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Research Article

Effect of Quercetin Primer and Diode Laser Irradiation on Microtensile Bond Strength of Universal Adhesive to Pulp Chamber Dentin: An *In Vitro* Study

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Abstract

Background: Dental bonding to pulp chamber dentin remains challenging due to its high organic content, altered collagen structure, and susceptibility to enzymatic degradation. Although universal adhesives containing functional monomers have demonstrated reliable bonding performance, adjunctive pretreatment strategies are often proposed to further enhance bond durability. Evidence supporting their effectiveness when used with universal adhesives in self-etch mode is limited.

Aim: To evaluate the combined effects of quercetin primer and diode laser irradiation on the Microtensile bond strength (MTBS) of universal adhesives to pulp chamber dentin.

Methods and Material: A total of 32 extracted human third molars were selected for the study. The samples were allocated into 4 experimental groups (n = 8/group) based on pretreatment protocol with quercetin primer and Laser irradiation: Group I, Group II, Group III, and Group IV. Then samples were subjected to Microtensile bond strength testing and failure mode analysis.

Statistical Analysis: Data were analysed using One way ANOVA, followed by Tukey's *post hoc* test for intergroup comparisons.

Results: The control group demonstrated highest mean MTBS (8.839 MPa) when compared to other groups. One-way ANOVA revealed a statistically significant difference among groups ($p = 0.019$). Tukey's post hoc test showed a significant difference only between Group I and Group IV ($p = 0.029$). Failure mode analysis revealed predominantly adhesive and resin-dentin interfacial failures.

Conclusion: The universal adhesive alone achieved optimal bonding to pulp chamber dentin, while quercetin primer and diode laser irradiation did not improve Microtensile bond strength of a universal adhesive applied in self-etch mode.

Keywords: Dentin Bonding; Self-Etch; Tensile Strength; Dentin; Flavonoids; Diode Laser

Introduction

The success of dental restorations relies on strength and durability of the bond between restorative materials and the dentin, especially within the challenging environment of the pulp chamber due to altered collagen structure, increased brittleness, enzymatic degradation or incomplete infiltration of adhesives [1]. Insufficient penetration of adhesive monomers results in exposed collagen fibrils that are highly susceptible to degradation by host-derived matrix metalloproteinases (MMPs) [1,2].

The hybrid layer, formed through micromechanical interlocking between adhesive monomers and exposed collagen fibrils, is regarded as most vulnerable component of the bonding interface, as poorly infiltrated collagen at its base is highly susceptible to degradation, which may result in failure of restorations or development of secondary caries [3,4]. To combat this issue, various approaches has been explored like ethanol-wet bonding, the use of MMP inhibitor, collagen cross-linking, and hybrid layer remineralization [5,6]. Among these, naturally derived collagen cross-linkers possessing antibacterial activity have been extensively studied for dentin bonding [7,8].

Quercetin is a natural flavanoid found in citrus fruit and exhibits multiple properties, including antioxidant, anti-inflammatory and anti-aggregatory activities.

In adhesive dentistry, dentin pretreatment with quercetin has been shown to inhibit matrix metalloproteinase activity within the hybrid layer, stabilize the collagen network, and enhance long-term durability of dentin adhesion [9,10]. Epasinghe., *et al.* has also observed that quercetin acts as a collagen cross-linker, leading to improved mechanical properties of demineralized dentin [11].

Previous studies have explored the role of laser irradiation as a supplementary technique to enhance adhesive performance. Diode lasers, in particular, have demonstrated a positive impact on the microtensile bond strength (MTBS) when applied after adhesive placement. These improvements have been attributed to enhanced adhesive polymerization, increased solvent evaporation, and favourable modifications at the adhesive–dentin interface, which may facilitate better monomer infiltration and hybrid layer formation [12-15]. However, no studies have evaluated the effect of quercetin as a dentin primer in combination with diode laser irradiation when universal adhesives are applied in self-etch mode.

10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) is considered the gold standard in adhesive dentistry, and it has been broadly used in the formulation of contemporary self-etch (SE) or universal adhesive systems [16].

Universal adhesives, also referred as multi-mode adhesives, are newest generation of dentin bonding systems designed to simplify clinical procedures while maintaining effective adhesion to enamel and dentin. These adhesives may be applied in self-etch, etch-and-rinse, or selective-etch strategies.

Their formulation combines hydrophobic and hydrophilic components within a single system, and a key characteristic of many universal adhesives is the presence of 10-MDP as functional monomers. Due to their versatility, simplified application, and enhanced bonding potential, universal adhesives have become widely adopted in restorative dentistry.

The presence of 10-MDP in adhesive systems are known to form strong and stable chemical bonds with hydroxyapatite and resulting water-insoluble MDP-calcium salts protects the underlying collagen matrix [17]. Several studies have demonstrated the superior performance of 10-MDP-based adhesives in terms of MTBS bond strength, interface stability, and nanolayer formation at the adhesive-dentin interface [12,18].

Therefore, present study aimed to evaluate the combined effect of quercetin primer and diode laser irradiation on microtensile bond strength (MTBS) of universal adhesives to pulp chamber dentin, with the objective of developing a more effective bonding protocol for use in clinical practice. Null hypotheses tested were: (1) Quercetin pretreatment doesn't affect the MTBS of universal adhesive to pulp chamber dentin after 24 hours of water storage; (2) There is no significant difference in MTBS of universal adhesive to pulp chamber dentin, with or without 970 nm diode laser irradiation.

Materials and Methods

The study received ethical clearance from the Institutional Ethics Committee (GDCH/IRB/IX-2024/RPN55-53). The study was conducted over a three-month period, from August 2024 to October 2024. A total of 32 human third molars indicated for extraction were procured from the Department of Oral and Maxillofacial Surgery. Sample size estimation was performed using G*Power software. Based on data obtained from a pilot study, a pooled standard deviation of 3.08 was used. Assuming an effect size of 0.8094, a significance level (α) of 0.05, a statistical power of 95%, and four study groups, the required sample size was calculated to be eight specimens per group. Following extraction, teeth were cleaned using an ultrasonic scaler and immersed in 0.5% chloramine-T solution until further preparation.

Preparation of experimental material

Quercetin powder 0.5 wt% (Sigma-Aldrich, St. Louis, MO, USA) was dissolved into 100 mL of 100% absolute ethanol. The mixture was continuously stirred at 37 °C in a water bath for 15 minutes to obtain a homogeneous primer solution, which was then stored under controlled cool storage conditions until use [Figure 1].

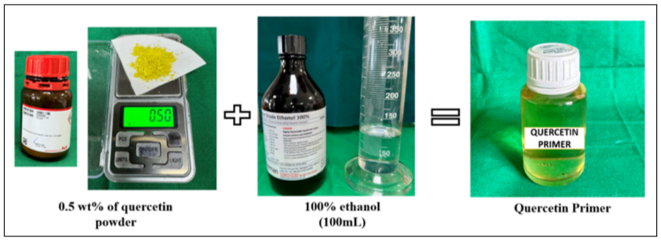

Figure 1: Preparation of Quercetin Primer.

Preparation of samples

Thirty-two third molars were used for the study. The samples were horizontally sectioned using a diamond disc (DFS Diamond Cutting Disc, Germany) and high-speed handpiece (Being, Lotus 401P-B2, China) 2 mm below cementoenamel junction and furcation area to expose pulp chamber. Pulp tissue was removed and the specimens were rinsed thoroughly with distilled water.

All specimens were subjected to immersion in 3% sodium hypochlorite (Prime Dental) for 20 minutes, followed by rinsing with distilled water. The specimens were randomly allotted to 4 groups (n=8/group) based on pretreatment with quercetin primer and irradiation with Laser:

- Group 1- Samples with no pretreatment (control-C)
- Group 2- Samples pretreated With Quercetin primer (QP)
- Group 3- Samples without Quercetin Primer pretreatment and with Laser irradiation (L)
- Group 4- Samples pretreated with Quercetin primer and Laser irradiation (QP +L)

Restorative protocol

Group I: Control group (C)

The pulp chamber of the samples was bonded with a Universal adhesive (Scotch Bond Universal adhesive 3M™, ESPE) using a microbrush in accordance with the manufacturer's recommendations. Adhesive was polymerized for 10 seconds with an LED curing unit (3M™ Elipar™, DeepCure-S LED curing light, 3M, India Ltd). Composite resin was subsequently placed into the pulp chamber in incremental layers of 2 mm thickness, with each increment light-cured for 10 seconds. The restored specimens were then maintained in distilled water at 37 °C for 24 hours [Figure 2: (Group I)].

Group II: Samples pretreated with quercetin primer (QP)

Samples were pretreated with quercetin primer using micro brush for 60 seconds. After gently blot-drying with filter paper, adhesive was applied followed by resin composite build up and storage in distilled water, same procedure was followed as mentioned for Group I [Figure 2: (Group II)].

Group III: Samples without quercetin primer pretreatment with laser irradiation (L)

Samples were bonded with Universal adhesive and subjected to laser irradiation before light polymerization. Laser irradiation was performed using a diode laser unit (IndiLase, Medsol, Honsur, India) operating at a wavelength of 970 nm, power output of 1 W, continuous wave mode, and non-contact application. A fiber tip of 400 μm diameter was used, and irradiation was performed in a circular motion from the center outward and then inward for 20 seconds. Following laser application, the adhesive was light-polymerized for 10 seconds followed by resin composite buildup and storage [Figure 2: (Group III)].

Group IV: Samples pretreated with Quercetin primer and Laser irradiation (QP+L)

Here, samples were pretreated with quercetin primer using micro brush for 60 seconds and blot-dried with filter paper. Adhesive was then applied and diode laser was employed similarly as mentioned in Group III. This was followed by light polymerization for 10 seconds and resin composite buildup followed by storage [Figure 2: (Group IV)].

Testing procedure

After storage in distilled water for 24 hours, sample were subjected to thermocycling for 1000 cycles between 5°C - 55°C with a dwell time of 60 seconds. Further they were sectioned at low speed using a diamond disc to obtain multiple beam-shaped sticks with a cross-sectional area of 1 ± 0.5 mm^2 and the mean MTBS value for each tooth was calculated. After this, the specimens underwent the following evaluations:

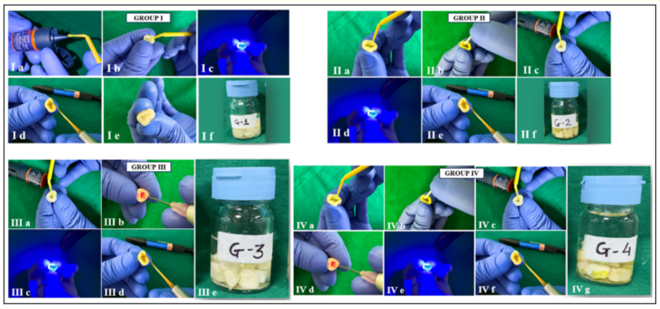

Figure 2: Restorative Protocol for each Group.

GROUP I (a-f): Control group with universal adhesive application.

GROUP II (a-f): Quercetin primer pretreatment followed by adhesive application.

GROUP III (a-e): Adhesive application followed by diode laser irradiation, and

GROUP IV (a-g): Combined quercetin pretreatment and diode laser irradiation prior to adhesive polymerization.

- Microtensile Bond Test (MTBS) using Universal Testing Machine
- Failure Mode Analysis using Stereomicroscope

Subjection of samples to mechanical analysis

Each specimen designated for MTBS testing was mounted onto a custom jig with the help of cyanoacrylate glue (3M™ Scotch-Weld™ Instant Adhesive CA8, India). Microtensile testing was then performed using a universal testing machine (ACME Engineers, UNITEST 10, Pune, India), applying load at the resin-dentin interface at a constant crosshead speed of 1 mm/min until fracture. The fractured specimens were subsequently examined under a stereomicroscope (Wuzhou New Found Instrument Co. Ltd., China; XTL 3400E) at 15× magnification to know the fracture pattern.

Statistical analysis

All statistical analyses were conducted using StataCorp. 2025. Stata Statistical Software. Descriptive statistics, including mean, median, and standard deviation, were used to summarize data. Shapiro–Wilk test was applied to assess data normality. Differences in mean microtensile bond strength among groups was analyzed using one-way ANOVA, then Tukey's post hoc test was used for pairwise comparison ($P \leq 0.001$). The distribution of failure patterns among groups was evaluated with Chi-square test.

Results and Discussion

Results

Based on the results of the current study, null hypothesis stating that Quercetin pretreatment does not affect the MTBS of universal adhesive to pulp chamber dentin following 24 hours of water storage and that there is no significant difference in MTBS of universal adhesive to pulp chamber dentin, with or without 970 nm diode laser irradiation was accepted.

Microtensile bond strength

Among experimental groups, Group I demonstrated highest mean MTBS value of (8.839 ± 3.014 MPa), followed by Group II (8.248 ± 1.484 MPa), Group III (6.649 ± 0.774 MPa), and Group IV (6.075 ± 1.402 MPa).

Descriptive analysis showed that the Group I exhibited the highest mean microtensile bond strength, while the quercetin and laser-treated groups demonstrated lower values. The standard deviations ranged from 0.774 to 3.014, indicating moderate variability in bond strength within the groups [Table 1]. Shapiro-Wilk test showed that Group I deviated significantly from normal distribution ($p = 0.019$), while remaining groups were normally distributed ($P > 0.05$).

Group	N	Mean	95% Confidence Interval		Median	SD	Min - Max	Shapiro Wilk normality test	
			Lower	Upper				W	p value
I	8	8.839	6.319	11.359	7.805	3.014	6.11 - 15.63	0.783	0.019
II	8	8.248	7.007	9.488	7.935	1.484	6.81 - 11.44	0.856	0.110
III	8	6.649	6.002	7.296	6.655	0.774	5.65 - 7.73	0.929	0.509
IV	8	6.075	4.903	7.247	6.055	1.402	4.19 - 8.05	0.913	0.377

Table 1: Results of the Microtensile bond strength to dentin.

One-way ANOVA demonstrated statistically significant difference in MTBS among the four groups (F = 3.925; p = 0.019) [Table 2], suggesting that the different pretreatment protocols had a significant impact on the bond strength.

One-way ANOVA	F	df1	df2	p value
Microtensile Bond Strength (MPa)	3.925	3	28	0.019

Table 2: One-Way ANOVA test.

These results suggest that the treatment protocol applied in Group I may offer superior adhesion performance to the pulp chamber dentin when compared to other groups and quercetin primer did not produce a statistically significant effect on bond strength. According to Post hoc comparisons only significant difference was between Group I (control) and Group IV (p = 0.029) [Table 3], showing that combined treatment (quercetin + laser) significantly reduced bond strength. All other group comparisons showed no statistically significant differences.

Pairwise Groups		Mean Difference	P value
I	II	0.591	0.92
I	III	2.19	0.11
I	IV	2.764	0.029*
II	III	1.599	0.334
II	IV	2.173	0.114
III	IV	0.574	0.926

Table 3: Tukey's post hoc test for intergroup comparison.

Failure mode analysis

The observed dentin fracture patterns were classified into cohesive failures within dentin or resin, adhesive failures, failures at the resin–dentin interface, or mixed failures. Failure mode analysis revealed group-dependent differences in fracture patterns [Figure 3]. Adhesive and interfacial failures predominated in the control and quercetin groups.

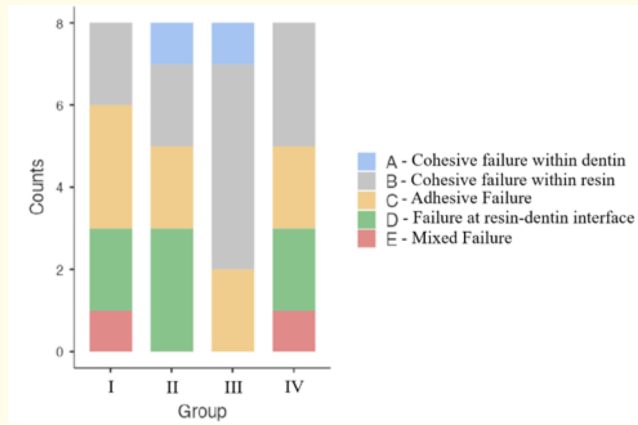

Figure 3: Mode of Failures.

Discussion

The present study assessed the influence of quercetin primer and diode laser irradiation, both individually and in combination, on microtensile bond strength (MTBS) of a universal adhesive to pulp chamber dentin. Contrary to initial hypothesis, the results indicated that neither quercetin primer nor diode laser irradiation improved bond strength compared to control group. Furthermore, combination of both treatments led to a statistically significant reduction in MTBS. The highest bond strength was observed in the control group, followed by quercetin-treated, laser-treated, and combined quercetin-laser groups (Group I > Group II > Group III > Group IV), indicating that adjunctive pretreatments did not enhance bonding.

The findings are not entirely in agreement with previous studies that reported beneficial effects of quercetin as a dentin pretreatment agent [9,10,19]. Quercetin has been widely documented for its antioxidant, antibacterial, and collagen cross-linking properties, and its role in inhibiting matrix metalloproteinases (MMPs), which contribute to hybrid layer degradation [9]. However, under the conditions of the present study, quercetin pretreatment did not significantly enhance bond strength to pulp chamber dentin. Similar findings were reported by Andrés Dávila-Sánchez., *et al.* who observed that although quercetin initially improved bond strength, this effect was not maintained after thermocycling due to structural alterations in quercetin under acidic conditions, compromising its collagen cross-linking ability over time [20].

The performance of the control group may be attributed to the use of Universal adhesive (3M ESPE), which contains 10-MDP as the functional monomer. This monomer is widely recognized for its ability to establish a stable and hydrolysis-resistant chemical bond with hydroxyapatite, forming MDP-Ca salts that reinforce the hybrid layer and improve adhesive durability. Such chemical bonding has been consistently demonstrated to play a crucial role in the stability and longevity of MDP-based adhesive interfaces. Furthermore, it has the potential to form an acid-base resistant zone at adhesive interface [17,21,22]. The superior performance of the control group suggests that the 10-MDP-based adhesive alone may be sufficient to provide strong and stable bonding to pulp chamber dentin, without the need for additional surface pretreatments [23,24]. This is especially relevant considering the inherently challenging nature of pulp chamber dentin.

Similarly, diode laser irradiation has been proposed to improve dentin, particularly when applied prior to adhesive curing. Previous studies evaluating diode laser irradiation have reported inconsistent findings. Maenosono., *et al.* Ramachandruni., *et al.* and Resaei-Soufi., *et al.* observed improved bond strength following laser application due to enhanced solvent evaporation and monomer infiltration, whereas other authors reported no additional benefit when universal adhesives were used [15–17,25].

Despite the proposed benefits of laser irradiation, Group III did not show a significant improvement in MTBS compared to the control group. This suggests that when a universal adhesive is applied in self-etch mode where adhesive-dentin interaction is already chemically optimized and its capable of achieving effective hybrid layer formation independently, laser irradiation may not provide additional bonding advantages. Zabeu GS., *et al.* and Rezaee M., *et al.* similarly reported that laser irradiation did not improve immediate dentin bond strength and could adversely affect the adhesive interface after aging [26,27]. Furthermore, the comparatively favourable performance observed with the universal adhesive was attributed to its MDP-based composition, which enhances the degree of conversion, promotes stable chemical bonding, and limits water-induced degradation over time.

Interestingly, the combination of quercetin pretreatment and diode laser irradiation (Group IV) resulted in significantly lower bond strength compared to control. This may be due to an adverse interaction between the quercetin-treated dentin surface and the thermal effects of laser irradiation, potentially leading to denaturation of collagen or interference with monomer infiltration. Instead of synergizing, these interventions may have disrupted the optimized interaction between the adhesive and dentin substrate. Similar studies have also reported limited or no additional bonding benefit of laser irradiation when used with MDP-based universal adhesives [28–30].

Overall, these findings of the present study suggest that additional pretreatments may not be necessary when using a high-performance universal adhesive in self-etch mode, as it can provide optimal bond strength independently, owing to its chemical formulation.

Conclusion

Universal adhesive used in self-etch mode demonstrated highest microtensile bond strength to pulp chamber dentin, likely due to chemical bonding capability of its 10-MDP monomer. Neither quercetin pretreatment nor diode laser irradiation offered any additional improvement in bond strength. Moreover, combination of quercetin and diode laser resulted in a significant reduction in MTBS, suggesting an unfavorable interaction when both approaches are employed together. These findings indicate that additional adjunctive procedures may be unnecessary when using a 10-MDP-containing universal adhesive and may, in some cases, negatively impact bonding. Further studies are required to clarify the long-term effects and optimal application protocol of such adjunctive techniques.

Acknowledgements

Nil.

Conflict of Interest

Nil.

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