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Examining the role of grape seed extract in enhancing collagen crosslinking and its effect on the shear bond strength of Universal adhesive to contaminated dentine

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Abstract

Aim of the study: The purpose of this study was to evaluate the effect of a collagen cross-linker, grape seed extract (GSE), applied at different times (1, 3, and 5 minutes) on the shear bond strength (SBS) of a universal adhesive bonding system applied to dentine after contamination with blood or blood and hemostatic agent (HA).

Material and method: Seventy-two teeth were divided into three main groups. Group A, the control group, consisted of 8 teeth treated only with Scotchbond Universal. The remaining teeth were divided into two contamination groups, B and C, with 32 teeth in each. Group B was contaminated with blood, while Group C was contaminated with both blood and HA. Each contamination group was further divided into four subgroups based on the timing of GSE application. One subgroup did not receive any GSE application, while the other three subgroups had an application of GSE for 1, 3, and 5 minutes, respectively. Then had adhesive application as in group A. After adhesive application and composite resin placement, samples were stored in distilled water at 37° for 24 hours and thermocycled for 500 cycles. SBS was measured using a universal testing machine.

Results: Group B3 (blood and GSE for 3 minutes) and Group C4 (blood, hemostatic agent, and GSE for 5 minutes) showed the highest SBS values at 12.43 MPa and 13.09 MPa, respectively.

Conclusion: GSE treatment for durations of 1, 3 or 5 minutes enhanced bond strength to contaminated dentine.

Keywords: Shear bond strength, Dentin contamination, Scotchbond Universal, collagen cross-linker

Introduction

Increasingly, composite resin is becoming a material of choice for dental restorations (Ravi et al., 2013). In the past 70 years, composite resin materials have significantly revolutionized restorative dentistry (Bayne et al., 2019). These materials have been developed progressively in formulation, properties, and aesthetics since they were initially introduced to dentistry (Samuel et al., 2009).

The achievement of micromechanical retention is the foundation behind an effective bond to dentine. This is achieved by resin penetration into partly demineralized dentine, which leads to the formation of a hybrid layer and tags (Rahal et al., 2012). The development of dental adhesives and the philosophy of minimally invasive dentistry have primarily shaped contemporary restorative dentistry (Alotaibi et al., 2024). There are two primary

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approaches that fulfill these criteria: the etch-and-rinse method and the self-etch method. In an etch-and-rinse technique, conditioning, rinsing, and priming steps are used for the complete removal of the smear layer. In the self-etch technique, the smear layer is incorporated into the hybrid layer (Saikaew et al., 2022).

Bonding to dentine still poses a challenge because of the variability and sensitivity to technique (Dey et al., 2016). In spite of the development of dentine adhesive systems that are more user-friendly and less technique-sensitive, contamination with salivary or blood-borne constituents during bonding procedures can still affect bond strength. A clean working area is essential, and must be established and maintained around the gingival margins when preparing and placing restorations. Hemostasis plays a significant role in order to maintaining the ideal contaminant-free environment (Taneja et al., 2017).

Blood contamination results in a high increase in protein content, which eventually brings severe changes in adhesion strength. The protein, together with macromolecules such as fibrinogen and platelets, forms a layer on the dentine surface. This layer prevents adhesion to the dentinal tubules themselves (Barakat and Powers, 1986; Haralur et al., 2019).

Hemostatic agents (HA) used to control bleeding. These agents have an acidic pH in general, ranging from 0.7 to 3, and possess a considerable hydrophilic nature, which might potentially interfere with different stages of the bonding process. Use of hydrophilic HA may bring change in dentine surface morphology, thereby affecting bond strength between adhesive resins (Mandouh and Alzayat, 2018). Although the application of HA has been shown to reduce adhesive bond strength to dentine, studies have reported that the bond strength in HA contaminated groups was higher compared to groups contaminated with blood alone (Ulusoy et al., 2011).

Enhancing the mechanical properties of collagen and its resistance to enzymatic breakdown involves increasing the formation of cross-links within and between molecules and microfibrils. Various collagen cross-linkers, both synthetic and natural, can achieve this when applied to the dentine surface before the bonding process (Han et al., 2003; Bedran-Russo et al., 2007; Cai et al., 2018).

Dentine pretreatment with collagen cross-linkers can be safely recommended as an effective chairside procedure to improve the

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bond strength of composite resin to dentine (Gajjela et al., 2017). There is evidence that GSE stands out as an effective natural cross-linker known for preserving bond strength over time; the most commonly used natural matrix metalloproteinase inhibitor was proanthocyanidin (PA) (6.5%) (Anumula et al., 2022).

In previous studies, GSE has typically been applied for 5-10 minutes to achieve optimal cross-linking effects on collagen (Gajjela et al., 2017; Manihani et al., 2023). However, a 5-10 minutes application may be impractical in clinical settings due to time constraints. Therefore, this study evaluates the effects of GSE application at 1, 3, and 5 minutes, aiming to determine if shorter application times can provide comparable improvements in bond strength, making the procedure more feasible for routine use.

Materials and Methods:

Teeth Collecting: the sample number was calculated utilizing G*Power with the power of the study set at 80%, an alpha error probability of 0.05 (two-sided), assuming an effect size f of 0.6 (where small = 0.1, medium = 0.25, large = 0.4). A total of seventy-two upper first premolars, extracted for orthodontic purposes, were obtained from patients aged 14 to 20 years. Study approval number was (…….). Each tooth was inspected under a 7X magnifying loupe to ensure it was intact and free from caries and cracks. Subsequently, they were cleaned using a rubber cup with pumice and rinsed thoroughly with distilled water applied via a triple syringe (Abdulrasool and Al-Shamma, 2019). These teeth were preserved in a 0.1% thymol solution to inhibit fungal and bacterial contamination (Al-Obaidi and Jasim, 2023).

Construction of Acrylic Block: A custom cubic silicone mold measuring 1.5 cm on each side was used to create acrylic blocks. The cemento-enamel junction was marked, then an additional mark 2 mm below the cemento-enamel junction, indicating the insertion depth of the teeth in the acrylic (Khalil and Al-Shamma, 2015; Hameedi and Gholam, 2023). Using a dental surveyor, each tooth was aligned to ensure the long axis was parallel to the surveyor rod (Al-Obaidi and Jasim, 2023).

Teeth Preparation: The measurement of the distance from the pit to the mesial marginal ridge was taken using a periodontal probe to determine the ridge height. One millimeter was added to this quantification and marked mesially on the side of the tooth for future sectioning (Khalil and Al-Shamma, 2015).

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The handpiece was fixed to a flat wood base with zip ties, and a 20 mL syringe was stabilized using adhesive tape for irrigation during cutting. A metal plate was used to control the level of cutting according to the marking on the tooth by adjusting the level of the metal positioned on the wood and locking it using a screw lock (figure 1).

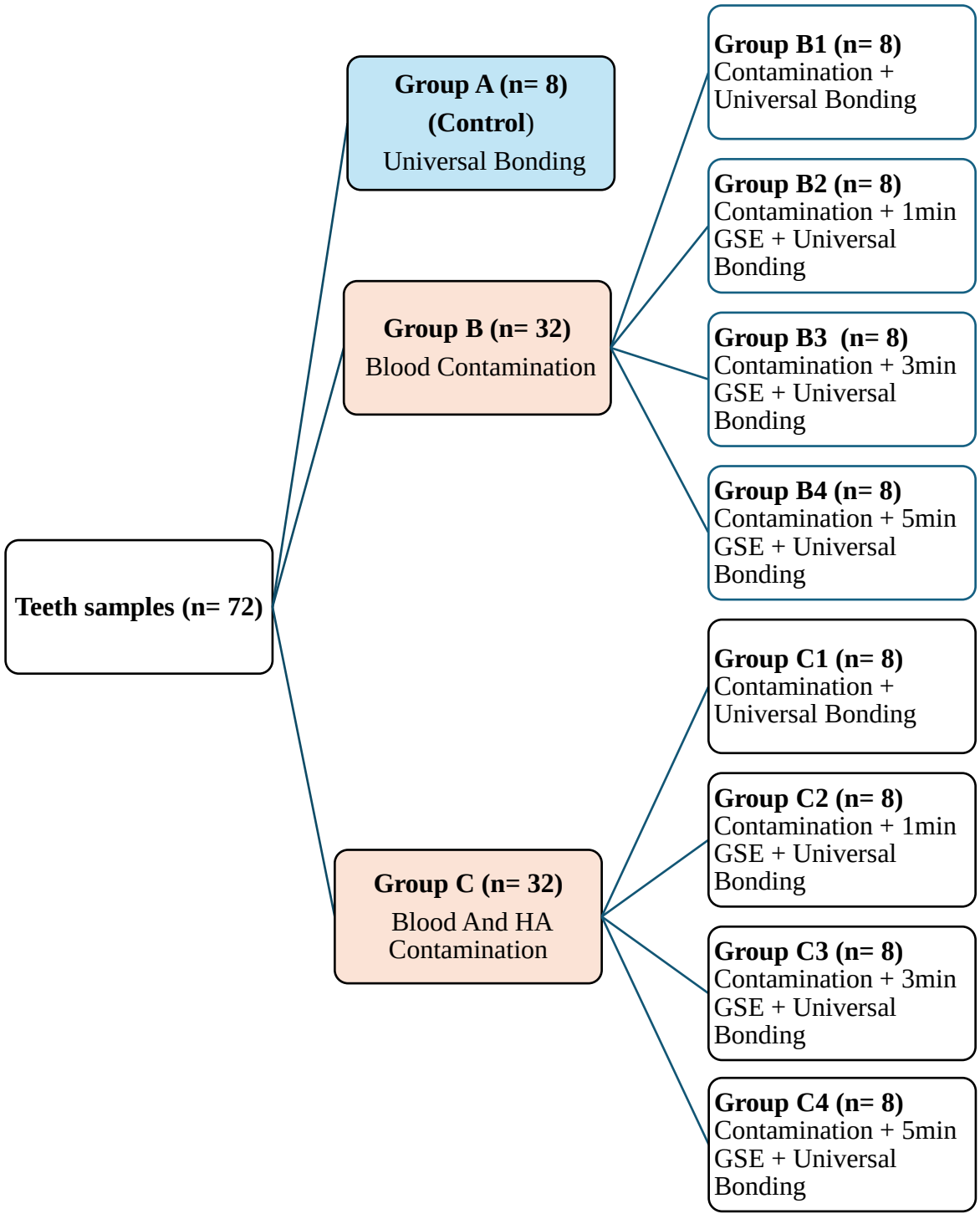

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Figure 1. Sample grouping

To expose a flat dentine surface for the purpose of study, the buccal and palatal cusps were both cut 1 mm below the mesial pit using a diamond disk mounted on a straight handpiece under running water (Abdulrasool and Al-Shamma, 2019; Al-Obaidi and Jasim, 2023).

The cut surface was examined using a 7X magnifying loupe to check for any remaining enamel (Al-Obaidi and Jasim, 2023).

Smoothing the cut surface of the teeth with 600-grit sandpaper, which was fixed on a flat wood marking a 10 cm in length by the used of red adhesive tape. The occlusal surface of each tooth was abraded against the surface of the abrasive paper. The grinding process was repeated four times on each surface (Khalil and Al-Shamma, 2015; Al-Obaidi and Jasim, 2023). The abrasive paper was moistened to avoid dryness of the dentine and ensure wet bonding (Manihani et al., 2023).

Sample Grouping: The Seventy-two teeth were divided into three main groups. Group A, the control group, consisted of 8 teeth treated only with Scotchbond Universal. The remaining teeth were divided into two contamination groups, B and C, with 32 teeth in each. Group B was contaminated with blood, while Group C was contaminated with both blood and HA. Each contamination group was further divided into four subgroups based on the timing of GSE application. One subgroup did not receive any GSE application, while the other three subgroups had application of GSE for 1, 3, and 5 minutes, respectively (figure 2).

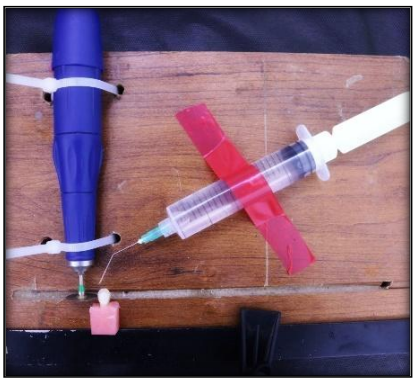

Figure 2. process of teeth sectioning.

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Group A: Group A served as the control group with no contamination or GSE application. In accordance with the manufacturer's instructions, the adhesive was meticulously applied for a duration of 20 seconds using a disposable microbrush. Following this, a 5-second air-drying period was implemented to facilitate solvent evaporation using a triple syringe positioned 1 cm away. The light cure (woodpecker, China), with an intensity of 1200 mW/cm², is positioned 1 mm away from the dentine with the help of a digital caliper, followed by a 10-second light-curing process.

Group B: For group B1 Fresh capillary blood was collected from the fingertip of the operator (figure 3). The freshly collected blood was promptly administered onto the surface of the samples using a microbrush and kept undisturbed for 1 minute (figure 4). Subsequently, the samples were rinsed with distilled water using a triple syringe for 1 minute and blotted dry with a paper point by wiping the surface with one paper point twice. For groups B2, B3, and B4 all groups had same blood contamination as in group B1, and then had GSE application for 1,3,5 minutes respectively. A weight of 6.5 g of GSE in the form of powder (Zazee Naturals, USA) was collected from the capsules and measured with an electronic digital scale. It was then dissolved in 100 mL of distilled water and thoroughly mixed with a cement spatula to make 6.5% of GSE (Gajjela et al., 2017; Manihani et al., 2023). The resulting pH was measured using a pH meter, and it was found to be 4.5. Subsequently, the solution was collected using a 2.5 mL syringe for ease of application. A drop of GSE was applied to a microbrush, which was promptly used to coat the surface of the samples. After allowing the application to remain undisturbed for 1, 3, or 5 minutes according to the specific group (figure 5), the samples were rinsed for 1 minute and then gently blot dried with a paper point. Then, all Group B subgroups had adhesive application the same as in Group A.

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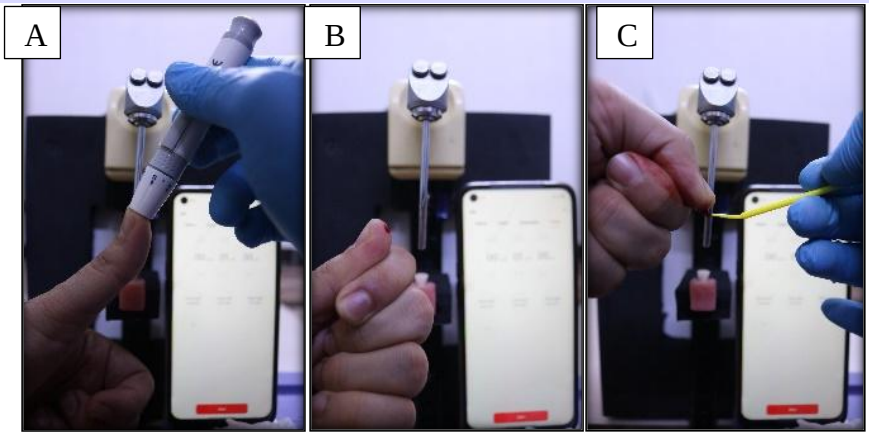

Figure 3. A: Puncture finger B: Squeezing blood C: Application of blood to micro brush.

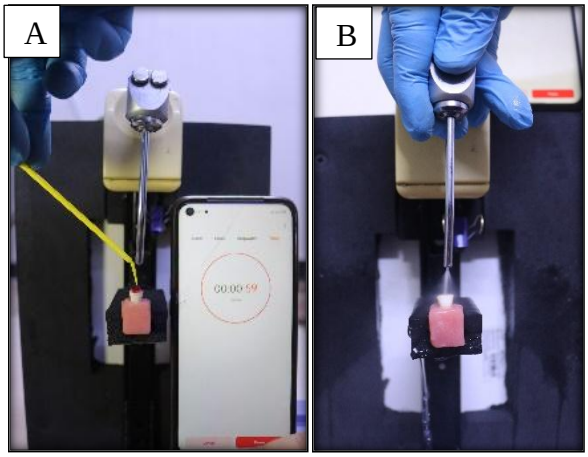

Figure 4. A: Application of blood to dentine surface B: washing of dentine surface.

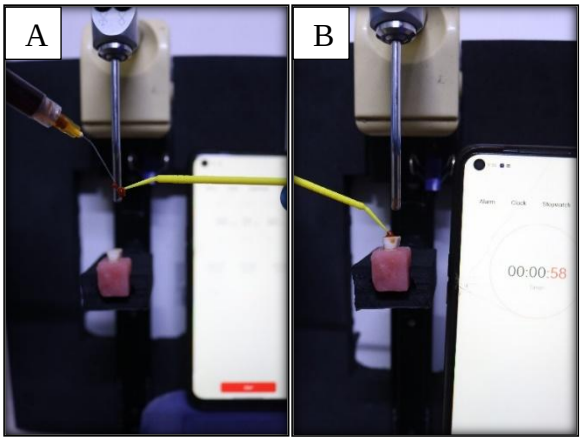

Figure 5. A: GSE application to micro brush B: Application of GSE to dentine surface.

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Group C: For Group C, all samples had blood contamination applied as in Group B. Subsequently, a drop of Ferric Sulfate (ViscoStat 20%) was applied to a microbrush, which was then immediately used to apply it to the surface of the samples (figure 6). The application was left undisturbed for 1 minute, followed by rinsing for 1 minute and blot drying using a paper point (Manihani et al., 2023). Group C1 had no GSE application, while Groups C2, C3, and C4 had GSE application similar to Groups B2, B3, and B4. Then, all Group C subgroups had adhesive application as in Group A.

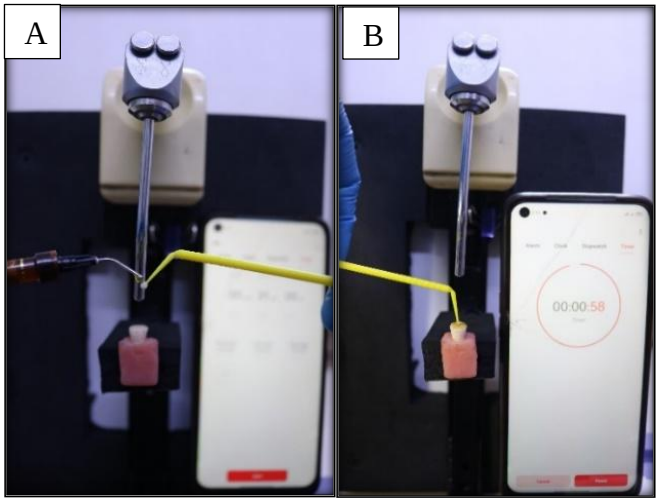

Figure 6. A: Hemostatic agent application to micro brush B: Application of hemostatic to. dentine surface.

A specialized teflon mold (figure 7) was crafted locally to standardize the procedure of composite application onto the dentine surface. This mold comprises several key components: a cylindrical translucent teflon structure designed to securely hold the acrylic block, a removable white teflon cover consisting of two semi-circular segments, each with a thickness of 2 mm, fastened to the cylinder using two screws, and a teflon bar featuring a central screw to affix the acrylic block against the teflon cover by controlling the vertical positioning of the tooth. Additionally, four strategically positioned screws encircling the cylinder serve to stabilize the acrylic block and guide the dentine surface in the horizontal plan to the 4 mm diameter hole in the teflon cover, facilitating precise placement of the composite material.

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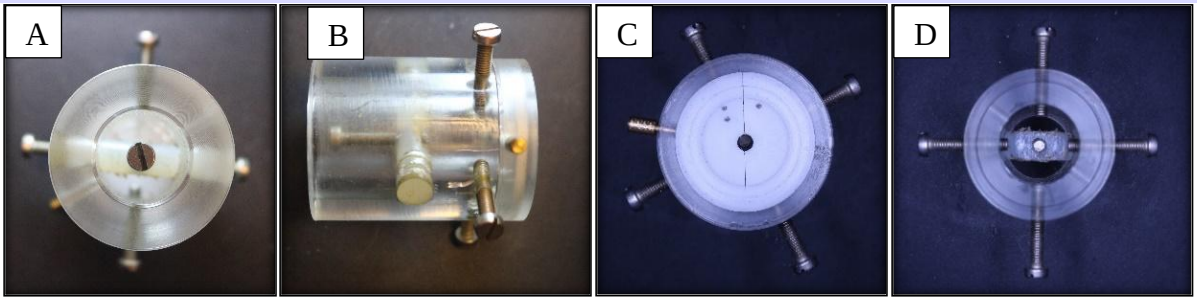

Figure 7. A-D: Difference angle view for the Teflon mold

Composite Application: Ash 49 was used to apply Filtek One Bulk Fill (3M-ESPE) composite in a one increment through the designated hole in the teflon mold cover. A celluloid strip was then placed over the composite, followed by a 50-gram load for 1 minute. After removing the load, The light curing device was positioned directly in contact with the celluloid strip for 40 seconds (Al-**Obaidi** and Jasim, 2023).

Storage and Aging Procedure: The samples were placed in distilled water and incubated at 37°C for 24 hours to allow for post-polymerization (Goncu and Yilmaz, 2022; Hashim and Abd-Alla, 2022). Using thermocycling device (figure 8) locally fabricated all samples were subjected to 500 cycles of thermocycling, with temperature fluctuations between 5°C and 55°C. Each cycle consisted of a dwell time of 20 seconds at each temperature extreme and a transfer time of 2 seconds (Goncu and Yilmaz, 2022).

Shear bond strength test**:** The SBS test was performed utilizing a computer-controlled universal testing machine (Tinius Olsen, Germany), which operated at a crosshead speed of 1 mm/min until failure occurred (Goncu and Yilmaz, 2022). The specimens were fastened in the jaw of the testing device and placed in a horizontal orientation. The straight-edged blade was aligned with the bonded interface, perpendicular to the longitudinal axis of the restorative material cylinder. This alignment ensured that the specimen cylinder was oriented at a 90° angle to the vertical plane of the blade (figure 9). The SBS were determined by dividing the maximum force at the point of fracture (measured in Newtons (N)) by the bonded area (measured in square millimeters)(mm^2), resulting in

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bond strength values expressed in megapascals (MPa).

$Shear\ Bond\ Strength$ = Fracture load (N) / Adhesion $Surface\ area$ (mm^2)

Surface area = πr^2

Data normality was confirmed using the Shapiro-Wilk test ($p > 0.05$), and Levene's test verified homogeneity of variance ($p > 0.05$). Descriptive statistics (mean SBS, ±SD, min, max) were reported. One-way ANOVA with Tukey's HSD post hoc tests identified significant group differences ($p < 0.05$), and independent samples t-tests compared subgroup pairs. Failure modes were analyzed using the Fisher exact test ($p < 0.05$). All analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA), with significance set at $p < 0.05$.

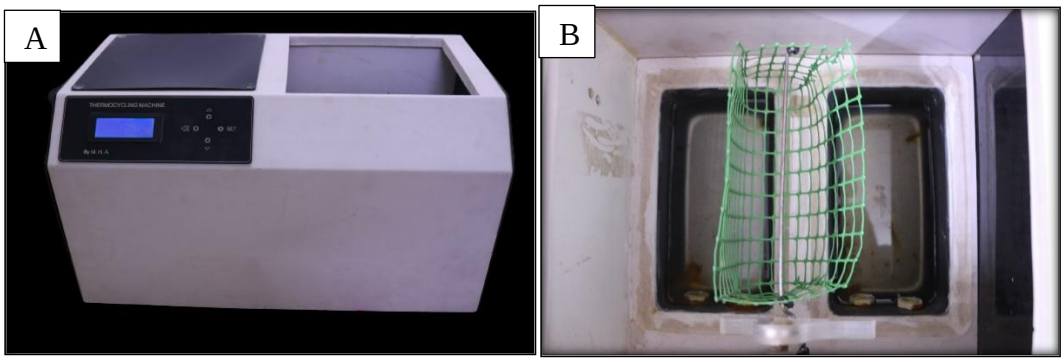

Figure 8. Thermocycling device A: Front view of the device B: Top view showing the two water baths and the sample basket

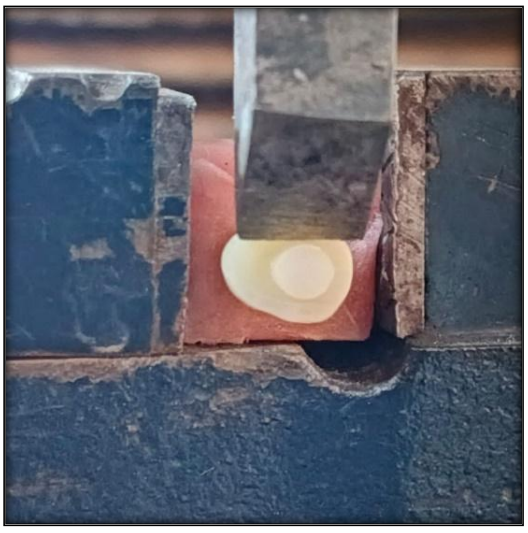

Figure 9. Universal testing machine sample in lower jaw of machine.

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Results:

The shear bond strength (SBS) test data were analyzed for normality using the Shapiro-Wilk test, which confirmed a normal distribution (P >0.05). Additionally, Levene's test was applied to assess homogeneity of variance, and the results indicated that all data were homogeneous (P > 0.05).

Descriptive Statistics The mean values of the SBS, standard deviations (±SD), minimum (Min), and (Max) of all groups expressed in (MPa) are shown in (Table1) (figure 11).

Table 1. Descriptive statistics of shear bond strength among groups.

Groups	No.	Mean	±SD	Min	Max
Group A (Adhesive)	8	12.14	2.86	7.16	17.11
Group B1 (Blood + Adhesive)	8	8.31	2.91	3.98	11.94
Group B2 (Blood + 1min GSE + Adhesive)	8	11.09	2.39	7.57	13.52
Group B3 (Blood + 3min GSE + Adhesive)	8	12.43	1.87	9.16	14.69
Group B4 (Blood + 5min GSE + Adhesive)	8	10.60	2.03	7.96	13.13
Group C1 (Blood + Hemostatic Agent + Adhesive)	8	7.06	2.01	3.58	9.94
Group C2 (Blood + Hemostatic Agent + 1min GSE + Adhesive)	8	9.15	1.41	7.96	11.54
Group C3 (Blood + Hemostatic Agent + 3min GSE + Adhesive)	8	11.79	3.33	5.18	16.74
Group C4 (Blood + Hemostatic Agent + 5min GSE + Adhesive)	8	13.09	1.71	9.55	14.71

No= number of teeth used, Mean= mean of the results, SD= standard deviation, Min= minimum result, Max= maximum result.

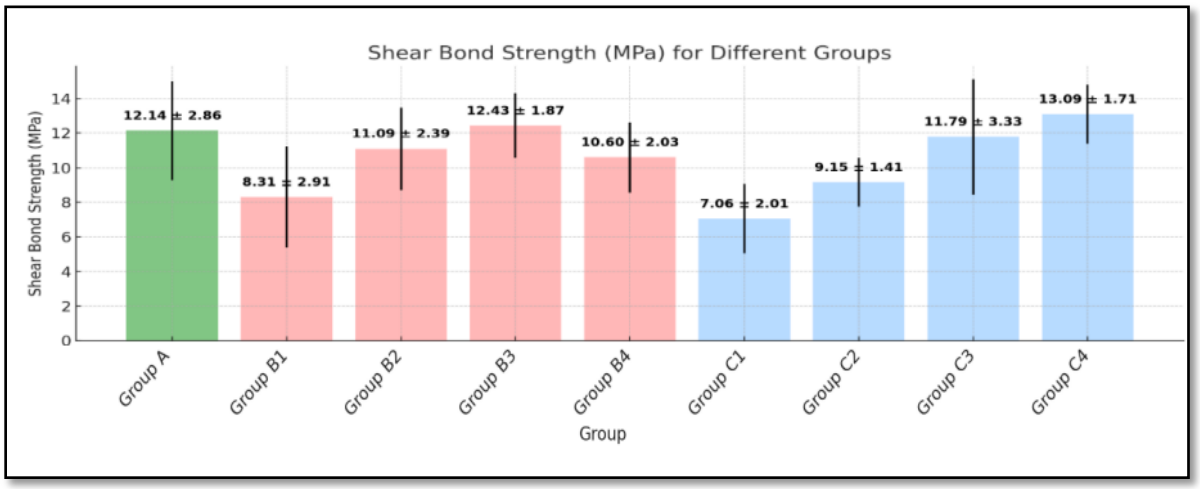

Figure 11. Bar chart showing the SBS for different groups. Each bar represents a different group, with the mean SBS value and SD indicated.

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One-way ANOVA revealed significant differences among the groups seen in (Table 2) (figure 12).

Table 2. One-way ANOVA comparing the mean SBS of the different groups.

Comparison Groups	F-statistic	P value	Significant (P < 0.05)
Groups A and B (A, B1, B2, B3, B4)	3.441	0.018	Significant
Groups A and C (A, C1, C2, C3, C4)	8.352	0.000	Significant
Groups B and C (B1, B2, B3, B4, C1, C2, C3, C4)	6.741	0.000	Significant

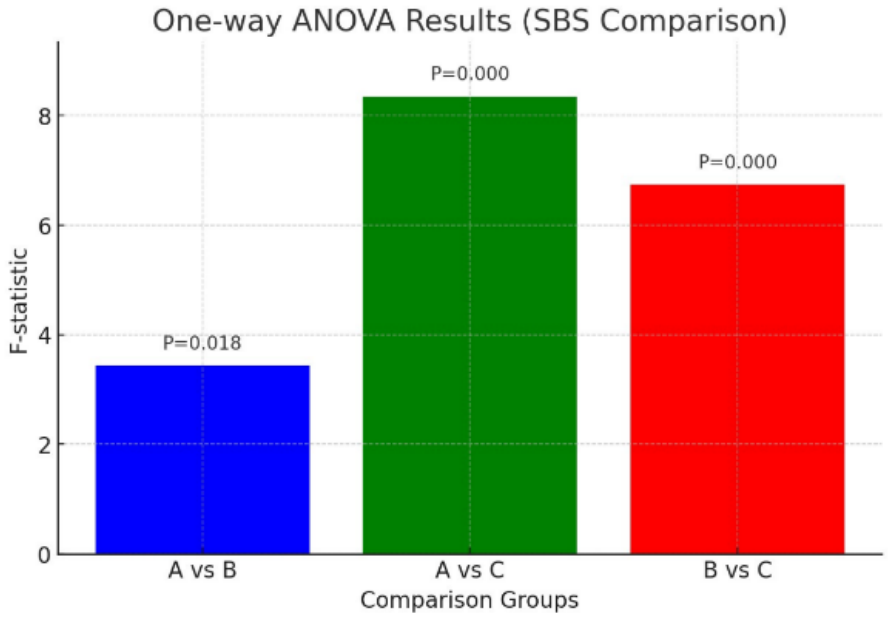

Figure 12. Bar chart showing the One-way ANOVA results, comparing the mean shear bond strength (SBS) among different treatment groups.

Tukey's HSD Post Hoc Test revealed Groups A and B: Statistically significant differences were observed between Group A and Group B1, as well as between Group B1 and Group B3 (P < 0.05). No significant (P > 0.05) differences were found among the other groups (Table 3) (figure 13).

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Table 3. Tukey's HSD post hoc tests for Group A and Group B subgroups.

Group 1	Group 2	Mean Difference	P value	95% CI Lower	95% CI Upper	Significant (P < 0.05)
A	B1	-3.828	0.032	-7.42	-0.235	Significant
A	B2	-1.041	0.918	-4.634	2.552	Non-Significant
A	B3	0.305	0.999	-3.288	3.898	Non-Significant
A	B4	-1.538	0.734	-5.13	2.055	Non-Significant
B1	B2	2.786	0.193	-0.807	6.379	Non-Significant
B1	B3	4.132	0.018	0.54	7.725	Significant
B1	B4	2.29	0.372	-1.303	5.883	Non-Significant
B2	B3	1.346	0.817	-2.247	4.939	Non-Significant
B2	B4	-0.496	0.994	-4.089	3.097	Non-Significant
B3	B4	-1.842	0.585	-5.435	1.75	Non-Significant

Lower= lower bound of the confidence interval, Upper= upper bound of the confidence interval.

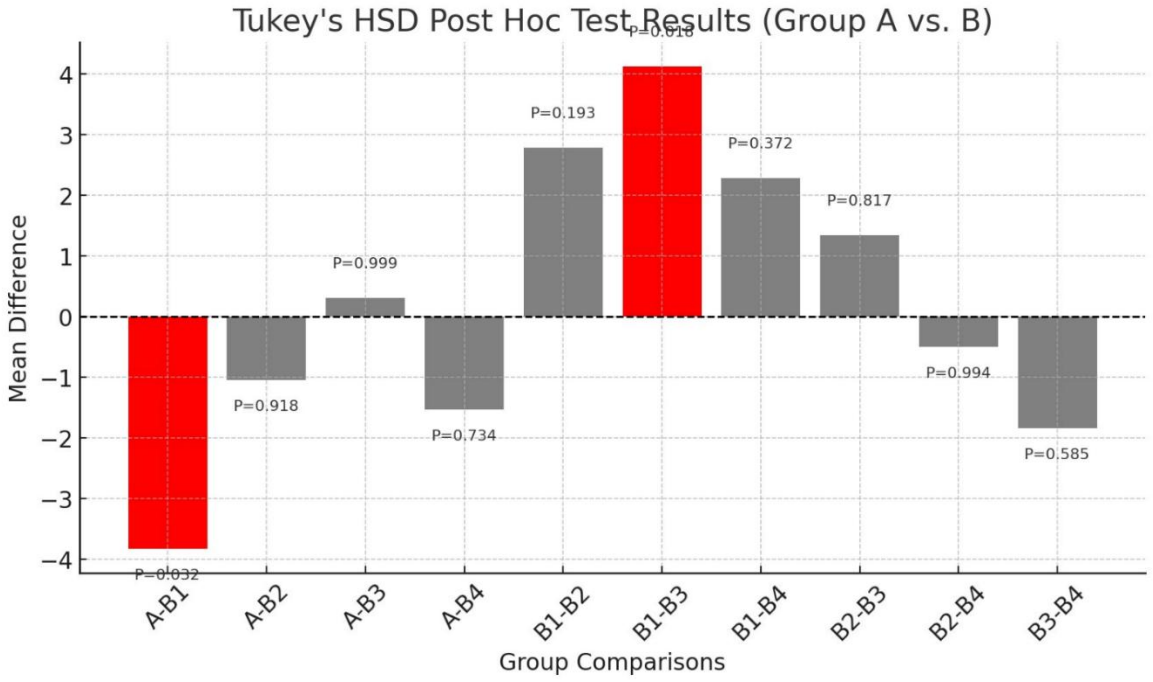

Figure 13. Bar chart illustrating the Tukey's HSD Post Hoc Test Results for Group A vs. B**.**

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Groups A and C: Significant differences were identified between Group A and Group C1, Group C1 and Group C3, Group C1 and Group C4, and Group C2 and Group C4 ($P < 0.05$). Other group comparisons showed no significant differences ($P > 0.05$) (Table 4) (figure 14).

Table 4. Tukey's HSD post hoc tests for Group A and Group C subgroups.

Group 1	Group 2	Mean Difference	P value	95% CI Lower	95% CI Upper	Significant (P < 0.05)
A	C1	-5.071	0.002	-8.557	-1.585	Significant
A	C2	-2.98	0.124	-6.466	0.506	Non-Significant
A	C3	-0.34	0.999	-3.826	3.146	Non-Significant
A	C4	0.954	0.933	-2.532	4.44	Non-Significant
C1	C2	2.091	0.433	-1.395	5.577	Non-Significant
C1	C3	4.731	0.004	1.245	8.217	Significant
C1	C4	6.025	0.000	2.539	9.511	Significant
C2	C3	2.64	0.212	-0.846	6.126	Non-Significant
C2	C4	3.934	0.021	0.448	7.42	Significant
C3	C4	1.294	0.822	-2.192	4.78	Non-Significant

Lower= lower bound of the confidence interval, Upper= upper bound of the confidence interval.

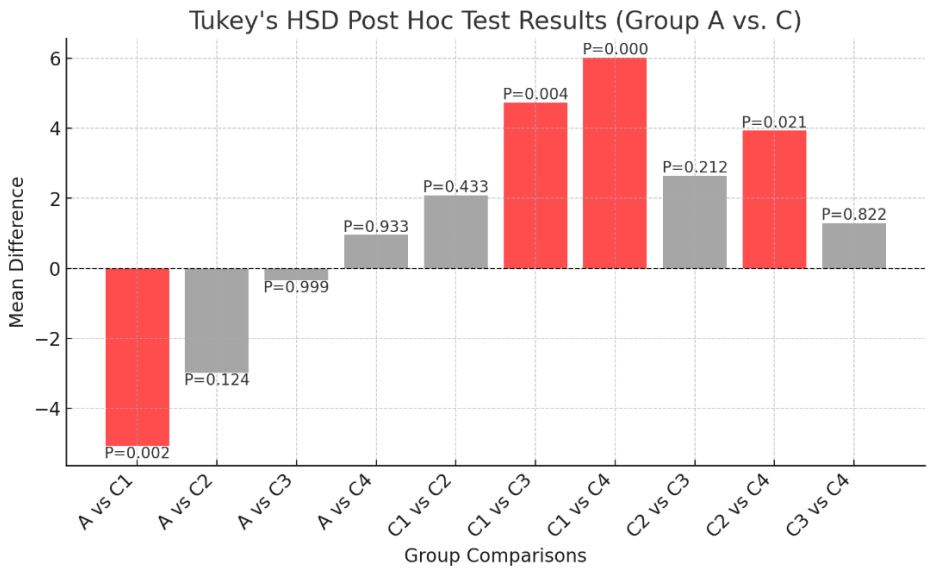

Figure 14. Bar chart illustrating the Tukey's HSD Post Hoc Test Results for Group A vs. C**.**

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The independent samples t-test compared the SBS between pairs of Group B and Group C subgroups ($P < 0.05$) (Table 5) (figure 15)

Table 5. Independent samples t-tests between pairs of group B and C subgroups.

Comparison	T-statistic	P value	Significant ($P < 0.05$)
B1 vs. C1	1.53	0.15	Non-Significant
B2 vs. C2	1.65	0.12	Non-Significant
B3 vs. C3	-0.51	0.62	Non-Significant
B4 vs. C4	-2.55	0.023	Significant

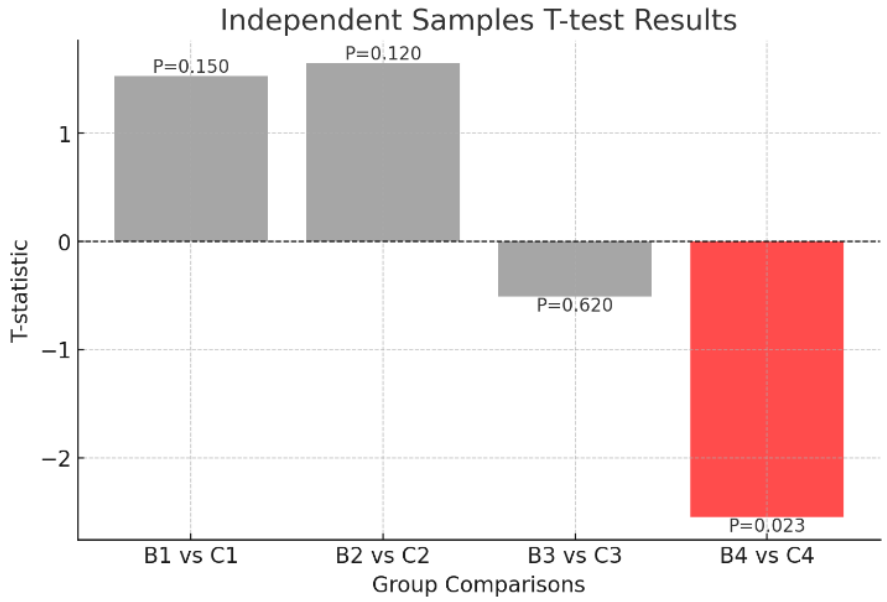

Figure 15. Bar chart depicting the Independent Samples T-test results comparing SBS between pairs of Group B and C subgroups treated with different conditions.

Mode of failure: After conducting shear testing, the samples that had been debonded were treated with Methylene-blue dye for a duration of five minutes. This was done in order to improve the distinction between the composite material and the tooth surface (Khalil and Al-Shamna, 2015). By utilizing a 7X (keeler England) magnifying loupe, two distinct failure types were observed (Figure 10). Adhesive failure is showed, where complete separation occurs between the composite and the tooth substrate at the adhesive surface, allowing the occlusal surface to absorb Methylene-blue dye.

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Mixed failures, where parts of the composite still adhere to the tooth surface, remain white (unpigmented) as they are incapable of absorbing the dye. The samples were statistically analyzed using the Fisher exact test to identify significant differences groups ($P < 0.05$) in the modes of failure, as shown in (Table 6) (Figure 16).

Table 6. Modes of failure of the different groups

Name of Group	Mode of Failure	Number	Percentage (%)	Fisher Test (P value)
Group A	Adhesive	6	75.00	0.132
	Mixed	2	25.00	
Group B1	Adhesive	8	100.00	0.0002
	Mixed	0	0.00	
Group B2	Adhesive	5	62.50	0.619
	Mixed	3	37.50	
Group B3	Adhesive	4	50.00	1.000
	Mixed	4	50.00	
Group B4	Adhesive	6	75.00	0.132
	Mixed	2	25.00	
Group C1	Adhesive	7	87.50	0.010
	Mixed	1	12.50	
Group C2	Adhesive	5	62.50	0.619
	Mixed	3	37.50	
Group C3	Adhesive	5	62.50	0.619
	Mixed	3	37.50	
Group C4	Adhesive	4	50.00	1.000
	Mixed	4	50.00	

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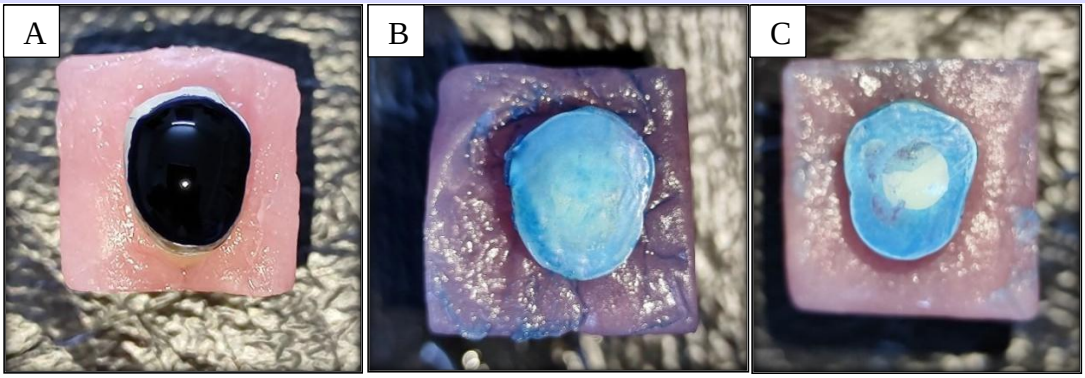

Figure 10. A: Staining samples with Methylene-blue B: Adhesive failure C: Mixed failure

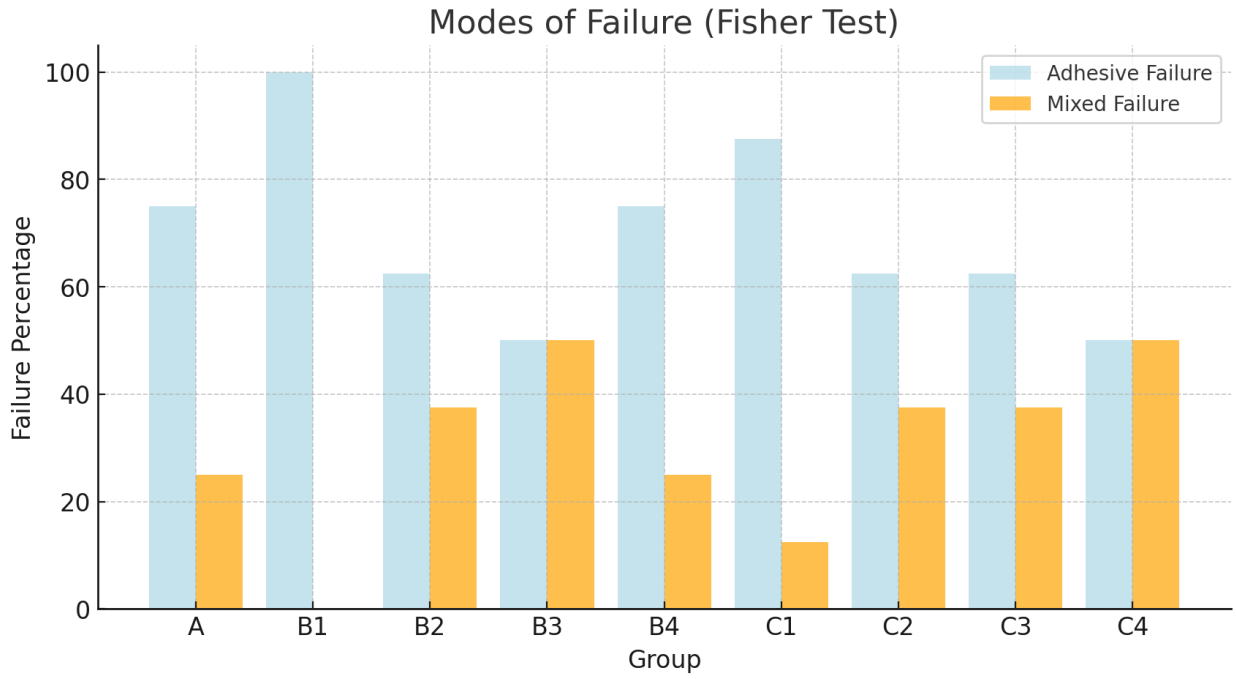

Figure 16. Bar chart displaying the Modes of Failure (Fisher Test Results) for different groups. The percentage of adhesive failure (light blue) and mixed failure (orange) is represented for each group.

Discussion:

Adequate adhesion requires a clean substrate (Unlu et al., 2015; Moharam et al., 2023). Although various isolation methods are used to prevent moisture contamination, avoiding contamination remains challenging, especially in clinical situations where cavity margins extend below the gingiva (Chang et al., 2010; Bourgi et al., 2023).

The strength of the bond between dentine and adhesive systems relies significantly on the integrity and mechanical properties of

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acid-demineralized collagen fibers (Fawzy, 2010; Hardan et al., 2021).

Maintaining collagen integrity is crucial for adhesion, and GSE's proanthocyanidins have been identified as potent natural collagen cross-linkers that may mitigate the negative effects of contamination. This study aimed to evaluate the impact of GSE on SBS of universal adhesives applied to contaminated dentine.

Wet bonding suggests that the tooth surface should remain moist before bonding to prevent the collapse of collagen fibrils. The destruction of collagen fibrils is a major factor contributing to adhesive failures (Manihani et al., 2023).

This study used blood and HA as contaminants on the dentine surface based on findings that saliva, blood, and HA all have a negatively impact on adhesive bond strength with saliva has the least effect whereas contamination with HA has the greatest effect (Koppolu et al., 2012; Kewlani et al., 2020). Therefore, it was expected that these contamination with blood and HA would significantly reduce SBS after contaminating the dentine surface.

Choosing fresh blood drawn at the time of study was to increase the accuracy and applicability of results in clinical practice settings. In laboratories where investigations are commonly done using ex-vivo models researchers often collect enough blood prior to restoration by adding anti-coagulants but this can greatly affect outcome measures (Dietrich et al., 2002). Anticoagulants mask natural interaction between blood and dentine which is observed in clinics, thereby concealing real effects caused by blood contamination during laboratory experiments. This underscores the role played by coagulation in bonding (Kaneshima et al., 2000; Dietrich et al., 2002).

In this study, blood and HA were used as contaminants. Each was applied for one minute and then rinsed for one minute. Previous authors, such as Brauchli et al. have suggested that a one-minute rinse is sufficient to effectively remove contaminants (Brauchli et al., 2010). Although a one-minute rinse with a triple syringe visibly removed the contaminants, the negative effects on bond strength persisted. The persistent negative effects on bond strength observed in other studies indicate that this duration may be insufficient for completely removing the residue, as shown by the reduced SBS in the water-rinsed group (Pucci et al., 2016; Haralur et al., 2019; Alzahrani et al., 2024).

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The choice of using GSE as a collagen cross-linker was guided by a systematic review and meta-analysis, which identified PA from GSE as the most efficient natural cross-linker. This study highlighted GSE's ability to preserve bond strength even after aging (Anumula et al., 2022).

Proanthocyanidins are abundant in natural sources like GSE, cocoa beans, pine bark extract, cranberries, lemon tree bark, and hazelnut tree leaves. GSE has 10% higher PA concentration, specifically PA B2-3'-0-gallate (Fine, 2000).

Testing SBS was used due to its straightforward nature. SBS testing is a widely employed method for quantifying bond strength, with extensive documentation on various conditions affecting SBS available in the literature (Odthon et al., 2015).

The control group exhibited a mean SBS of 12.14 MPa, which serves as the baseline for evaluating the effects of blood contamination, the combined contamination of blood and HA, and the application of GSE.

Among the groups contaminated with blood, the groups contaminated with blood alone showed a decrease in SBS compared with the control group. This reduction in bond strength aligns with the negative effects of blood contamination reported in the literature (Koppolu et al., 2012; Juneja et al., 2014; Taneja et al., 2017; Kewlani et al., 2020). This finding aligns with reports that suggest that water may not completely remove contaminants from the resin–dentine interface, potentially leaving residual blood components that could interfere with effective adhesion. (Kaneshima et al., 2000; Soares et al., 2007; Juneja et al., 2014). Furthermore, the reduction in bond strength associated with blood contamination could be linked to its high protein contents and macromolecules, such as fibrinogen and platelets, which may form a film on the dentine surface. This film has been proposed by studies as a potential barrier to the penetration of the adhesive system into dentinal tubules (Barakat and Powers, 1986; Kaneshima et al., 2000; Shaikh, 2017).

The groups contaminated with blood that received GSE treatment for 1, 3, and 5 minutes all demonstrated enhanced SBS, showing comparable results with the control group. The enhancement in SBS may be attributed to the effect of GSE on collagen cross-linking. The observed enhancement in SBS may be attributed to GSE's role as a natural collagen cross-linking agent, as

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previous studies have demonstrated that the application of such agents can significantly improve the mechanical properties and structural stability of the dentine matrix, thereby strengthening the bond between the adhesive and dentine (Aziz et al., 2023).

When comparing the blood contaminated groups treated with GSE to the blood-contaminated group without treatment, the 1 and 5-minute GSE treatments did not show any differences. However, the 3-minute GSE treatment resulted in a notably higher bond strength compared to the group with blood contamination alone. This suggests that the 3-minute exposure time was particularly effective in counteracting the negative effects of blood contamination on bond strength. With a 5-min treatment, the collagen may have been over-stabilized, leaving smaller interfibrillar spaces and less residual moisture negatively affecting bond strength.

Among the groups contaminated with both blood and HA, the group treated only with blood and the HA exhibited the low bond strength, which was noticeably lower than the uncontaminated group (Kewlani et al., 2020). The lower bond strength in the group contaminated with both blood and the HA may be attributed to the effect of the HA. According to Ayo-Yusuf et al. in their SEM-EDX study, HA were found to remove the smear layer, which normally obstructs the dentinal tubules. Additionally, due to their acidic pH (ranging from 0.8 to 3), these agents form an amorphous layer or granular precipitate on the dentine surface, potentially interfering with adhesion (Ayo-Yusuf et al., 2005). However, treating the contaminated dentine with GSE showed an improvement. The groups contaminated with blood and HA that received GSE treatment for 1, 3, and 5 minutes all demonstrated enhanced SBS, showing comparable results with the uncontaminated control group. The improvement in SBS may be attributed to the effect of GSE on collagen cross-linking, which appears to counteract the negative impact of dual contamination.

The groups contaminated with blood and HA that received GSE treatment for 1, 3, and 5 minutes all demonstrated enhanced SBS, showing results comparable to the control group. Although there was no difference between the blood and HA contaminated group and the group treated with GSE for 1 minute, differences were observed when comparing the contamination group with the 3-minute and 5-minute GSE

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groups. When comparing the 1-minute group with the 3-minute and 5-minute groups, a significant difference was noted only between the 1-minute and 5-minute treatments, highlighting the enhanced effect obtained with the 5-minute application. This may be attributed to the acidity of the HA, which could have exposed more collagen fibers, allowing for greater mechanical enhancement through the 5-minute cross-linking treatment, thereby improving bond strength.

When comparing the group contaminated with blood alone to the group contaminated with both blood and HA, there was no noticeable difference in bond strength across the untreated groups, nor between the groups that received GSE treatment for 1 minute or 3 minutes. However, when the comparison was made between the groups treated for 5 minutes, the group contaminated with both blood and HA showed a higher bond strength than the group contaminated with blood alone. This suggests that the 5-minute GSE treatment was more effective in enhancing bond strength in the presence of dual contamination.

The improvement in SBS observed in the GSE-treated groups is likely due to the cross-linking of collagen fibers, which strengthens the dentine matrix by enhancing its mechanical properties. Natural cross-linkers like PA found in GSE stabilize collagen through additional cross-links, improving dentine structure (Han et al., 2003; Bedran-Russo et al., 2007; Srinivasulu et al., 2013; Fawzy et al., 2017; El Gindy et al., 2023). Proanthocyanidins, particularly catechin, ent-catechin, epicatechin, and ent-epicatechin, play a key role in collagen stabilization by forming more cross-links (Han et al., 2003; Srinivasulu et al., 2013). Additionally, PA inhibit collagenase, promoting the conversion of soluble collagen to insoluble collagen, thereby further reinforcing the dentine (Han et al., 2003).

This study focused on the overall effects of GSE treatment on bond strength without investigating the specific underlying interactions between blood, hemostatic agents, and GSE at a molecular or structural level. Variations in treatment duration outcomes may be influenced by factors that were not assessed in this study, such as potential chemical interactions or time-dependent changes in adhesion dynamics. Further investigations are needed to explore these aspects and provide a more

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comprehensive understanding of the optimal application time for GSE in contaminated dentin.

Future research should focus on elucidating the molecular and structural interactions between GSE, blood contaminants, and hemostatic agents to better understand their collective impact on bond strength. Advanced analytical techniques, such as spectroscopy and electron microscopy, could be employed to investigate potential chemical interactions and adhesion dynamics at a microscopic level. Additionally, studies should assess the influence of varying application times on the effectiveness of GSE in contaminated dentin, considering time-dependent changes in bond stability.

Conclusions:

1- Blood and HA negatively affect the bond strength.

2- GSE treatment for durations of 1, 3 or 5 minutes, enhanced bond strength to contaminated dentine.

3- Increasing the time of application of GSE to 3 minutes in case of blood contamination led to better bond strength.

4- Increasing the time of application of GSE to 5 minutes in case of blood and HA contamination led to better bond strength.

Supplementary Material

None.

Author Contributions

Hussein Bachay: data curation, writing-original draft preparation. Hikmet A. Al-Gharrawi: Conceptualization, methodology, writing-review and editing.

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Data Availability Statement

Data are available from the authors upon reasonable request.

Conflict of interest

The authors reported that they have no conflicts of interest.

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