Original article

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

Salma A. Elnaili[1], Elnaihoum W. A[2], and Saied H. Alabidi[3]

[1, 2] Assistant lecturer, Department of prosthodontics, Faculty of Dentistry, University of Benghazi, Libya
[3] Professor, Department of prosthodontics, Faculty of Dentistry, University of Benghazi, Libya

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 37ºC 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 mutants*, 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 mutants*.

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

Corresponding author:
Salma A. Elnaili: Department of prosthodontics, Faculty of Dentistry, University of Benghazi, Libya
Email: salma82_2012@yahoo.com

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 CO_2 incubator for *Candida albicans* and *Streptococcus mutans* respectively at 37ºC 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 CO_2 incubator for *Candida albicans* and *Streptococcus mutans* respectively at 37ºC. 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)

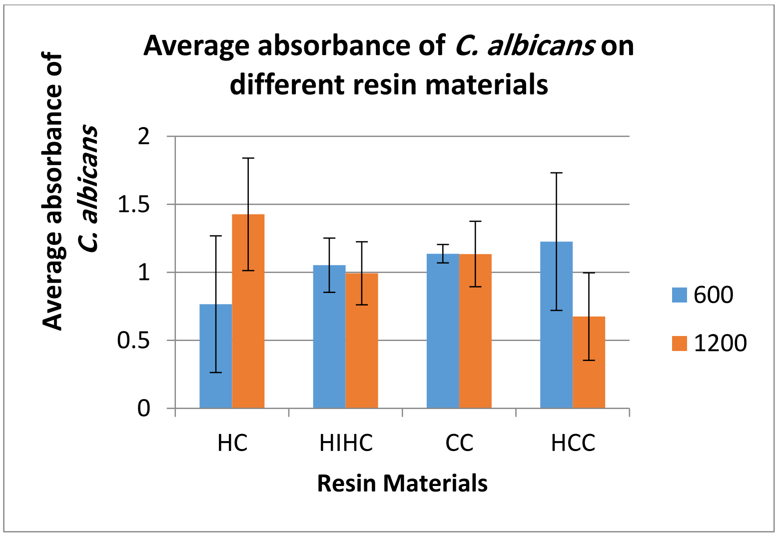


Figure (1): The chart demonstrates the average absorbance of *Candida albicans* on the different types of dentures with surface roughness 600 and 1200 as compared to the blank (the blank was subtracted).

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 (*C. albicans*)	P-value
HC	600 1200	0.765±0.502 1.4268±0.413	0.153
HIHC	600 1200	1.0527±0.1993 0.9935±0.231	0.754
CC	600 1200	1.137±0.067 1.135±0.240	0.989
HCC	600 1200	1.226±0.506 0.675±0.3216	0.1869

Table (2): The comparison between P values of *C. albicans* growth on different acrylic dentures with surface roughness 600 and1200

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

Assessing the growth of *S. mutants*

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*.

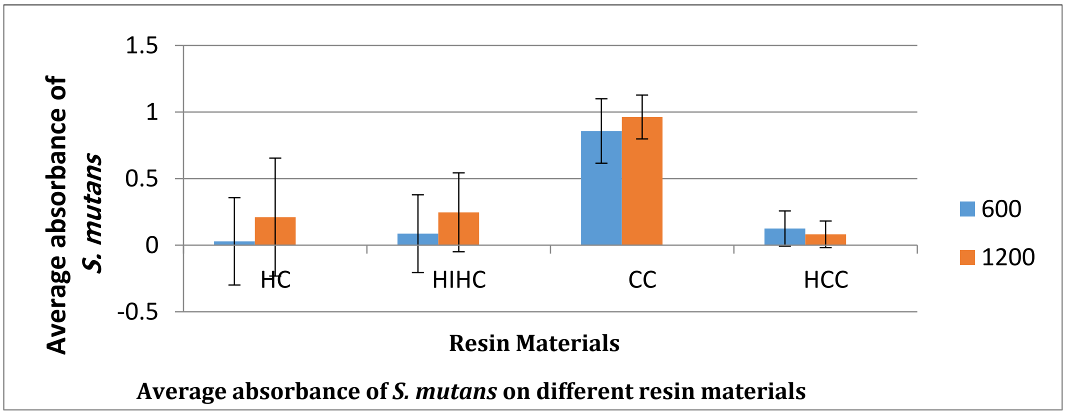


Figure (2): The average absorbance of *Streptococcus mutans* on different types of dentures with surface roughness 600 and 1200 as compared to the control group (blank was subtracted).

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

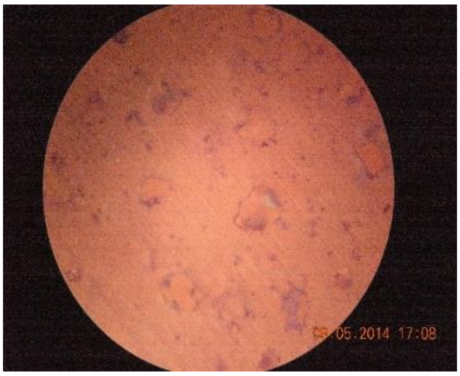
Materials	Surface roughness	Mean ± Standard deviation (*S. mutans)*	P-value
HC	600 1200	0.0294±0.327 0.211±0.443	0.598
HIHC	600 1200	0.0869±0.291 0.247±0.296	0.54
CC	600 1200	0.8577±0.2422 0.96±0.164	0.567
HCC	600 1200	0.125±0.132 0.0825±0.099	0.679

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

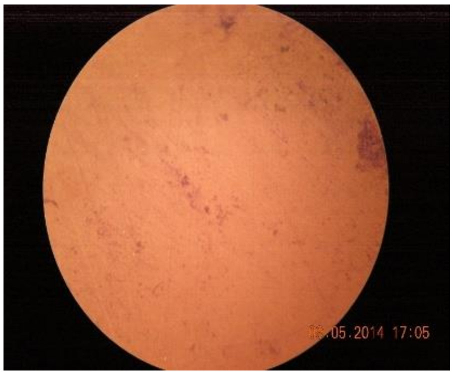
Materials at surface roughness 600	Mean ± Standard deviation	P-value	Materials at surface roughness 1200	Mean ± Standard deviation	P-value
Heat cure (HC) **High impact heat cure (HIHC)**	0.0294±0.327 0.0869±0.291	0.831 (NS)	**HC** **HIHC**	0.211±0.443 0.247±0.296	0.912 (NS)
Heat cure (HC) **Chemical cure (CC)**	0.0294±0.327 0.8577±0.2422	**0.024** **(S)**	**HC** **CC**	0.211±0.443 0.96±0.164	**0.05 (S)**
Heat cure (HC) **Heat cure clear (HCC)**	0.0294±0.327 0.125±0.132	0.6638 (NS)	**HC** **HCC**	0.211±0.443 0.0825±0.099	0.649 (NS)
High impact heat cure (HIHC) **Chemical cure (CC)**	0.0869±0.291 0.8577±0.2422	**0.024** **(S)**	**HIHC** **CC**	0.247±0.296 0.96±0.164	**0.021** **(S)**
High impact heat cure (HIHC) **Heat cure clear (HCC)**	0.0869±0.291 0.125±0.132	0.84 (NS)	**HIHC** **HCC**	0.247±0.296 00.0825±0.099	0.412 (NS)
Chemical cure (CC) **Heat cure clear (HCC)**	0.8577±0.2422 0.125±0.132	**0.010** **(S)**	**CC** **HCC**	0.96±0.164 0.0825±0.099	**0.001** **(S)**

Visualizing the growth of microorganisms

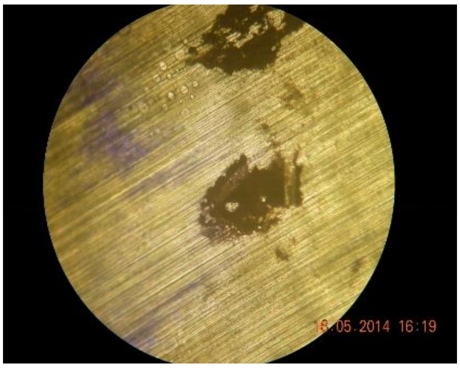
The microbial growths were exhibited by using light microscopes at 40x magnifications. The growth of *Candida albicans* on both HIHC600 and HC600 and the growth of *Streptococcus mutans* which appear as clumps on chemical cure as compared to the blank were exhibited in Figure 3 (A-D).



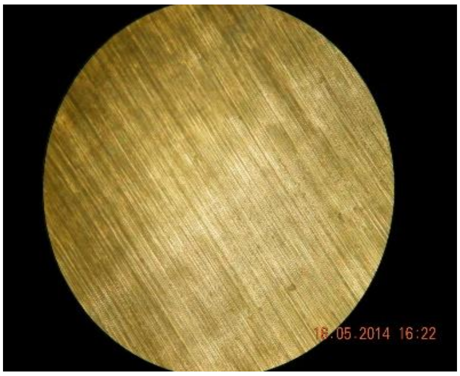
A. Growth of *candida albicans* on HC600



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. albican's* 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 muatns* 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 mutants*, 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 mutants only.

REFERENCES

1) Phillips RW, Anusavice KJ, Shen C, Rawls HR. Phillips' science of dental materials. Elsevier/Saunders; 2013.
2) Craig, R. G., Powers, J.M. Restorative Dental Materials. St. Louis: Mosby; 2002.
3) Charman KM, Fernandez P, Loewy Z, Middleton AM. Attachment of Streptococcus oralis on acrylic substrates of varying roughness. Lett appl microbiol. 2009 Apr;48(4):472-7.
4) Karaagaclioglu L, Can G, Yilmaz B, Ayhan N, Semiz O, Levent H. The adherence of Candida albicans to acrylic resin reinforced with different fibers. J Mater Sci: Mater Med. 2008 Feb;19(2):959-63.
5) Pereira T, Del Bel 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 Prosth. 2007 May 1;20(3).
6) Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. Candida-associated denture stomatitis. Med Oral Patol Oral Cir Bucal. 2011;16(2):e139-43.
7) Ebrahimi Saravi M, Zomorodian K, Vojdani M, Sattari M. Comparison of candidal and bacterial adherence to denture base acrylic resins. J Islam Dent Assoc Iran. 2013 Jul 10;25(3):169-74.
8) Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: Candida albicans and bacteria. FEMS microbiol. 2009 Oct 1;299(1):1-8.
9) Bamford CV, d'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF. StreptococcusGordoniii modulates Candida albicans biofilm formation through intergeneric communication. Infect immun. 2009 Sep;77(9):3696-704.
10) Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol. 1980 Jun;44(2):331-84.
11) Cohen B, Peach SL, RUSSELL RB. Immunization against dental caries. Med microbiol. 1983;2:255-94.
12) Salma A. Elnaili, David G. Patrick. Evaluation of the surface roughness of four different types of acrylic resin denture base materials: heat cure (HC), high impact heat cure (HIHC), heat cure clear (HCC), and clear chemical cure (CC). Part (1. Libyan J Sci & Tech. 2020;11:2 94-97.
13) Ozel GS, Guneser MB, Inan O, Eldeniz AU. Evaluation of C. Albicans and S. Mutans adherence on different provisional crown materials. J adv prostho. 2017 Oct 1;9(5):335-40.
14) Verran J, Boyd RD. The relationship between substratum surface roughness and microbiological and organic soiling: a review. Biofouling. 2001 Apr 1;17(1):59-71.
15) Soysa NS, Samaranayake LP, Ellepola AN. Diabetes mellitus as a contributory factor in oral candidosis. Diabet med. 2006 May;23(5):455-9.
16) Radford D, 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 Jan;10(1):99-116.
17) Nevzatoğlu EU, Özcan 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 Sep;11(3):231-6.
18) Jeganathan S, Lin CC. Denture stomatitis—a review of the etiology, diagnosis, and management. Aust dent J. 1992 Apr;37(2):107-14.
19) Cannon RD, Chaffin WL. Oral colonization by Candida albicans. Crit Rev Oral Biol Med. 1999 Jun;10(3):359-83.
20) Holmes AR, Cannon RD, Jenkinson HF. Interactions of Candida albicans with bacteria and salivary molecules in oral biofilms. J ind microbiol. 1995 Sep 1;15(3):208-13.
21) Millsap KW, Bos R, van der Mei HC, Busscher HJ. Adhesion and surface-aggregation of Candida albicans from saliva on acrylic surfaces with adhering bacteria as studied in a parallel plate flow chamber. Antonie Van Leeuwenhoek. 1999 May;75(4):351-9.
22) Waltimo T, Vallittu P, Haapasalo M. Adherence of Candida species to newly polymerized and water-stored denture base polymers. International J Prostho. 2001 Sep 1;14(5).
23) Webb BC, Thomas CJ, Whittle T. A 2-year study of Candida associated denture stomatitis treatment in aged care subjects. Gerodontology. 2005 Sep;22(3):168-76.
24) Verran J, Motteram KL. The effect of adherent oral streptococci on the subsequent adherence of Candida albicans to acrylic in vitro. J dent. 1987 Apr 1;15(2):73-6.
25) Branting C, Sund ML, Linder LE. The influence of Streptococcus mutans on adhesion of Candida albicans to acrylic surfaces in vitro. Arch Oral Biol. 1989 Jan 1;34(5):347-53.
26) Morgan TD, Wilson M. The effects of surface roughness and type of denture acrylic on biofilm formation by Streptococcus oralis in a constant depth film fermentor. J appl microbiol. 2001 Jul;91(1):47-53.
27) Faltermeier A, Rosentritt M, Müssig D. Acrylic removable appliances: Comparative evaluation of different postpolymerization methods. Am J Orthod Dentofacial Orthop. 2007 Mar 1;131(3):301-e16.
28) Gladwin MA, Bagby MD. Clinical Aspects of Dental Materials: Theory Practice and Cases. 2004.

Philadelphia: Lippincott Williams and Wilkins:154-6.
29) Busscher HJ, Cowan MM, Van der Mei HC. On the relative importance of specific and non-specific approaches to oral microbial adhesion. FEMS Microbiol. 1992 Jun 1;8(3-4):199-209.
30) Vacheethasanee K, Marchant RE. Nonspecific Staphylococcus epidermidis adhesion. In Handbook of Bacterial Adhesion 2000 (pp. 73-90). Humana Press, Totowa, NJ.
31) An YH, Friedman RJ, editors. Handbook of bacterial adhesion: principles, methods, and applications. Springer Science & Business Media; 2000 Jan 21.
32) Olsson J, Westergren G. Hydrophobic surface properties of oral streptococci. FEMS Microbiol. 1982 Dec 1;15(4):319-23.
33) Dankert J. Biomedical polymers: bacterial adhesion, colonization, and infection. CRC Crit Rev Biocompat. 1986;2:219-301.
34) Hogt AH, Dankert J, Feijen JA. Adhesion of Staphylococcus epidermidis and Staphylococcus saprophyticus to a hydrophobic biomaterial. J Gen Microbiol. 1985 Sep 1;131(9):2485-91.
35) Quirynen M, Dierickx K, van Steenberghe D. Effects of surface roughness and free energy on oral bacterial adhesion. In Handbook of Bacterial Adhesion 2000 (pp. 91-102). Humana Press, Totowa, NJ.
36) Whitehead KA, Rogers D, Colligon J, Wright C, Verran J. Use of the atomic force microscope to determine the effect of substratum surface topography on the ease of bacterial removal. Colloids Surf B: Biointerfaces. 2006 Aug 1;51(1):44-53.