

Original Research Article

A Study The Effect of Nd: YAG Laser on The Adhesion of *Candida albicans* and Some other Properties of Heat Cure Denture Base Material

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Abstract

Denture stomatitis is a disease associated with colonization of *Candida albicans* on a denture surface. In a review of literature, a variety of topics used for the management of this case. And this especially include: systemic drug, treatment with chemical disinfectant or irradiation of denture with plasma. With the introduction of laser and its several applications in medicine and dentistry .the present study used Nd: YAG laser for this purpose.

The present study evaluates the effect of diode pumped Nd: YAG laser (second Harmonic generation) on adherent *Candida albicans* on the surface specimen and other properties of heat cure acrylic including (Transvers strength, Impact strength, surface roughness surface hardness and FTIR test).

Diode pumped Nd:YAG laser type second harmonic generation have wave length 532nm & power 1 watt were used. Specimens were made from heat cure denture base material .Evaluation the properties of this material has been done before and after laser irradiation of the specimens for 5 & 10 min. The distance between the source of laser and specimens was 100cm .All data analyzed with the use of statistical test ($X \leq 0.05$).

Results of irradiation of heat cure specimens with Nd:YAG laser revealed a lower mean values ($M=66.1, SD=11.779, M=0.753, SD=0.415$) for transvers strength & surface roughness respectively at 10min. At the same time, the results appeared the highest mean values ($M=80.20, SD=11.3, M=6.21, SD=1.324$) for indentation hardness and impact strength respectively. Nd:YAG laser irradiation to heat cure specimens showed a decrease in the mean value of *Candida albicans* adherence test for two time: 10 & 5 min with statistically no significant difference at a level of ($p \leq 0.05$).

According to the results of the present study it can be concluded that Nd: YAG laser can be used for the reduction of *Candida albicans* adherence to heat cure with minimal adverse effect on some other properties of material tested.

Key words: Nd: YAG laser, heat Cure, *C. albicans*, Transvers strength, Impact strength.

الخلاصة

التهاب الفم المتعلق بطقم الاسنان هو مرض له علاقة بمستعمرات المبيضات الفطرية على سطح الطقم، عند مراجعة المصادر، يوجد انواع مختلفة من الطرق للسيطرة على المرض وخاصة المتضمنة الأدوية، المطهرات الكيميائية، او اشعاع الطقم بالبلازما. بظهور الليزر وتطبيقاته في الطب وطب الاسنان .الدراسة المقدمة تستعمل الاندياك ليزر لهذا الغرض .
الدراسة المقدمة تقيم تأثير اندياك ليزر على التصاقه الفطريات البيضاء على سطح العينة والخواص الاخرى للاكريل الحراري المتضمن (قوة الشد، قوة الصدمة، خشونة السطح، الصلادة).

استعمل اندياك ليزر الجيل الثاني بطول موجي ٥٣٢نم وقدره ١ واط. العينات معموله من مادة الطقم المطبوع حراريا .تم تقييم خواص هذه المادة قبل وبعد التعريض لليزر لمدة ٥ و ١٠ دقيقة.المسافة بين مصدر الليزر والعينة ١٠سم .كل البيانات حلت باستعمال الاختبار الاحصائي. $X \leq 0.05$.

اشعاع العينة المبلرة حراريا بالاندياك ليزر تظهر معدلات واطئة ($M=66.1, SD=11.779, M=0.753, SD=0.415$) لخشونة السطح وقوة المطاوعة على التوالي خلال ١٠ دقيقة. بنفس الوقت النتائج اظهرت اعلى معدلات بالنسبة للصلادة وقوة الصدمة. كما ان تعريض العينة المبلرة حراريا بالاندياك ليزر يظهر تقليل في معدلات قيم لالتصاق للفطريات البيضية للوقت ١٠ و ٥ دقيقة بدون اختلاف معنوي بمستوى ($p \leq 0.05$). استنادا لنتائج الدراسة من الممكن ان نستنتج ان الاندياك ليزر ممكن يستعمل في تقليل التصاق البيضات الفطرية باقل تأثير علىالخواص الاخرى للمادة المفحوصة.

الكلمات المفتاحية:اندياك ليزر,البلمرة الحرارية,البيضيّات الفطرية,قوة المطاوعة,قوة الصدمة.

Introduction

Adherences of *Candida albicans* to the impression surface of maxillary denture can cause candidiasis [1,2,3]. Denture can create an environment which serves as a reservoir of microorganisms that favor the localization and development of microorganisms [4,5]. The use of systemic antifungal agent might demonstrate an increase resistance of *Candida albicans* grown on denture surface such as fluconazole, nystatin & amphotericin B [6,7]. Other approaches for treatment of denture stomatitis include the use of topical application of antifungal to the affected prosthesis and mucosa. Pusateri et al used a surface coating of acrylic with Chlorhexidine or Hist5. This has a potential therapeutic application. Also it can effectively inhibits biofilms growth [8]. However, Al-Dwairi et al study demonstrated that exposure of poly methyl meth acrylate to nystatin can increase the surface roughness & wettability. On the other hand, fluconazole effect on the surface free energy might increase the adhesion of candida to poly methyl methacrylate material [9]. Some author used microwave irradiation of acrylic to reduce the adherence of *Candida albicans* on a denture surface [10-12]. Other used plasma irradiation for the same purpose. [13, 14].

Since the development of laser in 1960 [15]. A laser is widely used in a variety of application such as medicine, biology, and dentistry. It's application in dentistry is including: orthodontic, endodontic [16,17], oral and maxillofacial surgery, dental implant and periodontics dentistry [18]. Low laser therapy promotes healing of a wound, reduce pain and inflammation of onychomycosis [19,20] besides that low level of laser can be used in treatment of oral mucositis [21]. Basso et al [22] studied the effect of low laser therapy on microbial biofilms and the results revealed that biofilm growth is a dose dependent fashion, and there is a reduction in the formation of *Candida albicans* hypha. One kind of laser which was CO2 laser with an energy density rate $0.25/\text{cm}^2$ found to be effective against *E. coli*, *Staph aureus* and *Pseudomonas aeruginosa* [23]. Diode-pumped lasers has a stable frequency, efficiency in addition to improved reliability.

The present study was designed to evaluate the effect of diode pumped Nd: YAG laser (532nm) on: 1. Adhesion of *Candida albicans* present on the surface of heat cure denture base material in two different exposure time: 5 & 10 min. 2. Other properties of heat cure denture base material including transverse strength, impact strength, surface hardness and roughness.

Materials and Methods

Pilot study

The present study used diode pumped Nd:YAG laser light (second harmonic generation SHG green in color with a wave length of a 532nm and power 1watt .This type of laser was selected based on the results of absorption and transmission properties of heat cure after exposure to this type of laser for (5 and 10) min and as follow :

At this step thirty specimens of heat cure denture base material were prepared following a conventional procedure used in complete denture construction .The dimensions of the specimen were (45x10x2.5) mm, this was according to the manufacturer instruction of the device UV spectrophotometer (uvivisSP8001 Metertech). The absorption and transmission properties of heat cure specimens was tested for non-irradiated

and irradiated specimens with laser for 5&10min. Specimens were divided into ten specimens not expose to laser, the second ten specimens exposed to laser for 5 min, remaining 10 specimens irradiated with laser for 10 min.Then specimen was place in a suitable place in a device, and exposed to UV light of wave length from 300-800nm. Then the specimens were tested for the effect of absorption and transmission of this type of green color laser with properties mentioned previously on heat cure denture base materials.The results indicated that this type of laser can be used with this pink color heat cure denture base material, since that the amount of irradiation absorbed or transmitted at range (500-600) lambda nm approximate to each other and there were no marked differences from control as shown in fig 1 &-fig-2.

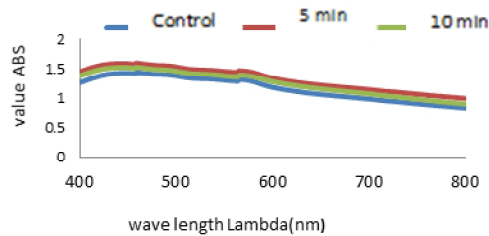


Fig 1-Absorption properties of heat cure before and after exposure to laser irradiation

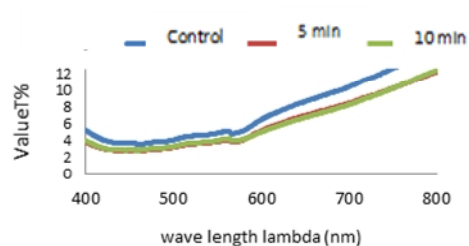


Fig 2-Transmission properties of heat cure before and after exposure to laser irradiation

In this study, the procedure for testing the effect of Nd YAG laser on adherent *Candida albicans* to the non-polish surface of heat cure and other properties of heat cure denture base materials describe as follow:

Preparation of Specimen and conditioning:

Metal patterns of different dimensions were used according to the type of test: for Indentation hardness test, surface roughness test and transverse strength test [24], the dimensions were (65X10X2.5) mm length, width and depth respectively.

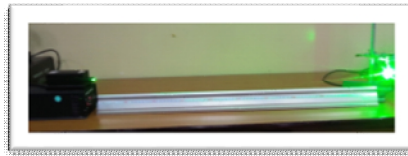
In case of impact strength test, the dimensions were (80 X 10 X 4) mm length, width and depth respectively [25], while for *Candida albicans* test, around disk of 50mm diameter & 2.4mm depth were used according to ADA specification No.12 [24]. For FTIR analysis, the dimensions of the specimens were (25X25X2.5) mm length, width and depth respectively .This was according to the manufacturer instruction of the device.

All metal pattern invested with dental stone (Zermack type III destine Italy and

silicone (Zermack) in a metal flask. Heat cure acrylic (super acryl plus, sofa Dental a.s.Markova) was manipulated (P/L) ratio 22gm/10ml, packed, pressed into the molds and polymerized according to the manufacturer instruction which was “immerse the flask in cold water, then rise temperature 70°C for 60min, then temperature was increased to 100°C for next 60 min in thermostatically controlled water bath”. polishing for one side of the specimens has been done with silicon carbide grid paper (350-600). Then polishing with a lath polishing machine. All specimens were prepared by the same operator and conditioned in distilled water for 48h (ADA specification No. 12) [24]. Then Irradiation of all specimens was performed using diode pumped Nd: YAG (Shanghai dream laser) before testing except that specimen used for *Candida albicans* test as follow:

Laser surface treatment

In this study, Laser light was applied in continues wave mode, green in color with a wave length of 532nm and 1watt power. Each specimen was exposed to laser irradiation for one side of the specimen which was the non-polish surface, and this represents the impression surface of the denture. Specimen is fixed in 15cm height and 100cm distance from the source of laser irradiation in a holder. A lens was placed between them, this would make the laser irradiation distribute to the whole area of the specimen not specified into dot site that can make a hole as shown in fig-3. At this step, a proper safety eye protection was worn by the operator. In the present study, all specimens were divided into three groups according to the laser irradiation time into the following: non-irradiated specimens (control), irradiated specimens for 5min and irradiated specimens for 10 min. After complete irradiation of the specimens, testing of specimens was performed as follow:



Testing procedures

For each test thirty specimens were prepared and divided into three groups: ten specimens for each subgroup, the 1st group was the none-irradiated group (control), the 2nd group was the irradiated with a laser for 5min, and the 3rd group was irradiated with laser for 10 min.

1. Transverse strength test

It is a three point bending test. And Instron universal testing machine (Instron Corporation, 1122) was used for testing the transverse strength of the

Specimens. Transverse strength measurements used in this study followed that specified in ISO1567 for denture base

resin. Each specimen was positioned on a bending fixture, consisting of 2 parallel supports (50)mm apart, the full scale load was 50kg, and the load was applied with a cross head speed of 1mm/min by a rod placed centrally between the supports making deflection until fracture occurred. Then stresses were calculated through the following equation [26].

$S=3PL/2bd^2$ s = transvers strength (N/mm²), P =load (N), L =Length (mm), d =depth (mm).

2. Impact strength test

It was conducted according to the procedure given by the ISO 179 for un-notched specimens with an impact testing instrument of charpy type (Impact tester LAYEE N. 43-1, INC. USA.). The specimen was supported horizontally in its ends and then struck by a free swinging pendulum which

3. Surface roughness test

This test has been done using a surface roughness tester (TR220 portable roughness tester, Beijing, Time high technology. Ltd, China. The surface roughness was derived from numerical values of the surface profile. R_a value (μm) described the surface roughness of the surface. It is defined as the mean value of all absolute distance of the roughness profile from the mean line within measuring distance. Mean values of three data were obtained and calculated from the non-polish surface which represent the impression surface.

4. Indentation hardness

To determine Indentation hardness values, A Durometer hardness tester from type shore D(hardness tester-th 210, time group Inc. Italy) was used for measurement of surface hardness, The device is supplied with indenter of 0.8 diameter that's taper to a cylinder of 1.6mm. Also indenter is a blunt pointed type that's attached to digital scale reading by a lever. This digital scale reading is graduated from 0-100unit, and the measurements were obtained directly from the scale [26]. The test has been used to evaluate the hardness of heat cure material before and after irradiation with

released from a fixed height in the middle. The maximum capacity of pendulum was 2joules. The charpy impact strength of the unnotched specimen was calculated in KJ/m² as given by the following equation: Impact strength = $\frac{E}{b.d} \times 10^3$ [27], Where E : is the impact absorbed energy in joules. B : is the width of the test specimens in millimeters and D : is the thickness in millimeters of the test specimen.

a laser for 5 and 10 min. Three measurements were obtained from the

non-polish surface at different site in each specimen (this was the same selected site in each specimen), then the average value of three data was obtained.

5. FTIR analysis

For this analysis, after complete conditioning of all specimens, they were divided into two group, the 1st group of specimens not irradiated with the laser and 2nd group irradiated with the laser for 10 min. At this step specimens were completely dry and ready to be place in a Fourier-transformed infrared spectroscopy device (FTIR) Shimadzu FTIR-8400S. (FTIR) Spectroscopy is a very useful tool for molecular characterization. Each chemical bond between the atoms of a material has one specific vibrational characteristic, which produces interference in electromagnetic waves, at highly specific wavelengths. As light is transmitted through the sample, chemical bonds can be identified with the use of an infrared (IR) bench detector. The Fourier transform algorithm produces a spectrum with characteristic bands over a wavelength range, providing a very accurate picture of molecular

structure directly from the surface of prepared specimens [26].

6. Candida adherence test (cell count)

This test started with the following step:

a. Isolation of *Candida albicans* A swab has been taken from the palatal surface of a patient with denture stomatitis. Then cultured in Sabouraud dextrose agar (this media prepared as Hareley et al [28]). Then after cooling antibiotic procaine penicillin & streptomycin was added to prevent bacterial growth [29].

b. Identification of *Candida albicans*

Identification of *Candida albicans* was done using four methods of identification starting with 1. Colony morphology: Colonies of *Candida albicans* appear smooth, moist and creamy in color with a yeast odor and typically medium sized 1.5-2 mm diameter which later develop into high convex, off-white and large colonies after 2 days [30] 2. Gram stain 3. Germ tube formation is the standard laboratory method for identifying *Candida albicans*. Germ tube are filamentous out growth from blastospores of *Candida albicans* 4. VITEK 2 systems product information: the yeast card YST card is used for the automated identification of the most significant yeast and yeast like organisms [31, 32].

c. preparation of *Candida albicans* suspension

In this study, sucrose broth was prepared according to Lennette et al. [33]. Then an electronic balance was used to weight 10 grams of sucrose broth. This was dissolved in 1000 ml of distilled water in a glass flask by gentle shaking on a flame. After that the broth was sterilized in autoclave for 15 min at 15 lb/inch. After sterilization, the broth was transferred into tubes and small inoculums from isolated yeast colonies were suspended in the broth. Thereafter, tubes were incubated at 37°C for 24 hours.

d. Preparation of *Candida albicans* suspension

Suspension of *Candida albicans* (1×10^6 cell/ml) was prepared and monitored by Neubauer chamber [34].

e. Adherence assay After complete conditioning of the specimens for 48 h. Thirty specimens were sterilized in autoclave for 2 min at 121°C. Then each specimen was placed in 20 ml of *Candida albicans* suspension (1×10^6 cell/ml) in sterile Petridish, left for 90 min at room temperature. After that specimen was ready for surface irradiation.

c. laser surface treatment Thirty specimens contaminated with *Candida albicans* were divided into three groups: twenty specimens out of thirty were irradiated with a laser for 5 & 10 min and this was done following the same procedure previously mentioned in 2.3 section. The remaining 10 specimens were non-irradiated control group. After that all specimens (irradiated and non-irradiated) were washed by dipping them in a sterile distilled water for 1 min with agitation so that all non-adherence cells were removed. After they were dried, adherent cells were fixed by methanol and stained with crystal violet, then all specimens examined under a light microscope (microscope AUSTRIA, with digital camera) with an illumination of the surface of specimens with 200X magnification.

d. Enumeration of *Candida albicans*

This was done via 15 fields of (0.2) mm²/field. The mean values for these 15 fields were obtained from non-polished surface of each specimen.

7. Statistical analysis

Statistical analysis of data was performed using SPSS software program version 17. ANOVA test was applied to determine any significance difference between different groups. Least significance difference test LSD was applied to determine the level of significance difference between groups at

a level of significance $p \geq 0.05$ (NS), $p \leq 0.05$ (S), $P < 0.01$ (HS).

Results

Table -1 shows the results of mean values and standard deviation of transverse strength, surface roughness test and the results of ANOVA test. In this table the lowest mean values of

transverse strength and surface roughness were (66.1MPa) & (0.753 μ m) respectively in specimens irradiated with a laser for 10 min. Also, it can be seen that there was a highly significance difference among a different group of specimens in each test whether transverse strength or surface roughness test.

Table 1: Mean, Standard deviation & ANOVA test for transverse strength (MPa) and surface roughness test (μ m)

Groups	N	Transverse strength		surface roughness	
		Mean	Std.	Mean	Std.
control	10	81.5	8.566	1.714	0.193
5 min	10	74.5	9.800	1.563	0.316
10min	10	66.1	11.779	0.753	0.415
ANOVA	F value	5.787		25.811	
	Sig	0.008		0.000	

Regarding the results of a least significance test, in table-2 there was a statistically significance difference among

all test groups except that between control group and 5min irradiation time group

Table 2: LSD Test among groups of transverse strength and surface roughness test

Group		Transverse strength			surface roughness		
		Mean Difference	P value	Sig.	Mean Difference	P value	Sig.
control	5min	7	0.134	NS	0.151	0.304	NS
	10 min	15.4	0.020	HS	1.961	0.000	HS
5min 10min		8.4	0.075	NS	0.810	0.000	HS

Impact strength and indentation hardness mean values of all groups tested are presented in table 3. In this table, there were no significance differences among the groups in each test. For all groups, the

mean values of indentation hardness and impact strength of specimens exposed to laser for 10min were higher than those exposed to laser for 5min and control groups.

Table 3: Mean, Standard deviation & ANOVA test for Impact strength KJ/m² and indentation hardness test

Groups	N	Indentation Hardness		Impact strength	
		Mean	Std.	Mean	Std.
control	10	76.00	6.514	5.70	0.627
5 min	10	78.00	7.363	5.99	0.874
10min	10	80.20	11.331	6.21	1.324
ANOVA	F value	0.588		0.689	
	Sig	0.562		0.511	

The results of FTIR test revealed no change in the chemical structure of the examined surface of heat cure specimen

after exposure to laser irradiation as shown in fig-3

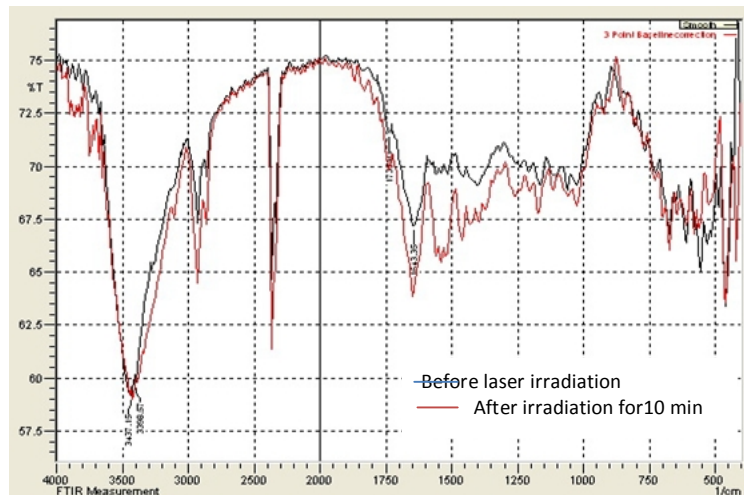


Figure 3: FTIR analysis for heat cure specimen before and after laser irradiation for 10 min

In Table 4-, the results of *Candida albicans* cell count test revealed that there was marked decrease in the mean values of this test with the lowest mean value (0.136) at 10 min exposure time. Also in the same table ANOVA test result revealed

that there was statistically significance difference between different groups of tested specimens. And the LSD test results in table 5- appeared a statistically non-significant difference between 5 and 10 min exposure time to a laser only.

Table4: Mean, standard deviation&ANOVA test for *Candida albicans* cell count test

Groups	N	Mean	Std.	F value	Sig
control	10	0.228	.065	4.732	0.017
5min	10	0.163	.072		
10min	10	0.136	.067		

Table 5:LSD test between different groups of heat cure specimens exposed to Nd:YAG laser for 5 and 10 min

Groups		No. of <i>C. albicans</i>		
		Mean Difference	P value	Sig.
control	5min	0.065	0.440	S
	10 min	0.092	0.006	HS
5min	10	0.027	0.388	NS

Discussion

Denture stomatitis can be treated with different modality of treatment and these include oral or topical antifungal drug .But this might have a side effects and systemic action from use chemical drug. With the introduction of laser and its many application in different part of science, especially in dentistry. A new approach for this research includes the use of Nd: YAG laser as a tool in reducing *Candida albicans* cell count contaminated heat cure acrylic surface, especially when it can be considered as a clean, safe and easy in method application in removal of *Candida albicans* from a denture surface. Also the present study evaluate the effect of this kind of laser on other properties of heat cure denture base material which include

impact strength, transverse strength, indentation hardness and surface roughness.

In the present study, the wave length of laser used was 532nm, this was because of the result in figure 1 &2 indicates that laser light (green beam) irradiated specimens for 5 & 10 min do not affect the color properties of a heat cure specimens. Also this results demonstrates that light absorption and transmission properties of heat cure resin specimen exposed to 200-800 appeared that no difference in the values of these properties, especially in the area from500-600.This can explain that amount of laser used in irradiation of a specimen, absorbed and reflected without significance change in color properties of

material as compared with control. In literature change in color properties of denture base materials is related to several factors: intrinsic and extrinsic factors, any one of these factors might affect the absorption and reflection properties of material or change the chemical structure of material and this might cause change in color properties of denture base material [35]. In this study laser do not cause change in chemical structure of material as in fig 3, and this might explain no change in color properties of material after irradiation with laser.

In the procedure of laser treatment, The distance between a source of laser and a non-polish surface of a specimen was 100cm and a lens place between them so that a laser beam irradiate all surface area of a specimen and not a spot.

Transvers strength and surface roughness

Effects of laser revealed that Nd: YAG laser causes a slight decrease in transvers strength although it was within the limitation of ADA specification. Also effect on surface roughness revealed a reduction in roughness this might be due to the slight etching effect on the surface.

Indentation hardness and Impact strength. Although the Nd: YAG laser cases a slight increase in indentation hardness and Impact strength, but still this effect had anon significance difference as compared with control despite of increase irradiation time. This might be attributed to that the maximum exposure time of 10 min might not have an adverse effect on the heat cue specimen, i.e. heat generation from

exposure to this type of laser not high enough that can change the properties of the material.

Candida albicans cell count test

According to the results of *Candida albicans* cell count test before and after exposure to laser, there is a decrease in the means value of *Candida albicans* cell count after exposure to Nd: YAG laser and the mean values of *Candida albicans* cell count deceased with increased exposure time to Nd: YAG this might be due to the amount of heat generation from exposure to laser can affect the viability of a microorganism which might lead to a reduction in the number of cell especially when a number decreased with increased exposure time.

This result agrees with the results obtained by Sennhenn-Kirchner et al who showed that diode light and Er: YAG laser light was efficient in decontamination of titanium and glass surface from *Candida albicans* biofilms grown on [36].

Also agree with the results of Alkayssi and Asaidy in 2002 in this study CO₂ laser was effective in killing microgram like *E. coli*, *Staph aureus* and *pseudomonas aeruginosa* [23].

Conclusion

Within limitation of this study, it can be concluded that Diode pumped Nd: YAG laser can be an effective tool used for reducing the numbers of *Candida albicans* on the non-polish surface of heat cure denture base material. A non-significance effects on Transverse strength, impact strength, surface hardness and surface roughness especially at a 5min exposure time.

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