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The Effect of *Aloe vera* Extract on Adherence of *Candida albicans* and Other Properties of Heat Cure Denture Soft Lining Material

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ABSTRACT

Objective: *One of the serious drawbacks of denture soft lining materials is colonization by Candida albicans that might eventually leads to denture stomatitis. This can be treated either systemically or locally. With the recent increase interest in medicinal plants, this study aimed to evaluate the effect of Aloe vera powder incorporated with heat cure acrylic soft-liner powder on the adherence of Candida albicans, shear bond strength and tear strength.* ***Methods:*** *According to the results of pilot study, two percentages (3% and 10%) of aloe vera powder was used. Candida adherence test, shear bond strength and tear strength tests were performed, also the long-term effect was evaluated after 2 and 4 weeks incubation in artificial saliva. All data was analyzed using SPSS software (version 24). Descriptive and inferential statistics, ANOVA test with post-hoc analysis was applied.* ***Results:*** *The results indicated that both concentrations of aloe vera showed a statistically highly significant decrease in Candida albicans cell count in comparison to control group, also a significant increase in shear bond strength and non-significant difference in tear strength of soft liner for the experimental groups. After 2 and 4 weeks incubation in artificial saliva, all experimental groups showed a statistically significant decrease in Candida albicans cell count and a statistically significant increase in shear bond strength and tear strength test.* ***Conclusion:*** *Incorporation of aloe vera powder with heat cure acrylic soft-liner powder helps to add an anti-candidal property to the soft liner, also this addition results in improvement in shear bond strength and tear strength.*

Keywords: Soft denture liners, Adherence test, *Candida albicans*, *Aloe vera*, Tear strength

Abbreviations: SDA: Sabouraud Dextrose Agar; SPSS: Statistical Package for Social Sciences; N: Newton, ANOVA: Analysis of Variance; Alo: *Aloe vera*; SD: Standard Deviation; Sig: significance

INTRODUCTION

Alveolar bone undergoes resorption which might cause a maladaptation of the prosthesis, this leads to patient discomfort. However, this maladaptation can be solved by relining of prosthesis [1]. Relining is defined as the procedures used to resurface the intaglio of a removable dental prosthesis with new base material, thus producing an accurate adaptation to the denture foundation area [2]. Soft lining materials can be divided into acrylic-and silicon-based groups and both groups are offered in auto-or heat-cured systems [3].

Soft denture liners should be easy to handle, easy to clean, tasteless, odorless, have minimal water absorption, minimal dimensional change, no change in color, acceptable aesthetics, and having a thickness of 2 mm to 3 mm with high bond strength to denture base. Disruption of this bond leads to the formation of an area that is difficult to clean and support the proliferation of fungi and bacteria [4]. As denture liners are in direct contact with oral tissue, they must be nonirritating, nontoxic, and incapable of supporting bacterial and fungal colonization [5].

The use of denture soft lining materials accompanied by several problems such as failure of bond between the soft liner and the denture base, loss of resiliency, color alterations, poor tear strength, formation of porosity and consequent plaque accumulation with *Candida albicans* colonization [3].

Candida albicans is the most important of the Candida species and can be found in all areas of the mouth also on the surface of dentures [6]. Denture stomatitis is the most common opportunistic infection among denture wearers [7]. In spite of being a multifactorial disease, the adherence of *Candida albicans* to fitting surface of dentures and to host cells is known as the first step in the beginning and propagation of denture stomatitis [8].

Different modality of treatment was used for denture stomatitis, these include systemic or local measures. A lot of scientific attention is paid to herbal agents, among the various currently available herbal agents and the most popular is *aloe vera* [9]. There are almost 500 species of the Aloe genus. *Aloe vera* is the most commonly used specie both medically and commercially [10]. *Aloe vera* is perennial succulents; it tolerates low water availability. *Aloe vera* has the ability to store in their tissue large volumes of water. *Aloe vera* has green fleshy leaves covered by a thick rind or cuticle, under which is a thin vascular layer covering an inner clear pulp [11]. The chemistry of the plant revealed the presence of more than 200 different biologically active substances [12], such as amino acids, anthraquinones, enzymes, hormones, minerals, salicylic acid, saponins, steroids, sugars and vitamins [13]. Some biologic effects of *aloe vera* includes wound healing, antimicrobial activity, anti-inflammatory effects, antioxidant effect, antiallergic activity and moisturizing effect [12,9,14,15].

It has been found that *aloe vera* has an effective antimicrobial activity, helpful in the management of gum diseases, reduces edema of the soft tissues and consequently this decreases gums bleeding. In addition to its strong antiseptic property that is helpful in treatment of periodontal pocket where normal cleaning is difficult. Also, it has antifungal properties that help in the treatment of denture stomatitis. Its antiviral properties help in the management of shingles (*Herpes Zoster*) and cold sores (*Herpes Simplex*) [15].

The present study aimed to get use from the anti-candida property of *aloe vera* to be incorporated with the powder of acrylic soft liner in different concentrations. And evaluation of this effect through Candida adherence test, tear strength test and shear bond strength test. In addition to test the long-term effect after two and four weeks incubation in artificial saliva.

MATERIALS AND METHODS

Pilot Study

Aloe vera powder (Biorigins Mystic moments, United Kingdom) was applied first in 1%, 2% and 3%. Then 6% was used and finally 10%. Two tests were performed, disk diffusion test and *Candida albicans* adherence test. Results of disk diffusion test showed no inhibition zone for control and experimental specimens. While for adherence test results showed decreased *Candida albicans* cell count for all experimental concentrations. Finally, suitable concentrations were selected (which were 3% and 10%).

Design of the study

Heat polymerized acrylic soft liner (Vertex, Netherlands) was used in this study. Pure organic *aloe vera* powder was incorporated into soft liner powder in two different percentages. A total of 270 samples were prepared and divided into three groups according to the tests to be performed and for each test, specimens were divided into 3 groups according to the time interval of performing the test.

Candida albicans Adherence Test

Specimen preparation

Plastic disc with 50 mm diameter and 2.4 mm thickness was invested into addition type of silicon impression material. Then silicon mold with the plastic pattern was invested in freshly mixed dental stone. For experimental specimens, an electronic balance (XT220A, Precisa, Germany) was used for weighing of *aloe vera* powder (3% and 10%) and the powder of soft lining material. An amalgamator device (Ivoclar Vivadent, Germany) was used for mixing of *aloe vera* powder with soft liner powder for 40 seconds. The soft lining material was mixed (taking into consideration that

the weight of *aloe vera* powder subtracted from the total weight of soft liner powder to get the accurate P/L), packed and cured as instructed by manufacturer. After complete curing, all specimens were finished and polished. Then a metal rod with sharp edges used to cut a circular soft-liner disc with the dimensions of 10 mm × 2 mm diameter and thickness respectively [16], as shown in Figure 1. All specimens were stored in distilled water in an incubator at 37°C for 24 hours before testing to eliminate any residual monomer [17].

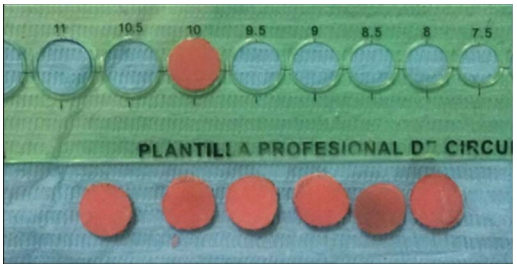

Figure 1 Soft liner specimens used for *Candida albicans* adherence test

Isolation and Identification of *Candida albicans*

Candida albicans was isolated from one patient with signs and symptoms of denture stomatitis. The swab was cultured on sabouraud dextrose agar (Salucea, Netherlands) and incubated at 37°C for 24 hours. Identification of the *Candida albicans* was done as follows: 1. Colony morphology which appeared as creamy, pasty, smooth and convex on SDA [18]. 2. Microscopical examination was done using Gram's stain method [19]. 3. Biochemical method used which includes API 20 C AUX system and API Candida system [20].

Evaluating the adherence of *Candida albicans*

Candida albicans suspension was prepared by taking small inoculum from identified *Candida albicans* and suspended in sabouraud dextrose broth (Oxoid, England) which was prepared according to manufacturer instructions. Then monitoring the concentration of the suspension by McFarland densitometer (Biomerieux, France) has been done until the required concentration was obtained which was equal to 0.5 McFarland standards.

Experimental and control specimens were putted in a sterile glass tubes containing the previously prepared *Candida albicans* suspension, and then incubated for 60 minutes at 37°C. After that the specimens were removed, washed with phosphate buffered saline for one minute and dried using absorbent paper. The adherent cells were fixed with methanol 80% for 30 seconds and stained with crystal violet for 1 minute [21]. Specimens were examined under inverted light microscope (Karl kolb, Germany) shown in Figure 2 using magnification power 40x [22]. The microscope was connected to camera and computer. Four standardized fields were counted on each specimen [23]. To standardize the measurement of adherent cells, filamentous forms were not counted, and budding daughter cells were counted as individual yeast [24]. All experiments were repeated on two separate occasions with duplicate determinations on each occasion [25].

This test procedure was repeated for specimens incubated in artificial saliva at 37°C after 2 and 4 weeks. Artificial saliva was prepared according to Burgmaier [26].

Shear Bond Strength Test

To evaluate shear bond strength of soft lining material to acrylic denture base, acrylic blocks with dimensions of (75 × 25 × 5) mm length, width and depth respectively with stopper about 3 mm depth were prepared using heat cure acrylic resin (Vertex, Netherlands) as shown in Figure 2 [27]. Preparation of acrylic block was done according to manufacturer's instructions.

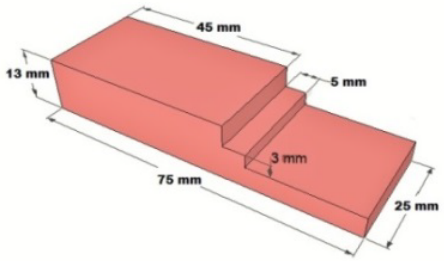

Figure 2 Schematic figure of pattern used for shear bond strength test

After that, two acrylic blocks put over each other leaving a space between them of dimensions (25 × 25 ×3) mm length, width and depth respectively for application of heat cure soft liner. The manufacturer instruction for the procedure of application soft lining material was followed.

The test was performed using Instron universal testing machine. All specimens were subjected to shear load with cross head speed (0.5 mm/min) with load of 100 kg. The maximum load required for failure was recorded and the value of shear bond strength was calculated according to (ASTM specification D-638m, 1986) formula:

Bond strength (N/mm^2)=F/A

Where, F=maximum load; A=cross sectional area

Test was repeated after 2 and 4 weeks for specimens stored in artificial saliva at 37°C.

Tear Strength Test

Specimens for tear strength test were fabricated according to (ASTM specification D-624, 2013) with dimension illustrated in the Figure 3.

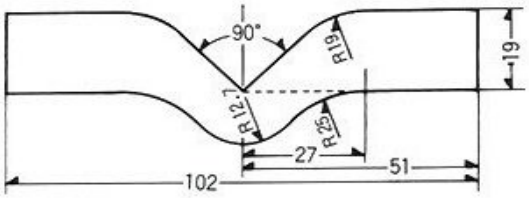

Figure 3 Pattern of tear strength test specimens

The test method measures the force per unit thickness required to initiate a tear using Instron universal testing machine.

After that the test was repeated for specimens stored in artificial saliva for 2 and 4 weeks for powder form of *aloe vera* application at 37°C.

Statistical Analysis

All data were analyzed using SPSS - version 24 computer software. Descriptive statistics which include mean, standard deviation, boxplot was used. Also, one-way ANOVA test was used for comparison among all groups. Post-hoc Bonferroni test was performed to examine the significance of difference between two independent means. P-value of ≤ 0.05 was considered statistically significant.

RESULTS

FTIR Analysis

FTIR results showed that there was no chemical reaction between acrylic soft-liner powder and *aloe vera* powder as shown in Figure 4.

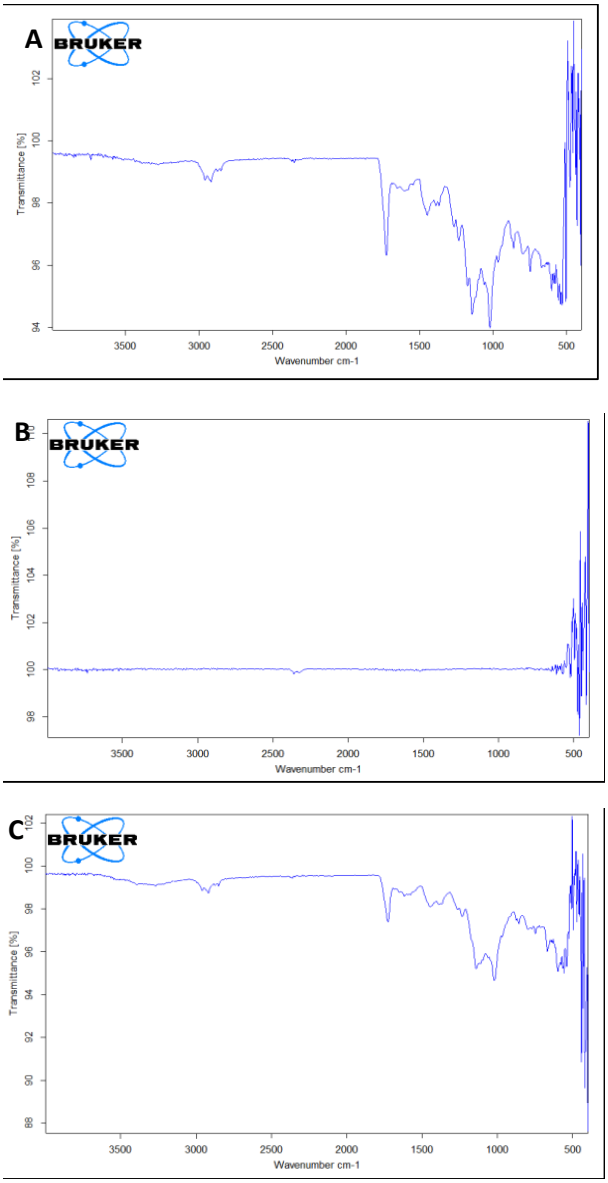

Figure 4 FTIR of (A) acrylic soft lining material, (B) *Aloe vera*, (C) acrylic soft liner/*aloe vera*

SEM Examination

SEM results for powder particles showed that soft liner particles were round with diameter of 15-43 µm, while *aloe vera* powder particles had irregular borders with size 6-30 µm as shown in Figure 5.

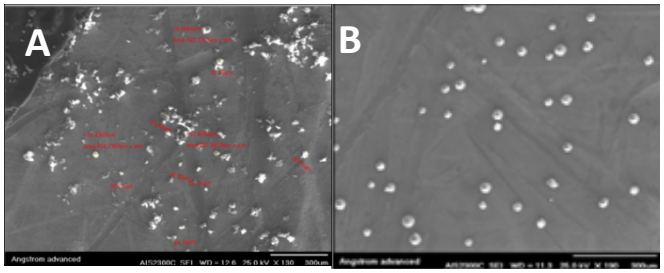

Figure 5 SEM images *aloe vera* powder (A) and for soft liner powder (B)

Candida albicans Adherence Test

Microscopical images for *Candida albicans* adherent cells to the surface of soft liner specimens are shown in Figure 6. The candida cells on the specimens appeared as round to oval cells and the crystal violet stains were retained by the candida [22]. In general, there was a remarkable reduction of *Candida albicans* that adhere to the surface of *aloe vera*-soft liner specimen compared to soft liner specimens.

Figure 6 Microscopical image for *Candida albicans* on soft liner specimen surface, arrow pointing to *Candida albicans* cell. A: control; B: alo 3%; C: alo 10%

Results of the statistical analysis showed a statistically significant decrease in *Candida albicans* count compared to control, the same was found for specimens stored in artificial saliva for 2 and 4 weeks as shown in Tables 1 and 2.

Table 1 Descriptive statistics with one-way ANOVA test of *Candida albicans* adherence test at different time intervals

Time interval	Groups	Mean (cells)	SD	ANOVA test for all groups		ANOVA test between groups	
				F-test	p-value	F-test	p-value
24 hours	Control	79.5	4.3	233.37	0.00	692.82	0.00
	Alo 3%	34.5	3.24				
	Alo 10%	24.1	2.92				
2 weeks in AS	control	79.8	5.49			264.49	0.00
	Alo 3%	37	4.11				
	Alo 10%	29	6.12				
4 weeks in AS	control	80.4	6.9			197.01	0.00
	Alo 3%	41.1	4.77				
	Alo 10%	33.3	5.16				

Table 2 Bonferroni test between groups at different time intervals of *Candida albicans* adherence test

Time interval	(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
24 hours	Control	Alo 3%	45.0000*	0.00
		Alo 10%	55.4000*	0.00
	Alo 3%	Alo 10%	10.4000*	0.00
2 weeks in AS	Control	Alo 3%	42.8000*	0.00
		Alo 10%	50.8000*	0.00
	Alo 3%	Alo 10%	8.0000*	0.007
4 weeks in AS	Control	Alo 3%	39.3000*	0.00
		Alo 10%	47.1000*	0.00
	Alo 3%	Alo 10%	7.8000*	0.015

For shear bond strength test, results show that *aloe vera* caused increased shear bond strength in both concentrations and at the different time intervals as shown in Tables 3 and 4.

Table 3 Descriptive statistics with one-way ANOVA test of shear bond (N/mm²) strength test at different incubation periods

Time interval	Groups	Mean (N/mm²)	SD	ANOVA test for all groups		ANOVA test between groups	
				F-test	P-value	F-test	P-value
24 hr.	Control	0.173	0.021	38.00	0.00	26.04	0.00
	Alo 3%	0.302	0.047				
	Alo 10%	0.288	0.055				
2 weeks in AS	Control	0.179	0.022			24.42	0.00
	Alo 3%	0.345	0.076				
	Alo 10%	0.334	0.066				
4 weeks in AS	Control	0.181	0.023			145.35	0.00
	Alo 3%	0.404	0.038				
	Alo 10%	0.391	0.035				

Table 4 Bonferroni test between all groups at different time intervals of shear bond strength test

Time interval	(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
24 hours	Control	Alo 3%	-0.1292800*	0.00
		Alo 10%	-0.1148800*	0.00
	Alo 3%	Alo 10%	0.0144	1.00
2 weeks in AS	Control	Alo 3%	-0.1658731*	0.00
		Alo 10%	-0.1557839*	0.00
	Alo 3%	Alo 10%	0.0100892	1.00
4 weeks in AS	Control	Alo 3%	-0.2233484*	0.00
		Alo 10%	-0.2103584*	0.00
	Alo 3%	Alo 10%	0.01299	1.00

Regarding tear strength test, statistical analysis results showed that that *aloe vera* caused increased tear strength, also tear strength increased after for 2 and 4 weeks immersion in artificial saliva as shown in Tables 5 and 6.

Table 5 Descriptive statistics with one-way ANOVA test of tear resistance test (N/mm) for all groups at different time intervals

Time interval	Groups	Mean (N/mm)	SD	ANOVA test for all groups		ANOVA test between groups	
				F-test	P-value	F-test	P-value
24 hr.	Control	6	0.816	22.58	0	14.76	0
	Alo 3%	7.093	0.739				
	Alo 10%	5.2	0.789				
2 weeks in AS	Control	6.94	0.849			18.11	0
	Alo 3%	7.92	0.512				
	Alo 10%	5.7	1.033				
4 weeks in AS	Control	7.593	0.905			26.16	0
	Alo 3%	9.2	0.949				
	Alo 10%	6.45	0.685				

Table 6 Bonferroni test between all groups of powder incorporation form at different time intervals of tear resistance test

Time interval	(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.
24 hours	Control	*aloe vera* 3%	-1.0930000	0.46
		aloe vera 10%	0.8	1.00
	Aloe vera 3%	*aloe vera* 10%	1.8930000*	0.009
2 weeks in AS	Control	*aloe vera* 3%	-0.9800000*	0.04
		aloe vera 10%	1.2400000*	0.007
	Aloe vera 3%	*aloe vera* 10%	2.2200000*	0.00
4 weeks in AS	Control	*aloe vera* 3%	-1.6072790*	0.001
		aloe vera 10%	1.1427210*	0.018
	Aloe vera 3%	*aloe vera* 10%	2.7500000*	0.00

DISCUSSION

The use of natural products as antimicrobial agents is considered advantageous over using systemic or local antibiotics which are synthetic products. Natural products are effective as bactericidal and fungicidal agents, in addition to their availability and low cost [28]. *Aloe vera* is well known for its important medicinal properties. *Aloe vera* is considered one of the richest natural plants for health [12]. In this study, *aloe vera* (*Aloe barbadensis*) powder was used. A spray dried powder obtained from the juice of the leaves of *aloe vera* plant. This type was selected because the whole leaf extract of *aloe vera* plant comprises the gel and the latex; both of them contain components that have anti-fungal properties that are useful in our study.

The results of incorporation *aloe vera* with the powder of heat cure acrylic soft liner with different concentrations revealed that *aloe vera* incorporation in 3% and 10% caused a statistically significant decrease in the mean values of *Candida albicans* count compared to control (*aloe vera* 0%). This might be due to the presence of novel protein of molecular weight 14 kDa within the constituents of *aloe vera*, which has antifungal and anti-inflammatory properties. The action of this protein is through inhibition of trypsin which shows protease inhibitory function [29]. Furthermore, *aloe vera* 10% has the lowest mean value and there was a statistically significant difference between *aloe vera* 3% and 10% and this might be due to increase concentration of *aloe vera* and increasing its effect. This was in agreement with Abduljabbar, et al. in 2016 who mentioned that *aloe vera* extract in alcoholic solvents induce significant inhibition in growth of *Candida albicans*, and this inhibitory effect increase as the concentration of *aloe vera* increase [30].

After 2 and 4 weeks incubation in artificial saliva, results showed that the means value of *aloe vera* 3% and 10% at the 2 weeks interval were a statistically higher than the first interval (24 hours), also the 4-week interval showed an increase in the mean values of *Candida albicans* cells count. This might be attributed to the increasing numbers of *Candida albicans* adherent cells, which might refer to the fact that soft lining materials when soaked in water or in an aqueous cleaning solution undergo two responses: the plasticizers and other soluble components are leached out and water or saliva are absorbed inside voids, which favor the colonization of yeasts and Candida [28].

Shear bond strength test results showed a statistically significant increase in experimental specimens (*aloe vera* 3% and 10%) compared to control. Since there was no chemical interaction between *aloe vera* and soft-liner powder as determined by FTIR. This effect could be explained by physical overlapping between *aloe vera* particles and soft-liner powder. Also this can appear clearly from SEM analysis, as there was a wide difference range between particle size of soft liner powder and *aloe vera* powder, also the irregular form of *aloe vera* particles and the round beads of soft liner which could enhance the bond strength by *aloe vera* acting as filler, filling spaces between soft liner particles and increasing surface area for adhesion with denture base material.

Mean values of *aloe vera* 3% (0.302 N/mm^2) was higher than 10% (0.288 N/mm^2) with a statistically non-significant difference. Similar difference also appeared after 2 and 4 weeks incubation period but with higher mean values.

This result agrees with Pisani, et al. who found that the bond strength of a soft-liner material increased for 60 days, even though it was statistically non-significant and explained this increase by the release of plasticizer which results in the increase of the rigidity of the material [31]. Also, our result agreed with Farzin who mentioned that bonding strength significantly increase for soft liners immersed in denture cleansers and distilled water [3].

Regarding tear strength test, results for the first interval (24 hours) showed that the tear strength increased for experimental group of specimens for both *aloe vera* 3% and 10% but statistically non-significant difference between control and experimental groups. This could be explained by the physical bonding between particles of *aloe vera* powder and soft-liner powder in addition to the differences in particle size and form of *aloe vera* and soft liner.

After 2 weeks of incubation in artificial saliva, there was a statistically significant increase in tear strength of experimental group as compared with control group. This was also observed after 4 weeks interval. And this result agrees with Sánchez-aliaga, et al., who found that the peel bond strength for soft liner material increased throughout the immersion time and this might be due to the release of plasticizer which in turn causes hardening and loss of viscoelasticity [32].

CONCLUSION

Aloe vera can be used as an effective natural herb against *Candida albicans* when incorporated with soft lining

material. The incorporation of *aloe vera* powder into acrylic soft lining material powder results in a statistically significant reduction of *Candida albicans* adherent cells and the effect seemed to be concentration dependent. Also, this incorporation result in increasing shear bond strength and tear strength. The preferable concentration was 3% for both *Candida albicans* adherence test and mechanical tests of this study.

DECLARATION

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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