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# Evaluation of Tensile Bond Strength of Zinc Containing and Zinc Free Denture Adhesives on Different Denture Base Resin Materials: An in Vitro Study

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ARTICLEINFO	ABSTRACT					
Article history: Received 05 July 2023 Received in revised form 21 August 2023 Accepted 25 August 2023 Available online 28 August 2023	<ul> <li>Background and aim: Denture adhesives augment the retention and stability of the complete denture. The includ studies have not directly compared tensile bond strength between zinc and zinc-free denture adhesives. This stu compared the tensile bond strength of zinc-containing and zinc-free denture adhesives on different denture bar resin materials at various intervals.</li> <li>Material and methods: Four groups of denture base resin materials (Acralyn H, Lucitone199- DB1, SR Ivoca DB2, Polytray-DB3) were fabricated using different polymerization techniques. Each group had ten specimens. The polytray-DB3 is the base result is a polytray bar of the base result is a polytray bar.</li> </ul>					
Keywords: Denture Bases Denture Retention Tensile Strength Tissue Adhesives	<ul> <li>control group consisted of resin cylinders coated with artificial saliva, while the test groups had denture adhesive applied between the test and control cylinders. Tensile bond strength was measured using a universal testing machine.</li> <li><b>Results:</b> The tensile bond strength values of Fixodent with DBI &amp;DB3 and DB2 &amp;DB3 at 5 min (P &lt; 0.01), 3 hours (P &lt; 0.01), and 6 hours (P &lt; 0.061 and P &lt; 0.020) alongside with DB1 &amp; DB2, DB1 &amp; DB3, and DB2 &amp; DB3 at 12 hours (P &lt; 0.01) were found to be statistically significant. The tensile bond strengths variations of Fittydent with DB1 &amp; DB3 and DB2 &amp; DB3 at 3 hours (P = 0.013, P = 0.012) and 6 hours (P &lt; 0.01), and DB2 &amp; DB3 at 12 hours (P=0.015), was statistically significant at 0.05 level.</li> <li><b>Conclusions:</b> The zinc-containing and zinc-free denture adhesives exhibited a significant increase in tensile bond strength compared to the control group (artificial saliva) at all time intervals.</li> </ul>					

# 1. Introduction

Retention is one of the most critical factors in complete denture fabrication. In obtaining optimal denture retention, biological, physical, and mechanical factors play an inevitable role.<sup>[1]</sup> Denture adhesives augment the retention and stability of the complete denture along with other factors, improving patient comfort and satisfaction.<sup>[2]</sup> Denture adhesives were used from the 18th century onwards, but they were mentioned in the dental literature only in the 19th century.<sup>[3]</sup> Although it is used worldwide, few references exist regarding denture adhesives. Dental practitioners believe that prescribing denture adhesives reflects their poor clinical skills, while others perceive them as helpful to denture retention, stability, and function. However, this attitude has changed, and dentists have started prescribing denture adhesives to patients who need extra psychological security, even in well-fitting and well-manufactured dentures.<sup>[4, 5]</sup> Denture adhesives are not used for making record bases. However, they aid in the retention and stabilizing of record bases, reducing tissue irritation since they act as a soft liner and as an adjunct in administering drugs to oral tissues.<sup>[4]</sup> Denture adhesive enhances the denture service by providing a cushioning effect and distributing force evenly over the denture-bearing region.<sup>[4, 6]</sup> Denture adhesive adsorbs water, swells by 50-150% by volume, and fills the space between the denture base and tissue.<sup>[3]</sup> This bio adhesion is provided by carboxyl groups, which are present in carboxy methyl cellulose (natural compound) and polyvinyl ether-maleic anhydride or PVM- MA (synthetic compound).<sup>[3,4]</sup> These two compounds were combined in order to compensate for the limitations of carboxy methyl cellulose. In the early 1970s, carboxymethylcellulose was combined with divalent calcium salts of PVM-MA to make denture adhesives to increase cohesive strength by forming a highly cross-linked matrix. In the 1980s1980, zinc was incorporated with calcium because zinc exhibited greater cohesive strength by producing stronger covalent bonds.<sup>[3]</sup> Denture adhesives are of soluble type (powder, creams, and paste) and insoluble type (wafers).<sup>[6]</sup> The cream adhesive swells by adsorbing water so that the viscosity increases than that of saliva and then spreads laterally, excluding air and saliva in between the surfaces, thereby enhancing retention.<sup>[4]</sup> Several studies reported that overuse of zinccontaining denture adhesives, especially combined with dietary supplements that contain zinc, can contribute to excess zinc deposition in the body.<sup>[7-10]</sup>

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Hence, dentists should focus on the reasons for using denture adhesives, the minimum amount required to achieve retention, and the risk factors associated with the overuse of denture adhesives. A direct comparison of tensile bond strength with zinc-containing and zinc-free denture adhesives has not been conducted. Hence, the purpose of the present study was to evaluate the tensile bond strength of zinc-containing and zinc-free denture adhesives on three denture base resin materials fabricated using different polymerization techniques at time intervals of up to 12 hours.

# 2. Material and methods *Specimen preparation*

Three different denture base resin materials, with ten specimens each, were processed by three different polymerization techniques for each group (Table 1). Acralyn H resin cylinders were used as a control against the denture base resin cylinders in all groups. The tensile bond strength of one zinc-containing denture adhesive (Fixodent) and one zinc-free denture adhesive (Fittydent) on three different denture base resin materials (Lucitone 199, SR Ivocap, Polytray) was investigated in the present study. Laboratory-prepared artificial saliva was taken as control (Table 1) against all the denture adhesives based on the report by Hara et al.<sup>[11]</sup>

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Ingredients	Weight
1.45 mM Calcium Chloride, Anhydrous (CaCl <sub>2</sub> ), MW 110.99	0.16g
5.4mM Pot. Phosphate Monobasic. (KH <sub>2</sub> PO <sub>4</sub> ), MW 136.09	0.74g
0.1M Tris-HCl, MW 156.60	15.66g
Adjust final pH using HCl or KOH	2.2g
Distilled H <sub>2</sub> O	1 Litre

# Table 1. Composition of artificial saliva.

#### Method of preparing denture base resin specimens.

One hundred cylindrical specimens with dimensions of 20mm height and 25mm diameter were made. A hole of 0.32 mm was drilled through the centre of the cylinder. A  $0.32\times$ 6mm round stainless-steel orthodontic wire was then luted with cyanoacrylate in the hole. A 3mm wire was left exposed at the surface that the Acralyn H resin cylinder would oppose. The Acralyn H resin

cylinders were also made similar to test cylinders, except the hole was kept free so that the 3mm pin exposed on the test cylinder would fit passively in the Acralyn H resin cylinder. This was done to ensure that the cylindrical specimens would separate only in the vertical direction when force was applied (Fig. 1).



Fig. 1. Test cylinder showing the alignment pin and Acralyn H cylinder with the matching hole to ensure vertical separation only during testing.

#### Method of testing tensile bond strength

In group A and group B (with denture adhesives), 0.2 grams of denture adhesive was sandwiched between the test cylinder and the Acralyn H resin cylinder (Figs. 2 and 3) since the appropriate amount of denture adhesive required to retain the maxillary denture according to study by Chew is 0.2 grams<sup>2</sup>. In group C (with artificial saliva), the resin test cylinder was coated

with a thin layer of artificial saliva (Fig. 4). The surface of the Acralyn H cylinder was left dry, and then the surface of the two specimens was positioned together. An approximate force of 12 N (1.2 kg) was then applied for 30 seconds to simulate a gentle biting force.<sup>[13]</sup>



Fig. 2. Test cylinders with Fixodent denture adhesive. a) Lucitine 199, b) Polytray, c) SR Ivocap.



Fig. 3. Test cylinders with Fittydent denture adhesive.



Fig. 4. Test cylinders with artificial saliva.

The specimens were placed in a sealed container with 100% hydration at 37°C until testing. The tensile bond strength was tested by American Dental Association specifications at 5 minutes, 3 hours, 6 hours, and 12 hours using a universal testing machine (Model: M-100) with a crosshead speed of 0.5mm/min with load to fracture measured in MPa (Figs. 5 and 6). After

measuring the tensile bond strengths with each adhesive, the specimens were washed with soap and tap water, dried with a paper towel, and then air dried. The same test cylinders were used for all measurements. Each test was repeated ten times, and a mean value was calculated.



Fig. 5. Test cylinders coated with Fixodent positioned in Instron machine.



Fig. 6. Test cylinders coated with Fittydent positioned in the Instron machine.

#### Statistical analysis

Categorical and quantitative variables were expressed as frequency (percentage) and mean  $\pm$  SD respectively. Independent t-test was used to compare quantitative parameters between categories. One-way ANOVA test (F-test) and Scheffe Multiple Comparisons (post hoc test) were carried out to compare quantitative parameters among categories. For all statistical interpretations, p<0.05 was considered the threshold for statistical significance. Statistical analyses were performed by using a statistical software package SPSS, version 20.0.

# 3. Results

The mean tensile bond strength among three denture bases between Fixodent and Fittydent at different time intervals are presented in Table 2 and illustrated in Fig. 7. The tensile bond strength of Fiitydent denture adhesive on Lucitone 199 (DB1) is higher than Fixodent denture adhesive at 3 hours, 6 hours, and 12 hours, since the P–value < 0.01 at 3 hours, 6 hours, and 12 hours, the comparison is considered statistically significant.

		F	Fixodent		F	ìittydent			
			1				T-test	P-value	
		Mean	SD	Ν	Mean	SD	Ν		
	1								
DB1	At 5 Minutes	1.46	0.37	10	1.57	0.36	10	0.64	0.532
	At 3 Hours	3.85	1.43	10	8.93	1.58	10	7.54	p<0.01
	At 6 Hours	4.11	0.52	10	11.54	1.58	10	14.12	p<0.01
	At 12 Hours	5.85	0.71	10	9.06	2.35	10	4.13	p<0.01
	At 5 Minutes	2.03	0.41	10	1.57	0.44	10	2.42*	0.026
DB2	At 3 Hours	3.94	0.54	10	8.95	0.62	10	19.28	p<0.01
	At 6 Hours	3.98	0.50	10	15.40	1.20	10	27.71	p<0.01
	At 12 Hours	4.35	0.38	10	7.38	0.92	10	9.58	p<0.01
	At 5 Minutes	3.72	1.07	10	1.50	0.44	10	6.07	p<0.01
DB3	At 3 Hours	9.02	1.65	10	7.28	1.06	10	2.8*	0.012
	At 6 Hours	4.77	0.73	10	7.73	0.97	10	7.7	p<0.01
	At 12 Hours	12.37	0.42	10	9.89	1.81	10	4.22	p<0.01

SD: Standard Deviation, N: Number of samples, t: Independent t-test.



Fig. 7. Comparison of tensile bond strength on resin blocks between Fixodent and Fittydent at different time intervals.

The tensile bond strength of Fiitydent denture adhesive on SR Ivocap (DB2) is higher than Fixodent denture adhesive at 3 hours, 6 hours, and 12 hours, since the P–value < 0.01 at 3 hours, 6 hours, and 12 hours, the comparison is considered statistically significant. Thus, the two denture adhesives showed statistically significant differences in their tensile bond strengths on Lucitone 199 and SR Ivocap at time intervals of 3 hours, 6 hours, and 12 hours (P<0.01), respectively. The tensile bond strength of Fixodent denture adhesive on Polytary (DB3) is higher compared to Fittydent denture adhesive at 5 min, 3 hours, and 12 hours. At 6 hours, the tensile bond strength

of Fittydent is higher than Fixodent on Polytray (DB3); since the P–value < 0.01 at 5 min, 6 hours, and 12 hours, the comparison is considered statistically significant. This proved that the two denture adhesives showed statistically significant differences in their tensile bond strengths at 5 min, 6 hours, and 12 hours (P<0.01). The results of ANOVA and Scheffe multiple comparison tests of tensile bond strength among resin blocks with fixodent and Fittydent at different time intervals are presented in Tables 3 and 4 and illustrated in Figs. 8 and 9.

Resin Block		Mean SD	N	F-value	P-value	Scheffe Multiple Comparisons					
						Pair	F-value`	P-value			
	DB1 (A)	1.46	0.37	10			A & B	1.7	0.210		
At 5 Minutes	DB2 (B)	2.03	0.41	10	28.61	p<0.01	A & C	26.4	p<0.01		
	DB3 (C)	3.72	1.07	10			B & C	14.9	p<0.01		
	DB1 (A)	3.85	1.43	10	51.76			A & B	0	0.989	
At 3 Hours	DB2 (B)	3.94	0.54	10		p<0.01	A & C	39.5	p<0.01		
	DB3 (C)	9.02	1.65	10			B & C	38.2	p<0.01		
At 6 Hours	DB1 (A)	4.11	0.52	10	5.18*	0.013	A & B	0.1	0.877		
	DB2 (B)	3.98	0.50	10			A & C	3.1	0.061		
	DB3 (C)	4.77	0.73	10				B & C	4.5*	0.020	
	DB1 (A)	5.85	0.71	10					A & B	20.2	p<0.01
At 12 Hours	DB2 (B)	4.35	0.38	10	653.44	44 p<0.01	A & C	381.9	p<0.01		
	DB3 (C)	12.37	0.42	10			B & C	578	p<0.01		

Table 3. Comparison of tensile bond strength among resin blocs with Fixodent at different time intervals.

SD: Standard Deviation, N: Number of samples.

Table 4. Comparison of tensile bond strength among resin blocs with Fittydent at different time intervals.

Resin Block		Mean SD	N	F-value	P-value	Scheffe Multiple Comparisons				
							Pair	F-value	P-value	
	DB1 (A)	1.57	0.36	10	0.09			A & B		
At 5 Minutes	DB2 (B)	1.57	0.44	10		0.913	A & C			
	DB3 (C)	1.50	0.44	10			B & C			
	DB1 (A)	8.93	1.58	10	6.89**			A & B	0	0.999
At 3 Hours	DB2 (B)	8.95	0.62	10		0.004	A & C	5.1*	0.013	
	DB3 (C)	7.28	1.06	10			B & C	5.2*	0.012	

	DB1 (A)	11.54	1.58	10			A & B	22.9	p<0.01
									1
At 6 Hours	DB2 (B)	15.40	1.20	10	90.1	p<0.01	A & C	22.2	p<0.01
	DB3 (C)	7.73	0.97	10			B & C	90.1	p<0.01
	DB1 (A)	9.06	2.35	10			A & B	2.2	0.131
At 12 Hours	DB2 (B)	7.38	0.92	10	5.07*	0.013	A & C	0.5	0.594
	DB3 (C)	9.89	1.81	10			B & C	4.9*	0.015

SD: Standard Deviation, N: Number of samples.



Fig. 8. Comparison of tensile bond strength among resin blocs with Fixodent at different time intervals.



Fig. 9. Comparison of tensile bond strength among resin blocs with Fixodent at different time intervals.

Comparing the tensile bond strength of Fixodent among three different denture base resin materials, there is statistically significant variation in their tensile bond strength at time intervals of 5 min, 3 hours, 6 hours, and 12 hours. The variation in tensile bond strengths of DB1 and DB2 at 5 min (P=0.210), 3 hours (P=0.989), and 6 hours (P=0.877) were statistically not significant. The tensile bond strength variations of DBI & DB3 and DB2 & DB3 at 5 min (P<0.01), 3 hours (P<0.01), 6 hours (P<0..061 and P<0.020) were found to be statistically significant. Comparing DB1 & DB2, DBI &DB3, and DB2 & DB3 at 12 hours (P<0.01), there was a statistically significant difference in their tensile bond strengths. Comparing the tensile bond strength of Fittydent among three different denture base resin materials, there are no statistically significant variations in their tensile bond strength at 5 minutes. However, at 3 hours (P=0.004), 6 hours (P<0.01), and 12 hours (P=0.013), the difference in the tensile bond strength is statistically significant. The variations in the tensile bond strength of DB1 and DB2 at 3 hours (P=0.999) and 12 hours (P=0.131) were not statistically significant, but at 6 hours (P<0.01), the variation in tensile bond strength is statistically significant. The variation in tensile bond strength of DB1 & DB3 and DB2 & DB3 at 3 hours (P=0.013, P=0.012) and 6 hours (P<0.01) was found to be statistically significant at 0.05 level. Comparing DB2 & DB3 at 12 hours (P=0.015), the variation in tensile bond strength is statistically significant at 0.05.

#### 4. Discussion

Rehabilitation of a completely edentulous patient is very demanding. For a successful outcome, proper retention, stability, and support are necessary prerequisites and of paramount importance. As a result, prosthodontists are very interested in various techniques to improve stability and retention. Dentists have hesitated to recognize adhesives' role in prosthetic dentistry, even though they are often used by denture users and promoted commercially. Denture adhesives can, however, be valuable in a dentist's toolbox if used correctly, as is now generally acknowledged. To understand how denture adhesives affect retention and stability, Tarbet and colleagues<sup>[12-14]</sup> counted the number of times patients' dentures dislodged while eating standardized quantities of food with and without denture adhesive, and according to their findings, utilizing an adhesive significantly reduced denture dislodgment. The dislodgement data confirmed the improvement in denture stability and retention with an adhesive, and the subjects stated that they had a more remarkable ability to chew, experienced less denture movement, and had more confidence and comfort. Denture adhesives are more retentive in paste form than in powder form. The effectiveness of the powder form of denture adhesive was first evaluated by Chew,<sup>[2]</sup> while Ghani et al.<sup>[15]</sup> later corroborated it. They showed that the liquid/paste form of denture adhesive made poorly fitting dentures almost as retentive as well-fitting ones. According to a survey of academic prosthodontists, dentistry adhesives are a beneficial adjunct in the provision of denture prosthesis services.<sup>[16]</sup> Education is essential for both patients and dentists to use denture adhesive properly and prevent overuse. The present study compared the retentive ability of zinc-containing and zinc-free denture adhesives on three different denture base materials at 5 minutes, 3 hours, 6 hours, and 12 hours. The materials were examined under the same controlled and constant experimental conditions. Thus, the null hypothesis was rejected since there were significant differences between the two denture adhesives, the three denture bases, and the four different times they were evaluated. The results of the present study demonstrated that all the adhesives tended to increase their effectiveness as time progressed, regardless of the denture base on which they were used. This is in agreement with a study conducted by Salman et al.,<sup>[17]</sup> Panagiotouni et al.,<sup>[18]</sup> Chowdhry et al.,<sup>[19]</sup> Ghani et al.,<sup>[15]</sup>

Kumar et al.,<sup>[20]</sup> who shared the same opinion. The present study also revealed that all denture adhesives (zinc-containing and zinc-free) showed improved retention values in comparison to those without adhesives (with artificial saliva) at all time intervals (5 minutes, 3 hours, 6 hours, and 12 hours). Similar findings were revealed from the studies conducted by Kumar et al.,<sup>[18]</sup> Chowdhry et al.,<sup>[19]</sup> Ghani et al.,<sup>[21]</sup> Kumar and Thombare,<sup>[20]</sup> and Salman and Ibrahim.<sup>[17]</sup> Apart from these studies, the study conducted by Chew[2] proved that the effectiveness of denture adhesives decreases over time due to the loss of sticky substances. DeVengencie's<sup>[22]</sup> research also revealed that the adhesive is more effective at initial placement and then diminishes over time. The present study also evaluated the retentive ability of zinc-containing and zinc-free denture adhesives on three different denture base resin materials (Lucitone 199, SR Ivocap, and Polytray) fabricated using conventional heat cure technique, injection moulding technique, and visible light cure technique. The intergroup analysis revealed that visible light cure denture base resin material (Polytray) showed greater retention than the other two denture base materials when Fixodent (zinc-containing) was employed as the denture adhesive at all time intervals. The SR Ivocap and Lucitone 199 denture base resin materials exhibited similar tensile bond strength values at all intervals. When Fittydent (zinc-free denture adhesive) was employed as the denture adhesive, both conventional light cure (Lucitone 199) and injection moulding (SR Ivocap) denture base resin materials exhibited similar tensile bond strength at 5 minutes, 3 hours, and 6 hours. The visible light cure denture base resin material exhibited the highest tensile bond strength at 12 hours with Fittydent. The Fittydent denture adhesive (zinc-free) was found to be more retentive than Fixodent denture adhesive on Lucitone 199 (DB1) and SR Ivoacap (DB2) at time intervals of 3 hours, 6 hours, and 12 hours. The tensile bond strength of Fittydent denture adhesive on Lucitone 199 and SR Ivocap was maximum at 6 hours and minimum at 5 minutes. Fixodent denture adhesive (zinc-containing) was more retentive than Fittydent on Polytray denture base resin material (visible light cure material) at 3 and 12 hours. Furthermore, the retentive ability of Fixodent was maximum at 12 hours and minimum at 5 minutes. Compared with artificial saliva, the retentive ability of zinc-containing and zinc-free adhesives found that denture adhesive poses a much greater retentive ability than saliva alone. Similarly, Ghani et al.<sup>[21]</sup> compared the retention of ill-fitting maxillary complete dentures with multiple denture adhesives at various intervals. They infer retention values with just saliva are tremendously lower than with adhesive. Previous studies focused on the effects of different types of denture adhesives when employed with heat-activated denture base resin materials. The focus of the present study was on the evaluation of the effect of 2 types of denture adhesive on three types of denture bases. However, the present study conflicted with the findings of previous studies, which concluded that there were significant differences in the mean retention values among denture base resin materials fabrication techniques. According to a study by AlRumaih et al.,[22] significantly higher retention values were recorded with milled denture bases than heat-activated resin bases without denture adhesive. However, denture adhesive did negatively affect the retention of milled complete dentures. Denture adhesives containing zinc face biocompatibility challenges and the possible danger of developing neurologic disorders such as hypercupremia and hyperzincemia. In evaluating hypocupremic patients, Hedera et al.<sup>[23]</sup> 100% of the patients with copper deficiency syndrome had swallowed significant amounts of zinc through denture adhesives. In the present study, zinc-free denture adhesive was more retentive than zinc-containing denture adhesive on denture base resin materials fabricated by conventional techniques. A study by Himli et al.<sup>[24]</sup> explores the creation of eco-friendly denture adhesives (EFDAs) using various native and modified natural

starches as fillers. EFDA, with its starch content, has promising potential as a natural denture adhesive, improving bonding, retention, and compatibility with the biological environment for dental applications. A novel hydrogel denture adhesive developed recently outperformed three existing commercial options, demonstrating superior initial bond strength in dry and wet conditions.<sup>[25]</sup> Artificial saliva, denture adhesives, and denture base material were study variables intended to mimic in vivo settings. A clinical denture adhesive bond strength value can be adversely affected by the absence of keratinized mucosa, normal saliva, muscle movements, and intaglio surface features of an actual denture base, among other essential but sometimes ignored elements. Denture adhesives do not work in the same manner when bound to keratinized mucosa as when bonded to acrylic resin. Although they have significant limitations, in vitro tests can be used to compare and assess the various denture adhesives already on the market and, in the process, serve as a benchmark for future clinical trials. Any denture adhesive's potential for success or failure depends critically on patient acceptance. In addition to strength and how long it will last, patients choose one product over another based on additional considerations like comfort, flavour, and convenience of application. If the adhesives are used as directed by the clinician, it is very safe and effective for ill-fitting dentures. Future studies should pave the way to include investigation utilizing multiple denture adhesives, additional fabrication techniques for denture bases, a wide range of time intervals, and longer seating times for denture bases.

# Limitations

The limitations of the present study include:

- The evaluation was done under in vitro settings, and variation in the results of the present study can be anticipated in in-vivo study settings due to differences in testing apparatus, study design, the difference in brands of denture adhesives employed, the difference in quantity and quality of saliva, degree of anxiousness, and apprehension of the patient.
- Only one form of zinc-containing and zinc-free adhesives were employed for the present study. Henceforth, the tensile bond strength of more adhesives needs to be investigated in the future.
- Only paste forms of denture adhesives were compared in the present study. Powder form and wafer forms were not taken into consideration.
- Denture adhesives do not work in the same manner when bound to keratinized mucosa as when bonded to acrylic resin.

Henceforth, the presented data of the current study may be interpreted with caution.

## 5. Conclusion

Within the limitations of the present study, the following conclusions were drawn:

1. The denture adhesives, both zinc-containing and zinc-free adhesives, exhibited a significant increase in tensile bond strength compared to control (artificial saliva) at 5 minutes, 3 hours, 6 hours, and 12 hours intervals.

2. Fittydent (zinc-free) denture adhesive exhibited the highest tensile bond strength with Lucitone 199 and SR Ivocap (Conventional heat cure and injection moulding technique) denture base resin materials at 5 minutes, 3 hours, and 6 hours.

3. Fixodent (zinc-containing) denture adhesive exhibited the highest tensile bond strength with Polytray (visible light cure technique) denture base resin material at 5 minutes, 3 hours, 6 hours, and 12 hours.

# **Conflict of Interest**

The authors declared that there is no conflict of interest.

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