Original Article

Effect of Some Disinfectant Solutions on the Adherence of *Candida albicans* to Three Types of Acrylic Resin Denture Base Materials

Seeran M. Ahmed[1*], *Nadia T. Jaffer*[1]

Abstract

Objectives: Microbial adhesion to denture surface is problematic for denture wearers, especially those with impaired dexterities. This study aimed to evaluate the effectiveness of two plant extracts (clove and Basil) in addition to Chlorhexidine on *candida Albicans* adherence to (heat, cold, light) activated acrylic denture base materials.

Methods: Two herbal extracted clove (Eugenia caryohyllata) and Basil (Ocimum basilicum) were prepared by drying, ground to powder, and extracted with ethanol chlorhexidine gluconate of 0.12% were used as three disinfectant solutions. One hundred and five square acrylic samples were prepared from (heat, cold, and light) activated acrylic resin denture base materials in dimensions of 10×10×2 mm for the study. The disk diffusion test was used to determine the susceptibility of *C. Albicans* to disinfectant solutions. The adhesion of yeast to the acrylic resin samples was measured with a light microscope by measuring the number of fungal cells in the corners of the square and expressed as no. of cells/mm². Statistical analysis was done by ANOVA to compare the mean of readings between disinfectant solutions and post-hoc t-test in which the p-value is less than 0.05.

Results: Statistically significant differences ($p<0.05$) of adhesion of *C.albicans* were found on the surfaces of three types of activated acrylic resin materials with means of 19.18, 46.25, and 26.50 cell/mm^{2}on heat, light, and cold cure respectively and after immersing the specimens on the disinfectant solutions regarding the highest effect of the disinfectant solutions was seen in chlorhexidine gluconate of 0.12% which demonstrate 3.11, 5.89 and 5.43 cell/mm^2 followed by Basil with means of 6.75, 13.57 and 10.11 cell/mm^2 on heat, light and cold cure acrylic resin specimens correspondingly, then the clove and finally distilled water.

Conclusions: Both clove buds and basil leaves extracted solutions exhibited high antifungal activity besides Chlorhexidine on the surface of denture base materials. The highest inhibition zone was seen in Chlorhexidine, followed by Basil, clove, and distilled water, respectively. The number of fungal cell adhesion to the three types of denture base was decreased in the three disinfectant solutions when compared to distilled water (control group).

Keywords: *Disinfectant solutions, acrylic denture base, C. Albicans.*

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Introduction

Adverse esthetic and biomechanical consequences accompany the loss of teeth. These problems are accompanied by reducing the person's ability for mastication and therefore disturbed nutritional state accompanied by defective speech and bad esthetics. Many patients seek a solution for their problems, and the conventional treatment for these cases is the construction of removable denture[1].

Poly (methyl methacrylate) is still the most predominantly used denture base material because of its excellent esthetics, ease of processing & repair, and economical[2]. However, it is not considered an ideal material because of its inferior physical and mechanical properties[3]. Polymerization of a PMMA-based dental resin is a different reaction that requires the activation of an initiator, such as benzoyl peroxide, which can then be decomposed by many different means, such as heat (heat polymerization) or microwave polymerization[4]or by addition of a chemical activator, such as dimethyl-toluidine, at moderate temperatures (autopolymerization) or light polymerization[5]. In the oral cavity, properties and functional values of acrylic resin-based products depend on its endogenous factors caused by polymerization (degree of conversion of their constituent monomers, methods, and the conditions of polymerization) as well as exogenous factors caused by conditions present in the oral cavity (saliva, bacteria, mastication)[6]. The degree of change depends on the size and quality of the restoration inserted, which, in turn, will affect the microbiology of the oral cavity. All surfaces in the oral cavity are covered by a pellicle of glycoproteins from saliva within seconds after cleaning[7]. Microorganisms can adhere to this layer and form biofilms organized as a consortium of bacteria, viruses, and fungi. *Candida albicans* (*C. Albicans*), which is considered to be strongly associated with denture stomatitis. This pathogen is naturally present in oral cavity flora and can form a biofilm that can adhere to oral mucosa or acrylic denture surfaces.

Denture hygiene is of utmost importance because dentures are used by the patients throughout the day and are in contact with the oral environment, including various microorganisms[8]. Three methods are advocated for cleaning of dentures that includes mechanical, chemical, and a combination of both. The mechanical method is routinely and widely used by the patients. Still, many elderly patients are not able to follow it because of a lack of compliance and poor motor coordination due to age. Hence, the use of chemical disinfectant solutions becomes a viable option for such patients[9]. These chemical agents are classified according to their composition and mechanism, namely, hypochlorites, peroxides, acids, enzymes, crude drugs, and disinfectant enzymes, neutral enzymatic peroxides solution[10]. Effervescent tablets yielding an alkaline peroxide dilution with water are the preferred denture cleansers because they can easily provide enough cleansing without damage to surface resins[11]. These effervescent tablets act differently as mechanisms against microbial flora; the Chemical agents being commercially available as chlorhexidine gluconate (CHX) solution. Also, there is an alternative treatment in using plant extracts and their essential oils to treat the adherence of microbial species to denture base material since plants contain biologically active compounds, many of which have been shown to have antimicrobial properties[12].

This study was carried out to evaluate the effectiveness of disinfectant solutions as chlorhexidine gluconate. Free plant extract solutions as clove, Basil on adhesion of *Candida albicans* on acrylic resin denture base (chemical, light, and heat) activated materials.

Materials and methods

Acrylic resin denture base specimens preparation:

A total of 105 samples of square prism-shaped with dimensions of 10×10×2 mm were fabricated for the study. Thirty-five specimens for each of denture base resin (heat, cold and light-activated denture resin), For heat and chemical cure acrylic resin the wax plate's specimens of would be prepared by cutting the sheet of modeling wax from Major, Italy company manually by wax knife and rule to proper dimensions on a clean-dry glass slab surface[13]. Each wax plate specimen was inserted into the investing stone, which then allowed to set for 1 hour before pouring the second layer of stone for flasking then wax elimination as seen in figure 1(A and B); compression molding technique used to transform the wax specimens to acrylic, powder (polymer) and liquid (monomer) from Veracril company (manufactured by New Stetis S.A. cr.53 no. 50-09 Guarne, Antioquia-Colombia) were mixed in a glass jar. The use of this 3:1 ratio minimizes the undesirable effects, the top of the glass jar is screwed on, the acrylic dough was packed into the flask using a hydraulic press, and the closing force was applied slowly until metal to metal contact of the flask s portions was obtained[14]. The flask was then placed under the clamp press and waited for 30 minutes before curing (for heat-cured materials), allowing the liquid to penetrate the powder thoroughly. While for the cold cure resin, the polymerization process was established at room temperature 25°C with the flask remained under the

clamp press for 24 hours. After bench cooling, the flask was opened, and the specimens were removed. The specimens (heat and cold cured specimens) were finished and polished as usual to the polish acrylic removable prosthesis[15]. While for light cure specimens (from Plaque Photo company made in Germany), a total of 35 specimens were cut using a surgical blade to the proper dimensions, then cured using the (curing machine) for four minutes[16] as seen in figure1(C). All specimens were placed in distilled water for 24 hours at 37°C to leach out any residual monomer[13], then sterilized by autoclave at 15 pounds/inch^{2}at 21°C for 15 minutes; after that, they were placed in the container that lid was tightly closed to prevent contamination and before adhesion experiment, the specimens immersed in 70% alcohol for 20-30 minute and washed in running D.W[16].

Preparing of Herbal extracted disinfectant solutions:

The clove (Eugenia caryohyllata), family of Myrtaceae and Basil (Ocimum basilicum), family of Lamiaceae, were used in this study. Basil leaves were washed with D.W. This was done to lessen the microbial load on the plant material. They were allowed to air dry and ground to a powdered form. The powder was extracted with 100% ethanol for 30 h. Three hundred grams of finely powdered basil leaves were mixed with 1 L of ethanol; then, it was centrifuged at 12000 rpm for ten minutes. The supernatant was collected, and the solvent was evaporated in a hot air oven at 50°C. From 300 g of powder dissolved in 1 L of ethanol, 18 g of residue (extract) was obtained. The extract obtained after processing was then desiccated and stored in an airtight container. The final solution was formulated by dissolving 0.4 g of extract in 10 ml of deionized water. The same method was done with clove buds samples except that these buds were already dried[17].

Disinfectant Solutions:

The chemical solution used was chlorhexidine gluconate of 0.12% (Kin S.A., Spain) according to the study of Rath and Singh[18], which show that adsorption of monolayer formed by low concentration CHX is more stable than the high multilayered concentration over the microbial cell wall and 0.12% chlorhexidine had the greatest antibacterial activity on both planktonic and biofilm-grown organisms[19]. In addition to these disinfectant solution used distilled water (D.W.)[20].

Isolation of *Candida albicans*

Five patients were selected randomly attending to prosthodontics department/college of the dentistry/ University of Duhok. Those patients were denture wearer for more than one year and had denture stomatitis with different intensities. The inner surface of the complete upper denture and the palatal mucosa was swabbed using disposable sterile cotton wool transporter jell swab, then the swab cultured immediately on sabouraud's dextrose agar and incubated at 37°C for 24-48 hrs[21].

Identification of *Candida* species:

Colonial morphology: Colonies are creamy to white, flat, or domed, and have a dry glistering or waxy surface[22].

Staining and microscopically characteristics: Using crystal violet staining, the candida species take gram-positive stain microscopically; it appears under a light microscope as spherical to budding oval cells[23].

Vitek2 test: Its smart design helps ensure better overall laboratory workflow with fewer repetitive tasks, higher safety, improved standardization, and rapid time-to-results and reporting[24].

Preparation of Phosphate Buffer Solution (P.B.S)

According to Zoccolotti *et al*.[25], the phosphate buffer solution (P.B.S) was prepared immediately by adding 28ml of monobasic sodium phosphate to 72 ml of dibasic sodium phosphate. The P.B.S has a pH of 7.2.

Susceptibility of *Candida albicans* to disinfectant solutions (disk diffusion method)

For Determination of Susceptibility of *C. Albicans* to the disinfectant solution used disk diffusion method: The disks have been prepared by sterilizing disk blank in the autoclave and then left in the three solutions containing the appropriate concentration of agents by immersing them inside a clean and closed container for about 24 hrs. A loop full of the yeast culture was taken and inoculated in 4 ml of brain heart infusion broth, mixed well then a swab from the broth was cultured on sabouraud agar, one control disk blank (immersed in D.W.), the four disks placed on the surface of the culture (used four Petri dishes sabouraud agar for this test) then incubated for 24 hrs at 37°C and the inhibition zone was measured[26].

Measurement of *C. albicans* adherence to acrylic resin denture base specimens

The thirty-five specimens of each type of acrylic resin base (heat cure, chemical cure, light cure) after sterilization of them were placed on three Petri dishes containing 4 ml of saliva used industry-specific artificial saliva according to Leung and Darvell[27]and Pavithra[28] incubated at 37°C for 30 minutes without agitation. A loop full of the yeast of *Candida Albicans* colons that cultured on sabouraud agar added on all specimens and retained to the Petri dishes and incubated without agitation for 1hr at 37°C. The petri dish was removed from the incubator, and the specimens were washed by dipping gently ten times in 100ml of P.B.S to remove the loosely adherent cells[29]. All the steps were carried out inside the sterilized closed hood. Specimens were then dried by lying horizontally inside the hood and immersed in 80% methanol for 30 seconds to fix the remaining C. *Albicans* cells. Specimens were allowed to dry by lying horizontally inside the hood. Seven specimens of each material were removed and stained by immersing in crystal violet for a minute[30] then were washed with P.B.S for 30 sec and air-dried; the remaining 28 specimens of each material were subdivided into four groups according to the disinfectant solution, each had seven specimens immersed in Petri dishes containing (chlorhexidine gluconate 0.12%, clove, Basil, D.W.) for 24 hours in room temperature as seen in figure 2. Specimens were allowed to dry inside the hood; then, they were stained, washed with P.B.S solution, and dried as mention before.

Examination of all stain specimens under the light microscope by examiner. Enumerate the number of adherent fungal cells in the corners of square shapes for each specimen, and the final result will express as the number of cells/mm^2[16].

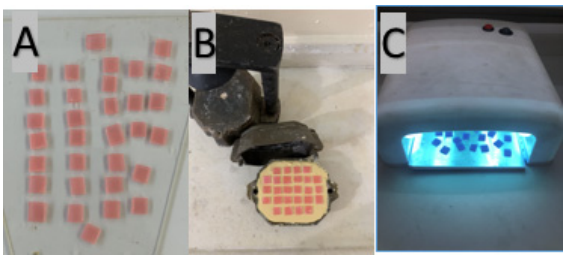

Figure 1: Acrylic denture resin preparation A: wax plate's specimens. B: Investing the wax specimens in the flask. C: Light cure specimens inside the curing machine.

Statistical Analysis

The average number of four readings of the number of *Candida Albicans* for each cure acrylic denture was determined in mean and standard deviation. The comparison of the mean of readings among different disinfectant solutions was determined in one-way ANOVA. The pairwise comparison of the mean of reading of the number of *candida Albicans* between different disinfectant solutions was examined in post-hoc t-tests (LSD). The difference in the inhibition zone made in the sensitivity test among the study group was examined in one-way ANOVA. The statistically significant difference in mean of readings was determined in a p-value of less than 0.05. The statistical calculations were performed in Statistical Package for Social Sciences version 25 (SPSS 25, IBM Corp; USA.

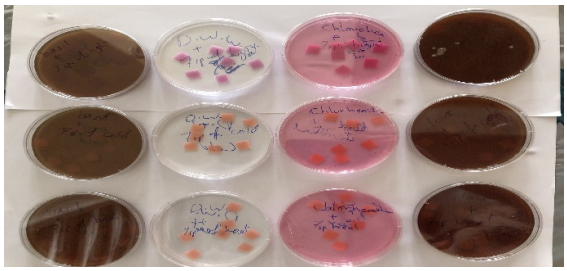

Figure 2: the specimens immersed in the disinfectant solutions

Results

Susceptibility of *C. albicans* to disinfectant solutions:

The study showed that the inhibition zone made in the Chlorhexidine group is significantly broader (10.25 mm) compared to other groups. The inhibition zone made in Basil has followed the Chlorhexidine (8.75 mm; $p<0.01$), as presented in Table 1.

Measurement of *C.albicans* adherence to acrylic resin denture base specimens:

The result showed in Table 2 and Figure 3 demonstrated the amount of adherence of *Candida Albicans* to heat cure acrylic denture resin specimens before and after immersing into different disinfectant solutions and compared between them, so the comparison exhibited that lowerest adhesion was in CHX 3.11 cell/mm^2 and greatest in D.W 11.89 cell/mm^2, but all the four solutions was a significant difference at the 0.05 level.

Table 3 and Figure 3 displayed the adhesion of *C. Albicans* to light-cured denture base specimens and the effectiveness of the disinfectant solutions; the outstanding one was CHX, followed by clove. Then, Basil, D.W showed the lowest effectiveness.in addition to that difference, among them was significant at the 0.05 level.

The study in Table 4 and figure 3 put on view the proximity of the effectiveness of the two disinfectant solutions clove and basil 9.18 and 10.11 cell/mm^2 respectively on cold cure acrylic resin specimens that came after CHX; the difference among all the four solutions was significant at the 0.05 level.

Table 1: Inhibition zones made by antibiotics sensitivity tests among disinfectant solutions.

Study Groups (n=4)	Statistics				
	Mean	SD	SE	Minimum	Maximum
Chlorhexidine	10.25	1.26	0.63	9.00	12.00
Distilled Water	6.38	0.48	0.24	6.00	7.00
Basil	8.75	0.50	0.25	8.00	9.00
Clove	7.63	0.48	0.24	7.00	8.00

The difference in the inhibition zones of the disinfectant solutions is statistically significant (P<0.001).

Table 2: Pairwise comparison of the mean of readings of different disinfectant solutions in heat cure acrylic denture.

Dependent Variable: Mean of Readings LSD							
disinfectant solutions n=7	Mean	SD	disinfectant solutions (I)	disinfectant solutions (J)	Mean Difference (I-J)	SE	p-value
Befor	19.18	10.49	Before	CHX	16.07*	2.69	**<0.001**
				Clove	11.04*	2.69	**<0.001**
CHX	3.11	1.00		Basil	12.43*	2.69	**<0.001**
				Distilled water	7.29*	2.69	**0.01**
Clove	8.14	1.86	CHX	Clove	-5.04	2.69	0.07
				Basil	-3.64	2.69	0.19
				Distilled water	-8.79*	2.69	**<0.001**
Basil	6.75	2.73	Clove	Basil	1.39	2.69	0.61
				Distilled water	-3.75	2.69	0.17
Distilled water	11.89	2.09	Basil	Distilled water	-5.14	2.69	0.07
The means of readings were statistically different among different disinfectant solutions (P<0.001).			*. The mean difference is significant at the 0.05 level. The bold numbers show the significant difference.				

Table 3: Pairwise comparison of the mean of readings of different disinfectant solutions in light cure acrylic denture.

Dependent Variable: Mean of Readings LSD							
disinfectant solutions n=7	**Mean**	**SD**	**disinfectant solutions (I)**	**disinfectant solutions (J)**	**Mean Difference (I-J)**	**Std. Error**	**p-value**
Befor	46.25	6.25	Before	CHX	40.36*	2.07	**<0.001**
				Clove	35.75*	2.07	**<0.001**
CHX	5.89	1.11		Basil	32.68*	2.07	**<0.001**
				Distilled water	25.07*	2.07	**<0.001**
Clove	10.50	1.74	CHX	Clove	-4.61*	2.07	**0.03**
				Basil	-7.68*	2.07	**<0.001**
				Distilled water	-15.29*	2.07	**<0.001**
Basil	13.57	2.71	Clove	Basil	-3.07	2.07	0.15
				Distilled water	-10.68*	2.07	**<0.001**
Distilled water	21.18	4.99	Basil	Distilled water	-7.61*	2.07	**<0.001**

Table 4: Pairwise comparison of the mean readings of different disinfectant solutions in cold cure acrylic denture.

Dependent Variable: Mean of Readings LSD							
disinfectant solutions n=7	**Mean**	**SD**	**disinfectant solutions (I)**	**disinfectant solutions (J)**	**Mean Difference (I-J)**	**SE**	**p-value**
Befor	26.50	9.17	Before	CHX	21.07*	2.41	**<0.001**
				Clove	17.32*	2.41	**<0.001**
CHX	5.43	1.93		Basil	16.39*	2.41	**<0.001**
				Distilled water	6.86*	2.41	**0.01**
Clove	9.18	1.20	CHX	Clove	-3.75	2.41	0.13
				Basil	-4.68	2.41	0.06
				Distilled water	-14.21*	2.41	**<0.001**
Basil	10.11	1.34	Clove	Basil	-.93	2.41	0.70
				Distilled water	-10.46*	2.41	**<0.001**
Distilled water	19.64	3.24	Basil	Distilled water	-9.54*	2.41	**<0.001**

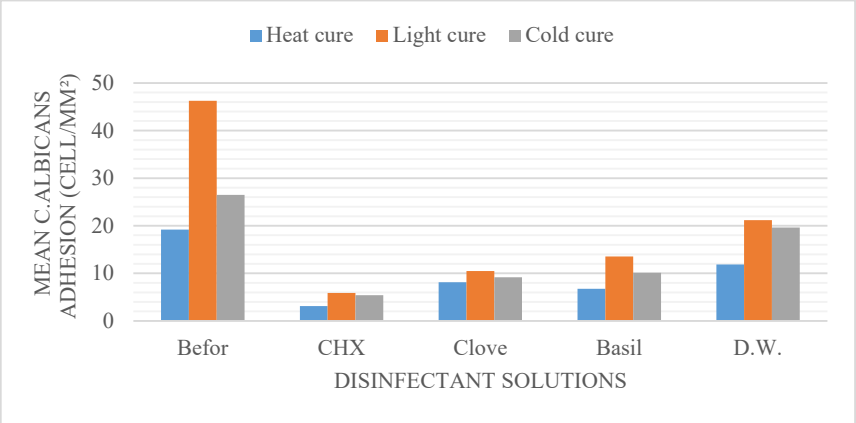

Figure 3: Pairwise comparison of the mean of readings of different disinfectant solutions in heat, light, and cold cure acrylic denture.

Discussion

In the oral cavity, most microorganisms can only survive if they adhere to non-shedding intraoral surfaces, including dental prosthesis. Surface characteristics of the acrylic resin, microorganism strain, and the mutual interaction of the two are among the most important factors affecting the colonization of microorganisms on tissue and denture surfaces. *C. Albicans* is the most prevalent opportunistic fungal pathogen in the oral cavity, usually observed in association with denture stomatitis. *C. Albicans* adheres to denture surfaces and proliferates. The adhesion of Candida direct associated with the surface roughness and of the acrylic resin[31].

The result of the present study was the first step when the yeast of *C. Albicans* was added to the three types of denture base specimens showed that the surface area of light cure acrylic specimens has highest adhesion of *Candida albicans* followed by cold cure specimens then the heat cure acrylic resin specimens of denture base materials that show less *candida albicans* adhesion as seen in Figure 3 and Tables 2,3 and 4 this result came in agreement with the result of recent study on the conventional polishing procedure on cold and heat denture base materials which summarized that all polished and smoothed specimens showed decreases the water sorption of the cold and heat-cured acrylic resins this occurs because the benefit of the polishing process which increased toughness opposite to that surface area of the rough acrylic specimen, so the polishing surface may influence surface smoothness. Rough surfaces mechanically retain moisture, which leads to more adhesion on its surface[15].

In the present study, uses of different disinfectant solutions to decrease the adhesion of biofilm of *C. Albicans* on three acrylic resins after immersing the specimens in those solutions for 24 hours at room temperature. It was found that the chemical agent used in this study to reduce the colonization of *Candida,* which was chlorhexidine gluconate of 0.12% had exhibited the highest significant anti-biofilm activity in the three types of acrylic resin specimens from the two other solutions as shows firstly in the disk fusion test, it formed the larger inhibition zone and after immersion the three types of acrylic resin specimens it had highest means difference among the two other herbal extracted disinfectant solutions as seen in Tables 2,3 and 4. As a result, showed a significant difference at the 0.05 level, the adhesion of *Candida* to heat cure specimens decrease. In contrast, in cold and light cure resin specimens, the adhesion of *Candida* changed to be a little higher. Generally, there was a clear decrease in adhesion after immersing in chlorhexidine gluconate. This was because the monolayer's adsorption formed by low concentration CHX is more stable over the microbial cell wall that reduced the activity of *C. Albicans* biofilms in vitro. Another study showed the efficient effect of 0.12% chlorhexidine as denture disinfectant as in Al-Irhayim study [16] that used a low concentration of CHX in compared with propolis, which is a natural product in denture base specimens and also with Parappa S. *et al*.[32] study that used more than one concentration of CHX and the two studies showed the highest result with this concentration which concluded

that: Chlorhexidine is considered to be the best for dental biofilm control and also used against various dental diseases like stomatitis, gingivitis, etc.

The two herbal extracted solutions that had used in the present study, one of them was clove bud extracted solution that had inhibition zone smaller than CHX also have a visible antifungal effect on the acrylic resin specimens after immersing them in this solution 24 hours at room temperature this result that also showed a significant difference ($P < 0.05$) is an agreement with the result of a recent study on antimicrobial and antifungal activities of spices which indicated that the clove bud extracted solution has an antifungal effect due to its physicochemical properties represented by an acid value that give the clove ability to destroy cell walls and membranes of microorganisms, and permeate the cytoplasmic membranes or enter the cells, then inhibit the normal synthesis of DNA and proteins. Eugenol, the major component of clove, has cell wall deterioration and cell lysis [33]. From the recent and present studies, clove bud was a clinically effective antifungal compound against fungal pathogens.

The second herbal extracted solution was basil leaves extracted solution, which had the smallest inhibition zone compared to CHX. The result of the present study revealed that this herbal extracted solution showed antifungal effect against *Candida albicans* on the surface of the specimens after immersing the heat, cold, and light cure acrylic specimens, as the microbial colonies account was reduced to less than 25 colonies Figure-1, this results which showed a significant difference ($P < 0.05$) similar to the result of a recent study on the microbial activity of Basil essential solution which concluded that basil leaves contain essential oil at a percentage of 0.2-1%, with the main components being linalool and estragole (methyl chavicol) which can inhibit acid-resistant of microorganism, in addition, it has an antioxidizing effect[34].

Conclusion

With the limitations of the study, it can be concluded that chlorhexidine gluconate 0.12% not only the excellent antifungal disinfectant solution also clove bud and Basil leaves extracted solutions emerged as the potent agents exhibiting high antifungal activity on the surface of the three types of acrylic denture base materials specimens as they show the broad effect on heat cure resin due to it 'smooth surface and they demonstrated pretty effect on both light and chemical acrylic resin specimens.

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