

## Efficacy of denture cleaners on the surface roughness and *Candida albicans* adherence of sealant agent coupled denture base materials

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This study investigated the effect of denture cleansers on the surface roughness and *Candida albicans* adherence of surface sealant agent coupled denture base resins. One hundred and twenty specimens were fabricated from 2 polymethyl methacrylate (PMMA) (Meliodent; Acron MC) and 1 polyamide (Deflex) denture base materials, coated with a sealant agent (Palaseal) and divided into 4 groups ( $n=10$ ) according to overnight cleaning procedures: distilled water (control), 5% sodium hypochlorite (NaOCl) and two different sodium perborate (Corega; Rapident). The surface roughness values were measured with a profilometer before ( $R_a0$ ) and after 90 days immersion in denture cleaners ( $R_a1$ ). Specimens were incubated with *Candida albicans* suspension and *Candida* colony-forming units (CFU) (Cfu/mm) were counted. Significant differences were found, between the  $R_a0$  and  $R_a1$  values of 5% NaOCl applied Acron MC, Deflex and also Rapident applied Deflex groups ( $p<0.05$ ). Denture cleaning procedures had no significant effects on the quantity of *Candida albicans*.

**Keywords:** Denture base material, Denture cleaners, Surface sealant agent, Surface roughness, *Candida albicans*

### INTRODUCTION

Polymethyl methacrylate (PMMA) is the most popular material in dentistry especially as a denture base material since 1937. PMMA has some advantages like ease of manipulation, repair and polish, low cost and acceptable esthetic properties. However, polymerization shrinkage, poor wear resistance, inadequate mechanical properties, and residual monomer content are the main limitations of the material<sup>1-3</sup>. Polyamide denture base material (nylon) that has been described in 1950, is an alternative to the acrylic resins in special situations such as repeated denture fracture and allergies<sup>3-4</sup>.

Surface roughness and surface free energy play a key role for the bacterial adhesion on to intra-oral hard surfaces and also for following dental plaque accumulation process<sup>5-6</sup>. It was reported in several studies<sup>5,7,8</sup> that rough surfaces are more inclined to bacterial adhesion and plaque accumulation than smooth surfaces. Dental restorations with rough surfaces are also more prone to staining and discoloration which may lead to reduced esthetics and acceptability of the restoration. Roughness average ( $R_a$ ) is a parameter which is universally recognized and the most used international parameter of roughness<sup>9</sup>. In recent studies, a threshold level of surface roughness ( $R_a=0.2$   $\mu\text{m}$ ) was indicated for the plaque accumulation. While no further reduction in the plaque accumulation was expected for the smooth surfaces below this

borderline, higher surface roughness resulted in the significant increase of bacterial retention and plaque accumulation<sup>10-12</sup>.

There are many types of polishing materials and techniques that are still available. Denture base materials have been traditionally polished by prepolishing with water and pumice and fine polishing with polishing paste or aluminum oxide particles containing polishing liquids, due to decrease the surface roughness at minimal level<sup>6</sup>. Alternatively, various chairside polishing kits and surface sealants have been developed to eliminate surface defects, irregularities, increase stain and wear resistance<sup>6,12,13</sup>. The success rates and longevity of these sealant agents are still vague.

For protecting the health of oral soft tissues and preventing denture stomatitis that especially induced by *Candida* species, removing the denture plaque by proper cleaning procedures is essential<sup>14,15</sup>. Dentures can be cleaned by mechanical and chemical methods and also by the combination of this two methods. Chemical cleaning method requires the immersion of dentures in variety of active agents such as enzymes, hypochlorite solutions, disinfectants, alkaline peroxides and diluted organic and inorganic acids<sup>15,16</sup>. However, it is important to know the adverse effects of this cleaners on both denture bases and coated sealant agent materials, in order to choose the most appropriate method. Therefore the aim of this study was to investigate the effect of different denture cleaners on the surface roughness and *Candida albicans* concentration values

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of sealant agent coupled denture base materials. The null hypothesis of this study was that the application of different denture cleaners or the type of denture base materials would not affect the surface roughness and *Candida* concentration values.

## MATERIALS AND METHODS

Two PMMA and one polyamide denture base materials, three denture cleansers and also one surface sealant agent were used in this study (Table 1). Forty disk-shaped (10×2 mm) specimens were prepared for each denture base material according to the manufacturers' instructions. Specimens were prepared as follows: (i) Meliodent-20 min/100°C in boiling water bath; (ii) Acron MC-3 min/500 W by microwave energy; (iii) Deflex-15 min/280°C by micro injection molding system. Before the polishing procedures, all specimens were ground-finished (100 revs/min, during 15 s) with 400 grit silicon carbide abrasive paper on a sanding machine (Phoenix Beta, Buehler) under water cooling. Then the specimens were coated with Palaseal surface sealant agent with a soft brush in an even thin layer in one direction avoiding air bubble formation. Twenty seconds later, the Palaseal coated specimens were polymerized for 90 s in a light polymerizing unit (Dentacolor XS, HeraeusKulzer). All specimens were ultrasonically cleaned in distilled water (Hygasonic, Dürr Dental) for 10 min, rinsed, and dried with air. The specimens of each base material group were then randomly divided into 4 subgroups (n=10) through the types of denture cleansers.

The surface roughness ( $R_a$ ) of each specimen was measured by using a contact profilometer (Perthometer M2, Mahr) before immersion in denture cleansers and

recorded as  $R_a0$ . Profilometer's resolution was 0.01  $\mu\text{m}$ , the interval (cut-off length) was 0.8 mm, transverse length was 5.5 mm and stylus speed was 1 mm/s. For each specimen 3 measurements were performed and the arithmetic mean values were used for the statistical analysis.

The denture base materials were then subjected to cleaning procedures. The specimens of control groups were immersed in a container with 200 mL of distilled water at room temperature. The test specimens were immersed in a container; with 200 mL of 5% sodium hypochlorite (NaOCl) solution; 200 mL distilled water with one Corega tablet; 200 mL distilled water with one Rapident tablet. Immersions were made to simulate daily hygiene routine for 3 months (90 days). Therefore to simulate nocturnal immersion (overnight) each 24 h corresponded to 3 immersions of 8 h per day. The cleaning solutions were changed three times a day. Each specimen was washed for 5 s and dried before immersed in a new solution<sup>17</sup>. Then the surface roughness measurements were repeated for each specimen and recorded as  $R_a1$ .

Prior to *Candida albicans* contamination and adhesion assay, each specimen was ultrasonically cleaned in distilled water (Hygasonic, Dürr Dental) for 10 min, rinsed and dried with oil-free air. *Candida albicans* (ATCC 90128) strain was cultured aerobically at 37 °C for 24 h on Sabouraud dextrose agar (SDA) (Oxoid). After 24 h of culture, *Candida albicans* strain was centrifuged (ThermoScientific SL8, Germany) at 6,000 rpm for 15 min and resultant cell pellets were washed three times with phosphate-buffered saline solution (PBS, pH 7.4) before being resuspended in PBS and adjusting to a final concentration of 0.5 MacFarland solution ( $1 \times 10^8$  cfu/mL). Each test specimen was

Table 1 Materials used in present study

Product	Code	Component	Manufacturer	Lot Number
Meliodent	HC	Conventional heat cure polymethyl methacrylate	Bayer UK	012373 12JUN170
Acron MC	MC	Microwave cure polymethyl methacrylate	GC	1212141 1112142
Deflex	TP	Polyamide	Nuxen	10616 CL 29
Palaseal	—	Methyl methacrylate, tris(2-hydroxyethyl)-isocyanurate-triacrylate, acrylizedepoxyoligomer, acrylates, acrylizedpolysiloxane	Heraeus-Kulzer	531
5% NaOCl	—	Sodium hypochlorite	Prime Dental	0507201-7
Corega	—	Potassium monopersulfate, sodium bicarbonate, sodium lauryl sulfoacetate, sodium perborate monohydrate, sodium polyphosphate	GlaxoSmithKline	HM2N
Rapident	—	Potassium persulfate, sodium carbonate, anhydrous citric acid, Sodium perborate monohydrate, sodium percarbonate copolymer, sodium lauryl sulfate, magnesium stearate, mint-apple flavor, sage-peppermint extract	L'Angelica	214507056

inoculated using 1 mL of *Candida albicans* suspension and incubated for 24 h at 37 °C in a horizontal shaker. After the adhesion phase, the nonadherent cells were removed from the specimen by washing three times with PBS. Subsequently specimens were transferred into 1 mL of PBS and vortexed for 60 s to resuspend all present microorganisms. 0.1 mL of this resuspensions were plated on Sabouraud dextrose agar and incubated aerobically in a horizontal shaker at 37°C for 48 h. After the incubation period, colonies were counted by the eye counting method and the logarithm of colony forming units (CFU) per millimeter (log cfu/mm) was then calculated<sup>18)</sup>.

Data were statistically analyzed (SPSS 20.0 V, SPSS, Chicago, IL, USA). Firstly, a normal distribution was obtained for variables by Levene's test of homogeneity.

Then the  $R_a$  and CFU results were separately analyzed by 2-way ANOVA test to evaluate the effects type of denture base material, cleaning procedure and their interactions. The mean  $R_a$  and CFU values were compared by Tukey's HSD multiple comparison test. The pairwise comparisons of  $R_{a0}$  and  $R_{a1}$  values were also compared by Independent Sample *t* test. Significance was evaluated at  $p < 0.05$  for all tests.

## RESULTS

According to the 2-way ANOVA results, while the type of denture base material and cleaning procedure were significant on CFU, these variables and their interactions also significant on  $R_a$  values ( $p < 0.05$ ) (Table 2). The mean  $R_{a0}$  and  $R_{a1}$  values and standard

Table 2 Two-way ANOVA results for comparison of surface roughness ( $R_a$ ) and *Candida* concentration values (cfu/mm)

Parameter	Source	SS	df	MS	F	<i>p</i>
$R_a$	Denture base material (A)	197,841,781.667	2	98,920,890.833	34.387	<0.001
	Cleaning procedure (B)	36,447,976.667	3	12,149,325.556	4.223	0.007
	A×B	107,631,518.333	6	17,938,586.389	6.236	<0.001
	Error	310,684,520.000	108	2,876,708.519	—	—
	Total	935,414,200.000	120	—	—	—
cfu/mm	A	0.115	2	0.057	7.864	0.001
	B	0.153	3	0.051	6.982	<0.001
	A×B	0.040	6	0.007	0.917	0.486
	Error	0.787	108	0.007	—	—
	Total	2.333	120	—	—	—

\* $p < 0.05$  indicates significant difference.

Table 3 Mean surface roughness values ( $\mu\text{m}$ ) and standard deviations at time 0 ( $R_{a0}$ ) and 90 days ( $R_{a1}$ ) for all groups

Denture Base Materials	Cleaning Procedure	$R_{a0}$			$R_{a1}$		
		Mean	SD	Sig	Mean	SD	Sig
HC	Cont	0.045	0.015	Aa	0.041	0.017	Aa
	NaOCI	0.112	0.167	Aa	0.122	0.049	Aa-c
	Corega	0.065	0.024	Aa	0.051	0.014	Aab
	Rapident	0.043	0.022	Aa	0.054	0.027	Aab
MC	Cont	0.055	0.040	Aa	0.047	0.018	Aab
	NaOCI	0.044	0.016	Aa	0.129	0.111	Ba-c
	Corega	0.067	0.018	Aa	0.062	0.048	Ab
	Rapident	0.079	0.047	Aa	0.143	0.135	Aa-c
TP	Cont	0.074	0.022	Aa	0.079	0.065	Aa-c
	NaOCI	0.084	0.027	Aa	0.172	0.120	Bbc
	Corega	0.063	0.027	Aa	0.121	0.102	Aa-c
	Rapident	0.066	0.053	Aa	0.197	0.147	Bc

\*The multiple comparisons of denture base material/cleaning procedure combination groups by Tukey HSD test were shown as small letters and pairwise comparisons (Independent Sample *t* test) among sinter groups shown as capital letters.

\*\*The values having same letters are not significantly different ( $p > 0.05$ ).

deviations (SD) of denture base material/cleaning procedure combination groups are shown in Table 3.

The  $R_a$  values (0.041 to 0.197  $\mu\text{m}$ ) of all test groups were smaller than plaque accumulation threshold level (0.20  $\mu\text{m}$ ). The Tukey's multiple comparisons showed

that there was no significant difference between the  $R_{a0}$  values of test groups ( $p>0.05$ ). When the same cleaning procedure applied denture base resins compared, significant differences was obtained between the HC and TP resin specimens that immersed in Rapident

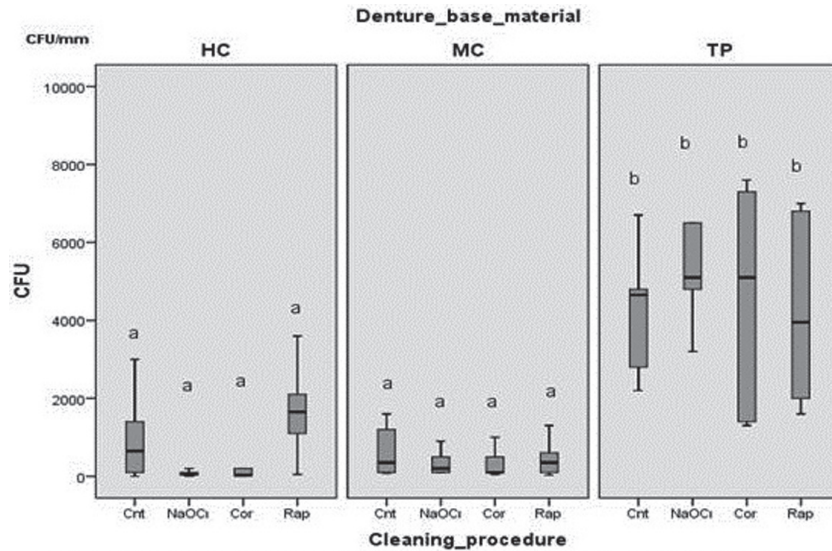


Fig. 1 Mean CFU values ( $\pm$ SD) of test groups. The multiple comparisons of denture base material/cleaning procedure combination groups by Tukey HSD test were shown as small letters.

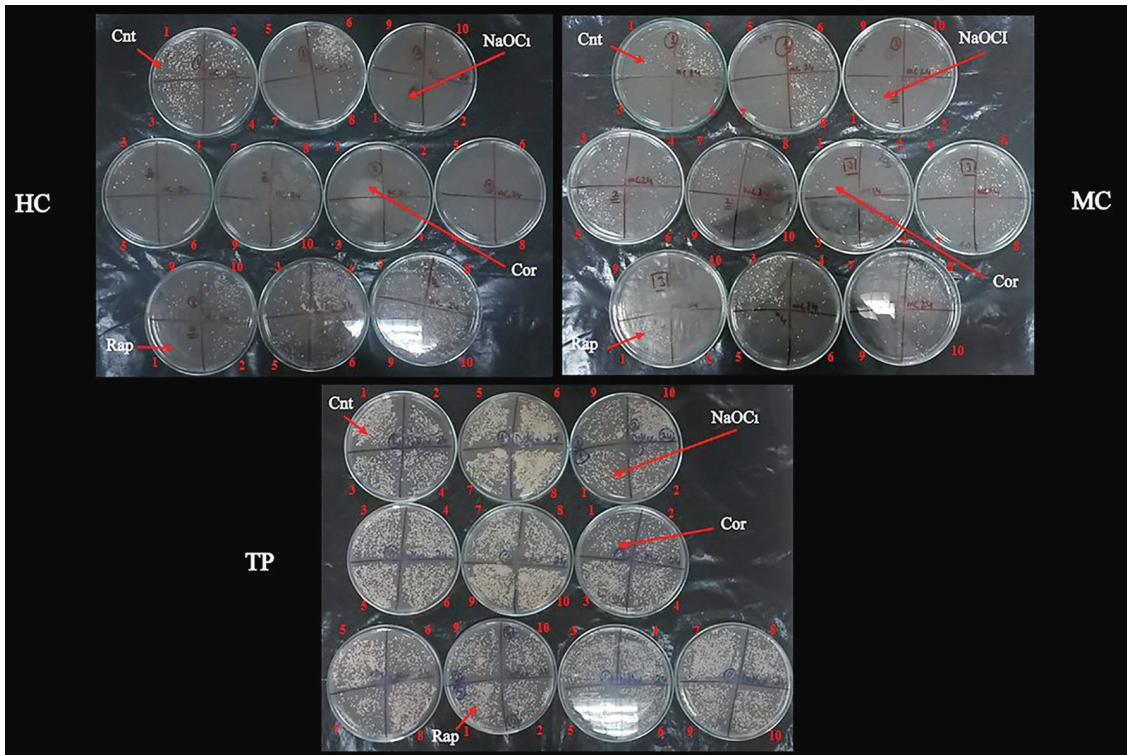


Fig. 2 *Candida albicans* colonies of denture cleansers applied HC, MC and TP denture base materials.

( $p=0.015$ ). The pairwise comparisons of  $R_a0$  and  $R_a1$  values was showed a significant increase for NaOCl applied MC ( $p=0.044$ ) and TP ( $p=0.04$ ) resin groups and Rapident applied TP ( $p=0.007$ ) resin group according to paired sample  $t$ -test.

The mean CFU values ( $\pm$ SD) of denture cleaners applied groups, which has incubated with *Candida albicans* suspension (ATCC 90128) for 24 h, are shown in Fig. 1. Statistically significant difference was observed between TP resin groups and the other test groups ( $p<0.05$ ). However there was no significant difference between different cleansing procedures applied denture base material groups ( $p>0.05$ ) (Fig. 2).

## DISCUSSION

The null hypothesis of this study was rejected. The type of denture base material and cleaning procedure were both significant on the surface roughness and *Candida* forming unit values.

The surface irregularities on denture base materials may act as a reservoir of infection and increase the possibility of hosting microorganisms even after the cleaning of dentures<sup>19</sup>. Rough surfaces make easier the penetration of bacterial and fungal cells on the denture base resins<sup>20</sup>. Coating the surface of the denture base material with sealant agents, which are recommended to improve surface smoothness of restorations through filling the micro-fissures and micro-defects, is an alternative application compared to conventional polishing with pumice and polishing pastes. However, surface sealant agents have some limitations, such as; low abrasion resistance, weak retention to the underlying material, exhibiting poor surface quality resulting from failure of spread that depends on probable high viscosity<sup>12,21,22</sup>. Palaseal surface sealant agent selected for this study is a light-cured sealer that adhere to all denture materials and has high resistance to solvents<sup>23</sup>. Sesma *et al.*<sup>24</sup> reported that sealant agent coupling (Palaseal) may improve the physical properties of dentures with smoother surface, decrease plaque adhesion and bacterial colonization, which results in good oral hygiene. Consistent with this results, no significant difference was found between the initial surface roughness values ( $R_a0$ ) of all tested denture base materials in present study, which had been coated with Palaseal sealant agent before. In addition, all of the  $R_a0$  values were lower than the threshold level of  $0.2 \mu\text{m}$ , indicated by Bollen *et al.*<sup>5</sup>.

Denture care is an important process that conduces to oral health and denture longevity<sup>16,17</sup>. Unclean dentures and poor oral hygiene are usually predisposing factors for *Candida* associated denture stomatitis<sup>14</sup>. Denture cleaning methods with soaking the dentures in a solution of some chemical agents, have advantages of efficient disinfection and easy usage when compared to the mechanical methods<sup>15,25</sup>. However it is an important criterion that the cleaners have no adverse effect on the physical and mechanical properties of denture base materials and artificial teeth while effective on removing

the organic and inorganic deposits; bactericidal and fungicidal<sup>26,27</sup>. In the present study, distilled water was determined as control since it is indicated for complete denture overnight immersions<sup>17,28</sup>. NaOCl solution that also used in the present study in the concentration of 5%, dissolves mucin, organic substances and shows antimicrobial activity by the action of hydroxyl ions and chloramination<sup>20,29</sup>. Several studies have reported satisfactory results for the antimicrobial activity of NaOCl solutions with different concentrations on denture base materials<sup>30–32</sup>. However, NaOCl has some disadvantages on denture base materials such as discoloration and an increase in surface roughness, depending on the concentration and immersion time<sup>17,33–35</sup>. It was also reported that NaOCl solutions may cause structural changes in the polymer matrix of acrylic resins. Thus consequently induce the deterioration on the surface layer and increase the roughness<sup>36,37</sup>. Consistent with this results, using 5% NaOCl denture cleaner was caused a significant increase on the surface roughness of the MC and TP denture base materials in the present study. However, 5% NaOCl also had no significant effect on the surface roughness of HC resin group disagreeing with the study results of Ma *et al.*<sup>38</sup>.

Alkaline peroxide effervescent denture cleansers may also be used for denture overnight immersions<sup>15,17</sup>. When an effervescent tablet dissolved in water, alkaline peroxide solution forms by the decomposition of sodium perborate. Oxygen bubbles that released from the solution enable the mechanical cleaning as well as chemical cleaning<sup>17,39</sup>. In the present study, while one of the tested sodium perborate (Corega) denture cleaner had no significant effect the surface roughness of base materials, the other one (Rapident) was significantly effected the surface roughness of polyamide denture base material ( $p<0.05$ ). Although both cleaning materials' active ingredient was sodium perborate, this result may be caused by the difference in the composition of the cleansers. The result for conventional heat-polymerized acrylic resin is in agreement with the study findings of Peracini *et al.*<sup>39</sup> and Paranhos *et al.*<sup>17</sup>.

Immersion times of the cleaning solutions that used in this study vary in different studies<sup>15,33,40</sup>. In the current study, the cleaning procedure simulated the nocturnal immersion (overnight) for 90 days. Dentists usually advise patients the overnight immersion, to remain their oral tissues in relax and to clean the dentures during sleeping period<sup>17</sup>.

*Candida albicans* has an ability to form biofilms on denture base materials and to cause denture-induced stomatitis<sup>41,42</sup>. This study evaluated the effectiveness of three denture cleaning agents on the surface characteristic and also adhesion of *Candida albicans*. Compared with HC and MC denture base material groups, the adherence and also quantity of *Candida albicans* was significantly higher on the TP denture base material groups. Several studies reported that the surface roughness can affect the microbial colonization of dentures<sup>19,41</sup>. In accordance with this results, surface

roughness values of TP resin groups were higher but not significant than other base material groups. In the current study, this situation may be responsible from the higher adherence ratios of TP denture base material groups. It was also resulted from present study that, denture cleaning procedures had no significant effect on the quantity of *Candida albicans*. This results may be depend on the fact that the surface roughness of all resin groups before and also after cleaning procedures were below the plaque accumulation threshold level of  $0.2 \mu\text{m}^5$ .

This study has some limitations. The specimens were polished by only one surface sealant agent. Under the same experimental conditions by using different sealant agent applications, denture cleaners and microorganisms, long term *in vivo* and *in vitro* investigations should be made.

### CONCLUSIONS

It was concluded from the results of the present study is that the sealant agent coupling may provide sufficient surface texture for all tested denture base materials. While the sodium perborate (Rapident) denture cleaner may be destructive for sealant agent coupled TP base material, the 5% NaOCl solution was for both MC and TP base materials. On the other hand, none of the denture cleaning procedures could significantly affect the quantity of *Candida albicans* on denture base materials.

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