



Original article

Interaction of *Candida Albicans* and *Streptococcus Mutans* with four different types of acrylic resin denture base materials Part (2)

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ABSTRACT

For denture base materials to be clinically accepted, they have to meet certain requirements such as superior mechanical and chemical properties, having a natural appearance, being easy to construct, easy to repair as well as biocompatibility and resistance to adhesion of microorganisms. Microbial adhesion to denture base materials may lead to oral diseases such as candidiasis. The present in-vitro study was aimed to assess the adherence of *Candida albicans* and *Streptococcus mutans* to four different types of acrylic dentures; Heat Cure (HC), High Impact Heat Cure (HIHC), Heat Cure Clear (HCC) and Clear Chemical Cure (CC).

Materials and Methods: 25 discs measuring 11 mm in diameter and 3 mm in thickness were fabricated for each type of acrylic resin. All samples were polished with different roughness parameters, including 600 and 1200 surface polishers. *Candida albicans* was cultured in Sabouraud dextrose broth (Sigma-Aldrich) while, *S. mutans* was cultured in a Columbia blood agar. They were then placed in an aerobic or CO₂ incubator for *Candida albicans* and *Streptococcus mutans* respectively at 37°C overnight. The absorbance of the crystal violet stain in the de-staining solution was measured. Subsequently, the samples were removed, fixed on a glass slide, and lastly viewed under the light microscope [Nikon (ECLIPS TS100)] at magnification 40x.

Results: The highest average absorbance of *C. albicans* was shown in HCC600 and HC1200. Whereas, there was no significant difference in the P-value of *C. albicans* growth on the different surfaces of acrylic resins. Regarding the adhesion of *Streptococcus mutans*, CC had much more average absorbance than the other three heat cure types. When these materials were compared by ANOVA single factor, the data statistically showed a significant difference in the capacity of attachment between heat cure and chemical cure.

Conclusion: The acrylic denture surface roughness by its nature has a large impact on the colonization of denture base, specifically by *Streptococcus mutans*.

Keywords: Microbial adhesion, *Candida albicans*, *Streptococcus mutans*, denture base, surface roughness.

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INTRODUCTION

The demand of patients to replace their missing teeth has dramatically increased. Despite the fact that implants are now commonly used as one of the major prosthetic devices for tooth replacement, dentures are still the most common choice of teeth replacement.¹ However, for denture base materials to be clinically accepted, they have to meet some requirements such as sufficient strength to withstand

the force of mastication, adequate durability, superior mechanical and chemical properties, natural appearance, good adhesion to metal, plastic, and porcelain, easy to construct as well as biocompatibility and resistance to adhesion of microorganisms.²

The dentures' tissue surface usually has microporosities and micropit areas that allow for the accumulation of microorganisms; rather than other areas in the dentures.³ There are many factors which

may affect surface roughness such as the material utilized, the polymerization technique as well as the fiber incorporation into the material.^{4,5} Also, many types of microorganisms adhere to the denture surfaces such as bacteria like *Streptococcus mutans* and fungi such as *Candida albicans* species, and nonalbicans species such as *Candida glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. dubliniensis*.⁶ However, *Candida albicans* is considered the most common microorganism that adheres to dentures. *Candida albicans* is a fungus present in the oral cavity of a healthy individual as a normal commensal organism. Under systemic and local factors, mainly poor oral hygiene, *C. albicans* becomes pathogenic leading to oral atrophic candidosis.⁷ Despite the fact that *Candida* species are the main pathogen of denture stomatitis, bacteria such as *Streptococcus mutans*, *Actinomyces species*, and *Fusobacterium species* are also involved in the denture biofilms.^{8,9,10} *Streptococcus mutans* was first described by Clark who isolated these bacteria from the carious lesion in 1924.¹¹ *Streptococcus mutans* is a spherical Gram-positive bacterium belonging to the lactic acid and the phylum Firmicutes groups of bacteria which has eight serotypes from A to H, and the most common serotypes isolated from the human plaque was C.^{11,12} This study aimed to assess the difference in adhesion of *Candida albicans* and *Streptococcus mutans* according to materials type (four different types of acrylic denture; heat cure, high impact heat cure, clear heat cure, and clear chemical cure) and according to the surface roughness of each type (two different surface roughness for each type) to detect which type of acrylic resin has the least adhesion of oral microorganisms.

MATERIALS AND METHODS:

Materials

This study is an experimental study design. 25 samples of each different type of acrylic resins measuring 11 mm in diameter and 3mm in thickness were made. All samples were polished by p600. Then, half of each type was polished again by grinding paper p1200.¹²

Microbial growth (*Streptococcus mutans* and *Candida albicans*)

Candida albicans was cultured in Sabouraud dextrose broth (Sigma-Aldrich) while *S. mutans* was cultured in a Columbia blood agar; they were then placed in an aerobic or CO₂ incubator for *Candida albicans* and *Streptococcus mutans* respectively at 37°C overnight.

Measuring absorbance of crystal violet stain in the destaining solution

To assess the absorbance of crystal violet stain in the destaining solution, three experiments for each microorganism; *Streptococcus mutans* and *Candida albicans* were carried out. For each experiment, a couple of colonies of *S. mutans* and *C. albicans* were obtained from the Department of Microbiology, University of Sheffield, UK. They were placed in separate bottles containing Brain Heart Infusion (BHI) and yeast nutrient broth for *S. mutans* and *C. albicans* respectively, and then they were placed in an incubator for 24 hours. Furthermore, 4 samples of each type (3 for the growth of microorganisms and one as standard (blank)) were used; they were placed in an autoclave overnight to be ready for the growth of the microorganism. The samples were removed by forceps and placed in sterile plates, then one ml of the microbial suspension of (optical density) OD 0.05 was added to 3 wells, whereas the BHI was added without microorganisms to the one which was used as a standard; afterward, the plates were stored in an aerobic or CO₂ incubator for *Candida albicans* and *Streptococcus mutans* respectively at 37°C. The microbial suspension and BHI were removed, and the acrylic samples were gently transferred to a fresh plate. In the next phase, 200 µl of phosphate-buffered saline was used to wash the biofilm-coated wells of microtiter plates, then they were left to dry for 45 min. Following that, 0.4% aqueous crystal violet solution (200 µl) was added to stain each of the washed wells for 45 min. Subsequently, each well was washed three times with 350 µl of sterile distilled water and destained with 200 µl of 95% ethanol immediately. They remained there for 45 min. Afterward, 100 µl of the destaining solution was transferred to a new well and the amount of absorbance was assessed with a microtiter plate reader [FLUO Star Galaxy (2000 BMG Lab technologies)] at 570 nm.

Assessment the microbial growth by using a light microscope

After the experiment was over, the samples were removed, fixed on a glass slide, and then viewed under the light microscope [Nikon (ECLIPS TS100)] magnification 40x. In the end, pictures of 40x magnification by using (COOLPIX P5100) were taken.

Statistical analysis

For data statistical analysis, ANOVA single factor was utilized to compare the adherence of the microorganisms on four different types of acrylic denture base materials; a P-value of 0.05 or less was considered significant.

RESULTS

Assessing the adhesion of oral microorganisms in different surface roughness

To examine the growth of microorganisms (*Candida albicans* and *Streptococcus mutans*) on different types of acrylic resins, 100 µl of the destaining solution was measured with a microtiter plate reader [(FLUO Star Galaxy (2000 BMG Lab technologies))] at 570 nm.

Assessing the growth of *Candida albicans*

The average absorbance of *Candida albicans* growth (the blank was subtracted) on different types of acrylic resins are displayed on the chart Figure (1). It showed that HCC600 has the highest average absorbance of *C. albicans*, which was approximately 1.2. Chemical cure 600 (CC600) had the second-highest average, then HIHC600 came afterward whereas, HC600 had the lowest average absorbance which was about 2/3 as high as HCC600. In contrast, one can see that HCC1200 and HC1200 had the opposite average absorbance; it was as great as

HCC600 and HC600. HC1200 was about twice as high as HCC1200 while, there was no significant difference in the average absorbance of HIHC1200 and CC1200 as compared to 600 surface roughness. Furthermore, the Standard deviation of *Candida albicans* growth on four acrylic resins with different surface roughness was calculated (Table 1). In addition, the P-value of *C. albicans* growth on each type of acrylic resins that has different surface roughness was statistically analyzed (Table 1). The P-value of the different materials with the same surface roughness are compared to each other as illustrated in Tables (2). The ANOVA single factor test revealed that the P-value of the *Candida albicans* adhesion on the different surfaces of acrylic resins was not significant; this is evident when the same materials were compared with different roughness or when different materials were compared with the same roughness (Tables 1, 2)

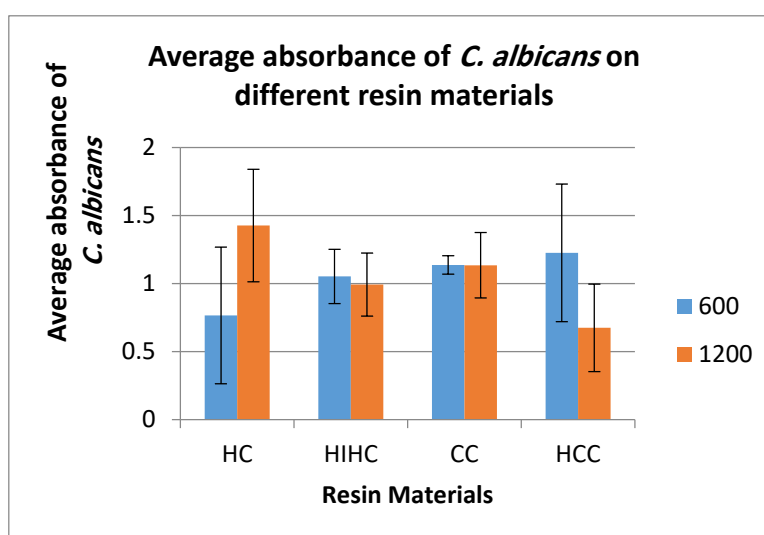


Figure (1): The chart demonstrates the average absorbance of *Candida albicans* on the different types of dentures with surface roughness 600 and 1200 as compared to the blank (the blank was subtracted).

Table (1): The Mean, Standard deviation, and P-value of *Candida albicans* growth on four acrylic resins with different surface roughness.

Materials	Surface roughness	Mean ± Standard deviation (<i>C. albicans</i>)	P-value
HC	600	0.765±0.502	0.153
	1200	1.4268±0.413	
HIHC	600	1.0527±0.1993	0.754
	1200	0.9935±0.231	
CC	600	1.137±0.067	0.989
	1200	1.135±0.240	
HCC	600	1.226±0.506	0.1869
	1200	0.675±0.3216	

Table (2): The comparison between P values of *C. albicans* growth on different acrylic dentures with surface roughness 600 and 1200

Materials at surface roughness 600	Mean $\pm$ Standard deviation	P-value	Materials at surface roughness 1200	Mean $\pm$ Standard deviation	P-value
Heat cure (HC)	0.765 $\pm$ 0.502	0.409	HC	1.4268 $\pm$ 0.413	0.188
High impact heat cure (HIHC)	1.0527 $\pm$ 0.1993	(NS)	HIHC	0.9935 $\pm$ 0.231	(NS)
Heat cure (HC)	0.765 $\pm$ 0.502	0.2734	HC	1.4268 $\pm$ 0.413	0.349
Chemical cure (CC)	1.137 $\pm$ 0.067	(NS)	CC	1.135 $\pm$ 0.240	(NS)
Heat cure (HC)	0.765 $\pm$ 0.502	0.326	HC	1.4268 $\pm$ 0.413	0.06
Heat cure clear (HCC)	1.226 $\pm$ 0.506	(NS)	HCC	0.675 $\pm$ 0.321	(NS)
High impact heat cure (HIHC)	1.0527 $\pm$ 0.1993	0.526	HIHC	0.9935 $\pm$ 0.231	0.53
Chemical cure (CC)	1.137 $\pm$ 0.067	(NS)	CC	1.135 $\pm$ 0.240	(NS)
High impact heat cure (HIHC)	1.0527 $\pm$ 0.1993	0.61	HIHC	0.9935 $\pm$ 0.231	0.236
Heat cure clear (HCC)	1.226 $\pm$ 0.506	(NS)	HCC	0.675 $\pm$ 0.321	(NS)
Chemical cure (CC)	1.137 $\pm$ 0.067	0.777	CC	1.135 $\pm$ 0.240	0.118
Heat cure clear (HCC)	1.226 $\pm$ 0.506	(NS)	HCC	0.675 $\pm$ 0.321	(NS)

Assessing the growth of *S. mutans*

The result was illustrated in Figure (2). Chemical cure acrylic resin in both CC600 and CC1200 exhibit a greater amount of average absorbance, which constituted (0.857733, 0.962867) respectively, than the heat cure (HC, HIHC, and HCC). HC and HCC reveal reverse absorbance in 600 and 1200 surface roughness. For instance, HC600 had the lowest average absorbance which was virtually 1/5 HC1200 whereas, HCC1200 had the least absorbance, which was approximately 1/3 as high as HCC 600. Furthermore, HIHC1200 has increased by double as compared with HIHC 600. In addition, the Standard

deviation of *S. mutans* growth on four types of acrylic resins which have different surface roughness was calculated in Table (3). The P-value of *S. mutans* growth on each type of acrylic resins that has different surface roughness was statistically analyzed (Table 3). The P-value of the different materials with the same surface roughness are compared to each other as illustrated in Tables (4). The ANOVA single factor test revealed that the P-value of *S. mutans* was significant solely when comparing chemical cure denture base (CC) with three other different types of heat cure in both 600 and 1200 surface roughness (Tables 4). Therefore, the Chemical cure had the highest adhesion of *Streptococcus mutans*.

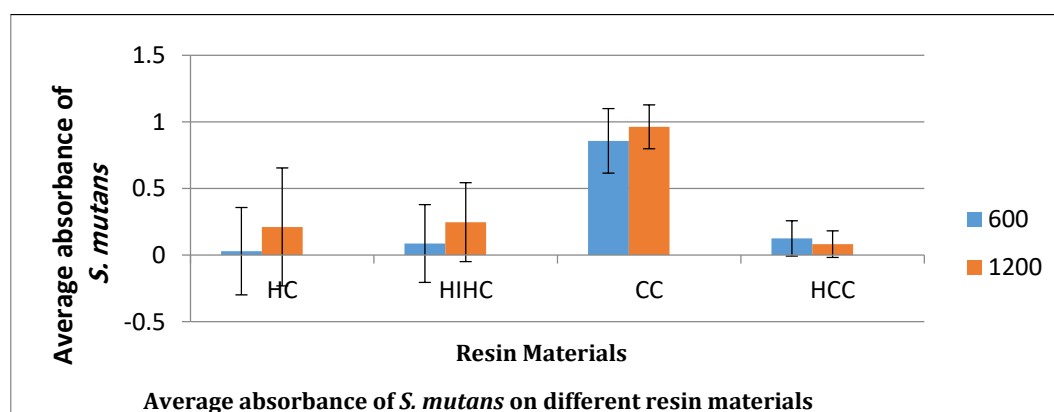
**Figure (2):** The average absorbance of *Streptococcus mutans* on different types of dentures with surface roughness 600 and 1200 as compared to the control group (blank was subtracted).

Table (3): The Mean, Standard deviation, and P-value of *Streptococcus mutans* growth on four types of acrylic resins which have different surface roughness.

Materials	Surface roughness	Mean $\pm$ Standard deviation (<i>S. mutans</i>)	P-value
HC	600	0.0294 $\pm$ 0.327	0.598
	1200	0.211 $\pm$ 0.443	
HIHC	600	0.0869 $\pm$ 0.291	0.54
	1200	0.247 $\pm$ 0.296	
CC	600	0.8577 $\pm$ 0.2422	0.567
	1200	0.96 $\pm$ 0.164	
HCC	600	0.125 $\pm$ 0.132	0.679
	1200	0.0825 $\pm$ 0.099	

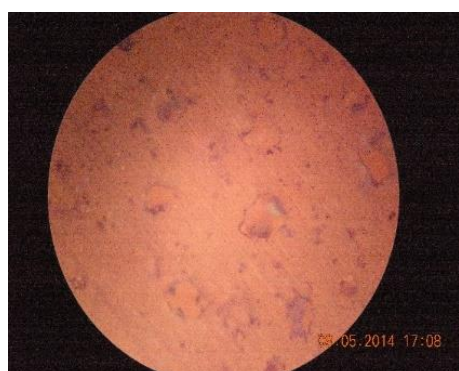
Table (4): Comparison between P-value of *S. mutans* growth on different acrylic dentures with surface roughness 600 and 1200

Materials at surface roughness 600	Mean $\pm$ Standard deviation	P-value	Materials at surface roughness 1200	Mean $\pm$ Standard deviation	P-value
Heat cure (HC)	0.0294 $\pm$ 0.327	0.831	HC	0.211 $\pm$ 0.443	0.912
High impact heat cure (HIHC)	0.0869 $\pm$ 0.291	(NS)	HIHC	0.247 $\pm$ 0.296	(NS)
Heat cure (HC)	0.0294 $\pm$ 0.327	0.024	HC	0.211 $\pm$ 0.443	0.05 (S)
Chemical cure (CC)	0.8577 $\pm$ 0.2422	(S)	CC	0.96 $\pm$ 0.164	
Heat cure (HC)	0.0294 $\pm$ 0.327	0.6638	HC	0.211 $\pm$ 0.443	0.649
Heat cure clear (HCC)	0.125 $\pm$ 0.132	(NS)	HCC	0.0825 $\pm$ 0.099	(NS)
High impact heat cure (HIHC)	0.0869 $\pm$ 0.291	0.024	HIHC	0.247 $\pm$ 0.296	0.021
Chemical cure (CC)	0.8577 $\pm$ 0.2422	(S)	CC	0.96 $\pm$ 0.164	(S)
High impact heat cure (HIHC)	0.0869 $\pm$ 0.291	0.84	HIHC	0.247 $\pm$ 0.296	0.412
Heat cure clear (HCC)	0.125 $\pm$ 0.132	(NS)	HCC	0.0825 $\pm$ 0.099	(NS)
Chemical cure (CC)	0.8577 $\pm$ 0.2422	0.010	CC	0.96 $\pm$ 0.164	0.001
Heat cure clear (HCC)	0.125 $\pm$ 0.132	(S)	HCC	0.0825 $\pm$ 0.099	(S)

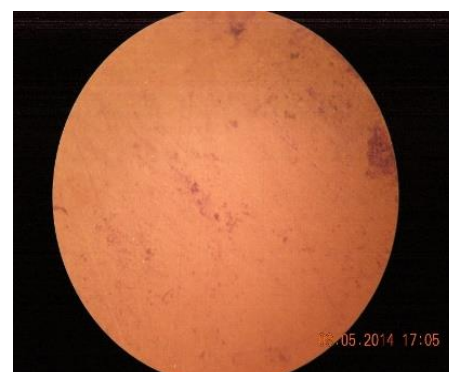
Visualizing the growth of microorganisms

The microbial growths were exhibited by using light microscopes at 40x magnifications. The growth of

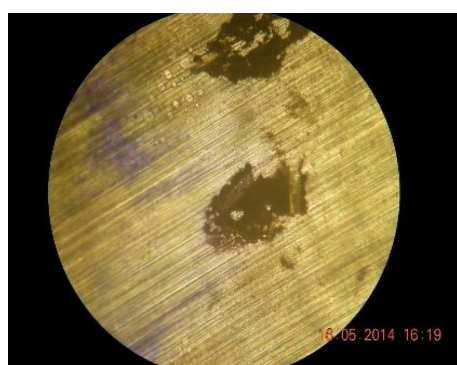
Candida albicans on both HIHC600 and HC600 and the growth of *Streptococcus mutans* which appear as clumps on chemical cure as compared to the blank were exhibited in Figure 3 (A-D).



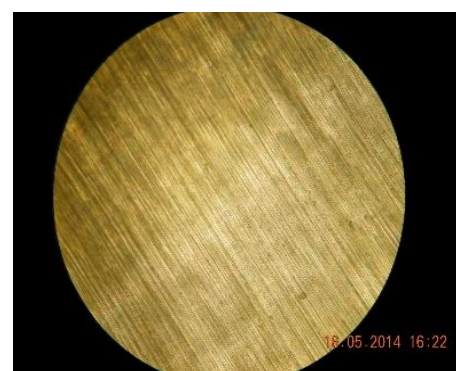
A. Growth of *candida albicans* on HC600



B. Growth of *candida albicans* on HC600



C. Growth of *streptococcus*



D. Blank chemical cure

Figure 3 (A-D): Figures (A-C) demonstrated the growth of microorganisms, whereas Figure D showed the blank surface

DISCUSSION

In several research studies concerning the *C. albicans* and *S. mutans* adhesion mechanisms to the acrylic resins denture base, the material types and surface roughness of the materials, are considered as major factors that play a major role in the direct adherence mechanism.¹³ However, understanding the exact attachment mechanism of *C. albicans* has yet to be identified.⁷ According to Anusavice (2003), the decrease in the surface roughness of the denture, results in a decrease in the friction which in turn reduces the abrasion impact on the soft tissue of the patient. Moreover, the study indicates that the high rough surface results in an increase in the stain as well as in the adhesion of the microorganisms on the surface.¹⁴ However, *C. Albicans* adherence to acrylics resins denture base, and to the subsequent formation of biofilm, is considered as a significant factor in denture-induced stomatitis development.¹⁵

Regarding the attachment of *Candida albicans* (in vitro) on acrylic resins, there was no difference between the average absorbance (optical density) of HIHC and CC in the different surface roughness (600,1200), whereas HC and HCC revealed reverse profiles. However, even though the P-value of the average surface roughness of HIHC, CC, and HCC was statistically significant, there was no difference in the adhesion of *Candida albicans* between these three materials and insignificantly, HC. In other words, the result concerning the adhesion of *C. albicans* especially to chemical cure material is contrary to what to have been expected according to the profilometry. A previous study which was undertaken by Radford et al (1999) demonstrated that fewer *C. albicans* was observed on the smooth surface rather than on the rough surfaces.¹⁶ Also, another study demonstrated the colonization of *C. albicans* on the denture surface.¹⁷ The reason for the difference between the current study and the

previous one was probably that this study was carried out under different conditions.¹⁶

The presence of *Candida* species, within the oral cavity, adhesion to the oral mucosa, and biofilms development on the surface of the denture are associated with mild to severe physio-pathological effects. *Candida*-induced stomatitis has a certain range according to the classifications of Newton.¹⁸ This infection is caused by *C. albicans*' cell attachment to the denture impression surface, which depends on the non-specific factors such as the surface charge and hydrophobicity which are related to the materials, and the specific factors (receptor-ligand binding) which are related to the microorganisms.^{19,20,21} Additionally, the chemical and physical compositions of the acrylic denture base have a positive effect on the adhesion and colonization of the yeast.

Various chemical materials may affect the *Candida* attachment level. It has been shown that the immersion of acrylic resin base in water increases *Candida* adhesion by reducing the level of residual monomer.²² Previous clinical studies have shown that there is a close relation between denture hygiene procedures frequency and the *Candida* infection.²³ Therefore, those patients are exposed to more risks concerning denture sore mouth than other people. Furthermore, as noted by Verran and Motteram (1987), *Candida albicans* cannot be attached to the denture base materials that have not been already preincubated with streptococcus.²⁴ Likewise, Branting et al (1989) outlined that *C. albicans*' adhesion to the acrylic resins was increased when *S. mutans* was incubated on the acrylic dentures.²⁵

On the other hand, the interaction of *Streptococcus mutans* on the different materials, there was no significant difference concerning the average absorbance between 600 and 1200 surface polishers. It can be seen that Chemical cure (CC) had by far the highest absorbance compared to the other three heat cure types. The findings are similar to those of the previous study which is undertaken by Morgan and Wilson (2001)²⁶ who demonstrated that the adhesion of the chemical cure was colonized by a high amount of bacteria (*Streptococcus oralis*) as compared to that of the heat cure. The reason for the difference in the attachment of *Streptococcus mutans* on the various types was probably attributed to the difference in the processing conditions used, resulting in dissimilarity in the nature and porosity of the surface of both heat cure and chemical cure materials.²⁶ Furthermore, the chemical cure denture base materials exhibit higher surface irregularities that have lower strength compared with the conventional heat cure materials because of the difference in the physical nature.^{27,28}

The formation of plaque is based on the microorganism's retention capacity, and therefore on acquired pellicle cohesive strength. The non-specific properties of substratum, especially hydrophobicity,

have the highest effect.²⁹ The non-specific adhesion of the bacteria in low shear stress environments is affected mainly by substratum hydrophobicity.³⁰ In general, hydrophilic substrata were preferred to bacteria with hydrophilic surfaces, and the hydrophobic substrata were preferred to bacteria with hydrophobic surfaces.³¹ Regarding *Streptococci*, the strain hydrophobicity was reduced dramatically, resulting in a loss in its properties of adhesion, when it was sub-cultured in vitro.³² Another factor that affects the attachment of *Streptococci* is the surface charge. Concerning surface charge, bacteria are invariably negatively charged in an aqueous environment like human saliva.³³ Even though high surface energy is usually characterized by hydrophilic bacteria, the bacteria being hydrophobic may have these properties.³⁴ In addition to the hydrophobicity and surface charge, Surface Free Energy (SFE) is another factor that affects the adhesion of bacteria. In most cases, the higher the substratum surface free energy, the more the colonization of bacteria will be.³⁵

However, it can be seen that there is a difference regarding the adherence between *C. albicans* and *S. mutans*. The reason could be that the surface characteristics of the bacterial cell are different from the fungal ones. In other words, the bacterial cell is smaller in size as compared to the yeast; therefore, they behave differently.³⁶ Also, extracellular polymers can play another key factor. In comparison, the fungal and bacterial biofilms, the bacterial biofilm extracellular polymers have lower levels of galactose and glucose and higher carbohydrate and protein content.⁷ However, this study does not simulate the oral environment (in-vitro study). Furthermore, the fitting surface of the denture is not polished; therefore, it would be better to study the adhesion of microorganisms on the surface without any polishing.

CONCLUSIONS

Within the limitation of this study, the study showed the highest average absorbance of *C. albicans* in HCC600 and HC1200 whereas there was no significant difference in the P-value of *C. albicans* growth on the different surfaces of acrylic resins. Regarding the adhesion of *Streptococcus mutans*, CC had much more average absorbance than the other three heat cure types. When these materials were compared by ANOVA single factor, the data showed a statistically significant difference in the capacity of attachment between heat cure and chemical cure. Thus, according to this study, the acrylic denture surface roughness by its nature has a large impact on the colonization of denture base by *Streptococcus mutans* only.

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