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Original research

DETERMINATION OF THE EFFECTIVE TIME OF DENTURE CLEANSER TABLETS ON THE REMOVAL OF *CANDIDA ALBICANS* ON DENTURE BASE RESINS

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ABSTRACT

Objective: The aim of this study was to determinate of the effective time of different denture cleanser tablets on the removal of *Candida albicans* on various base resins.

Materials and Methods: Conventional heat cured resin, high impact resin, autopolymerized resin, and polyamide resin were used. 160 samples were prepared for each resin type. The biofilm of *C. albicans* was formed on the resins and then exposed to alkaline peroxide tablet and enzymatic tablets for 3, 5, 10, 20, 40, 80, 160 and 200 minutes. Cell viability was assessed by MTT test. 3-way ANOVA was used for statistical analysis.

Results: The effect of resin type, tablet type and application time on cell viability of *C. albicans* were found to be significant (p<0.05). Cell death was at least 65% on the resins even at the minimum time. Furthermore, as the duration of administration of both tablets increased, cell viability in all resins tended to decrease. The administration of both tablets on all resins for 10 min resulted in approximately 80% cell death. Additionally, the most significant antimicrobial activities of the tablets were determined at 20th minutes. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet.

Conclusions: Alkaline peroxide and enzymatic cleanser tablets showed remarkable anticandidal activity for all resins. The patients with risk of *C. albicans* infection should keep their prostheses in cleanser tablets for at least 20 minutes. Furthermore, the anticandidal effect tended to increase with prolonged exposure time.

Keywords: Denture bases, denture cleansers, candida albicans, cell survival.

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INTRODUCTION

The most commonly used base resin material in dentistry is polymethylmethacrylate (PMMA) resin materials. However, it has some disadvantages such as low flexural and impact strength.[1] Therefore, alternative materials such as polyamide resin and high-impact resins have been developed to obtain a denture base with better mechanical properties. High impact acrylic resin has high resistance to falls[2] and polyamide thermoplastic resin is more elastic than PMMA.[3] In cases where the heat cured PMMA denture base needs to be repaired for reasons such as fracture and tooth replacement, autopolymerized PMMA resins are used.

The oral cavity has a rich and diverse microorganisms.[4] Although the denture base resins appeared to be visually smooth, they were shown to have small pits for the attachment of microorganisms when examined under a microscope.[5] Therefore, denture base resins provide a suitable area for the growth of biofilm.[5-11] Microorganisms hold less on polished surfaces than rough surfaces.[12]

C. albicans is the most common microorganism of denture plaque and causes prosthetic stomatitis in patients with a removable prosthesis.[7,13] The interactions between microorganisms contribute to the development of biofilm communities containing mixed microorganisms.[14] In this way, the developed biofilm is more complex and difficult to remove.[6-8] *Candida* species adhere to the internal or external surfaces of the denture base resin with hydrophobic and electrostatic forces and can be transferred to distinct parts of the oral mucosa after adherence.[15]

In the absence of oral hygiene, microorganisms cause many systemic diseases other than oral diseases.[13] The microbial plaque on the prosthesis must be effectively removed to prevent these diseases. Various methods of denture cleaning have been proposed, including mechanical, chemical, or a combination of mechanical and chemical. Mechanical cleaning includes brushing, while chemical cleaning includes various disinfectants, solutions, and denture cleansing tablets.[16, 17]

Tooth loss generally increase with age[18], and the individuals using removable prostheses are mostly elderly individuals.[19] With increasing age, motor function slows down, and movement limitation occurs. Therefore, mechanically cleaning the dentures is difficult for the elderly, disabled or patients with motor dysfunction.[6] Mechanical cleaning by brush may also cause scratches on the base, which may lead to the attachment of more microorganisms.[6] Therefore, chemical cleaning which can be achieved by using denture cleansers should be considered. Hence, denture cleansing tablets are recommended for the elderly and patients with motor function impairment.[20,21]

Numerous studies have been conducted to determine the effectiveness of cleansing tablets to remove various microorganisms on prostheses.[7,8,22] These studies showed that denture cleanser tablets had significant anticandidal activity, but *C. albicans* biofilm on the resin could not be eliminated entirely. In the studies performed, anticandidal activity was evaluated for random times. However, the application time of the tablets may also be significant in removing microorganisms. No standardization for the duration of application of the tablets exists, and varying periods, from three minutes to overnight usage, are recommended by the manufacturer.

This study aimed to firstly evaluate the effect of two types of heat cured PMMA resins; autopolymerized PMMA and polyamide resin, on *C. albicans* biofilm formations, and secondly to determine the application time effect of two types of denture cleanser tablets for periods ranging from 3 to 200 minutes to remove *C. albicans* biofilm formations. No comprehensive research had previously been conducted to determine the effective duration of tablets used to remove *C. albicans.*

The null hypotheses were that: 1) resin type would affect the amount of microorganism attachment, 2) denture cleanser tablet type would affect the amount of microorganism attachment, and 3) the cell viability of microorganisms would decrease with an increasing application time of tablets.

MATERIAL AND METHODS

Sample preparation

Four different types of resins were used in the study including heat cured conventional PMMA resin (QC-20, Dentsply, Addleston, UK), high impact PMMA resin (Acron-hi, Kemdent, Swindon, UK), autopolymerized PMMA resin (Meliodent Cold; Heraeus Kulzer, Hanau, Germany), and polyamide resin (Deflex, Nuxen SRL, Buenos Ares, AR). The samples were prepared in the form of a disc with a diameter of 10 mm and a thickness of 2 mm following the instructions of the manufacturers (n=160 per resin). The type of resins used in the study are shown in Table 1.

Table 1 Description of tested denture base materials

Trade Name	Material	Processing Method	Manufacturer
Acron-hi	PMMA	Heat cure compression molded	Kemdent, Swindon, UK
Deflex	Polyamide	Injection molded	Nuxen SRL, Buenos Ares, Argentina
Meliodent-cold	PMMA	Cold cure compression molded	Heraeus Kulzer, Hanau, Germany
Qc-20	PMMA	Heat cure compression molded	Dentsply, Addleston, UK

All resin samples were left in the distilled water for 24 hours for residual monomer release after the polymerization. The samples were then smoothen with 600, 800 and 1,000 grit of sandpaper. A profilometer device (Taylor Hobson, Surtronic 25, Leicester, UK) was used to assess the surface roughness (Ra). Measurements were made from three different points of each resin surface, and these readings were averaged. The Ra of all samples were standardized at 0.32 ± 0.02 μm. Before microbial contamination, the sterilization of the samples was performed using an ultrasonic device (Pro-Sonic 600, Sultan Healthcare, Hackensack, NJ) in a distilled water at 50 °C, at 28 kHz for 10 minutes.

Microorganism cultures and growth conditions

C. albicans (ATCC 1023) strain was incubated for 24 h at Sabouraud dextrose agar at 35°C. Twenty-four hours later, the fungal concentration was prepared using RPMI-2% glucose broth and counted using the Neubauer chamber and trypan blue dye to obtain a final concentration of 1-5x10^{6}cell/mL. To produce fungal biofilm on resin samples, the resin and cells were transferred to 24-well polystyrene microtiter plates containing RPMI-2% glucose broth and allowed to grow for 48 hours at 35 °C in a shaker incubator at 150 rpm.

Test tablets

Alkaline peroxide denture cleanser tablet (CoregaTM, GlaxoSmithKline Healthcare, İstanbul, Türkiye) and neutral enzymatic denture cleanser tablet (Polident3minTM, GlaxoSmithKline Healthcare, Moon Township, PA) were prepared following the manufacturer's instructions. The cleansing tablets used in the study are shown in Table 2. For cleaning working solutions, each tablet was dissolved in 150 mL of warm distilled water, and the solution was used immediately.

Table 2. Denture cleanser tablets used in the study

Trade Name	Denture cleanser	Ingredients	Manufacturer
Polident 3 min™	Enzymatic tablet	Sodium perborate, potassium monopersulfate, proteolytic enzyme, detergent and effervescent base	GlaxoSmithKline Healthcare, Moon Township, USA
Corega™	Alkaline peroxide tablet	Potassium monopersulfate, sodium bicarbonate, sodium lauryl sulfoacetate, sodium perborate monohydrate, sodium polyphosphate	GlaxoSmithKline Healthcare, Istanbul, Turkey

Susceptibility testing

The number of living cells was determined by MTT analysis. MTT analysis was performed according to AFST-EUCAST standards. The MTT stock solution (5 mg MTT/ml distilled water) was sterilized by filtration and kept at -20°C until use.

Firstly, the biofilm was grown as previously described. Following incubation of 48 hours, the old medium in the wells was removed, and the cells in the biofilm were exposed to 200 µL of the alkaline peroxide tablet solution and enzymatic tablet solution for 3, 5, 10, 20, 40, 60, 80, 160 and 200 minutes. The cleanser solutions were then replaced with fresh RPMI-2% glucose liquid medium containing MTT (final concentration, 0.5 mg/mL). The mixture was incubated with shaking for 4 hours (150 rpm at 35°C). After the incubation period, 180 µL of the medium was removed. 30 µL of Sorenson 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 660 nm to 560 nm as the reference range. Percentage of viability was calculated using Microsoft Excel software (Washington, USA).

Statistical analysis

Three-way ANOVA was used to compare the continuous data between/among groups. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY).

RESULTS

This study evaluated the antimicrobial activity of two different denture cleansing tablets against *C. albicans* on four different resins and determined the cell viability percentages corresponding to the times of treatment with the tablet solution ranging from 3 to 200 minutes. The mean values and standard deviations of cell viability on acrylic resins for all applied times of cleanser solutions are shown in Table 3. Both tablets were effective against *C. albicans* on all denture base resins at all application times. Cell death occurred in at least 65% of the microorganisms on the resins even at the 3rd minute. The administration of both tablets on all resins for 10 min and over resulted in approximately 80% cell death. As the duration of administration of both tablets increased, a tendency to decrease in cell viability was observed on all resins. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet. Resin type was found to be effective in *C. albicans* adhesion (p<0.05). Cell viability values for alkaline peroxide tablet and enzymatic tablet for 200 minutes were 8% and 10% for high impact resin, 8% and 12% for polyamide resin, 8% and 11% for autopolymerized resin, 8% and 8% for conventional heat cured resin, respectively. Regardless of time and type of tablet, cell adhesion from high to low was observed in autopolymerized resin, polyamide resin, high impact resin, and heat cured resin, respectively.

Cell viability values of microorganisms cultured on high impact resin

Comparison of times for alkaline peroxide tablets

The cell viability of *C. albicans* on high impact resin exposed to alkaline peroxide tablet was 32% at the 3rd minute and decreased to 30% at the 5th minute, 21% at the 10th minute,10% at the 20th minute (p<0.05) (Table 3) (Figure 1). There was no statistical difference between the cell viability at 20th, 40th, 80th, 160th and 200th minutes (p>0.05). Thus, optimal treatment time for high impact resin to be exposed to alkaline peroxide tablet can be considered as 20 minutes (10%).

Comparison of times for enzymatic tablets

When cell viability results of *C. albicans* on high impact resin exposed to enzymatic tablet were evaluated, the cell viability was 35% at the 3rd minute, decreased to 31% at the 5th minute and 13% at the 10th minute (p <0.05) (Table 3) (Figure 2). The comparisons of 10th vs. 20th, 20th vs. 40th, and 80th vs. 160th, and 200th minutes were found to be insignificant (p>0.05). However, the cell viability at the 200th minute was 10%. Although there is a statistically significant difference between the 40th minute and 200th minute, the difference is not clinically significant. The optimal treatment time for high impact resin can be considered as 40 minutes (12%) for enzymatic tablets.

Table 3. The cell viability of *C. albicans* for all application periods on all resins

Resins	Time (minute)	(Cell viability %) Corega™ (n=10) Mean±SD	Polident 3 min™ (n=10) Mean±SD	Total Mean±SD
High impact resin	3	32.28±0.38(a,x,1)	35.14±0.40(a,y,1)	33.71±1.68 (a,1)
	5	29.73±0.31(a,x,2)	31.13±0.33(a,y,2)	30.43±0.86 (a,2)
	10	21.25±0.29(a,x,3)	13.22±0.36(a,y,3)	17.23±4.65 (a,3)
	20	9.53±0.35(a,x,4)	12.58±0.38(a,y,34)	11.05±1.78 (a,4)
	40	9.15±0.42(a,x,4)	12.01±0.27(a,y,4)	10.58±1.72 (a,45)
	80	8.96±0.25(a,x,4)	10.89±0.32(a,y,5)	9.92±1.15 (a,5)
	160	8.77±0.39(a,x,4)	10.14±0.34(a,y,5)	9.45±0.84 (a,56)
	200	8.39±0.36(a,x,4)	9.76±0.41(a,y,5)	9.07±0.87 (a,6)
	Total	16.01±9.87(a,x)	16.86±9.83(a,y)	16.43±9.7 (a)
Polyamide resin	3	38.93±0.31(b,x,1)	35.41±0.35(a,y,1)	37.17±2.05 (b,1)
	5	31.29±0.36(b,x,2)	34.35±0.38 (b,y,1)	32.82±1.79(b,2)
	10	21.75±0.22(a,x,3)	13.14±0.25(a,y,2)	17.44±4.98(a,3)
	20	8.77±0.27(ab,x,4)	12.77±0.31(a,y,23)	10.77±2.33(a,4)
	40	8.58±0.28(ab,x,4)	12.39±0.34(a,y,234)	10.48±2.22(a,45)
	80	8.39±0.33(ab,x,4)	12.01±0.42(b,y,234)	10.2±2.11(a,45)
	160	8.19±0.37(ac,x,4)	11.64±0.34(b,y,34)	9.92±2.01(a,5)
	200	8±0.36(a,x,4)	11.56±0.36(b,y,4)	9.78±2.08(b,5)
	Total	16.74±11.99(b,x)	17.91±10.14(b,y)	17.32±10.94 (b)
Autopolymerize resin	3	33.2±0.22(a,x,1)	30.79±0.26(b,y,1)	31.99±1.42 (c,1)
	5	24.42±0.32(c,x,2)	28.53±0.36(c,y,2)	26.48±2.39(c,2)
	10	23.66±0.35(b,x,2)	23.28±0.39(b,x,3)	23.47±0.36(b,3)
	20	8±0.25(b,x,3)	21.4±0.27(b,y,4)	14.7±7.74(b,4)
	40	7.97±0.4(b,x,3)	19.52±0.33(b,y,5)	13.74±6.68(b,5)
	80	7.81±0.37(b,x,3)	12.54±0.35(b,y,6)	10.18±2.75(a,6)
	160	7.62±0.26(c,x,3)	11.75±0.29(b,y,67)	9.69±2.4(a,6)
	200	7.62±0.32(a,x,3)	11.26±0.39(b,y,7)	9.44±2.12(ab,6)
	Total	15.04±10.03(c,x)	19.88±7.33(c,y)	17.46±8.98(b)
Conventional heat cured resin	3	35.11±0.3(c,x,1)	34.58±0.35(a,x,1)	34.84±0.42(d,1)
	5	31.29±0.36(b,x,2)	29.14±0.44(c,y,2)	30.21±1.28(a,2)
	10	9.15±0.22(c,x,3)	11.71±0.32(c,y,3)	10.43±1.51(c,3)
	20	8.96±0.46(ab,x,3)	10.89±0.41(c,y,34)	9.92±1.15(c,34)
	40	8.77±0.26(ab,x,3)	10.14±0.34(c,y,45)	9.45±0.84(c,45)
	80	8.58±0.28(ab,x,3)	9.01±0.31(c,x,56)	8.79±0.38(b,56)
	160	8.39±0.46(ac,x,3)	8.63±0.36(c,x,6)	8.51±0.32(b,6)
	200	8.35±0.25(a,x,3)	8.26±0.27(c,x,6)	8.3±0.29(c,6)
	Total	14.82±11.01(c,x)	15.29±10.04(d,y)	15.06±10.37(c)

*a, b, c: Polyamide vs. PMMA, intergroup comparison for interaction and main effects.
x, y: Comparison of denture cleanser tablets for interaction and main effects.
1, 2, 3, 4, 5, 6: Comparison of times for interaction and main effects.
Three-way ANOVA was used, $p<0.05$ was considered significant. Different letters indicate difference with statistical significance.

Cell viability of microorganisms cultured on polyamide resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on polyamide resin exposed to alkaline peroxide tablet were evaluated, the cell viability of the 3rd minute decreased from 39% to 31% at the 5th minute, to 21% at the 10th minute and 9% at the 20th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistical difference between the cell viability of 40th, 80th, 160th and 200th minutes of cell viability compared to 20th minutes ($p>0.05$). Besides, cell viability at the 200th minute was 8%. The optimal treatment time for polyamide resin for alkaline peroxide tablet can be considered as 20 minutes (9%).

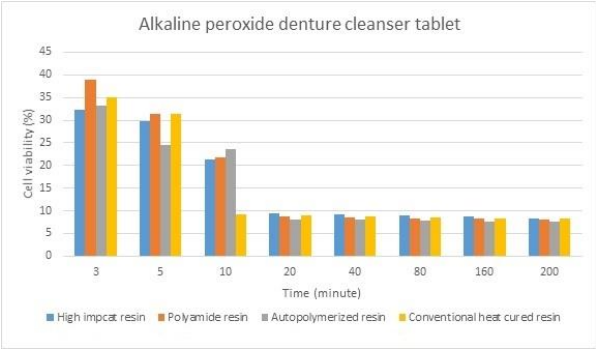

Figure 1: The graphic illustration of the time-dependent efficacy of alkaline peroxide denture cleanser tablets in removal of *Candida albicans* on denture base resins

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on polyamide resin exposed to enzymatic tablet were evaluated, cell viability at 3rd minute decreased from 35% to 13% at the 10th minute ($p<0.05$). No statistical differences were observed in the comparison of 3rd vs. 5th, 10th vs. 20th, 40th, 80th, 20th vs. 40th, 80th, 160th, and 160th vs. 200th ($p>0.05$) (Table 3) (Figure 2). Although there is a statistically significant difference between the 10th minute and 200th minute, this difference does not have clinical importance and can be neglected. Optimal treatment duration for polyamide resin in the enzymatic tablet can be considered as 10 minutes with 13% cell viability.

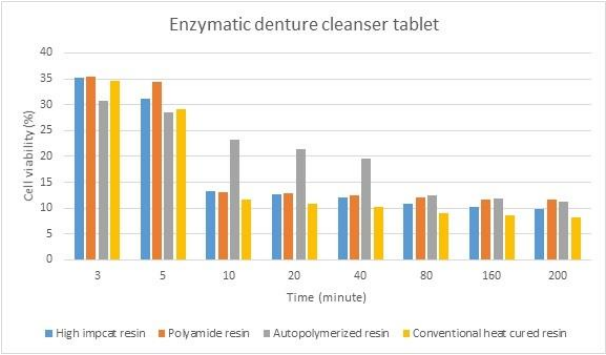

Figure 2: The graphic illustration of the time-dependent efficacy of enzymatic denture cleanser tablets in removal of Candida albicans on denture base resins

Cell viability of microorganisms cultured on autopolymerized resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on autopolymerized resin exposed to alkaline peroxide tablet were evaluated, the cell viability at the 3rd minute decreased from 33% to 24% at the 5th minute and 8% at the 20th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistically significant difference in cell viability between 5th vs.10th, 20th, 40th, 80th, 160th, and 200th minute ($p>0.05$). The optimal treatment time for autopolymerized resin in alkaline peroxide tablet can be considered as 20 minutes (8%).

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on autopolymerized resin exposed to enzymatic tablet were evaluated, cell viability at the 3rd minute decreased from 30% to 28% in the 5th minute, to 23% at the 10th minute, to 21% at the 20th minute, to 19% at the 40th minute, 12% at 80th minute and to 11% at 200th minute ($p<0.05$) (Table 3) (Figure 2). There was no statistical difference in comparisons of 80th vs. 160th minute and 160th vs. 200th minute ($p>0.05$). Although there is a statistically significant difference between the 80th minute and 200th minute, the difference is not clinically significant. The optimal treatment time for autopolymerized resin can be considered as 80 minutes (12%) for enzymatic tablets.

Cell viability values of microorganisms cultured on heat cured resin

Comparison of times for alkaline peroxide tablets

When cell viability results of *C. albicans* on heat cured resin exposed to alkaline peroxide tablet were evaluated, the cell viability at the 3rd minute decreased from 35% to 31% at the 5th minute, and 9% at the 10th minute ($p<0.05$) (Table 3) (Figure 1). There was no statistically significant difference between cell viability at 10th, 20th, 40th, 80th, 160th and 200th minute ($p>0.05$). Furthermore, cell viability at the 200th minute was 8%. The clinically optimal treatment time for heat cured resin for alkaline peroxide tablet can be considered as 10 minutes (9%).

Comparison of durations for enzymatic tablets

When cell viability results of *C. albicans* on heat cured resin exposed to enzymatic tablet were evaluated, the cell viability at 3rd minute decreased from 35% to 29% at 5th minute, to 12% at 10th minute, 10% at 40th minute, and to 7% at 160th minutes ($p<0.05$). There was no statistical difference in comparisons of 10th vs. 20th minute, 20th vs. 40th minute, 40thvs. 80th, 160th and 200th minutes ($p>0.05$) (Table 3) (Figure 2). Although there is a statistically significant difference

between the 20th minute and 200th minute, this difference is not clinically significant and the clinically optimal treatment time for heat cured resin for enzymatic tablet can be considered as 20 minutes (11%).

Comparison of tablets

Comparison of tablets for high impact resin

The tablets statistically differed for all application times (p<0.05) (Table 3). The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 10th minute.

Comparison of tablets for polyamide resin

The tablets statistically differed for all application times (p<0.05) (Table 3). The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 3rd minute.

Comparison of tablets for autopolymerized resin

The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 3rd minute. However, there was no statistically significant difference between the tablets at the 10th minutes (p>0.05) (Table 3).

Comparison of tablets for heat cured resin

The anticandidal efficiency of alkaline peroxide tablet was higher than the enzymatic tablet for all the times except for the 5th minute. However, there was no statistically significant difference between the tablets at the 3rd, 80th, 160th and 200th minutes (p>0.05) (Table 3).

DISCUSSION

The present study determined the anticandidal efficacy of two different denture cleanser tablets against *C. albicans*. The effective time of different denture cleanser tablets in the removal of *C. albicans* on various base resins was determined on four different types of denture base resins. All hypotheses were accepted. The type of the acrylic resin, type of denture cleanser tablet and duration of the tablet treatment were found to be effective on cell viability of *C. albicans*. Both tablets showed a high anticandidal effect at all the tested durations. Cell death was seen at least 65% on the resins even at the minimum time. The optimal treatment duration for the alkaline peroxide tablet and enzymatic tablets were 20 and 40 minutes for high impact resin, 20 and 10 minutes for polyamide resin, 20 and 80 minutes for autopolymerized resin, and 10 and 20 minutes for heat cured resin. However, the anticandidal effect tended to increase with prolonged exposure time.

Rough surfaces are known to cause increased microbial colonization.[23, 24] Initially, the roughness of the resin surfaces was standardized to 0.32±0.02 μm in order to evaluate the anticandidal effect of alkaline peroxide denture cleanser and neutral enzymatic peroxide denture cleanser on four different denture base resins. Polyamide resin surfaces were generally reported to be rougher than PMMA resins.[8, 25] Although the surface roughness of all the resins were equalized, the cell viability percentages on the resins showed differences according to the resin groups. Regardless of the tablet type and duration, the minimum cell viability was observed in the conventional heat cured resin group. Conventional heat cured resin was followed by high impact resin, polyamide resin and autopolymerized resins respectively.

It has been suggested that the primary factor in the connection of microorganisms to the denture base surfaces is the surface roughness.[24] However, the results of the present study showed that not the only factor that is effective in the amount of *C. albicans* that is formed on the resin is surface roughness and that resin type plays an important role in the attachment of microorganisms. As in this study, it has been shown by other in vitro studies that resin variety is effective in the attachment of microorganisms.[8,10,26] Physical and chemical properties of denture base resins may play a role in the colonization of microorganisms.[27-29] In addition to the physical and chemical properties of the material, characteristic of the microorganism is also effective in the adhesion of microorganisms to the resin surface.[10,30] In this context, Radford et al. suggested that the adherence of *C. albicans* to the prosthetic base resin was due to the hydrophobicity of the organism.[28]

The manufacturers have recommended the minimum time required for antimicrobial activity of their denture cleanser tablets. The recommended

minimum time for Polident 3 min™ enzymatic tablet is 3 minutes, while the recommended minimum time for Corega™ alkaline peroxide tablet is 5 minutes. However, the dentures may be exposed to the tablet solution overnight; but, it has been shown that dentures that have been kept in the denture cleansing agent overnight is damaged.[31, 32] It is essential to determine the optimal duration of administration of these tablets to reduce or eliminate oral microorganisms without damaging the prosthesis. Therefore, in order to determine the most effective duration of tablets in our study, periods ranging from 3 to 200 minutes were studied. In light of the results of the present study, it was determined that the type of cleansing tablet and the application time were also crucial in the reduction or destruction of microorganisms. Tablet solutions significantly reduced the microorganisms on the resins during all their application times. This result also confirms the results from previous studies[6, 8, 26, 33] Both denture cleanser tablets were shown to exhibit high anticandidal activity overall base resins at application times of 10 minutes or more. As the duration of administration increased, cell viability tended to decrease. The cell viability of all resins was approximately 30% after 3 minutes of administration in both tablets, and after 200 minutes of administration, all resins had less than 10% cell viability. However, *C. albicans* could not be removed entirely. Denture cleanser tablets have an antimicrobial effect, but *C. albicans* biofilms show high antifungal resistance.[34, 35] This result confirms that *C. albicans* is a persistent infectious agent. In many studies, as in the present study, denture cleanser tablets have a significant reduction in cell viability of *C. albicans*, but not all microorganisms have been removed.[6-8, 10, 36]

According to the results of the present study, it was found that the type of tablet was as crucial as the application period of the tablets in the reduction of microorganisms. After 3 minutes exposure of the resins to the alkaline peroxide tablet solution, cell viability percentages were determined as high impact resin-autopolymerized resin-conventional heat cured resin-polyamide resin from small to large. After 3 minutes exposure of the resins to the enzymatic tablet solution, cell viability percentages were determined as autopolymerized resin-conventional heat cured resin-high impact resin-polyamide from small to large.

It was determined that the alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet. These results showed that the antimicrobial activity of denture cleanser tablets varied according to the resin type. Several studies have been conducted to evaluate the efficacy of denture cleanser tablets in the removal of *C. albicans* on denture base resins.[8-10] As in the present study, Hayran et al. reported that the antimicrobial activity of the alkaline peroxide tablet on high impact resin and polyamide resin was higher than the enzymatic tablet.[10] Fernandes et al. reported that the alkaline peroxide tablet was more effective on the conventional heat cured PMMA based resin and the enzymatic tablet was more effective on the polyamide-based resin.[22] In another study, it was emphasized that enzymatic tablet was more effective on conventional heat cured PMMA based resins than polyamide based resins.[8] However, it is not possible to compare the other studies directly with this study because of the variety of the resins studied, the different types of tablets used, the varying application times and the selection of different methods in the evaluation of cell viability.

This study showed that both alkaline peroxide and neutral enzymatic denture cleanser tablets exhibit potent antimicrobial activity over all the prosthetic base resins studied and for all selected application times. Cell viability tended to decrease as the application time increased from 3 minutes to 200 minutes. However, complete inhibition of *C. albicans* biofilm formed on all resins for both tablets could not be achieved within any time. Although *C. albicans* is a persistent infectious agent, observed death at least 65% of the cells on all resins in a short period such as 3 minutes indicates the antimicrobial efficacy of the tablets. The results also showed that cell viability varies depending on the resin type, tablet type and the duration of administration of the tablets.

CONCLUSIONS

It is an advantage for patients to perform the cleaning procedure of removable prosthesis in a short time without damaging the prosthesis. The results showed the significant anticandidal activity of alkaline peroxide and enzymatic denture cleanser tablets on all resins. As the duration of administration of both tablets increased, a tendency to decrease in cell viability was observed on all resins. Cell death was at least 65% on the resins even at the 3rd minute. The administration of both tablets on all resins for 10 min resulted in approximately 80% cell death. However, the most significant antimicrobial activities of the tablets were determined at 20th minutes. Keeping the prostheses in a tablet solution for more than 20 minutes did not cause significant changes in cell viability. The alkaline peroxide tablet on all base resins for application periods of 3 to 200 minutes was generally more effective than the enzymatic tablet.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

Protez Kaide Rezinleri Üzerindeki Candida Albicans'ın Uzaklaştırılmasında Protez Temizleyici Tabletlerin Etkin Sürelerinin Belirlenmesi

ÖZ

Amaç: *Bu çalışmanın amacı, çeşitli protez kaide rezinleri üzerindeki Candida albicans'ın uzaklaştırılmasında farklı protez temizleyici tabletlerin etkin sürelerinin belirlenmesidir.* ***Gereç ve Yöntemler:*** *Çalışmada geleneksel ısıyla polimerize olan rezin, yüksek çarpma dayanıklı rezin, otopolimerize rezin ve poliamid rezin kullanıldı. Her rezin tipi için 160 numune hazırlandı. C. albicans'ın biyofilmi rezinler üzerinde oluşturuldu ve daha sonra 3-5-10-20-40-80-160-200 dakika boyunca alkalin peroksit tablet ve enzimatik tablete maruz bırakıldı. Hücre canlılığı MTT testi ile değerlendirildi. İstatistiksel analiz için 3 yönlü ANOVA kullanıldı.* ***Bulgular:*** *Rezin tipi, tablet tipi ve uygulama süresinin rezinler üzerindeki C. albicans'ın hücre canlılığı üzerine etkisi anlamlı bulundu (p<0,05). Minimum sürede bile rezinlerde en az %65 hücre ölümü meydana geldi. Bununla birlikte, her iki tabletin uygulama süresi arttıkça, tüm rezinlerdeki hücre canlılığı azalma eğilimi gösterdi. Rezinlerin her iki tablete de 10 dakika maruz bırakılması tüm rezinlerde yaklaşık %80 hücre ölümüne neden oldu. Bununla birlikte, tabletlerin en önemli antimikrobiyal aktiviteleri 20. dakika da görülmüştür. Alkalin peroksit tablet, tüm protez kaide rezinleri üzerinde 3 ila 200 dakikalık uygulama süreleri için genel olarak enzimatik tabletten daha etkili olmuştur.* ***Sonuçlar:*** *Alkalin peroksit ve enzimatik temizleyici tabletler, tüm rezinler için kayda değer bir antikandidal aktivite göstermiştir. C. albicans enfeksiyonu riski taşıyan hastalar protezlerini temizleyici tabletlerde en az 20 dakika tutmalıdır. Bununla birlikte, antikandidal etki tablet süresinin uzaması ile artma eğilimindedir.* ***Anahtar Kelimeler****: Protez kaideleri, protez temizleyicileri, kandida albicans, hücre canlılığı.*

REFERENCES

1. Jagger DC, Harrison A, Jandt KD. The reinforcement of dentures. J Oral Rehabil 1999;26:185-94.

2. Rodford R. The development of high impact strength denture-base materials. J Dent 1986;14:214-7.

3. Yunus N, Rashid A, Azmi L, Abu-Hassan M. Some flexural properties of a nylon denture base polymer. J Oral Rehabil 2005;32:65-71.

4. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318-22.

5. Barbeau J, Seguin J, Goulet JP, de Koninck L, Avon SL, Lalonde B et al. Reassessing the presence of Candida albicans in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:51-9.

6. Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil 2002;29:300-4.

7. Lucena-Ferreira SC, Ricomini-Filho AP, Silva WJ, Cury JA, Cury AA. Influence of daily immersion in denture cleanser on multispecies biofilm. Clin Oral Investig 2014;18:2179-85.

8. Freitas-Fernandes FS, Cavalcanti YW, Ricomini Filho AP, Silva WJ, Del Bel Cury AA, Bertolini MM. Effect of daily use of an enzymatic denture cleanser on Candida albicans biofilms formed on polyamide and

poly(methyl methacrylate) resins: an in vitro study. J Prosthet Dent 2014;112:1349-55.

9. Ferreira MA, Pereira-Cenci T, Rodrigues de Vasconcelos LM, Rodrigues-Garcia RC, Del Bel Cury AA. Efficacy of denture cleansers on denture liners contaminated with Candida species. Clin Oral Investig 2009;13:237-42.

10. Hayran Y, Sarikaya I, Aydin A, Tekin YH. Determination of the effective anticandidal concentration of denture cleanser tablets on some denture base resins. J Appl Oral Sci 2018; 26:e20170077.

11. İnan H, Turgut S, Tamam E, Bağış B. Evaluation of microbial accumulation of different base materials. Cumhuriyet Dent J 2014;17:151-58.

12. Jagger DC, Al-Akhazam L, Harrison A, Rees JS. The effectiveness of seven denture cleansers on tea stain removal from PMMA acrylic resin. Int J Prosthodont 2002;15:549-52.

13. Nikawa H, Hamada T, Yamamoto T. Denture plaque-past and recent concerns. J Dent 1998;26:299-304.

14. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W. Interspecies interactions within oral microbial communities. Microbiol Mol Biol Rev 2007;71:653-70.

15. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of Candida species to plastic surfaces. Infec Immun 1985;50:97-101.

16. de Souza RF, de Freitas Oliveira Paranhos H, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. Cochrane Database Syst Rev 2009:CD007395.

17. Karakis D, Akay C, Oncul B, Rad AY, Dogan A. Effectiveness of disinfectants on the adherence of Candida albicans to denture base resins with different surface textures. J Oral Sci 2016;58:431-7.

18. Karaaslan F, Dikilitaş A, Yiğit U. Oral health status and associated factors in a subpopulation of Turkish patients. Cumhuriyet Dent J 2019;22:167-75.

19. Dolan TA, Gilbert GH, Duncan RP, Foerster U. Risk indicators of edentulism, partial tooth loss and prosthetic status among black and white middle-aged and older adults. Community Dent Oral Epidemiol 2001;29:329-40.

20. Ozkan YK, Ucankale M, Ozcan M, Uner N. Effect of denture adhesive on the micro-organisms in vivo. Gerodontology 2012;29:9-16.

21. 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.

22. de Freitas Fernandes FS, Pereira-Cenci T, da Silva WJ, Filho AP, Straioto FG, Del Bel Cury AA. Efficacy of denture cleansers on Candida spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent 2011;105:51-8.

23. Berger JC, Driscoll CF, Romberg E, Luo Q, Thompson G. Surface roughness of denture base acrylic resins after processing and after polishing. J Prosthodont.2006;15:180-6.

24. Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of Candida albicans to denture-base materials with different surface finishes. J Dent 1998;26:577-83.

25. Kawara M, Iwata Y, Iwasaki M, Komoda Y, Iida T, Asano T et al. Scratch test of thermoplastic denture base resins for non-metal clasp dentures. J Prosthodont Res 2014;58:35-40.

26. da Silva FC, Kimpara ET, Mancini MN, Balducci I, Jorge AO, Koga-Ito CY. 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.

27. Sesma N, Lagana DC, Morimoto S, Gil C. Effect of denture surface glazing on denture plaque formation. Braz Dent J 2005;16:129-34.

28. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med 1999;10:99-116.

29. Pereira-Cenci T, Cury AA, Cenci MS, Rodrigues-Garcia RC. In vitro Candida colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria. Int J Prosthodont 2007;20:308-10.

30. Nevzatoglu EU, Ozcan M, Kulak-Ozkan Y, Kadir T. Adherence of Candida albicans to denture base acrylics and silicone-based resilient liner materials with different surface finishes. Clin Oral Investig 2007;11:231-6.

31. Handa RK, Jagger DC, Vowles RW. Denture cleansers, soft lining materials and water temperature: what is the effect? Prim Dent Care 2008;15:53-8.

32. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials

caused by denture cleansers in vitro. J Oral Rehabil 2003;30:243-50.

33. Fernandes CB, Aquino DR, Franco GC, Cortelli SC, Costa FO, Cortelli JR. Do elderly edentulous patients with a history of periodontitis harbor periodontal pathogens? Clin Oral Implants Res 2010;21:618-23.

34. Mukherjee PK, Chandra J. Candida biofilm resistance. Drug Resist Updat 2004;7:301-9.

35. Ramage G, Saville SP, Wickes BL, Lopez-Ribot JL. Inhibition of Candida albicans biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol 2002;68:5459-63.

36. Hahnel S, Rosentritt M, Burgers R, Handel G, Lang R. Candida albicans biofilm formation on soft denture liners and efficacy of cleaning protocols. Gerodontology 2012;29:e383-91.