11,12,14, 16,22,23), *A.actinomycetemcomitans*, the cause of periodontal infections, nephritis, arthritis, etc. (5,7,8,26), *Streptococci* cause of caries, pneumonia, periodontitis, etc. (4,6,9,11,12, 20), *S.aureus*, cause of hospital infections (9,11), and *E.coli* (27,28). To eliminate biofilm formed by these microorganisms, various methods of denture cleaning methods, including mechanical brushing, disinfectant solutions, and denture cleansing tablets have been introduced (15, 21-23, 29-32). The significant advantage of the denture cleansers is the chemical cleaning of the denture in elderly patients who lack motor functions (22,24,33). However, studies are lacking in terms of the optimal duration to provide optimal efficacy against biofilm and selection of resin type, which might further aid antimicrobial effect (25,34). Also, most of the previous studies were performed on *C.albicans*, and other common oral and non-oral pathogens were either not covered or not studied as much as *C.albicans* (15,22,23). Also, despite reported efficacy against tested microorganisms, the course of antimicrobial effect with time was not evaluated either. Therefore, the present study aimed to evaluate the time-dependent effect of an enzymatic tablet on six microorganisms cultured in two different prosthetic base resins.

MATERIAL and METHODS

Specimen preparation

The denture base resins used in the present study were a heat-polymerized PMMA (QC-20; Dentsply) resin and polyamide thermoplastic resin (Deflex; Nuxen SRL). 480 samples from each resin, totally 960 equally sized round samples (8 durations x 2 resins x 6 microorganisms=96 test, ten samples for each test=960 samples in 10mm diameter and 2mm thickness) were prepared. The polymerization of the resins was carried out considering the manufacturers' recommendations, and all samples were abraded and polished to have a surface roughness (Ra) of 0.32±0.02 µm measured using a profilometer (Surtronic 25; Taylor Hobson). Three readings were performed for each sample, and a mean value was calculated as Ra. All resin samples were immersed in distilled water for 24 h for residual monomer release (35) and cleaned in sterilized distilled water with using an ultrasonic device (Pro-Sonic 600; Sultan Healthcare).

Culture and growth conditions of microorganisms

Three Gram-positive bacteria *S.aureus* (ATCC 25923), *S.mutans* (ATCC 35668), *S.gordonii* (NCTC 7870), two Gram-negative bacteria *E.coli* (ATCC 35218), *A.actinomycetemcomitans* (ATCC 33384) and one yeast *C.albicans* (ATCC 10231) were used in the present study. The *C.albicans* strain was maintained on solid Sabouraud dextrose agar in an incubator at 35°C. Twenty-four hours later, fungal inocula were prepared using RPMI-2% glucose broth and were counted using a Neubauer chamber and trypan blue stain to obtain a final concentration of $1–5x10^6$ cells/mL. To produce fungal biofilm on the sample resins, the cells were transferred to the polystyrene microtiter plates with 24 wells containing RPMI-2% glucose liquid medium and allowed to grow at 35°C for 48 h in a shaker incubator at 150 rpm. As for bacterial cultures, a total of 5 bacterial cultures were grown in solid Mueller Hinton agar supplemented with 5% defibrinated sheep blood strains. Bacterial inocula were prepared using Luria-Bertani broth (for *E.coli*), trypticase soy broth (for *S.aureus*, *S.gordonii*, and *A.actinomycetemcomitans*), and brain heart infusion broth (for *S.mutans*) cultures and suspensions were adjusted to 0.5 McFarland standard turbidity ($1.5 x 10^8$ CFU/mL). To produce bacterial biofilm on the sample resins, all bacteria were cultured at the polystyrene microtiter plates with 24 wells for 24 h at 35°C. After cultivation, the sample disks with biofilm were rinsed twice with 1 ml Dulbecco's phosphate buffered saline to remove the loosely attached bacteria and bacterial debris.

Test tablets

As the denture cleanser tablet, Polident 3 min™ enzymatic denture cleanser tablet (Polident 3 min; GlaxoSmithKline) was prepared according to the manufacturer's instructions and used. For working solutions, each tablet was dissolved in 150 mL of warm distilled water, and the solution was handled immediately.

Cell viability (CV) assay

An MTT assay was performed according to AFST-EUCAST guidelines. An MTT stock solution (5 mg of MTT/mL of distilled water) was filter sterilized and kept for at -20°C until use. First, the biofilm was grown as described previously. After a 48 h incubation, the old medium in the wells was removed, and the cells were treated with 200 µL of the cleanser working solutions of Polident 3 min™ for 3rd, 5th, 10th, 20th, 40th, 80th, 160th, and 200th minutes. Then, the cleanser solutions were replaced with fresh RPMI-2% glucose liquid medium containing MTT (final concentration, 0.5 mg/mL). The mixture was incubated for four h on a shaker incubator (150 rpm at 35°C). After the incubation period, 180 µL of the medium was removed, 30 µL of Sorenson's buffer and 150 µL of DMSO were added to the well, and the plate was vortexed for 5 minutes. The optical density of the sample and blanks (DMSO with Sorenson's buffer) was measured with a spectrophotometer at 560 nm, with 690 nm as a reference interval. The percent of CV was calculated using Excel software.

Scanning electron microscopy (SEM) imaging

SEM imaging was performed to evaluate surfaces of the samples treated with denture cleanser with different durations. Further sample treatment was required for SEM imaging. Firstly, the samples were washed twice with DPBS and then fixed in 2.5% glutaraldehyde in a phosphate buffer for a 16 hour. And shortly after samples were re-

fixed in 2% osmium tetroxide for two h then dehydrated through ethanol rinses (30, 50, 90, 95 and 100%). Lastly, samples were mounted and sputter-coated with gold. The surface topography with adhered microorganisms was examined using SEM (LEO 440; Zeiss).

Statistical analysis

Statistical analysis was performed with software (SPSS Statistics 19; IBM). All data were tested for normality with One Sample KS test, and after three-way, ANOVA was used to compare the continuous data between/among groups. For post hoc, the Tukey test was used. All data were presented as mean and standard deviation and percentage, either of which is appropriate.

RESULTS

The present study evaluated the antimicrobial efficacy of a denture cleanser tablet against *C.albicans*, *S.mutans, S.gordonii, A.actinomycetemcomitans*, *S.aureus*, and *E.coli* and determined the CV is corresponding the tablet treatment durations ranging from 3 to 200 minutes. All tested durations were effective against all tested microorganisms with at least 65% cell death even at the 3rd minute in both denture base resins. Detailed comparisons were presented in Table 1 and Table 2.

CV values of the microorganisms cultured on polyamide resin (Figure 1).

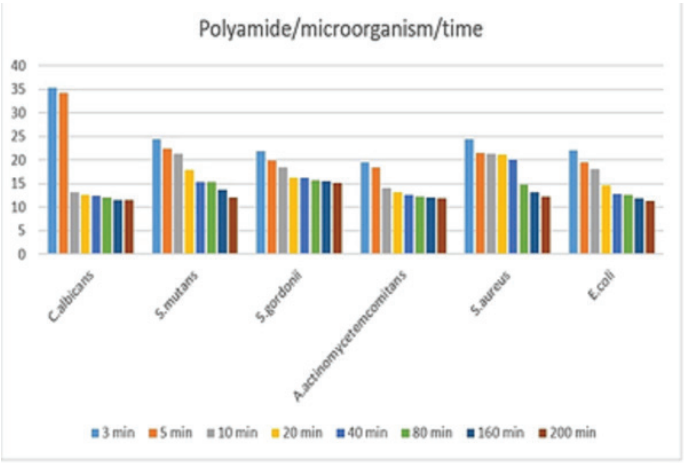

Figure 1. The graphics illustration of CV values of polyamide resin/microorganism/time

Comparisons of durations (Table 1 and Table 2)

When the CV results of *C.albicans* on polyamide resin were evaluated, the CV was 35% at the 3rd minute and decreased to 13% at the 10th minute, and 11% at 160th minute ($p<0.05$). The comparisons of 3rd vs. 5th, ten vs. 20th, 40th, and 80th, and 40th vs. 80th, 160th, and 200th minutes were found insignificant ($p>0.05$). The clinically optimal treatment time can be considered as the 10th minute, and the difference between 10th (13%) and 160th (11%) is not clinically significant, though it is statistically significant.

For *S.mutans*, every tested duration except for 40th vs. 80th was statistically different from each other, with a low but constant decrease in CV starting from 3 minutes to 200 minutes ($p<0.05$). The CV values reached at 20th minute in *C.albicans* was achieved at the 200th minute in *S.mutans*. However, the CV at the 3rd minute was 24%, and this value has clinical relevance and is clinically acceptable.

For *S.gordonii*, the CV value of 20th minute was 16% and similar to longer durations ($p>0.05$) while significantly higher than those of the 3rd, 5th, and 10th minutes, which all had significantly different CV values ($p<0.05$). Similar to *S.mutans*, *S.gordonii* also had low CV level as 21% at the 3rd minute, which is also clinically acceptable as treatment duration.

As for *A.actinomycetemcomitans*, the CV value at the 3rd minute was 19% and decreased to 18%, 14%, 13%, 12%, 12%, 12%, and 11%, in respected durations of 5th, 10th, 20th, 40th, 80th, 160th, and 200th minutes. The statistically significant differences were observed with the 3rd, 5th, 10th, and 20th minutes ($p<0.05$). Duration over 20 minutes made no difference ($p>0.05$); however, like *S.mutans* and *S.gordonii*, the optimal duration for this microorganism was also 3rd minute.

Non-oral pathogens *S.aureus* and *E.coli* exhibited also low CV values at the 3rd minute (24% and 22%, respectively). CV decreased with increasing time in both microorganisms. Though being more evident in *E.coli*, CV levels of both bacteria had a steady decrease from 3rd minute to 200th minute. For *S.aureus*, the difference between 5th vs. 10th and 20th, 20th vs. 30th, 160th vs. 200th minutes were similar ($p>0.05$), and other comparisons were statistically significant ($p<0.05$). For *E.coli*, 40th vs. 80th and 160th, and 160th vs. 200th minute were similar ($p>0.05$), and other differences were found significant ($p<0.05$).

Comparisons of microorganisms (Table 1 and Table 2)

At the 3rd minute, all oral pathogens had a significantly different CV ($p<0.05$) the highest being *C.albicans* and with decreasing order, *S.mutans, S.gordonii*, and *A.actinomycetemcomitans* ($p<0.05$). *S.aureus* had a similar CV with *S.mutans,* and *E.coli* has a similar CV with *S.gordonii* ($p>0.05$), and both were significantly different from other microorganisms ($p<0.05$).

At the 5th minute, similar to the 3rd minute, the highest value belonged to *C.albicans*, and the lowest was in *A.actinomycetemcomitans* ($p<0.05$). All comparisons apart from *S.gordonii* vs. *E.coli* were found significant ($p<0.05$).

At 10th minute, *C.albicans* and *A.actinomycetemcomitans* had similar CV values (13% and 14%, respectively, $p>0.05$) and lower than *S.mutans* and *S.gordonii* (21% and 18%, respectively, $p<0.05$) which has also significant difference ($p<0.05$). Comparisons of *S.aureus* vs. *S.gordonii* and *S.mutans* vs. *E.coli* had similar CV values within ($p>0.05$), but the values were significantly different from other microorganisms ($p<0.05$).

At 20th minute, *C.albicans* and *A.actinomycetemcomitans* had similar CV values (12% and 13%, respectively, $p>0.05$) and the differences apart from these two microorganisms were significantly higher ($p<0.05$).

At 40th minute, *C.albicans*, *A.actinomycetemcomitans*, and *E.coli* had significantly lower CV compared to the other microorganisms (p<0.05). *S.mutans* and *S.gordonii* had similar CV levels (p>0.05), but their values were lower than *S.aureus* (p<0.05).

At 80th minute, *C.albicans*, *A.actinomycetemcomitans*, and *E.coli* had significantly lower CV compared to the other three microorganisms (p<0.05) which had similar CV among each other (p>0.05).

At 160th minute, *S.gordonii* had the significantly highest CV with 15% (p<0.05). *C.albicans*, *A.actinomycetemcomitans*, and *E.coli* had significantly lower CV compared to S.mutans (p<0.059, and *S.aureus* had similar CV with *A.actinomycetemcomitans* and *S.mutans* (p>0.05).

At the 200th minute, *S.gordonii* had the significantly highest CV with 15% compared to the other microorganisms (p<0.05). Other differences were insignificant (p>0.05).

CV values of the microorganisms cultured on PMMA resin (Figure 2).

Comparisons of durations (Table 1 and Table 2)

The CV of 3rd-minute application against *C.albicans* was 34% and decreased with time. All differences apart from 10th vs. 20th, 20th vs. 40th, 40th vs. 80th, and 80th vs. 160th and 200th were statistically significant (p<0.05). The optimal application time can be considered as the 10th minute, which exhibited a drastic decrease from 29% to 11%.

For *S.mutans*, The CV of the 3rd-minute application was 25% and decreased with time. All differences apart from 5th vs. 10th, 40th vs. 80th and 160th, and 80th vs. 160th and 200th were statistically significant (p<0.05). The optimal application time can be accepted as the 3rd minute with 75% cell death, which is clinically acceptable.

For *S.gordonii*, the 3rd-minute application exhibited 23% CV, and CV decreased with time. All differences apart from 20th vs. 40th and 160th vs. 200th minute were statistically significant (p<0.05). Likewise, S.mutans, 3rd minute CV value is an acceptable value for clinical application.

As for *A.actinomycetemcomitans*, 3rd and 5th minute CV values were similar (25% and 24%, respectively, p>0.05), and higher than those of the longer durations (p<0.05). Also, the differences between 20th vs. 40th, and 80th vs. 160th and 200th minutes were found insignificant (p>0.05).

For non-oral pathogens *S.aureus* and *E.coli*, 3rd minute CV values were 24% and 23%, respectively. For *E.coli*, the differences between 3rd vs. 5th and 160th vs. 200th minutes were insignificant (p>0.05) while other differences were statistically significant (p<0.05). For *S.aureus*, CV comparisons of 5th vs. 10th, 20th vs. 40th, 40th vs. 80th, and 80th vs. 160th and 200th were similar (p>0.05). Other differences were significant (p<0.05). For all microorganisms other than *C.albicans*, the 3rd-minute application provided at least 75% cell death and can be considered as a beneficial application time.

Comparisons of microorganisms (Table 1 and Table 2)

When the efficacy of 3rd-minute application was compared among the microorganisms, as observed with polyamide resin, *C.albicans* exhibited the significantly highest CV values (34%) compared to the other microorganisms (p<0.05). *A.actinomycetemcomitans* and *S.mutans* showed similar CV (p>0.05), but the values were still higher than those of the S.gordonii, *S.aureus*, and *E.coli* (p<0.05), which had similar CV among each other (p>0.05).

5 minute and 10-minute tablet treatments also provided same difference pattern as observed with the 3rd-minute application. *C.albicans* exhibited the significantly highest CV value at the 5th minute and the lowest value at the 10th minute (29% and 11%, respectively) compared to the other microorganisms (p<0.05). *A.actinomycetemcomitans* and S.mutans exhibited similar CV (p>0.05), but the values were still higher than those of the S.gordonii, *S.aureus*, and *E.coli* (p<0.05), which had similar CV among each other (p>0.05).

In 20 minute application, *C.albicans* exhibited the significantly lowest CV (10%) and *A.actinomycetemcomitans*, *S.gordonii*, and *S.mutans* had significantly higher CV (19%, 18%, and 18%, respectively) compared to the *S.aureus* and *E.coli* (17% and 16%, respectively).

As for the 40-minute application, the significantly lowest value was observed in *C.albicans* again (p<0.05). *A.actinomycetemcomitans*, *S.aureus* and *S.gordonii* (18%, 17%, and 17%, respectively) had similar CV among each other (p>0.05) but significantly higher (p<0.05) than those of the *E.coli* and *S.mutans* which had a similar CV between each other (p>0.05).

80th-minute values were significantly lowest in the *C.albicans* (9%) compared to the other groups (p<0.05). *A.actinomycetemcomitans*, *S.gordonii,* and *S.mutans* exhibited higher CV than *E.coli* and *S.aureus* (p<0.05). The differences among the *A.actinomycetemcomitans*, S.gordonii, and S.mutans and *E.coli* and *S.aureus* were insignificant (p>0.05).

As for 160th minute values, the CV of *A.actinomycetemcomitans*, *S.aureus* and *S.mutans* (15%, 16%, and 15%, respectively) were significantly higher than *C.albicans* (8%), *E.coli* (11%), and *S.gordonii* (13%) (p<0.05). The difference among the *C.albicans*, *E.coli*, and *S.gordonii* were also significant (p<0.05).

200th minute CV values exhibited the similar difference pattern as found in the 160th minute application. The CV of *A.actinomycetemcomitans* and *S.aureus* (15% and 15%, respectively) were significantly higher than *C.albicans* (8%), *E.coli* (11%), and *S.gordonii* (13%) (p<0.05). S.mutans had CV similar to *A.actinomycetemcomitans*, *S.aureus*, and *S.gordonii* (p>0.05) but higher from *C.albicans* and *E.coli* (p<0.05). The difference among the *C.albicans*, *E.coli*, and *S.gordonii* were also significant (p<0.05). Polyamide vs. PMMA resin (Figure 3A, Figure 3B -SEM)

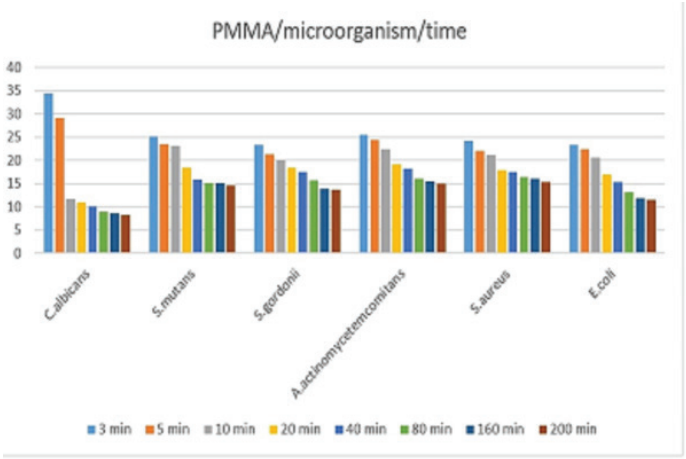

Figure 2. The graphics illustration of CV values of polymethyl methacrylate resin/microorganism/time.

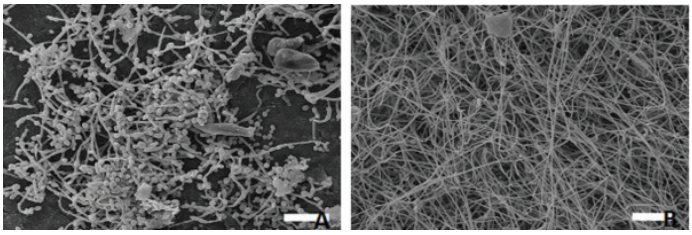

Figure 3A. Scanning electron microscopy images of the adhesion and spread of *C.albicans* on the polyamide resin, after three minutes of subjection to denture cleanser tablet. SEM showed that *C.albicans* had greater adhesion and spread on the resin. The bar is two µm -

Figure 3B. Scanning electron microscopy images of the adhesion and spread of *C. albicans* on the PMMA resin, after three minutes of subjection to denture cleanser tablet. SEM showed that *C. albicans* had greater adhesion and spread on the resin. The bar is two µm

Table 1. The cell viability of microorganisms for all application periods on both resins

Resins/Cell viability (%)	Time (minutes)	*C.albicans*	*S.mutans*	*S.gordonii*	*A.actinomycetemcomitans*	Total
	3	35.41±0.35^{a,p,1}	24.4±0.28^{a,x,1}	21.78±0.38^{a,y,1}	19.52±0.31^{a,z,1}	24.61±5.34^{a,1}
	5	34.35±0.38^{a,p,1}	22.53±0.32^{a,x,2}	19.9±0.26^{a,y,2}	18.4±0.36^{a,z,1}	22.68±5.63^{a,2}
	10	13.14±0.25^{a,z,2}	21.03±0.27^{a,x,3}	18.43±0.24^{a,y,3}	14.08±0.35^{a,z,2}	17.71±3.32^{a,3}
	20	12.77±0.31^{a,z,2}	17.91±0.3^{a,x,4}	16.29±0.43^{a,y,4}	13.22±0.24^{a,z,23}	15.99±3.03^{a,4}
Polyamide	40	12.39±0.34^{a,y,23}	15.43±0.41^{a,x,5}	16.22±0.33^{a,x,4}	12.58±0.37^{a,y,34}	14.93±2.^{9a,5}
	80	12.01±0.42^{a,y,23}	15.39±0.36^{a,x,5}	15.77±0.25^{a,x,4}	12.28±0.4^{a,y,34}	13.82±1.63^{a,6}
	160	11.64±0.34^{a,p,3}	13.85±0.24^{a,x,6}	15.66±0.32^{a,y,}4	12.09±0.29^{a,zp,34}	13.05±1.47^{a,7}
	200	11.56±0.36^{a,x,3}	12.16±0.28^{a,x,7}	15.13±0.34^{a,y,}4	11.9±0.36^{a,x,4}	12.41±1.34^{a,8}
	Total	17.91±10.14^{a,x}	17.84±4.25^{a,x}	17.4±2.32^{a,y}	14.26±2.91^{a,z}	16.9±5.39 ^{a}
	3	34.58±0.35^{b,z,1}	25.16±0.24^{b,x,1}	23.28±0.44^{b,y,1}	25.53±0.38^{b,x,1}	25.98±4.13^{b,1}
	5	29.14±0.44^{b,p,2}	23.65±0.22^{b,x,2}	21.4±0.42^{b,y,2}	24.4±0.36^{b,x,1}	23.88±2.67^{b,2}
	10	11.71±0.32^{b,z,3}	23.2±0.31^{b,x,2}	20.05±0.36^{b,y,3}	22.41±0.28^{b,x,2}	19.85±3.97^{b,3}
	20	10.89±0.41^{b,p,34}	18.58±0.26^{a,xy,3}	18.51±0.32^{b,xy,4}	19.22±0.31^{b,x,3}	17±2.96^{b,4}
PMMA	40	10.14±0.34^{b,z,45}	15.92±0.3^{a,x,4}	17.5±0.33^{b,y,4}	18.36±0.37^{b,y,3}	15.81±2.87^{b,5}
	80	9.01±0.31^{b,p,56}	15.21±0.25^{a,x,45}	15.69±0.29^{a,xy,5}	16.07±0.3^{b,xy,4}	14.29±2.71^{b,6}
	160	8.63±0.36^{b,p,6}	15.24±0.31^{b,x,45}	13.89±0.35^{b,y,6}	15.96±0.28^{b,x,4}	13.62±2.8^{b,7}
	200	8.26±0.27^{b,p,6}	14.64±0.28^{b,xy,5}	13.7±0.24^{b,x,6}	15.02±0.25^{b,y,4}	13.09±2.63^{b,8}
	Total	15.29±10.04^{b,p}	18.95±4.24^{b,x}	18±3.37^{b,y}	19.62±3.92^{b,z}	17.94±5.47 ^{b}

C.albicans: Candida albicans, S.mutans: Streptococcus mutans, S.gordonii: Streptococcus gordonii, A.actinomycetemcomitans: **Aggregatibacter actinomycetemcomitans**

***a,b: Polyamide vs. PMMA, intergroup comparison for interaction and main effects. x,y,z,p: Comparison of materials for interaction and main effects. 1,2,3,4,5,6,7,8: Comparison between methods for interaction and main effects. Three-way ANOVA was used. The Bonferroni test was used for multiple comparisons. p<.05 was considered significant. Different letters indicate difference with statistical significance**

Table 2. The cell viability of microorganisms for all application periods on both resins

Resins/Cell viability (%)	Time (minutes)	*C.albicans*	*S.mutans*	*S.gordonii*
Polyamide	3	22.15±0.26^{a,y,1}	24.4±0.29^{a,x,1}	24.61±5.34^{a,1}
	5	19.52±0.32^{a,x,2}	21.4±0.27^{a,y,2}	22.68±5.63^{a,2}
	10	18.06±0.28^{a,x,3}	21.51±0.36^{a,y,2}	17.71±3.32^{a,3}
	20	14.6±0.31^{a,x,4}	21.14±0.35^{a,y,23}	15.99±3.03^{a,4}
	40	12.8±0.35^{a,x,5}	20.16±0.38^{a,z,3}	14.93±2.9^{a,5}
	80	12.65±0.39^{a,x,5}	14.83±0.34^{a,y,4}	13.82±1.63^{a,6}
	160	11.98±0.28^{a,x,56}	13.07±0.33^{a,x,5}	13.05±1.47^{a,7}
	200	11.41±0.31^{a,x,6}	12.28±0.39^{a,x,5}	12.41±1.34^{a,8}
	Total	15.4±3.88^{a,x}	18.6±4.38^{a,y}	16.9±5.39 ^{a}
PMMA	3	23.28±0.42^{b,x,1}	24.03±0.32^{a,x,1}	25.98±4.13^{b,1}
	5	22.53±0.4^{b,x,1}	22.15±0.34^{b,x,2}	23.88±2.67^{b,2}
	10	20.65±0.39^{b,x,2}	21.1±0.35^{a,x,2}	19.85±3.97^{b,3}
	20	16.93±0.35^{b,x,3}	17.87±0.33^{b,x,3}	17±2.96^{b,4}
	40	15.39±0.36^{b,x,4}	17.57±0.38^{b,y,34}	15.81±2.87^{b,5}
	80	13.22±0.37^{a,x,5}	16.52±0.24^{b,y,45}	14.29±2.71^{b,6}
	160	11.83±0.29^{a,x,6}	16.14±0.26^{b,y,5}	13.62±2.8^{b,7}
	200	11.53±0.27^{a,x,6}	15.39±0.28^{b,y,5}	13.09±2.63^{b,8}
	Total	16.92±4.58^{b,x}	18.85±3.07^{a,y}	17.94±5.47^{b}

E.coli: Escerichia coli, S.aureus: Staphylococcus aureus

***a, b: Polyamide resin vs. PMMA resin, intergroup comparison for interaction and main effects. x, y: *E.coli vs. S.aureus*, comparison of materials for interaction and main effects. 1, 2, 3, 4, 5, 6, 7, 8: Differences regarding the durations. Three-way ANOVA was used. The Bonferroni test was used for multiple comparisons. p<.05 was considered significant. Different letters indicate the difference with statistical significance**

For *C.albicans* and *A.actinomycetemcomitans*, all durations were significantly different when polyamide resin and PMMA resin were compared (p<0.05). However, for *C.albicans*, CV values in polyamide resin were significantly higher than those of the PMMA resin while for *A.actinomycetemcomitans*, it was the opposite since the PMMA resin CV values were higher than those of the polyamide resin CV values (p<0.05). Based on these CV values, *C.albicans* seems to be more resistant to polyamide resin while *A.actinomycetemcomitans* were sensitive.

S.mutans had also significantly higher CV values on PMMA resin after 3rd, 5th, 10th minutes applications (p<0.05) while 20th, 40th, and 80th minute results were similar to polyamide resin (p>0.05), and again CV values of 160th and 200th minutes were higher than those of the equivalent polyamide resin values (p<0.05).

For *S.gordonii*, the difference pattern was same with *A.actinomycetemcomitans* with all durations of PMMA resin had higher CV values compared to polyamide resin except the 80th minute, which had a similar CV when polyamide resin and PMMA resin were compared (p>0.05).

For *S.aureus*, polyamide resin caused significantly lower CV at 5th, 20th, 40th, 80th, 160th, and 200th minutes in polyamide resin compared to PMMA resin (p<0.05), and the values of 3rd and 10th-minute applications were similar (p>0.05).

For *E.coli*, PMMA resin also caused higher CV values to the 80th minute (p<0.05). The CV values of 80th, 160th, and 200th minutes were similar between the polyamide resin and PMMA resin (p>0.05).

DISCUSSION

The present study determined the antimicrobial efficacy of a denture cleanser tablet against six microorganisms, including oral and non-oral pathogens. The time-dependent efficacy of cleanser tablet on both resin types was also determined. All tested durations were effective and provided at least 75% cell death even in the shortest duration as a 3rd minute in both resins except for *C.albicans*. *C.albicans* exhibited 65% and 66% cell death in the 3rd minute and 5th minute in polyamide resin and 66% and

71% in PMMA resin respectively. The clinically acceptable treatment durations can be considered as three minutes for *S.mutans*, *S.gordonii*, *A.actinomycetemcomitans*, *S.aureus*, and *E.coli* and 10 minutes for *C.albicans*. Nevertheless, the longer the tablet was used, the more effective the effect was increased despite the reduction in the acceleration of the effect.

In the reduction or elimination of microorganisms, the application time of cleanser tablets affects the results as much as the disinfection method (15,21,22,31,32). Manufacturers specify the minimum duration for the inhibition of the microorganisms, and as for the tablet used in the present study, the recommended minimum duration was three minutes, which is also covered in the present study. However, determination of the optimal application time of these tablets is essential for reducing or eliminating oral microorganisms especially for microorganisms which might be resistant against cleansers such as *C.albicans* as reported in previous studies (12,14,22). Furthermore, the resistance of the microorganism might be different on different denture base resins as observed in the present study shown as total values in Table 1. *A.actinomycetemcomitans* were found to be significantly more resistant on PMMA resin compared to the polyamide resin, and all other microorganisms except for *C.albicans* and *S.aureus* exhibited significantly high resistances on PMMA resin and sensitivity to polyamide resin. *S.aureus* had similar CV values in both resins, and as for *C.albicans*, the resistance was on polyamide resin, and *C.albicans* was found to be sensitive on PMMA resin.

Furthermore, individual total CV values of the microorganisms were also significantly different apart from *C.albicans* and *S.mutans*, which had similar resistance on polyamide resin. On the other hand, in PMMA resin, *S.mutans* had similar resistance with *S.aureus*, and other microorganisms had significantly different total CV values. A possible reason for the attachment of a microorganism to denture surfaces was suggested to be the surface roughness (36).

In terms of different adherence observed in various resins, Freitas-Fernandes et al. reported that PMMA resin caused increased microorganism attachment compared to polyamide resin (23). Abuzar et al. analyzed the unpolished and polished polyamide resin and PMMA resin surfaces and demonstrated higher Ra in polyamide resin even in both and polished and unpolished surfaces. A possible disintegration of mold surface resulted from high temperature and pressure during production might increase microorganism attachment (10, 37). A polyamide as a denture material has crystalline polymer structure with a low melting temperature and overheating, and the inappropriate rapid cooling process might disrupt the material structure (10,38) on the other hand PMMA has an amorphous structure and deterioration of the material with heat or other causes is harder than polyamide (39). Also, the polarity of the resin was reported to affect antimicrobial efficacy, especially in PMMA resin against *C.albicans* (31). Polarity or the resin-surface charge result from residual monomers in PMMA resin decreases the adhesion of microorganisms and prevent biofilm formation (22).

In contrast, surface free energy was found irrelevant in terms of the antibacterial effect of denture resins (31). Durkan et al. demonstrated the deterioration of polyamide resin surface, increased the hardness of the resin and Ra after 20 days of denture cleanser application (40). This could also lead to increased *C.albicans* colonization, which is also evident in the present study since the CV of *C.albicans* were higher in the polyamide resin.

The present study showed that the enzymatic denture cleanser tablet exhibited strong antimicrobial activity for all application times for all microorganisms on both of the denture base resins. The cell viability decreased as the application time was increased from 3 to 200 minutes. Although a reasonable proportion existed between increased time and reduced cell viability for both of the resins, complete inhibition of biofilm-forming microorganisms in both resins was not achieved at any duration; nonetheless, complete inhibition is not necessarily required for the long-term safe, comfortable and healthy use of dental prostheses. Furthermore, even in the 3-minutes of application, the minimum cell inhibition was 76%, except for *C.albicans*, which is known to be difficult to remove and can rapidly multiply (23-25). Microorganisms other than *C.albicans* tend to exhibit a slight decrease with time. However, *C.albicans* had drastic declines after 10 minutes in both polyamide resin and PMMA resin from 65% CV to 88% CV. The adhesion of the *C.albicans* was apparent on the SEM images obtained after the 3-minute cleanser application (Figure 3A, Figure 3B-SEM).

The results also showed the effect of the application time on a CV which was found to vary depending on both resin and microorganism type. Although the cell inhibition on the resins increased with time, no significant change in total CV occurred after 40-minutes for polyamide resin and 80-minutes for PMMA resin. Regardless of the duration, overall CV was higher for *C.albicans* and lowered for *S.mutans, S.gordonii*, *A.actinomycetemcomitans*, *S.aureus*, and *E.coli* in PMMA resin and vice versa for polyamide resin as reported in previous studies (23,24,31,34,36). Regardless of the microorganism type, all durations caused a significant decrease in the CV; however, the increase in cell death after 10 minutes does not have clinical importance since 10 minutes of the application provided substantial efficacy. Considering that maintaining prosthesis is a challenging issue for elderly patients, and they might not keep track of the time of the cleanser application, shorter durations for cleaning might be beneficial in their daily life. Moreover, the fact that polyamide resin caused lower bacterial attachment and higher antimicrobial activity, patients who are not prone to *C.albicans* infections can safely use polyamide as a denture material. However, patients with the risk of

C.albicans infection need to treat dentures at cleanser tablets at least 10 minutes. The antimicrobial activity of the denture cleanser tablet was evident in both resins against all microorganisms. The effect against *C.albicans* has particular importance since it is the most common cause of stomatitis related to use of prosthesis and the tablet cleanser effectively reduced 89% of the cells within 10 minutes in PMMA resin, which was also reported in previous studies (23,31,34).

The present study has two significant limitations. First is the monobacterial biofilm layer since the biofilm in the present study composed of one microorganism and was not a mixed biofilm. Mixed biofilms would be beneficial to mimic real-life conditions. The latter is the duration. The antimicrobial activity of the tablet was determined between 3 and 200 minutes. Further durations would have been evaluated. However, 200th minute provided 85 to 92% cell death; therefore, additional durations do not have clinical importance. However, further studies are required to investigate the antimicrobial activity and duration of administration of the denture cleanser tablets on the mixed biofilms of these microorganisms on other types of denture base resins.

CONCLUSION

The results demonstrated the antimicrobial activity of the enzymatic denture cleanser tablet on all microorganisms, and the activity tends to increase in time with a descending acceleration. Regardless of the microorganism type, all durations caused a significant decrease in the CV. A high antimicrobial effect after the 40-minutes for the polyamide resin and after the 80-minutes for a PMMA-based resin was observed; however, the increase in cell death after 10 minutes does not have clinical importance since 10 minutes of the application provided significant efficacy. Considering that maintaining prosthesis is a challenging issue for elderly patients, and they might not keep track of the time of the cleanser application, shorter durations for cleaning might be beneficial in their daily life. The recommended minimum duration was 3 minutes for the tablet studied in the present study, and the results revealed that this is not valid for *C.albicans*, which required 10 minutes for a significant reduction in the CV.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: The study does not require ethical approval.

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