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ORIGINAL RESEARCH

Evaluation and Comparison of Effect of Delmopinol Application on Adherence of *Candida albicans* on Denture Fitting Surface on Three Types of Acrylic Resin: An *in vitro* Study

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ABSTRACT

Purpose: Denture-related stomatitis is probably the most common form of oral candidiasis and its reported prevalence varies widely ranging up to 65%. In this *in vitro* study, we evaluate the effect of delmopinol application on *Candida albicans* adherence on heat-cured acrylic resin, cold-cured acrylic resin and microwave-cured acrylic resin.

Materials and methods: A total of 40 specimens of each type of acrylic resin were made; 20 specimens of each type were contaminated before delmopinol treatment and 20 specimens were contaminated after delmopinol treatment. The each specimen in each tube was individually transferred to a spectrophotometer at 530 nm wavelength in order to measure the turbidity degree, through the transmittance. Aliquots of 10 µl of each tube was then collected and inoculated into agar Sabouraud plates containing 500 mmol/l of sucrose, which was incubated for 24 hours at 37°C, in order to check microbial growth. Two-way ANOVA analysis of variance test and post-hoc Turkey's test were carried out to ascertain the level of significance ($p < 0.001$) of various observations.

Results: Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1) 1440.80, colony forming units (CFU) ($\times 10^6$/ml), cold-cured acrylic resin specimens (B1) 833.30 CFU ($\times 10^6$/ml) and microwave-cured acrylic resin specimens (C1) 944.70 CFU ($\times 10^6$/ml) was significantly higher than the mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2) 550.85 CFU ($\times 10^6$/ml), cold-cured acrylic resin specimens (B2) 341.45 CFU ($\times 10^6$/ml) and microwave-cured acrylic resin specimens (C2) 451.50 CFU ($\times 10^6$/ml).

Conclusion: In case of contamination after delmopinol application, heat-cured acrylic resin showed maximum reduction in adherence of *C. albicans* in the study.

Keywords: *Candida albicans*, Denture-induced stomatitis, Heat-cured acrylic resin, Cold-cured acrylic resin, Microwave-cured acrylic resin, Delmopinol.

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INTRODUCTION

The synthetic acrylic resins have a long, clinically proven history of use for dentures since they exhibit adequate physical, mechanical and esthetic properties;[1] however, they are susceptible to microbial adhesion, leading to denture stomatitis.[2] Denture itself is considered to be a 'plaque applicator,' by holding plaque masses in contact with the oral mucosa for extended periods of time.[3]

Light and electron microscopic studies have revealed that denture plaque has essentially the same structure as dental plaque on natural teeth. The composition of microbial flora of denture plaque also resembles that of dental plaque, except for an increased number of Candida species which accompanies denture-induced stomatitis.[4]

Denture-related stomatitis, which is probably the most common form of oral candidiasis and its reported prevalence, varies widely ranging up to 65%.[5] It is an inflammatory process of the mucosa has a multifactorial etiology,[6] but one important etiologic factor is the presence of numerous yeasts, usually *Candida albicans*, on the fitting surface of the denture. Denture stomatitis can cause soreness and palatal inflammatory papillary hyperplasia and may lead to poorly fitting dentures in the future.[7]

The adherence of *C. albicans* to solid surfaces, such as acrylic resin has been thought to be an essential prerequisite for successful colonization, subsequent plaque formation and development of pathogenesis.[8]

There are several factors which affect *C. albicans* adhesion on acrylic resin surface; quality and quantity of saliva, wettability of acrylic resin surface, surface properties of acrylic resin, pH, cell surface hydrophobicity and oral bacteria.[5]

Management of Candida-associated denture stomatitis is complex due to its multifactorial etiology. Current treatment includes control of denture plaque, removal of dentures at night, use of antifungal,[3] antiseptic mouth rinses, denture soaks, removal of denture trauma and microwave irradiation.[9]

The most important aspect of control of this interface is good denture hygiene.[10] A routine denture cleaning regimen should be designed to remove and prevent reaccumulation of microbial plaque and also to remove mucin, food debris, calculus and exogenous discoloration. In principle, mechanical cleansing is an effective means of improving denture cleanliness but the major disadvantage is loss of

surface details, which ultimately cause loss of denture retention. Chemical denture cleansers (antiseptic and disinfecting agents) might be an important additive to mechanical cleansing, especially among geriatrics and/or handicapped patients.

The morpholinoethanol derivative delmopinol is a third generation of chemical plaque-control agents. Plaque studies involving delmopinol revealed that the nascent biofilm was loosely adherent [11] and that there was a significant reduction in the proportion of dextran-producing Streptococci.[12] There was no colonization by Candida or major shift in bacterial composition in the active group nor was there any decrease in susceptibility to delmopinol.[13,14]

Delmopinol is a tertiary amine surfactant that is used to counteract dental plaque formation and dissolves newly formed plaque. The adsorption of delmopinol is complex and strongly influenced by pH and concentration[15] and adsorbed on both hydrophobic and hydrophilic surface.[16]

There are very few study reported in literature showing effect of delmopinol on deposition of plaque on natural teeth,[11-16] but there is no study reported in literature showing effect of delmopinol on deposition of plaque on acrylic resin denture fitting surface. Therefore, it is pertaining to taken up an *in vitro* study for evaluation and comparison of effect of delmopinol application on heat-cured, cold-cured and microwave-cured acrylic resin on *C. albicans* adherence.

MATERIALS AND METHODS

Preparation of Acrylic Resin Specimens

Prefabricated acrylic resin sheet (Polytek Industries, Indore, Madhya Pradesh, India) was used to fabricate dies having 10 × 10 × 5 mm dimensions, were invested in flasks, and mold was obtained (Figs 1 and 2).

Thin layer of acrylic resin separating media film (Acralyn-H, Asian Acrylates, Mumbai, India) was applied and packed with plastic stage heat-cured acrylic resin (Acralyn-H, Asian Acrylates, Mumbai, India). The flasks were pressed slowly with a hydraulic pressure until the excess material extruded from the borders and then screwed. Polymerization procedure was performed; according to the cycle described by the manufacturer's instructions and specimens were cooled at room temperature for 30 minutes and left in tap water for 15 minutes. For preparation of cold-cured denture base acrylic resin specimens (DPI-RR Cold Cure, The Bombay Burmah Trading Corporation, Ltd, Mumbai, India), the adequate amount of the polymer and the monomer was mixed, packed and bench cured according to manufacturer instructions. For preparation of microwave-cured denture base acrylic resin specimens (Maarc, Shiva Products, Mumbai, India) 4.5 ml of the monomer is taken into mixing jar and 15 cc of polymer was added.

Fig. 1: Stone mold for preparation of specimens in hanau flask

Fig. 2: Stone mold for preparation of specimens in FRP flask

Microwave Curing Cycle

First stage: For 13 minutes (13:00) at 90 W (low wattage).
Second stage: For 1 minute and 30 seconds (1:30) at 500 W (high wattage).

Total duration of curing cycle is 14:30 min.

Forty specimens of dimension 10 × 10 × 5 mm of each acrylic resin (heat-cured acrylic resin, cold-cured acrylic resin and microwave-cured acrylic resin) were fabricated (total 120 specimens) and were removed after opening the flasks. No finishing and polishing procedure was done in order to simulate the tissue surface of a complete denture. Only remaining excess was removed with the aid of 320-grit wet sandpaper.

Microbiological Test

Before culture of *C. albicans* on acrylic resin plate, all specimens are sterilized by immersion in 2% gluteraldehyde (PKS Pharma Pvt Ltd, Karnataka, India) for 30 minutes to

prevent the contamination and placed in sterilized container. The specimens were immersed in distilled water for 24 hours (Hindustan Pharmaceuticals, Barauni, Bihar, India) in order to promote the maximum water sorption to prevent, when in culture, the occurrence of distortion and the release of residual monomer after polymerization. Pure culture of *C. albicans* was grown on agar Sabouraud plates (Himedia laboratory Pvt Ltd, Mumbai, India) containing 500 mmol/L of sucrose at 25°C. After 24 hours, the colonies were suspended in tubes containing 5 ml of brain heart infusion (BHI) broth (Himedia Laboratory Pvt Ltd, Mumbai, India). The cell suspension in each tube was adjusted spectrophotometrically at 800 nm (OD 800) to match the transmittance of 90 T [equivalent to 0.5 McFarland scale = 1.5 × 108 colony forming units (CFU)]. The *C. albicans* (ACTT-10231) strain was obtained from Department of Microbiology, Calcutta. Next, the specimens were placed into the tubes containing BHI plus inoculums and remained for 11 hours at 37°C in order to favor an initial colonization of the acrylic resin surfaces and colony count was calculated at this stage. Each specimen was first washed with saline (Hindustan Pharmaceuticals, Barauni, Bihar, India) after immersion in the contaminated culture broth. Saline excess was removed with a gentle compression of sterile gauze. Then, the disinfection step was performed by application of delmopinol (Sinclair Pharmaceuticals Limited, UK marketed by Wockhardt Limited in India) for 48 hours. Each specimen was then washed again with saline and the excess was removed with sterile gauze. It was then transferred to individual tubes containing 5 ml of BHI broth. After 24 hours of incubation, the tubes were individually transferred to a spectrophotometer at 530 nm wavelength in order to measure the turbidity degree (Table 1), through the transmittance. Aliquots of 10 µl of each tube was then collected and inoculated into Agar Sabouraud plates containing 500 mmol/l of sucrose, which was incubated for 24 hours at 37°C, in order to check microbial growth. The purity of the positive cultures was confirmed by gram staining, by colony morphology on agar plates.

RESULTS

Turbidity degree is inversely proportional to the *C. albicans* adherence to the all three types of acrylic resin surface. Two-way analysis of variance test (Table 2) and post hoc Tukey's test (Table 3) for materials were carried out to ascertain the level of significance ($p < 0.001$) of various observations. Following results were drawn from this *in vitro* study:

1. Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1), cold-cured acrylic resin specimens (B1) and microwave-cured acrylic resin specimens (C1) was 1440.80, CFU ($\times 10^6$/ml), 833.30 CFU ($\times 10^6$/ml) and 944.70 CFU ($\times 10^6$/ml) respectively (Table 4).

Table 1: Value of colony forming units ($\times 10^6$/ml) obtained in all types of acrylic resin specimens

S. no.	*Heat-cured acrylic resin specimens (group A)*		*Cold-cured acrylic resin specimens (group B)*		*Microwave-cured acrylic resin specimens (group C)*	
	Group A1 (n = 20) Contamination after delmopinol application	*Group A2 (n = 20) Contamination before delmopinol application*	*Group B1 (n = 20) Contamination after delmopinol application*	*Group B2 (n = 20) Contamination before delmopinol application*	*Group C1 (n = 20) Contamination after delmopinol application*	*Group C2 (n = 20) Contamination before delmopinol application*
1.	1432	502	816	306	922	409
2.	1421	550	825	328	982	410
3.	1436	555	847	394	930	432
4.	1400	560	812	388	910	400
5.	1440	542	814	344	948	453
6.	1422	547	812	387	981	442
7.	1430	598	823	321	972	449
8.	1421	541	832	346	963	472
9.	1499	536	822	335	976	435
10.	1470	572	820	342	963	496
11.	1460	567	812	348	942	421
12.	1455	556	815	344	913	407
13.	1450	549	823	346	923	468
14.	1439	562	818	342	912	480
15.	1422	573	834	332	919	496
16.	1427	569	845	306	911	458
17.	1467	592	856	320	945	490
18.	1458	512	890	327	956	449
19.	1447	516	893	338	953	474
20.	1420	518	857	335	973	489

2. Mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2), cold-cured acrylic resin specimens (B2) and microwave-cured acrylic resin specimens (C2) was 550.85 CFU ($\times 10^6$/ml), 341.45 CFU ($\times 10^6$/ml) and 451.50 CFU ($\times 10^6$/ml), respectively (see Table 4).
3. Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1), cold-cured acrylic resin specimens (B1) and microwave-cured acrylic resin specimens (C1) was significantly higher ($p < 0.000$) than the mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2), cold-cured acrylic resin specimens (B2) and microwave-cured acrylic resin specimens (C2) (Tables 4 and 5).
4. Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1) was significantly higher ($p < 0.000$) than cold-cured acrylic resin specimens (B1) (Tables 3, 4 and 6).
5. Mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2) was significantly higher ($p < 0.000$) than cold-cured acrylic resin specimens (B2) (Tables 3, 4 and 6).

Table 2: Two-way analysis of variance analysis

Source	*Type III sum of squares*	*df*	*Mean square*	*F-value*	*p-value*
Corrected model	16341491.37	5.00	3268298.27	4915.38	0.00
Intercept	69391062.53	1.00	69391062.53	104361.09	0.00
Material	3569754.72	2.00	1784877.36	2684.38	0.00
Group	11718750.00	1.00	11718750.00	17624.48	0.00
Material* group	1052986.65	2.00	526493.33	791.82	0.00
Error	75800.10	114.00	664.91		
Total	85808354.00	120.00			
Corrected total	16417291.47	119.00			

Table 3: *Post hoc* Tukey's test for material

	Heat-cured acrylic resin	*Cold-cured acrylic resin*	*Microwave-cured acrylic resin*
Heat-cured acrylic resin		$p < 0.000$	$p < 0.000$
Cold-cured acrylic resin	$p < 0.000$		$p < 0.000$
Microwave-cured acrylic resin	$p < 0.000$	$p < 0.000$	

Table 4: Mean and standard deviation of degree of turbidity for all tested groups

Materials	*Group*	*N*	*Mean*	*Standard deviation*
Heat-cured acrylic resin	Delmopinol → contamination	20	1440.80	22.84
	Contamination → delmopinol	20	550.85	25.44
Cold-cured acrylic resin	Delmopinol → contamination	20	833.30	24.46
	Contamination → delmopinol	20	341.45	24.13
Microwave-cured acrylic resin	Delmopinol → contamination	20	944.70	25.54
	Contamination → delmopinol	20	451.50	31.41

Table 5: Comparison of groups

Group	*Mean*	*Standard error*	*95% confidence interval*		*ANOVA*
			Lower bound	*Upper bound*	
Delmopinol → contamination	1072.93	3.33	1066.34	1079.53	$p < 0.000$
Contamination → delmopinol	447.93	3.33	441.34	454.53	

ANOVA: Analysis of variance

Table 6: Comparison of materials

Materials	*Mean*	*Standard error*	*95% confidence interval*	
			Lower bound	*Upper bound*
Heat-cured acrylic resin	995.83	4.08	987.75	1003.90
Cold-cured acrylic resin	587.38	4.08	579.30	595.45
Microwave-cured acrylic resin	698.10	4.08	690.02	706.18

6. Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1) was significantly higher ($p < 0.000$) than microwave-cured acrylic resin specimens (C1) (Tables 3, 4 and 6).
7. Mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2) was significantly higher ($p < 0.000$) than microwave-cured acrylic resin specimens (C2) (Tables 3, 4 and 6).
8. Mean of the turbidity degree of contamination after delmopinol application for microwave-cured acrylic resin specimens (C1) was significantly higher ($p < 0.000$) than cold-cured acrylic resin specimens (B1) (Tables 3, 4 and 6).
9. Mean of the turbidity degree of contamination after delmopinol application for microwave-cured acrylic resin specimens (C2) was significantly higher ($p < 0.000$) than cold-cured acrylic resin specimens (B2) (Tables 3, 4 and 6).

DISCUSSION

Although polymethyl methacrylate resin are the most commonly used material for denture base for partial and complete denture prosthesis as they provide acceptable color, esthetics, characteristics, function and easy of processing,[1] the adherence of plaque produced specially on the fitting surface has been a matter of serious concern.[17]

Both *in vivo* and *in vitro* studies, regardless of simplicity or complexity of machinery or measurement method have constantly demonstrated the accumulation of plaque on denture surface.[7,17-19]

The process by which denture accumulates plaque stain and calculus is apparently similar to that process which take place on natural tooth surface.[18] Denture hygiene is a important element in maintenance of tissue health, which unfortunately, has always been a neglected aspect. [3, 20] The initial stage of plaque formation involves attachments of microorganism to this pellicle surface,[10] followed by adherence of bacteria to each other, mediated by the component of the plaque matrix, causing the microorganism to coalesce and form large bacterial masses.

Candida is most commonly associated with denture plaque.[20-23] These yeasts are present in the saliva of a majority of denture wearers and display an affinity for adherence to acrylic resin.

Chandra et al,[24] showed that the development of this yeast on acrylic resins happens in three distinct stages: Initial stage up to 11 hours from the colonization, when some microcolonies begin to be formed; intermediated stage from 12 to 30 hours after colonization, when extracellular material begin to accumulate over colonies and maturation stage from 38 to 72 hours after colonization, when Candida colonies become totally involved by the extracellular matrix forming a biofilm. They also concluded that antifungal resistance increases during biofilm development, as the extracellular matrix acts as a barrier to action of the antifungal.

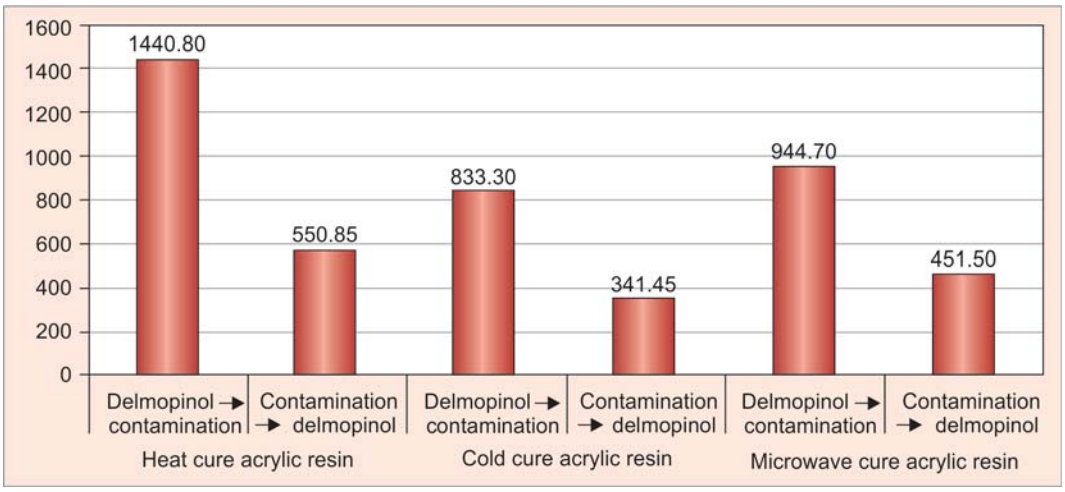
Moran et al.[13] in 2006 with the meta-analyses of studies of 0.2% delmopinol mouth rinse as an agent to gingival health and plaque control measures and concluded that delmopinol 0.2% mouthwash is effective as an adjunct measure for reducing plaque burden and indices of gingivitis, whether or not it is used under supervision.

Eley[14] in 1999 studied a trial of 0.1% and 0.2% delmopinol hydrochloride mouth rinses as adjuncts to normal oral hygiene has been carried out. The microbiological effects were investigated on plaque collected at 12, 24 and 36 weeks. There were no consistent effects on the microscopical or total counts. However, there was a significant reduction in the proportion of dextran-producing Streptococci in the active compared with the control group throughout treatment. There was no colonization by Candida or major shift in bacterial composition in the active group nor was there any decrease in susceptibility to delmopinol. Thus, delmopinol seems to mediate its plaque inhibitory and anti-inflammatory effects without causing a major shift in bacterial population apart from the reduction in dextran-producing Streptococci.

The present study was a comparative evaluation of effectiveness of delmopinol on Candida adherence on three types of acrylic resin: heat-cured, cold-cured and microwave-cured acrylic resin.

Mean of the turbidity degree of contamination after delmopinol application for heat-cured acrylic resin specimens (A1), cold-cured acrylic resin specimens (B1) and microwave-cured acrylic resin specimens (C1) was significantly higher than the mean of the turbidity degree of contamination before delmopinol application for heat-cured acrylic resin specimens (A2), cold-cured acrylic resin specimens (B2) and microwave-cured acrylic resin specimens (C2) (Graph 1). The results of present study were consistent with the findings of Moran et al,[18] that 0.2% delmopinol as a mouthwash reduces plaque on natural teeth surface.

Therefore, it can be concluded that adherence of *C. albicans* is less with the contamination after delmopinol application of the acrylic resin specimens as compared to the contamination before delmopinol application. These



Graph 1: Mean of degree of turbidity for all tested groups

findings can be correlated with the finding of Eley[14] that delmopinol is truly a surfactant agent and has no antibacterial and antifungal effect.

Mean of the turbidity degree of contamination before and after delmopinol application for heat-cured acrylic resin specimens was significantly higher than cold-cured acrylic resin specimens as said by Berger et al,[25] that surface roughness of cold-cured acrylic resin is greater than heat-cured acrylic resin.

Mean of the turbidity degree of contamination before and after delmopinol application for heat-cured acrylic resin specimens was significantly higher than microwave-cured acrylic resin specimens as said by Berger et al,[25] that surface roughness of microwave-cured acrylic resin is greater than heat-cured acrylic resin.

Mean of the turbidity degree of contamination before and after delmopinol application for microwave-cured acrylic resin specimens was significantly higher than cold-cured acrylic resin specimens as said by Berger et al,[25] that surface roughness of cold-cured acrylic resin is greater than microwave-cured acrylic resin.

Adherence of microorganisms and debris is favored by rough or otherwise irregular surface topography.[26-29] Surface irregularities provide an increase in surface area and expansion in the number of niches not readily cleansed by action of tongue or other orofacial musculature. It has been shown that choosing an appropriate type of smooth acrylic resin could lead to reduced biofilm formation.[30]

The surfactant action of delmopinol is greatest for heat-cured acrylic resin than microwave-cured acrylic resin and lesser for cold-cured acrylic resin whether the acrylic resin specimens were treated with contamination before and after delmopinol application.

Though, care has been taken to simulate this *in vitro* study to clinical conditions, but variation may be occurring *in vitro*. Also, owing to a small sample size, the result may not truly represent the population and further *in vitro* investigation in this field with a larger sample size may be required.

CONCLUSION

Following conclusions were drawn from this *in vitro* study:

1. *Considering the material:* Heat-cured acrylic resin showed maximum reduction in adherence of *C. albicans* followed by microwave-cured acrylic resin and further followed by cold-cured acrylic resin.
2. *Considering application method:* Contamination after delmopinol application showed maximum reduction in adherence of *C. albicans* when compared with contamination before delmopinol application.

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