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Evaluation of Candida Albicans Attachment with Two Types of Denture Base (Heat Cured Acrylic & Flexible Resin) Polished By Different Polishing Materials

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الخلاصة

الأهداف: تهدف هذه الدراسة الى تقييم تأثير ثلاثةانواع من مواد التلميع (البومس، البومس مع منظف سائل، ومعجون التلميع العالمي) تاثيرها على نوعين من مواد قاعدة الطقم (راتنجألاكريليكِ الحراريِ ومادِة طقمِ الأسنان المرنِ) ودراسة تاثيرها على خشونة السطح وبالتالي تأثيرها على نمو وارتباط فطرِ المبيّضات **.المواد وطرائق العمل:** تم إعداد ستون (90) عينه بابعاد (3سم عرض، 3سم طول، 1 سم سمك)، تم تقسيمها الى مجموعتين رئيسيتين وحسب نوع مادة الطقم المستخدمة (45عينة من الاكريليكالمبلمر حراريا و 45 عينة من مادِة طقمِ الأسنان المرنِ). كل مجموعة رئيسية قسمت إلى ثلاث مجموعات فرعية وفقا لنوع مادة التلميع المستخدمة في هذه الدراسة، 15 عينة في كل مجموعة وكالاتي: مجموعة ا – (لمعثْ بالبومسِ)= (مجموعة قياسية)مجموعة ب– (لمعثْ بالبومسِ مع منظف سائل) مجموعة ج– (لمعثْ بمعجونِ التلميعِ العالميِ)تم قياس خشونة السطح لكل عينة. بعد ذلك تم احتضان العينات في وسط زرعي يحتوي على فطرِ المبيّضات لمدة 72 ساعة في 30 درجه مئوية **.النتائج:** أن راتنجِ ألاكريليكِ المبلمرحراريا كان الاكثر نعومه من مادِة طقمِ الأسنان المرنِ وان السطوح الملمعة بمادة (البومس) كانت الأكثر نعومه ولكلا مادتي قاعدة الطقم من اسطح العينات الملمعة ب (معجون التلميع العالمي) و(البومس مع المنظف السائل) ولذلك كان التصاق المبيضات على الأسطح الخشنه أكثر من الأسطح الملساء الناعمة. وظهرت اختلافات احصائية كبيرة في عدد فطرِ المبيّضات وفقا لمواد التلميع المستخدمة في هذه الدراسة.

ABSTRACT

Aims: This study aims to measure the effect of three different types of polishing materials (pumice, pumice with soap solution and universal polisher paste) on the surface roughness and the growth of Candida Alicans (C.A) on two types of denture base materials (heat cured acrylic and flexible thermoplastic resin). **Materials and Methods**: Ninety (90) samples with dimensions (3cm length, 3cm width & 1cm thickness) were prepared & divided into two main groups (45 samples in each) according to the type of base material, heat cured & flexible. Each group was subdivided into three subgroups (15 samples in each) according to the type of polishing materials used in this study: Group1: Polished with pumice (control group), Group 2: Polished with (pumice with soap solution), Group 3: Polished with universal polisher paste. Roughness was measured to all samples by Profilometer Tester. After that the samples were incubated in media containing C.A. for 72 hr. at 300 C to count and compare the value of C.A. growth & adhesion.**Results**: the statistical analysis of roughness test between the heat cured & flexible showed a highly significant differences between samples polished with pumice, t-test=10.594, $p<0.01$, and significant differences for both groups polished by (pumice + soap solution) & (polishing paste), t-test= 4.651 & 3.173 with $p<0.05$ respectively. And for both types (Heat cured & Flexible), samples polished with pumice have the lowest number of viable cells count of C.A. (54, 67 respectively) **Conclusions**: The heat cured acrylic resin samples had a smoother surface than flexible samples. (Pumice) produced smoother surface than (universal polishing paste) and (pumice +soap solution), therefore, C.A. adhesion were higher than samples polished with pumice.

Key words: Candida Albicans, flexible thermoplastic resin, pumice, polisher paste.

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INTRODUCTION

Acrylic resin is used for the fabrication of most dental prostheses and orthodontics appliances. Thermoplastic resins have been used in dentistry for over 50 years. [1,2] Applications for thermoplastics resin originally involved various dental prostheses. [3]

Yeast of the genus Candida are commonly present in the plaque, their adhesion to the surface of the denture may cause Candidosis infections to the patient.[4] Smooth and polished surfaces of the prosthesis play a major role for the patient comfort and denture longevity.[5]

Failed to maintain adequate hygiene and surface roughness of the denture have been shown to be associated with high level of oral Candida colonization[6]. Proper finishing and polishing of dental materials are important aspects of clinical restorative procedures[7].

The rough acrylic resin surfaces increase the probability of bacterial accumulation, and Candida adhesion than smooth surface.[8] During denture construction, all factors including powder/ liquid ratio, handling and inclusion of acrylic resin as well as curing, finishing and polishing are fundamental.[9] The choices of finishing and polishing techniques to achieve the optimum smoothness of restorations have been the subjects of a number of studies. One of these studies compared the retention of C.Albicans on smooth, rough acrylic resin and silicon surfaces. They found an increase in the surface roughness facilitated yeast retention and infection on silicon and acrylic resin surfaces. Therefore should be kept to minimum.[10]

Candida has been isolated not only from the oral cavity but also from the tissue fitting surface and the outer surface of denture.[11] This has been explained by high affinity of Candida to adhering and subsequently colonizes denture acrylic resin material.[12]

Generally ,polishing of dental materials consist of gradual elimination of rough layers from the rough surface ,the objective of the procedure is to produce an adequately smooth and glossy surface and there by prevent bacterial adhesion. [13] The surface of the resins used in the construction of the prosthesis can be finished and polished using variety of techniques and different materials.[14]

In this study an attempt to improve the surface smoothness of denture base material which was done by study the effects of different polishing materials in order to reduce the adhesion of C.A., So the aims of this study are to measure the effect of three types of polishing materials (pumice, pumice+soap solution and universal polisher paste) on the surface roughness & growth of C.A. for two types of denture base materials (heat cured acrylic and flexible thermoplastic resin)

MATERIALS & METHODS

Ninety (90) samples with dimensions (3cm length, 3cm width & 1cm thickness) were prepared. They divided into two main groups according to the type of denture base material (45 samples for each), heat cured acrylic resin (Major base2/Italy) & thermoplastic flexible resin (Valplast plastic bag- flexible resin; FDA, MSDs, ISO, USA).

Each group was subdivided into three subgroups (15 samples for each) according to the type of polishing materials used in this study:

Group1: Polishing with pumice (steribim super, pumice fine grade; QD, England).

Group2: Polishing with pumice + soap solution (Al-Wazir liquid detergent; Al-Wazir Company-Jordan).

Group 3: Polishing with universal polisher paste (IvoclarVivadent; Germany).

All samples were finished by stone bur to remove all excessive materials for two minutes with low speed 1500 rpm and low pressure then Tungsten carbide bur for two minutes with low speed 1500 rpm and low pressure. After that sand paper for one minute with low speed 1500 rpm and low pressure with continuous water cooling.

Finally the samples were polished with 3 different materials used in this study. The amount of water added to each of these polishing materials (pumice and pumice + soap) was 2 ml measured by using plastic disposable syringe, and all samples were polished for two minute with low speed 1500 rpm and low pressure. According to the polishing paste;

we used 2 ml of paste measured by using plastic disposable syringe; polishing was done for 2 minutes and with low speed 1500 rpm and low pressure.

Polishing was accomplished by using bristle brush and rag wheel with selected polishing materials in lathe polishing machine a glass surface was obtained by using chamois baff and polishing soap on dental lathe using low speed(1500 rpm] with regard to continuous cooling with water to avoid over heating[15].

-Measuring Surface Roughness:

The surface of the test specimen was analyzed with surface roughness tester (Profilometer-Digital, China, TR200) to study the effects of finishing and polishing agent on the mircogeometry of the test surface.

Diamond stylus of the profilometer was moved about 4mm across the surface of the acrylic specimen. According to the manufacture instructions of the device, the vertical displacement of the stylus is measured as the surface variations, usually measuring from10 nm to 1mm the height position of diamond stylus is converted to a digital signal which is stored and displayed a 2mm distance separated each reading and all measures were carried out by the same researcher. Two readings were recorded for each specimen and the mean value for each specimen was the average of two readings. All the specimens were examined after finishing & polishing. The results were expressed in micrometer.

-Preparation of Candida Albican:

Thirty two and a half (32.5) grams of sabourauds dextrose agar were weighed by using precision electronic balance and dissolved in 500 ml of distilled water in a glass flask by magnetic stirrer. After being completely dissolved, the media was sterilized in autoclave at 15 IB, 121 0C for 15 min. The culture medium was cooled, and then 1 ml of procaine penicillin and 2 ml of streptomycin were added to the medium as broad spectrum antibiotics to prevent bacterial growth.

The culture media was transferred into Petridishes and kept it in cool place, reactivation of Candida Albican by brain heart infusion (BHI) by taking 1ml of solution from(BHI)and culturing on sabourouds dextrose media (fungal media) incubating the plate on 30ºC for 2days harvesting the colonies with distal water several dilutions(6 dilution)culture the Candida on sabourouds dextrose media 7*105 was done Concentration centum(culture) C.A. on acrylic samples fina lly the acrylic samples were incubated on 30ºC for (2-3) days. Results were analyzed by calculating numbers of colonies, × numbers of fungal cells (C.A.) *colony =1×10¹read the results inhabitation over samples polishing with (pumice, pumice +soap solution and polisher paste).

Identification of Candida Albican: Identification of C.A. was done according to its colony morphology on sabourouds dextrose media which is specific media for fungi (fungal media), [16] and to the microscopically examination and Gram stain. Germ tube formation:Germ tube are filamentous outgrowth that arise from blast spores of C.A., this was carried out according to Milne (1996) [17], through lightly touching one representive colony with a loop then was suspended in one ml of human serum and incubated at (30-37)˚C for 2 hours after which it was examined under the light microscope to identify germ tube production of C.A.

The statistical tests were applied: Descriptive statistics; mean and Standard deviation were calculated for each variable, for each group. ANOVA test was applied to see significant difference among groups' & Duncan multiple rang test also used to show the differences between groups. T-test was applied to see the trend of different beverages within the group.

RESULTS

Table (1) shows the descriptive statistics of groups: mean, S.D, min and max., values of the roughness test of (heat cured acrylic and flexible resin) samples polished with different polishing materials (pumice, polishing paste, and pumice +soap solution).The flexible resin had higher value of roughness in comparison to heat cured acrylic. The maximum mean value of roughness between groups was recorded by flexible samples polished with (polishing paste =1.2607), while the heat acrylic polished with (pumice) recorded the minimum mean value of roughness =

0.296. Figure (1) shows mean values of the Roughness Test (µm) of heat cured acrylic samples groups, while Figure (2) shows values of the Roughness Test (µm) of flexible resin samples groups which shows the maximum value of roughness was in samples polished with (polishing paste = 1.2607) and minimum value was in samples polished with (pumice) for both heat acrylic =0.296 and flexible resin =0.8464.

Table (1): (Mean, S.D, Max., Min.) values of the Roughness Test of (heat cured acrylic and flexible resin) samples polished by (pumice, polishing paste and pumice + liquid soap)

	Samples polished with Pumice		**Samples polished with Polishing Paste**		**Samples polished with (Pumice+ liquid soap)**	
	Heat cured acrylic	Flexible resin	Heat cured acrylic	Flexible resin	Heat cured acrylic	Flexible resin
Mean(µm)	0.296	0.8464	0.954	1.2607	0.61	0.9677
SD	0.087	0.116	0.237	0.118	0.181	0.141
Max.	0.464	1.017	1.251	1.465	1.049	1.190
Min.	0.230	0.668	0.572	1.127	0.404	0.732

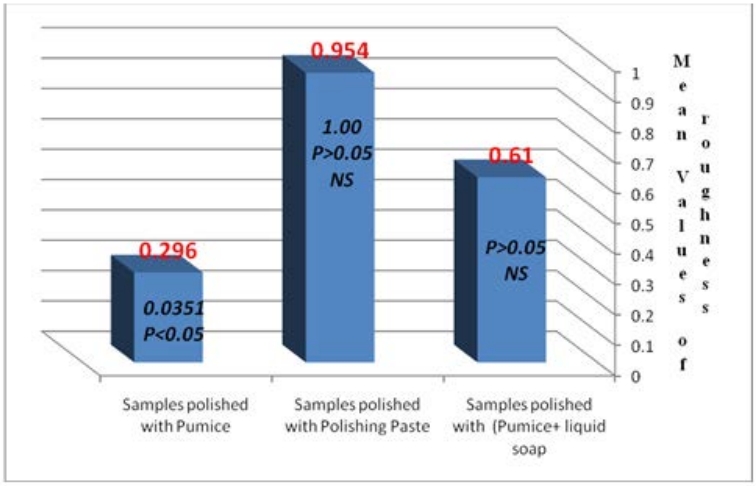

Figure (1): Mean values of the surface roughness (µm) of heat cured acrylic samples groups written above the bars & Duncan multiple range test written on the bars.

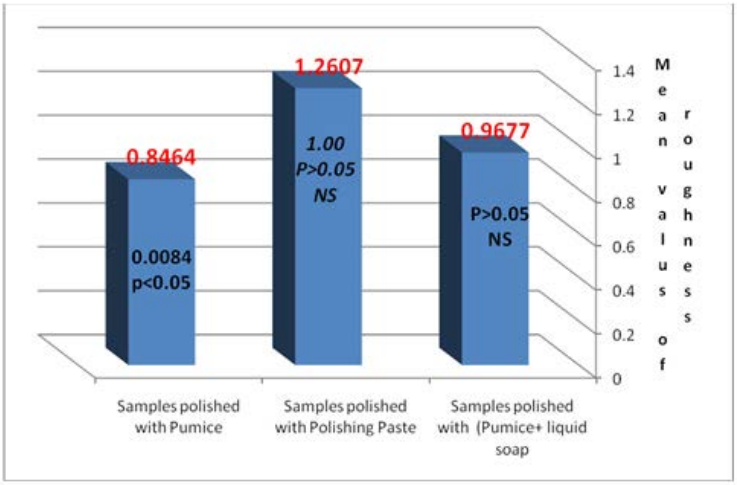

Figure (2): Mean values of the surface roughness (µm) of flexible resin samples groups written above the bars & Duncan multiple range test written on the bars.

The descriptive of groups: Mean, S.D, min., max., values of the number of viable cells count of Candida Albicans (C.A.) on (heat cured acrylic and flexible resin) samples polished by (pumice, polishing paste and pumice + liquid soap) isshown in Table (2). The maximum value of the mean of viable cells count of C.A. was recorded by flexible resin polished by (pumice + liquid soap =2450), while the heat acrylic polished with (pumice) recorded the minimum mean value of viable cells count of C.a.=54.

Table (2): (Mean, S.D, Max., Min.) values of viable cells counts of Candida Albicans on (Heat cured acrylic and flexible resin) samples polished by (pumice, polishing paste and pumice + liquid soap)

	Samples polished with Pumice		**Samples polished with Polishing Paste**		**Samples polished by (Pumice+ liquid soap)**	
	Heat cured acrylic	**Flexible resin**	**Heat cured acrylic**	**Flexible resin**	**Heat cured acrylic**	**Flexible resin**
Mean(µm)	54	67	580	429	109	2450
SD	19.5	23.5	220.1	171.1	38.4	761.9
Max.	80	100	900	600	180	4000
Min.	20	40	300	100	60	2000

Table (3) shows ANOVA test for roughness value among groups. For (heat cured) shows that there was a highly significant differences, $p<0.05$; F-test=49.852. A highly significant differences are showed too for (Flexible), $p<0.05$;F-test=81.045.

Table (3): ANOVA test for roughness values of heat cured acrylic and flexible resin.

	F-test	**P-value**	**Sig**
Heat cured acrylic	49.852	P<0.05	HS
Flexible resin	81.045	P<0.05	HS

In Table (4) &Figure (1), Duncan multiple range tests for roughness values of heat cured acrylic appear that, there was a significant difference between samples polished with pumice; S=0.0351, P<0.05; and a no significant difference for both groups polished with polishing paste and (pumice + liquid soap), NS=1.00 ; P>0.05;while Table (5) &Figure (2), shows the Duncan multiple range tests for roughness values of flexible samples and appear that, there was a significant difference between samples polished with pumice; S= 0.0084 and no significant difference for both groups polished with polishing paste and (pumice + liquid soap), NS=1.00; P>0.05 .

Table (4): Duncan multiple range tests for roughness values of (Heat cured acrylic).

	Subset for alpha=0.05	
	Samples polished with Pumice	**Samples polished with Polishing Paste**
Samples polished with Pumice	54.0	-
Samples polished with (Pumice+ liquid soap)	109.0	-
Samples polished with Polishing Paste	-	580.0
sig	0.0351 S	1.00 NS

Table (5): Duncan multiple range tests for roughness values of (Flexible resin).

	Subset for alpha=0.05	
	Samples polished with Pumice	**Samples polished with Polishing Paste**
Samples polished with Pumice	67.0	-
Samples polished with (Pumice+ liquid soap)	429.0	-
Samples polished with Polishing Paste	-	2450.0
sig	0.0084 S	1.00 NS

Table (6) shows ANOVA test for viable cells counts among groups. For (heat cured) shows that there was a highly significant differences, $p<0.05$; F-test=33.308, also a highly significant differences for (Flexible), $p<0.05$; F-test=28.537.

Table (6): ANOVA test for viable cell counts of heat cured acrylic and flexible resin.

	F-test	**P-value**	**Sig**
Heat cured acrylic	33.308	P<0.05	HS
Flexible resin	28.537	P<0.05	HS

In Table (7) &Figure (3), Duncan multiple range tests for viable cells counts of C.A. on heat cured acrylic appear that, there was a significant difference for all (pumice=0.049, polishing paste=0.048 and (pumice + liquid soap solution)=0.049) groups , P<0.05.and this result was repeated in Table (8) &Figure (4), that shows the Duncan multiple range tests for viable cells counts of C.A. on flexible samples and appear that, there was also a significant difference for all groups, pumice=0.046 & polishing paste= 0.043, and (pumice + liquid soap solution), P<0.05 .

Table (7): Duncan multiple range tests for viable cell counts of (Heat cured acrylic).

	Subset for alpha=0.05		
	Samples polished with Pumice	**Samples polished with Polishing Paste**	**Samples polished with (Pumice+ liquid soap**
Samples polished with Pumice	0.2969	-	-
Samples polished with (Pumice+ liquid soap)	-	0.610	-
Samples polished with Polishing Paste	-	-	0.954
sig	P<0.05	P<0.05	P<0.05

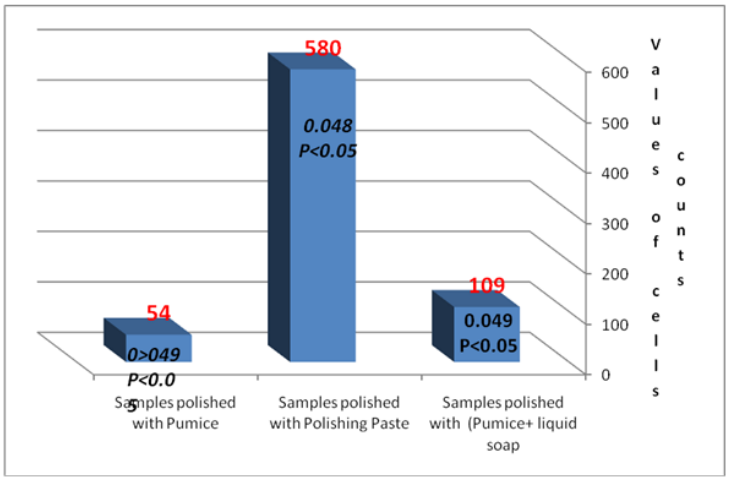

Figure (3): Values of viable cells count of Candida Albican on heat cured acrylic samples groups written above the bars & Duncan multiple range test written on the bars.

Table (8): Duncan multiple range tests for viable cell counts of (Flexible resin).

	Subset for alpha=0.05		
	Samples polished with Pumice	**Samples polished with Polishing Paste**	**Samples polished with (Pumice+ liquid soap**
Samples polished with Pumice	0.846	-	-
Samples polished with (Pumice+ liquid soap)	-	0.967	-
Samples polished with Polishing Paste	-	-	1.2607
sig	P<0.05	P<0.05	P<0.05

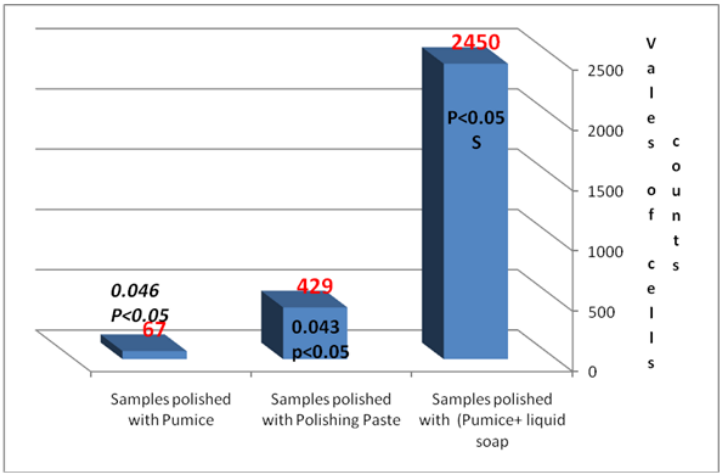

Figure (4): Values of viable cells count of Candida Albican on flexible samples groups written above the bars & Duncan multiple range test written on the bars.

Table (9) appears the LSD of the Roughness Test between groups shows a highly significant differences between all groups of heat cured acrylic samples, P<0.01, and all groups of flexible resin except between samples polished by (pumice) & samples polished by (pumice + liquid soap), that there was only a significant differences. P<0.05

Table (9): LSD for the Roughness Test & viable cells counts of Candida Albicans.

	P-value Roughness Test				P-value viable cells count			
	Heat cured acrylic	**mean diff.**	**Flexible resin**	**mean diff.**	**Heat cured acrylic**	**mean diff.**	**Flexible resin**	**mean diff.**
Pumice & Polishing Paste	P<0.01 (HS)	-0.658	P<0.01 (HS)	-0.4143	P<0.01 (HS)	-526	0.949 (NS)	-362
Pumice & (Pumice+ liquid soap)	P<0.01 (HS)	-0.314	0.048 P<0.05 (S)	-0.1213	P>0.05 (NS)	-55	0.226 (NS)	-2383
Polishing Paste & (Pumice+ liquid soap)	P<0.01 (HS)	0-344	P<0.01 (HS)	0.293	P<0.01 (HS)	471	0.298 (NS)	-2021

In the same table, table (9), the LSD for viable cells counts on heat cured acrylic sample shows a highly significant differences between samples polished with (pumice) & samples polished with (polishing paste)P<0.01, while there was no significant differences between samples polished with (pumice) & samples polished with (pumice + liquid soap)P>0.05, and there was a highly significant differences between samples polished with (polishing paste) & samples polished with (pumice+ liquid soap)P<0.01. While the LSD for flexible resin shows that there were no significant differences between all samples.

Figure (3) shows the values of viable cells counts of C.A. on heat cured acrylic samples while figure (4) shows the values of viable cells count of C.A. on flexible samples groups. Both figures show that the samples polished with (pumice) recorded the lowest value of C.A. growth, heat cured acrylic=54 & flexible resin =67, but in heat acrylic the samples polished with (polishing paste) show the highest value of C.A. account=580 while for flexible resin group the samples polished with (pumice + liquid soap) show the highest value of C.A. account=2450

In Table (10), t-test of roughness values between heat cured and flexible show a highly significant differences between; heat cure samples and flexible samples polished with (pumice) =10.594, P<0.01; while it shows a significant differences between heat cured and flexible, for both groups polished with (polishing paste) =3.173, P<0.05; & (Pumice + liquid soap) =4.651, P<0.05.

Table (10): T-test of roughness value between (heat cured acrylic & flexible resin)

	Samples polished with Pumice	**Samples polished with (Polishing Paste)**	**Samples polished with (Pumice+ liquid soap)**
t-test	**10.594**	**3.173**	**4.651**
P-value	**P<0.01**	**P<0.05**	**P<0.05**
Sig	**HS**	**S**	**S**

DISCUSSION

During denture construction, obtaining successful acrylic resin dentures with highly smooth and highly polished surfaces are of almost importance for patient comfort and denture longevity, the denture surface must be the smoothest possible for good aesthetical results, oral hygiene and low plaque retention.[18] Surface roughness and the surface free energy play a key role

in Candidal attachment; changes in these clinically important variables might have a significant influence on bacterial adhesion and retention. The results of the microbiological study have showed that there was a highly significant difference between the Candidal attachment to the flexible resin and heat cure acrylic samples with different polishing materials. The polishing material that show lower surface roughness value have less number of Candidal cells attachment (Table 1,2) and this can be explained that materials with the roughest surface may serve as reservoir, with surface irregularities providing an increased microorganism retention and protection from shear forces.[19]

Rough surface has irregularities inducing adhesion of Candida and bacteria. These superficial defects such as voids and micro cracks on surface were possible sites for Candidal adhesion. [20] The results of this study agree with the finding of Tylar and Radford, [21, 22] when they did an artificial roughness on acrylic surface and found that adherence of Candida significantly increase with increase surface roughness. Also agree with Hammoudi[23] who found that increase in surface roughness due to different polishing materials facilitate Candida retention in acrylic resin .

Also, in this study, samples that polished with pumice has the lowest roughness value than samples polished with other materials for both heat and flexible resin, this result is agree with Rahal *et al*.[24] who concluded that mechanical polishing (pumice) cause lower surface roughness values on acrylic resin denture base .

The results showed that the mean average surface roughness of acrylic surfaces measured after polishing with universal polishing paste was higher in comparable with mean average surface roughness of acrylic surfaces measured after polishing with pumice and (pumice with liquid soap solution), Table (10). This has been stressed upon by the manufacturer who stated that loose abrasive particles in the paste mass may do so. In this study polishing paste produced less adequate smoothness of acrylic surfaces because of poor abrasive capacity of the polishing paste. This is consistent with the results of Şen *et al*.,Kuhar and Funduk [25,13] who demonstrated that acrylic resin specimens polished with polishing paste, clearly reduced all surface ridges and elevated areas but failed to smooth deeper areas and pits in the surface. Many studies examined the effects of various denture base resin surface textures on the adherence of microorganism. [26]

CONCLUSIONS

The acrylic resin samples polished with pumice produce a smoother surface than flexible samples and the (pumice) produce smoother surface than (universal polishing paste groups) and (pumice + soap solution groups) in both heat cured acrylic and flexible resin groups, therefore, C.A. adhesion in (universal polishing paste groups) and (pumice + liquid soap solution groups) were higher than the group polished with pumice.

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