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RESEARCH ARTICLE

# Evaluation Of Microleakage Of Composite Restoration After Pretreatment With Collagen Cross Linkers Between Two Adhesive Systems: An In-Vitro Study

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**Abstract:** Microleakage at the tooth-restoration interface is a significant concern in restorative dentistry, often resulting in marginal discoloration, secondary caries, and pulpal inflammation. The hybrid layer, crucial for bond integrity, is susceptible to enzymatic degradation. Incorporating collagen cross-linkers like proanthocyanidins (PA) has shown promise in enhancing bond durability and reducing microleakage. **Aim:**To evaluate and compare the microleakage of composite restorations pretreated with collagen cross-linkers using self-etch and total-etch adhesive techniques.

**Materials.and.Methods:**This in-vitro study involved 40 extracted human premolars, divided into four groups:

Group A: Self-etch adhesive
Group B: Total-etch adhesive
Group C: Self-etch + PA pretreatment
Group D: Total-etch + PA pretreatment

Standardized Class V cavities were prepared and restored with composite resin. Samples underwent thermocycling (1000 cycles) to simulate oral conditions.Samples will then be coated with Nail varnish leaving only restorations and 1mm peripheral exposed. Teeth will be immersed in rhodamine B dye for 24 hours. Specimens will then be rinsed and sectioned buccolingually through the center of restorations with a slow speed diamond disk. Confocal microscopy will be used to assess the microleakage. **Results:**Group C showed lower microleakage scores in the enamel margins and group D showed lower microleakage scores in the dentin margins. **Conclusion:**Pretreatment with proanthocyanidin-rich grape seed extract potentially reduces microleakage in composite restorations, with total-etch techniques showing enhanced marginal integrity. Self etch adhesives also have shown adequate seal to prevent microleakage. Proanthocyanidin pre-treatment can be an approach to improve the longevity and clinical performance of adhesive restorations

## INTRODUCTION

One of the significant advancements in restorative dentistry is the development of resin-based composite technology.[1] Compared to amalgam restorations, composite restorations offer distinct advantages such as better preservation of tooth structure and strong adhesive properties.[1] The use of composite resins is particularly indicated for the restoration of cervical cavities.[1]Microleakage remains a major concern in composite restorations, as marginal gaps can lead to secondary caries, postoperative sensitivity, and restoration failure due to polymerization shrinkage and inadequate sealing at the tooth–restoration interface.[2]

For smear layer removal, instrumentation must be supplemented with irrigation [2].

In composite restorations, the adhesive strategy is one of the important factors which determines the success of the restoration. The primary objective of adhesive systems is to ensure an effective seal between the restorative material and the tooth substrate(enamel and dentin), thereby establishing a durable bond.[3] Based on their bonding approach and application technique, adhesive systems are categorized as: (i) **etch-and-rinse adhesives**, which completely eliminate the smear layer and are applied in two or three steps; and (ii) **self-etch adhesives**, which alter and integrate the smear layer into the hybrid layer, utilizing it as part of the dentin substrate, and can be applied in either two or a single step.[3]

Dentin is a mineralized, collagen-rich tissue composed of inorganic apatite crystallites embedded within an extracellular matrix (ECM) that contains dentin-specific proteins and various enzymes. Among these enzymes, matrix metalloproteinases (MMPs) have attracted significant interest due to their potential involvement in bond degradation.[3,4] Matrix metalloproteinases (MMPs) are endogenous enzymes that depend on $Zn^{2+}$ and $Ca^{2+}$ ions for their activity and possess the ability to degrade nearly all components of the extracellular matrix (ECM).[4] During adhesive procedures, the acid etching or the acidic monomers creates exposure of dentin matrix which is followed by the impregnation of resin to form the hybrid layer.[4] The stability of this layer is important for the longevity of composite restoration.[4]

Both in vivo and in vitro studies have demonstrated that hybrid layers (HLs) formed by dentin bonding systems are unstable in aqueous environments due to hydrolytic degradation of the resin components and collagen fibrils, which progressively deteriorate over time.The degradation of the hybrid layer can be attributed to multiple factors, with the most critical being the hydrolysis of both the resin component and the exposed, non-hybridized collagen.[4] Additionally, the activation of matrix metalloproteinases (MMPs) and their enzymatic activity, which can break down type I collagen fibrils within the hybrid layer, play a key role in this process.[5,6,7]

Various natural and synthetic agents capable of enhancing collagen cross-linking have been employed to improve bond durability. Among them, proanthocyanidins (PAs)—oligomeric flavonoids abundantly present in grape seeds, pine bark, cranberries, lemon tree bark, and hazelnut leaves—have shown promising potential.[5] In dentistry, grape seed extract offers multiple therapeutic benefits, including antibacterial (both bactericidal and bacteriostatic) and antiplaque effects, anti-erosive properties, remineralization potential, collagen cross-linking ability, dentin biomodification, use as an endodontic irrigant, therapeutic application in periodontitis, and anticancer activity against oral malignancies.[8] Many studies have shown that pretreatment of dentin with proanthocyanidins have significantly improved the bond strength.[8]

The aim of this study was to evaluate the microleakage of composite resins after pre-treatment with collagen crosslinker and to compare the microleakage with use of total etch technique and self etch technique in enamel and dentin margins.

## Materials and methods

<u>Sample preparation</u>

Forty extracted premolar teeth, free from anomalies or caries and removed for orthodontic purposes, were included in this study (significance level $\alpha = 0.05$). The teeth were cleaned using an ultrasonic scaler (Varios2 Ultrasonic Scaler, NSK, Japan) to remove external debris and stains, stored in 0.5% thymol solution for one week, and subsequently kept in normal saline to prevent dehydration. Standardized Class V cavities, measuring 3 mm in width, 1.5 mm in depth, and 1.2 mm in height, were prepared on the facial surface parallel to the cementoenamel junction using a high-speed cylindrical diamond bur. The occlusal margin of each cavity was positioned in enamel, while the cervical margin was located in dentin. The cavities were rinsed with water for 20 seconds and air-dried with an air syringe.

<u>Bonding protocols</u>

The Adper single bond 2 (3M) was used for total etch technique and Clearfil SE2(kuraray) was used for self etch technique.

Group 1: total etch

Cavity was etched with 37% phosphoric acid for 15 seconds, washed, dried and 4th generation bonding agent was applied and cured for 20 seconds. This was followed by composite restoration using a nanohybrid composite (Ivoclar tetric N ceram).

Group 2: self etch

Clearfil SE2 primer was applied on the cavity for 20 seconds, dried with mild air blow. Clearfil SE2 bond was applied followed by gentle airflow and light cured for 20 seconds. This was followed by composite restoration using a nanohybrid composite (Ivoclar tetric N ceram).

Group 3: total etch with PA pre treatment

Cavity was pre-treated with 6.5% proanthocyanidin for 1 minute and washed. This was followed by composite restoration done using total etch technique as described in group 1.

Group 4: self etch with PA pre treatment

Cavity was pre-treated with 6.5% proanthocyanidin for 1 minute and washed. Composite restoration done using two step self etch according to manufacturer instructions as described in group 2.

<u>Microleakage evaluation</u>

All samples were subjected to 1000 cycles of thermocycling following which they were immersed in 0.5% rhodamine dye for 48 hours.

Specimens were rinsed and the specimens were sectioned buccolingually through the center of each restoration using a cutting instrument. Microleakage was then evaluated at both the occlusal enamel margin and the cervical dentin margin using confocal laser scanning microscopy.

0: No microleakage found

1: Dye penetration by one-third of the cavity depth

2: Dye penetration by two-thirds of the cavity depth

3: Dye penetration by more than two-thirds of the cavity depth

4: Dye penetration reaching the axial wall or towards the tooth pulp

Finally, the mean score of microleakage was calculated for each group and statistical analysis was done using the Kruskal wallis test and Dunn's post hoc test.

## Results

Table 1 shows the mean microleakage score at the enamel margin was highest in Group 2, which recorded a value of $2.70 \pm 1.89$. This was followed closely by Group 1, which showed a mean score of $2.10 \pm 1.85$. Group 4 exhibited a lower mean score of $1.20 \pm 1.69$, while Group 3 demonstrated the least microleakage with a mean of 0.50 ± 0.97. All groups had a minimum score of 0.0, indicating that some samples showed no microleakage, while the maximum score reached 4.0 in Groups 1, 2, and 4, and 3.0 in Group 3. The difference in microleakage scores among the four groups was statistically significant (p< 0.001), suggesting that the treatment protocols or materials used in these groups influenced the extent of microleakage at the enamel margin.

Comparison of mean Microleakage scores at Enamel Margin b/w 4 groups using Kruskal Wallis Test

| Groups | N | Mean | SD | Min | Max | p-value |
|---|---|---|---|---|---|---|
| Group 1 | 10 | 2.10 | 1.85 | 0.0 | 4.0 | <0.001* |
| Group 2 | 10 | 2.70 | 1.89 | 0.0 | 4.0 | |
| Group 3 | 10 | 0.50 | 0.97 | 0.0 | 3.0 | |
| Group 4 | 10 | 1.20 | 1.69 | 0.0 | 4.0 | |

Table 1: mean microleakage scores in enamel margins

When comparing microleakage scores at the enamel margin, Group 1 showed a significantly higher mean score than Group 3, with a mean difference of 1.60 and a p-value of 0.04.
Similarly, Group 1 had a significantly greater score than Group 4, with a mean difference of 0.90 and a p-value of 0.02. However, the difference between Group 1 and Group 2 was not statistically significant (p=0.31).
Group 2 exhibited significantly higher microleakage than Group 3, with a mean difference of 2.20 and a p-value of 0.01. The comparison between Group 2 and Group 4 also revealed a significant difference, with Group 2 showing greater microleakage (mean difference of 1.50, p=0.03).
No statistically significant difference was observed between Group 3 and Group 4 (p=0.42), indicating that these two groups had comparable microleakage scores at the enamel margin.

Table 2 shows the mean microleakage score at the dentinal margin was highest in Group 1, which recorded a value of 2.90 ± 1.52. Group 2 followed with a mean score of 1.80 ± 1.99, while Group 3 showed a slightly lower mean of 1.20 ± 1.62. Group 4 exhibited the least microleakage, with a mean score of 0.50 ± 1.08. All groups included subjects with a minimum score of 0.0, indicating complete absence of microleakage in some cases. The maximum score reached 4.0 in Groups 1, 2, and 3, and 3.0 in Group 4. The observed differences in microleakage scores among the four groups were statistically significant (p< 0.001), suggesting that the interventions or materials used had a marked impact on microleakage at the dentinal margin.

Comparison of mean Microleakage scores at Dentinal Margin b/w 4 groups using Kruskal Wallis Test

| Groups | N | Mean | SD | Min | Max | p-value |
|---|---|---|---|---|---|---|
| Group 1 | 10 | 2.90 | 1.52 | 0.0 | 4.0 | <0.001* |
| Group 2 | 10 | 1.80 | 1.99 | 0.0 | 4.0 | |
| Group 3 | 10 | 1.20 | 1.62 | 0.0 | 4.0 | |
| Group 4 | 10 | 0.50 | 1.08 | 0.0 | 3.0 | |

Table 2: mean microleakage scores at dentin margins

At the dentinal margin, Group 1 demonstrated significantly higher microleakage scores compared to Group 3 and Group 4. The mean difference between Group 1 and Group 3 was 1.70, with a p-value of 0.02, while the difference between Group 1 and Group 4 was even more pronounced at 2.40 (p=0.002). However, the comparison between Group 1 and Group 2 did not yield a statistically significant result (p=0.18).
Group 2 showed significantly greater microleakage than Group 4, with a mean difference of 1.30 and a p-value of 0.03. No significant differences were observed between Group 2 and Group 3 (p=0.43), nor between Group 3 and Group 4 (p=0.28), indicating that these latter groups had relatively similar microleakage scores at the dentinal margin.

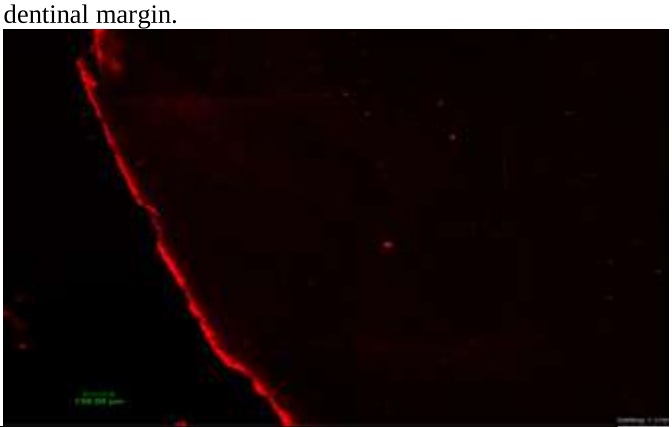

Fig 1: CLSM image of group 4(self etch and PA)

## Discussion

Microleakage scores were assessed at both the enamel and dentinal margins across four experimental groups: The Total-Etch group (Group 1), the Self Etch group (Group 2), the Total-Etch with Proanthocyanidin group (Group 3), and the Self Etch with Proanthocyanidin group (Group 4). Distinct patterns emerged in the distribution of microleakage across these groups, highlighting the influence of both bonding strategy and the incorporation of Proanthocyanidin.

At the enamel margin, the Self Etch group exhibited the highest degree of microleakage, followed closely by the Total-Etch group. These two groups consistently showed greater leakage compared to their counterparts that incorporated Proanthocyanidin. The Total-Etch with Proanthocyanidin group demonstrated the least microleakage, indicating a potential synergistic effect between the etching protocol and the antioxidant treatment. The Self Etch with Proanthocyanidin group also showed reduced leakage relative to the conventional Self Etch group, though not to the same extent as the Total-Etch with Proanthocyanidin group.

In this study, the significant difference of enamel margin microleakage between self etch group and total etch groups indicates the importance of acid application on enamel margins for reduction of microleakage.[14] This requires a ph of 0.5 to 1 for optimal enamel etching where as the ph of clearfil SE adhesive is 2 which is not sufficient to create adequate bonding with the enamel.[14]

Research indicates that omitting phosphoric acid etching leads to increased microleakage at enamel margins.[15,16] Similarly, the present study demonstrates that enamel microleakage is significantly reduced when Clearfil SE Bond is combined with pre-treatment using proanthocyanidins.

At the dentinal margin, the trend was even more pronounced. The Total-Etch group recorded the highest microleakage, suggesting that the conventional etching approach may have compromised the seal at the dentin interface. The Self Etch group followed with moderately high scores, while the Total-Etch with Proanthocyanidin group showed further reduction in leakage. Notably, the Self Etch with Proanthocyanidin group exhibited the lowest microleakage at the dentinal margin, underscoring the potential benefit of combining a milder etching strategy with antioxidant reinforcement in preserving dentin integrity.

The significant reduction in microleakage scores in the self etch and proanthocyanidin group can be attributed to:

Presence of MDP monomer in clearfil SE bond which inhibits MMP.[17]

And forms an acid base resistant zone which is more stable..[17]

antioxidant treatment with the use of Proanthocyanidins promotes collagen cross linking which prevents bond degradation over time.[5]

The activation of matrix metallo proteinases is responsible for degradation of the hybrid layer.[17] The effect of 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) on biodegradation of the hybrid layer could be the reason for reduced microleakage in dentinal margins with the self etch adhesive groups. 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), a key functional monomer in modern dental adhesives, contains a hydrophobic carbon-chain spacer and can partially demineralize the dentin surface while simultaneously interacting with hydroxyapatite (HAP) to form a water-stable, acid-resistant 10-MDP-Ca salt. This salt organizes into nanolayered, highly ordered structures that help protect and stabilize the dentin–resin bonding interface, reducing its susceptibility to degradation.[17]

PA strengthens the exposed dentinal collagen through its cross-linking action and dehydrates the fibrils, creating a more favorable substrate for hybridization and ultimately forming a stable, long-lasting hybrid layer. Also, PA as a non-specific MMP inhibitor, it can protect exposed collagen fibrils to MMP degradation over time.[18]

The mildly acidic pH 2.0 of the Clearfil SE primer creates mild etching rather than harsh etching(with 37% phosphoric acid) and prevents excessive decalcification of the dentin and collapse of the collagen fiber network, ensuring better penetration of the bond component and the formation of a uniform hybrid layer.

Also the presence of HEMA in the clearfil SE adhesive promotes the permeation of monomers and bonding resin into demineralized collagen network.[19]

This study infers that, the microleakage at enamel margins can be reduced by adequate etching of enamel and microleakage at dentinal margins can be reduced by conditioning dentin with crosslinkers like proanthocyanidins and using gold standard self etch adhesives containing functional monomers that enhance the quality of bond interface.

The limitations of this study are mainly the in vitro nature of the study and the functional stress loading like cyclic loading and chewing simulation was not incorporated.

## Conclusion

Within the limitations of this in-vitro study, pretreatment of dentin with proanthocyanidin-rich grape seed extract significantly reduced microleakage in composite restorations, particularly when combined with self-etch adhesives. The incorporation of PA enhanced collagen stability and improved the quality of the hybrid layer, contributing to better marginal integrity at both enamel and dentin interfaces. Total-etch adhesive systems demonstrated improved performance at enamel margins, especially when used with PA pretreatment, while self-etch systems combined with PA showed the greatest reduction in dentinal microleakage. These findings suggest that the use of collagen cross-linkers such as PA, along with appropriate adhesive strategies, may help improve the longevity and clinical reliability of adhesive restorations

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