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Evaluation of surface roughness and Candida albicans attachment on light cured and heat cured acrylic denture base resin using Corega, Fittydent and Lacalut denture cleansers

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Abstract

Background: Denture cleansers are the most widely used by the patients to maintain clean denture as the presence of Candida albicans on the surfaces of denture-base acrylic resins is strongly related to the development of oral stomatitis but the cleansers may have harmful effect on some properties of the denture. So the aim of this study is to evaluate and compare the effect of different denture cleansers on the surface roughness and adherence of the Candida albicans on the light and heat cured acrylic resin materials.

Materials and methods: Eighty specimens were made from two different denture base materials for each test. Forty specimens were made of light cured acrylic and forty specimens were made of heat cured acrylic resin. Each material group was subdivided into four subgroups according to the type of the denture cleansers and the distilled water that was used as control group. Antimicrobial activity and the surface roughness test were measured for each specimen to show the effect of each denture cleansers on surface of the denture base materials.

Results: The results showed that there were highly significant differences in the antimicrobial activity before and after the use of denture cleansers while there were no significant differences in surface roughness between before and after immersion in the denture cleansers for both acrylic specimens. While the surface roughness and the *Candida albicans* attachment to light cured acrylic was significantly higher than that of heat cured acrylic specimens.

Conclusion: It was concluded that the *Candida albicans* attachment was effected by the immersion in the denture cleansers while the roughness of acrylic materials was not affected by immersion in denture cleansers as well as it was found that the *Candida albicans* attachment and the surface roughness of the light cured was higher than the heat cured acrylic denture base.

Key words: ***Candida albicans*, surface roughness, denture cleansers, acrylic denture base.**

Introduction

Favorable denture base material is needed for fabricating long standing and biological acceptable dentures [1]. Acrylic resins have been used to produce dentures more than 60 years. Polymethyl methacrylate (PMMA) polymers have been referred as conventional base materials and one of

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the most widely used denture base material with numerous advantages [2].The light-curing systems offered simpler laboratory procedures and less risk of allergic reactions as they do not contain methyl metacrylate monomer [3]. Furthermore, poor fracture resistance of early light-cure systems was improved by entrance of a new material on the market [4]. As well as it is more effective and time efficient because the visible light cured (VLC) is polymerized by means of visible light [5]. Therefore, the visible light-cured resin has been developed and used for many prosthodontic applications [6] and many medical workers worldwide nowadays turn to use this material [7].

On the other hand, in human mouth the dentures prepare an optimal environment for adhesion and multiplication of both pathogenic and non-pathogenic organisms and the increasing use of removable dentures have caused increasing the denture related infections [8]. Such as poor hygiene favors *Candida albicans* infection [9] because the inner surface of dentures acts as a *C.albicans* reservoir, which is responsible for the progression and maintenance of the infection [10]. According to Ramage, 11% to 67% of complete denture users present candidiasis [11]. Therefore, indication of denture cleansing is of paramount importance. Also it is important to point out that the cleaning agents employed must be not toxic or irritate the mucosa, preferentially bactericidal and fungicide and must be harmless for the denture [10]. The rate at which deposits accumulate on dentures may vary between individuals and can be affected by factors such as surface texture and porosity of the denture base material, duration for which the dentures are worn, and the denture-cleansing regimen adopted by the wearer so the irregularities and porosities present on denture surfaces offer a favorable niche to retain stain and microbial plaque [12]. The surface roughness is of particular clinical relevance since it can affect the biofilm formation or make its removal difficult [13].A previous study concluded that surface roughness favored colonization by the microorganisms, contributing indirectly to tissue injury [14]. Therefore management of denture related infections is challenging and the infected dentures generally need to be disinfected [15] .The dentures can be cleaned mechanically, chemically or by the combination of both methods, the mechanical methods may be ineffective, and thus demand alternative means such as chemical cleansing is necessary [16]. Several disinfectants have been suggested for the disinfection of dentures. The best disinfectant should fulfill most of the requirements of the ideal agent while not causing any kind of alteration in the structure of the denture [17].Therefore the efficacy of chemical denture cleansers dislodging food debris, and tobacco stains from prosthodontics surface has been reported previously [18] but the cleansers and cleaning methods may have harmful effect on the plastic or metal component of the denture so the dentist must able to recommend a denture cleanser that is effective non deteriorative to denture material and safe for patient use [19].

Since the chemical method for disinfection of dentures is widely used by patients and as it is one of the processes for the treatment of candidiasis, this study were designed to evaluate the antifungal action of 3 the different commercial denture cleaners: fittydent, corega and lacalut against the adhesion of the *Candida albicans* and their effect on the surface roughness of the light and heat cured acrylic resin materials. As well as compare between the adhesion of the *Candida albicans*

and the surface roughness of light cured and the conventional heat-cure acrylic resins denture base materials.

Materials and methods

Chemical composition and types of denture cleansers and denture resins used in this study are listed in (Table 1) (Figure1).

Preparation of the sample:

The heat cured acrylic was mixed in a powder/liquid ratio 3:1 by volume according to manufactures instruction the mixture left covered until dough stage [20]. The mixture was packed into stone mold previously coated with separating medium and then the two halves of the flask were closed together and placed under hydraulic pressure for 5min to get metal to metal closure and flow of the resin throughout the mould space, then transferred to the water bath. For curing the specimens in short cycle fasting technique involves 74ºC for 1.5 hours and then increases the temperature of water bath to boiling 100 ºC for 1hour [21]. Then the flask was left to cool slowly for 30min and the specimens were removed from the mould. For light cured acrylic specimens preparation the sheet of the light cured acrylic resin was taken out of its light proof packing and positioned into the mould after coated with separating medium. The material was adapted well in the mould and excess material was removed by cutting with sharp wax knife. The curing of the material by using light curing unit for 5min following manufacturers' instruction then specimen was removed from the mould and inverted and then exposed to light cured unit again for additional 5min to insure complete polymerization [22].The specimens were finished first by tungsten carbide bur to remove the flashes. For smoothing the stone bur at low speed used first followed by silicon carbide paper (grades 120 to 600) with continuous water cooling to avoid over heating, and then polished with rouge and wool brush on dental lathe. The space between brush and specimens 2mm and the time of polishing was 2min for each specimen [23].

Preparation of denture cleanser solutions:

The solution was prepared for the each denture cleansers by dissolving one tablet in 200 ml of warm water 40ºC according to manufactures' instruction.

Surface roughness test:

For the surface roughness test the specimens were prepared by metal pattern was constructed with dimensions of (65mm x 10mm x 2.5mm) length, width and thickness respectively [21] was used to form eighty specimens:forty light cured and forty heat cured acrylic resin, and then each material group divided into four groups according to type of the denture cleansers and distilled water, each group consists from 10 specimens. Portable roughness tester (profilometer device) (Figure 2) was used to study the surface roughness of each specimen. The specimens were placed on bench under the device and the analyzer (stylus) in contact with sample surface pass along the length of the specimen which is moved for a distance 11mm according to apparatus design. The data were collected from screen part of the device represented as surface roughness value(Ra) in micrometer(µm).The values obtained in two measurements the first one before soaking and the second one after soaking in denture cleanser solutions for7days period (5hour/day in denture cleanser and then specimens removed from the cleanser and soaked in distilled water)except the control group the

specimens were soaked in distilled water for7days and the2nd measurement was done at8th day [24].

Adherence of *Candida albicans* test:

For the *C.albicans* test the specimens were prepared with the dimension of (10mm x10mmx2mm) length, width and thickness respectively [25] was used to form eighty specimens:forty light cured and forty heat cured acrylic resin and then each material divided into four groups according to type of the denture cleansers and distilled water, each group consists from10specimens. Pure cultures of *C.albicans(CA18)*strain were grown on Agar Sabouraud plates (Himedia,India) at 25°C.After 24hrs, the colonies were suspended in tubes containing 5ml of brain heart infusion (BHI) broth, take1ml of solution from the(BHI)and culture on the Sabouraud dextrose media then incubate the plate on 30°C for 48hrs then collecting the colonies with distilled water to make serial dilution after dilution with this solution, a final yeast suspension of approximately10^6 *C.albicans* per milliliter was prepared [25]. Next, the specimens were ultrasonically cleansed in sterile distilled water for 20 minutes prior to biofilm formation to remove any contaminants and artifacts from their surfaces [26]. then the specimens were placed into the tubes containing BHI plus inoculums and remained for 48-72 hrs at 37°C in order to favor colonization of the acrylic resin surfaces. The specimens were distributed in the test groups according to the type of the denture cleansers treatment that were immersed according to the manufacturer instruction and the control group the specimens were immersed in the distilled water only. Each specimen was then washed with saline and the excess was removed with sterile gauze then the swab samples were taken from each specimens transferred into tube containing of 1ml of normal saline and only portions (0.1 ml) of dilutions was taken ad spread on the Sabourauds dextrose agar medium and plates were incubated for 48hrs at 37°C.Plates with colonies of *C.albicans* were counted and the results were expressed in cells count/ml to check microbial growth [26] that was calculated before and after use of the denture cleansers (Figure 3).

Statistically all data were collected and analyzed using SPSS program version 21. The data were analyzed descriptively and comparison between two groups was done using t-test, and ANOVA test for assessing differences between more than two groups, with significant level set at $p<0.05$.

Results

Surface roughness measurements:

The descriptive analyses of the results were presented in (Table 2). The mean values of the surface roughness for light cured acrylic were higher than those for heat cured acrylic in general for all studied groups as shown in figure (4). The results of the effect different types of the denture cleansers on the surface roughness of the light and heat cured acrylic in comparison to the distilled water were illustrated in (Table 3). Two-way analysis of variance test (ANOVA) showed there were significant differences between different types of the denture cleansers compared to the distilled water for both light cured and heat cured acrylic groups. The LSD test showed there were significant differences in the surface roughness between the control group and the groups of the denture cleansers but there were non significant differences between the groups of the different denture cleansers for light cured groups while for the heat cured groups

there were non significant differences in the surface roughness between control group and different denture cleansers but there were significant differences between denture cleansers groups (Table 4). For comparison of the surface roughness values of presoaking and post soaking in either distilled water and the denture cleansers the t-test indicated there were no significant differences between presoaking and post soaking for all groups (Table 5).In the comparison of the surface roughness values of the light cured and heat cured acrylic resin the result showed there was highly significant differences between the light cured and the heat cured for all studied groups as shown in (Table 6).

Candida albicans adhesion measurement:

The descriptive analyses of the results were presented in (Table 7). The mean values of the *C.albicans* measurements for light cured acrylic were higher than those for heat cured acrylic for all studied groups as shown in (Figure 5).The results of the effect different types of the denture cleansers on the *C.albicans* adherence on the surface of the light and heat cured acrylic resin in comparison to those were immersed in distilled water were illustrated in (Table 8). Two-way analysis of variance test (ANOVA) showed there were highly significant differences between different types of the denture cleansers compared to the distilled water for all acrylic groups. The LSD test showed there were highly significant differences between the control group and the groups of the denture cleansers but there were non significant differences between the groups of the different denture cleansers (Table 9). For comparison of the *C.albicans* adherence of presoaking and post soaking in either distilled water and different denture cleansers, the t-test indicated there were highly significant difference between all groups (Table 10). In the comparison of the *C.albicans* adherence of the light cured and heat cured acrylic the results showed there were highly significant differences between the light and the heat cured for all studied groups (Table 11).

Discussion

The maintenance of clean denture prosthesis is important for health of the patient and to maintain an esthetic, odor free appliance and to keep the patient mouth free of denture stomatitis [27].

Surface roughness measurements:

Surface roughness is the factor in the entrapment of microorganism on acrylic surface, significantly higher number of microorganism cells was observed on roughened surface than on smooth surface [28].In this study the results of the effect different types of the commercial denture cleansers on the surface roughness of the light and heat cured acrylic resin showed there were significant differences in comparison to those were immersed in distilled water in all tested groups this results agreed with the study that was reported lower surface roughness measurements when the acrylic resin samples were immersed in a commercial cleanser [29] ,and also agreed with other study that showed the alkaline peroxide effervescent denture cleansers should be used with caution because it cause significant changes in the surface roughness of the heat-polymerized acrylic resin[30]. While the result of this study disagreed with HATIM et al.2003 study that was showed acrylic surface smoothness was not effected even the samples immersed for one years in denture cleansers solutions [31]. Also disagreed

with other study showed there was no change in the surface roughness of the light cured denture base material when immersed in denture cleansers compared to that immersed in distilled water [32].The result of the comparison of the surface roughness values of the presoaking and post soaking in different denture cleansers showed there was no significant difference for both light and heat cured acrylic groups this result agreed with results other studies that found that there were no significant differences in the surface roughness of the acrylic resin between before and after cleaning procedure that applied to acrylic specimens [24,33].In the comparison of the surface roughness values of the light cured and heat cured acrylic resin the result showed the specimens of light cured were highly significant than that specimens prepared by heat cured acrylic this result agreed with other study that showed the light cured specimens were significantly more surface roughness than that specimens prepared by water bath [34] this result may be due to light cure specimens not kept under pressure during polymerization results in voids with in the material that giving the brittle nature of the light cured acrylic resin denture base [35]. In addition to the absences of water during polymerization which lead to the reduction in degree of conversion and create linear polymeric chains, more extensive surface degradation could be present that assumes the water used during the polymerization could interface with important physical properties such as surface roughness [36].

Candida albicans adhesion measurement:

Oral environment temperature and the acquired pellicle formed over dentures promote *Candida albicans* adhesion to resin materials, indicating the need of an adequate plaque control for maintaining oral health [37].The results of this study showed the *Candida albicans* adherence on the light cured acrylic was higher than the heat cured acrylic this in agreement with the results of the study revealed that there were significant differences between specimens of heat cure acrylic in contrast of light cure acrylic material, in which light cure specimens had a higher value of *Candida* adherence to the surface of the specimens [38],which might be due to their high value of surface roughness because the increased roughness associated with surface irregularities, such as cracks and pits that was provide a larger surface area and a more environment for biofilm to develop microorganisms [39].On the other hand, in present study showed all the types of the denture cleansers used in this study had effect on reducing the *C.albicans* adherence to both types of the acrylic denture base this agreed with ULUDAMAR et al 2010, who found that Fittydent was found to be more effective than Polident and Efferdent in reducing *C.albicans* after 60min of immersion because Alkaline peroxides are the most used denture cleansers, but did not completely eliminate them [40]. All the denture cleansers used in this study showed the same result this may be attributed to methodology, the composition of cleanser, and the disinfection times nearly the same. Also agreed with other study that found the use of denture cleansers definitely reduced the microbial numbers as compared to plain manual cleansing methods in complete dentures [41].But disagreement with VIEIRA et al 2010, who found that alkaline peroxide denture cleansers were not effective in removing *Candida spp*. biofilm from denture liner surfaces and preventing biofilm recolonization [26]. And also disagreed

with other study that was showed the use of the effervescent agent used according to the manufacturer's instructions was not effective in removing *C. albicans* colonies [10].

With limitation of this study, it is concluded that the *Candida albicans* attachment is effected by the immersion denture cleansers. the denture base materials both light cured and heat cured did not reveal any clinical significant surface changes in the roughness after being immersed in any type of the denture cleansers solutions but there is difference in roughness when immersed in different denture cleansers in comparison to distilled water. On the other hand, it is found that the *Candida albicans* attachment and the surface roughness of the light cured is higher than the heat cured acrylic denture base.

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Figure (1): denture cleansers used in this study.

Figure (2): Portable roughness tester.

Figure (3): *C. albicans* colonies on Sabourauds dextrose agar.

Table 1: The materials used in this study.

Product	Composition	Manufacturer
Rodax	PMMA denture base material.	W.P. dental, Germany
Megatray	VLC denture base material.	Megadenta, Germany
Fittydent	Sodium perborate, sodium bicarbonate, potassium monopersulphate, trisodium phosphate.	Fittydent International GmbH, Pinkafeld, Austria
Corega	Sodium carbonate, potassium caroate, citric acid, sodium carbonate peroxide, sodium bicarbonate, sodium benzoate, sodium lauryl sulfoacetate.	Block Drug Company, Inc., USA
Lacalut	Sodium bicarbonate, potassium peroxo-monosulphate, citric acid, sodium lauryl sulfoacetate.	DR.Theisis Naturwaren Gmb H, Homburg/ Gremany

Table (2): Descriptive statistics of surface roughness (µm) for each group.

Studied groups			No.	Mean ± SD	Mini. value	Max. value
Group A (control group)	Light cure	Presoaking	10	3.570± 0.253	3.143	3.966
		Postsoaking	10	3.553 ± 0.354	3.025	3.999
	Heat cure	Presoaking	10	0.715± 0.184	0.478	1.057
		Postsoaking	10	0.704± 0.182	0.436	0.938
Group B (fittydent)	Light cure	Presoaking	10	2.578± 0.816	2.040	3.477
		Postsoaking	10	3.215 ± 0.473	2.707	3.992
	Heat cure	Presoaking	10	0.912 ± 0.171	0.641	1.156
		Postsoaking	10	0.776± 0.204	0.508	0.996
Group C (Corega)	Light cure	Presoaking	10	2.663 ± 0.632	1.726	3.672
		Postsoaking	10	3.153 ± 0.393	2.482	3.627
	Heat cure	Presoaking	10	0.912± 0.171	0.641	1.156
		Postsoaking	10	0.625± 0.300	0.431	1.441
Group D (laculut)	Light cure	Presoaking	10	3.366± 0.417	2.669	3.942
		Postsoaking	10	3.372 ± 0.532	2.359	3.921
	Heat cure	Presoaking	10	0.719 ± 0.219	0.431	0.983
		Postsoaking	10	0.824 ± 0.124	0.626	0.970

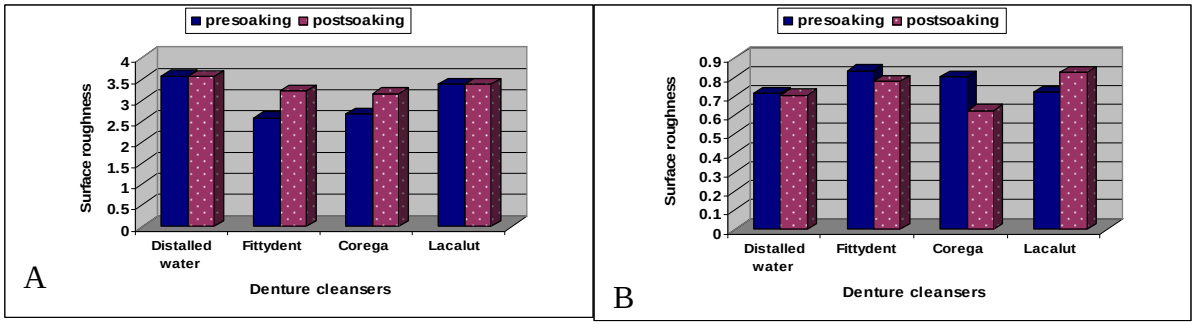

Figure (4): Bar chart shows the surface roughness of: A. Light cured acrylic. B.The heat cured acrylic.

Table (3): ANOVA test between the surface roughness values of the groups A,B,C,D for the light cured groups and the heat cured groups .

Acrylic denture base	Denture cleansers	Mean ± SD	F-test	P-value	Sig.
Light cured	Group A (control group)	3.553 ± 0.354	4.693	0.047	S
	Group B (fittydent)	3.215 ± 0.473			
	Group C(Corega)	3.153 ± 0.393			
	Group D(laculut)	3.372 ± 0.532			
Heat cured	Group A (control group)	0.704± 0.182	4.918	0.045	S
	Group B (fittydent)	0.776± 0.204			
	Group C(Corega)	0.625± 0.300			
	Group D(laculut)	0.824 ± 0.124			

*P<0.05 significant

Table (4): LSD test for comparison of the significance between the groups A,B,C,D for the light cured groups and the heat cured groups .

	Light cured		Heat cured	
	P-value	Sig.	P-value	Sig.
Group A & Group B	0.048	S	0.451	NS
Group A & Group C	0.047	S	0.411	NS
Group A & Group D	0.367	NS	0.05	NS
Group B & Group C	0.757	NS	0.048	S
Group B & Group D	0.433	NS	0.616	NS
Group C & Group D	0.049	S	0.043	S

*P<0.05 significant **P> 0.05 Non significant

Table(5):T-test of surface roughness values between presoaking and postsoaking groups within same material.

		Light cured			Heat cured		
		t-value	P-value	Sig.	t-value	P-value	Sig.
Group A (control group)	Presoaking	0.103	0.920	N S*	0.160	0.877	N S*
	Postsoaking						
Group B (fittydent)	Presoaking	1.139	0.284	N S*	1.439	0.184	N S*
	Postsoaking						
Group C (Corega)	Presoaking	0.736	0.480	N S*	1.534	0.159	N S*
	Postsoaking						
Group D (laculut)	Presoaking	0.028	0.976	N S*	0.318	0.758	N S*
	Postsoaking						

*P> 0.05 Non significant

Table (6): T- test of surface roughness values between the same subgroups (presoaking and post soaking groups) within different material.

Studied groups			t-value	P-value	Sig.
Group A (control group)	Presoaking	Light cure	29.176	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	22.465	0.00	HS*
		Heat cure			
Group B (fittydent)	Presoaking	Light cure	11.288	0.000	HS*
		Heat cure			
	Postsoaking	Light cure	16.298	0.000	HS*
		Heat cure			
Group C (Corega)	Presoaking	Light cure	9.883	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	7.144	0.00	H S*
		Heat cure			
Group D (laculut)	Presoaking	Light cure	42.27	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	12.999	0.000	H S*
		Heat cure			

Table (7): Descriptive statistics of *C.albicans* adherence to the surface of light and heat cured acrylic groups measured in cells count×$10^{\wedge 6}$/ml.

Studied groups			No.	Mean±SD	Mini. value	Max. value
Group A (control group)	Light cure	Presoaking	10	11.8±2.699	9	17
		Postsoaking	10	10.7±2.750	8	16
	Heat cure	Presoaking	10	4.1±1.663	2	7
		Postsoaking	10	2.8±1.135	1	5
Group B (fittydent)	Light cure	Presoaking	10	11.5±2.635	9	18
		Postsoaking	10	1.0±0.471	0	2
	Heat cure	Presoaking	10	4.4±1.897	2	8
		Postsoaking	10	0.6±0.516	0	1
Group C (Corega)	Light cure	Presoaking	10	10.8±2.097	9	15
		Postsoaking	10	1.10±0.567	0	2
	Heat cure	Presoaking	10	3.6±1.505	1	6
		Postsoaking	10	0.7±0.483	0	1
Group D (laculut)	Light cure	Presoaking	10	10.5±2.953	7	16
		Postsoaking	10	1.0±0.417	0	1
	Heat cure	Presoaking	10	4±2.108	1	8
		Postsoaking	10	0.6±0.516	0	1

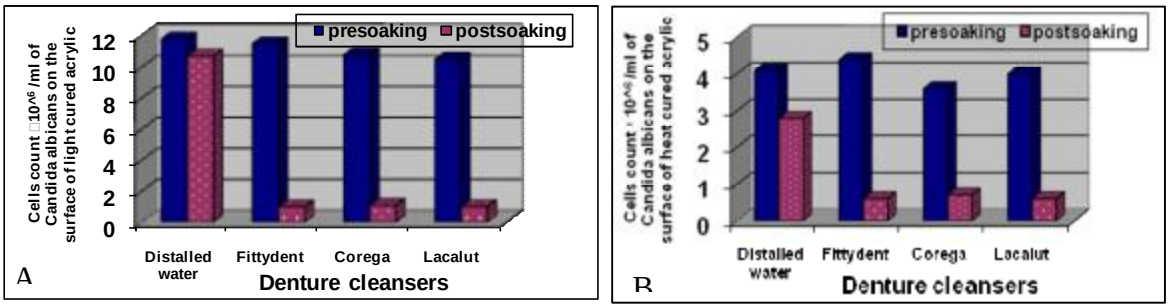

Figure (5): Bar chart shows cells count of the *C. albicans* from surface of A. Light cured acrylic. B. heat cured acrylic.

Table (8): ANOVA test for comparison the number of *Candida albicans* mean values of the groups A,B,C,D for the light cured groups and the heat cured groups .

Acrylic denture base	Denture cleansers	Mean ± SD	F-test	P-value	Sig.
Light cure Postsoaking	Group A (control group)	10.7±2.750	27.355	0.000	HS*
	Group B (fittydent)	1.0±0.471			
	Group C(Corega)	1.10±0.567			
	Group D(laculut)	1.0±0.417			
Heat cure Postsoaking	Group A (control group)	2.8±1.135	22.791	0.000	HS*
	Group B (fittydent)	0.6±0.516			
	Group C(Corega)	0.7±0.483			
	Group D(laculut)	0.6±0.516			

*P<0.01 High significant

Table (9): LSD test for comparison of the significance between the groups A,B,C,D for the light cured groups and the heat cured groups .

	Light cured		Heat cured	
	P-value	Sig.	P-value	Sig.
Group A & Group B	0.000	HS	0.000	HS
Group A & Group C	0.000	HS	0.000	HS
Group A & Group D	0.000	HS	0.000	HS
Group B & Group C	0.744	NS	0.758	NS
Group B & Group D	1.000	NS	0.357	NS
Group C & Group D	0.744	NS	0.211	NS

*P<0.01 High significant **P> 0.05 Non significant

Table(10): T-test comparing the *C. albicans* cells count/ml between presoaking and postsoaking groups within same material.

		Light cured			Heat cured		
		t-value	P-value	Sig.	t-value	P-value	Sig.
Group A (control group)	Presoaking	11.000	0.000	HS	2.899	0.018	S*
	Postsoaking						
Group B (fittydent)	Presoaking	14.017	0.000	HS	6.219	0.000	HS**
	Postsoaking						
Group C (Corega)	Presoaking	14.182	0.000	HS	5.513	0.000	HS**
	Postsoaking						
Group D (laculut)	Presoaking	9.474	0.000	HS	4.735	0.001	HS**
	Postsoaking						

*P<0.05 Significant **P<0.01 Highly Significant

Table (11): T- test for comparison of *C.albicans* cells count between the same subgroups (presoaking groups and postsoaking groups) within different material.

Studied groups			t-value	P-value	Sig.
Group A (control group)	Presoaking	Light cure	8.374	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	11.185	0.000	H S*
		Heat cure			
Group B (fittydent)	Presoaking	Light cure	6.843	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	2.449	0.037	S**
		Heat cure			
Group C (Corega)	Presoaking	Light cure	8.565	0.000	H S*
		Heat cure			
	Postsoaking	Light cure	2.449	0.037	S**
		Heat cure			
Group D (laculut)	Presoaking	Light cure	5.278	0.001	H S*
		Heat cure			
	Postsoaking	Light cure	2.449	0.037	S**
		Heat cure			

*P<0.01 Highly Significant **P<0.05 Significant