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Original Article

Dentistry Section

Comparison of Microleakage of Resin-based Composite Restoration on Pretreating Dentin with Chlorhexidine and Grape Seed Extract by Dye Penetration Technique: An In-vitro Study

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ABSTRACT

Introduction: Durability of dentin-resin adhesion relies on the integrity of the collagen network within the hybrid layer. However, this collagen is susceptible to degradation by Matrix Metalloproteinases (MMPs) and cysteine cathepsins, particularly in areas insufficiently infiltrated with resin. To enhance collagen stability and reduce degradation, various cross-linking agents such as Chlorhexidine (CHX) and Grape Seed Extract (GSE) have been explored. This study investigates whether these agents, when applied to etched dentin, can reduce microleakage in composite restorations.

Aim: To evaluate and compare the effect of 2% CHX and 6.5% GSE as dentin pretreatment on microleakage in composite restorations.

Materials and Methods: The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, KMCT Dental College, Manassery, Mukkam, Kozhikode, Kerala, India in January 2025. Twenty-four extracted, non carious human premolars extracted for therapeutic or periodontal reasons were randomly divided into two groups (n=12 each). Standardised Class V cavities were prepared with cervical margins in dentin. Group 1 was pretreated with 2% CHX, and Group 2 with 6.5% GSE after acid etching. Cavities were then restored using a 5th generation bonding agent and composite resin. Samples underwent 1500 thermocycles, followed by immersion in 2% methylene blue dye for 24 hours. Longitudinal sections were evaluated for microleakage under a stereomicroscope and scored from 0 (no leakage) to 4 (penetration including pulpal floor). Data were analysed using Statistical Package for Social Sciences (SPSS) version 20.0, using an Independent t-test with $p<0.05$ considered statistically significant.

Results: Both groups exhibited varying degrees of microleakage. The GSE group showed a lower mean score (1.83±1.33) compared to the CHX group (2.66±1.30), with three GSE samples showing no leakage. However, the difference between the groups was not statistically significant (p=0.13).

Conclusion: Within the limitations of this in-vitro study, pre-treatment of etched dentin with CHX or GSE did not significantly eliminate microleakage in composite restorations, despite some specimens in the GSE group showing zero leakage.

Keywords: Collagen cross-linking agents, Collagen fibre degradation, Hybrid layer, Proteinase inhibitor, Stereomicroscopic study

INTRODUCTION

The long-term dentin adhesion is a direct result of the stability and integrity of the collagen fibrils within the resin-dentin hybrid layer [1]. MMPs present in dentin as proenzymes are activated after etching with phosphoric acid [2]. Literature reports that proteases, particularly MMPs and Cysteine cathepsins, are responsible for hydrolysing collagen fibres, especially those that are not optimally infiltrated with resin within the hybrid layer in dentin bonding. This hydrolysis weakens the hybrid layer, which is a crucial interface between the tooth structure and the restorative material and is responsible for the bonding of the restoration [3].

By cross-linking dentin collagen, the dentin's physical properties may be improved, providing greater resistance to degradation by endogenous proteases and improving the bond strength [4-6]. Some agents, including CHX, carbodiimide, glutaraldehyde, and proanthocyanin, are being investigated for their collagen cross-linking and anti-proteolytic properties [5].

The CHX acts both as a cross-linking agent and an inhibitor of MMPs in dentin, enhancing the longevity of resin-dentin adhesion. It is thought to work by binding to the active sites of MMP enzymes responsible for breaking down collagen, the main structural component of dentin. Through this inhibition, CHX helps maintain the collagen framework within the hybrid layer created during bonding, contributing to a stronger and longer-lasting bond [7].

Proanthocyanidins (PAs) are naturally occurring bioflavonoids predominantly present in sources such as grape seed, pine bark, cranberries, lemon tree bark, and leaves of the hazelnut tree [8]. In-vitro studies have demonstrated that grape seed-derived PAs can effectively cross-link collagen and improve bond strength following dentin pretreatment in the total etch technique [9,10].

The presence of an uninfiltrated zone or a ghost hybrid layer is more evident in the total etch technique due to the deeper acid penetration forming thicker areas of demineralisation [11]. These zones leave nanospaces, which allow fluid permeation. If unchecked, it eventually leads to restoration failure or even pulpal disease. Thus, a microleakage study would be clinically relevant in evaluating the effects of these collagen-stabilising materials, which should be able to eliminate microleakage while increasing the bond strength.

Therefore, the present study aimed to assess the ability of dentine pretreatment agents, CHX and GSE, to eliminate microleakage in composite restorations when applied to etched dentine. The objective of the study was to compare and evaluate the microleakage

of composite resin restoration following pretreatment of the etched dentin with CHX and GSE.

The null hypothesis of the present study is that pre-treatment of etched dentin with CHX or grapeseed extract does not eliminate the microleakage in composite resin restorations.

The alternative hypothesis stated that there is a significant difference in the degree of microleakage between composite restorations pretreated with 2% CHX and those pretreated with 6.5% GSE.

MATERIALS AND METHODS

An in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, KMCT Dental College, Manassery, Mukkam, Kozhikode-673602, Kerala, India. The study was carried out over a duration of one month, commencing from the first week of January 2025, after obtaining approval from the Institutional Ethics Committee (IEC No. KMCTDC/IEC/C/2024/75).

Sample size calculation:

$$n = \frac{(Z\alpha/2+Z\ 1-\beta)2 \times 2(\sigma)^2}{d^2}$$

σ= Pooled standard deviation obtained from previous study [12],

=3.5

$$\sigma 2 = \frac{(n1-1)\ \sigma 1\ 2 + (n2-1)\ \sigma 2\ 2}{n1+\ n2\ -2}$$

d= accuracy of the estimate=4

Zα/2=Normal deviate for two tailed hypothesis=1.96

Z1- β=0.84 (Type II Error)

Substituting the values

$$n = \frac{(1.96+0.84)2 \times 2(3.5)2}{42}$$

n=12

Sample size for one group is 12.

Inclusion and Exclusion criteria: Non carious premolars freshly extracted for therapeutic or periodontal reasons were selected for study, and teeth having caries or developmental anomalies on the crown were excluded.

Study Procedure

A total of 24 freshly extracted intact permanent premolars were collected and equally distributed into two groups [Table/Fig-1]. Class V cavities (2 mm deep, 4 mm {Mesiodistal (M-D) width, and 3 mm Occlusal-Gingival (O-G) width) were prepared on the buccal aspect [Table/Fig-2]. The cervical margins of the cavities were in dentin, 1 mm apical to the Cementoenamel Junction (CEJ). Cavity walls were etched with 37% phosphoric acid for 15 seconds and rinsed with water for 20 seconds, and gently blotted dry [Table/Fig-3].

- **Group 1: Chlorhexidine (CHX) (n=12):** 2% CHX was applied onto the cavity with an applicator tip for 60s and excess removed with a new applicator tip [Table/Fig-4] [13].
- **Group 2: Grape Seed Extract (GSE) (n=12)**

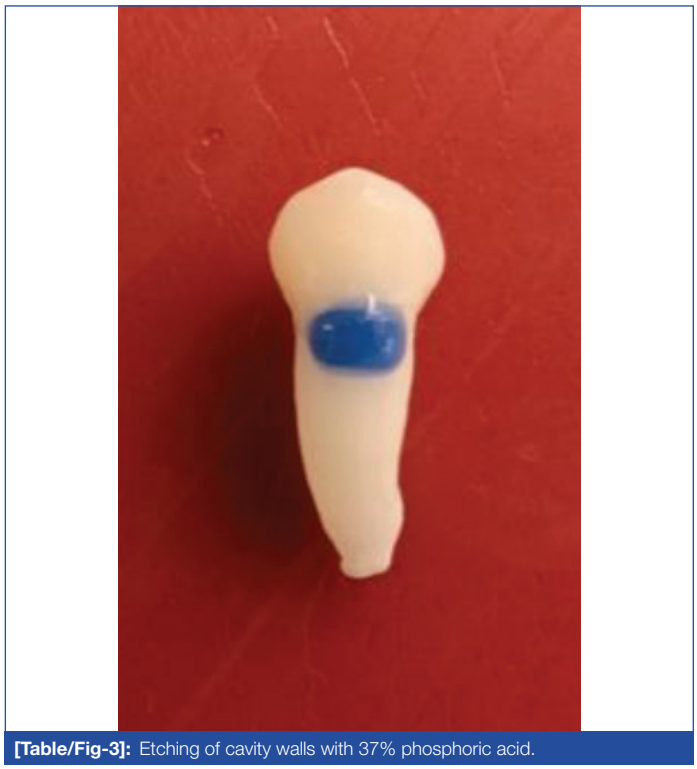
Solution preparation: The GSE capsules (Inlife GSE) are available in the market as capsules containing 400 mg of powder. A total of 650 mg of GSE powder was weighed and mixed with 100 mL of distilled water.

Protocol for use: A 6.5% GSE (Inlife GSE) was applied onto the cavity for 30s, then rinsed and blot dried [Table/Fig-5-8] [14].

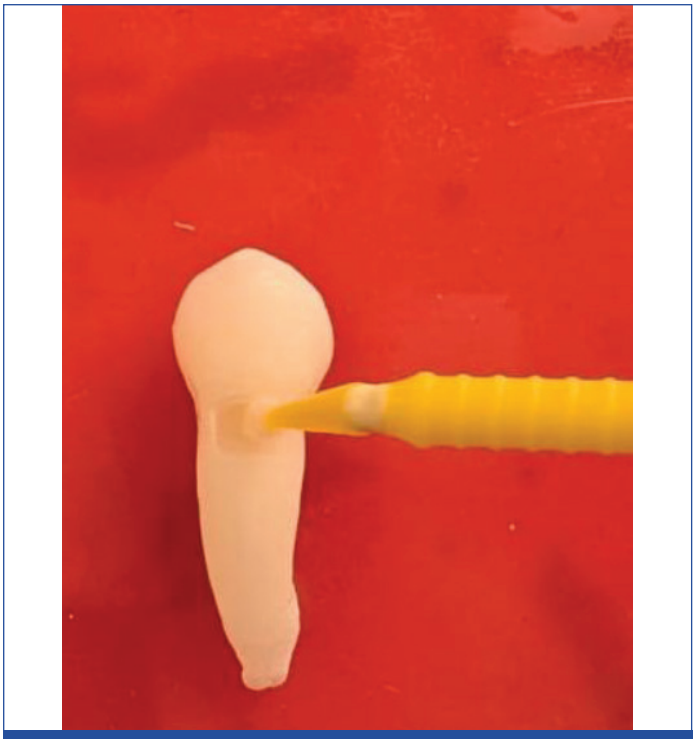
Restorative procedure: Bonding agent (Ivoclar Vivadent Te-Econom Bond Bonding Agent) was applied to the cavity with a micro applicator tip for 15 seconds, then gently blow dried for five seconds and cured for 15 seconds. Thereafter, the cavity was filled with composite (Ivoclar Vivadent Tetric N-Ceram Syringe) with a layering technique and cured for 40 seconds and polished with Shofu polishing discs [Table/Fig-8].

[Table/Fig-1]: Twenty-four extracted premolars.

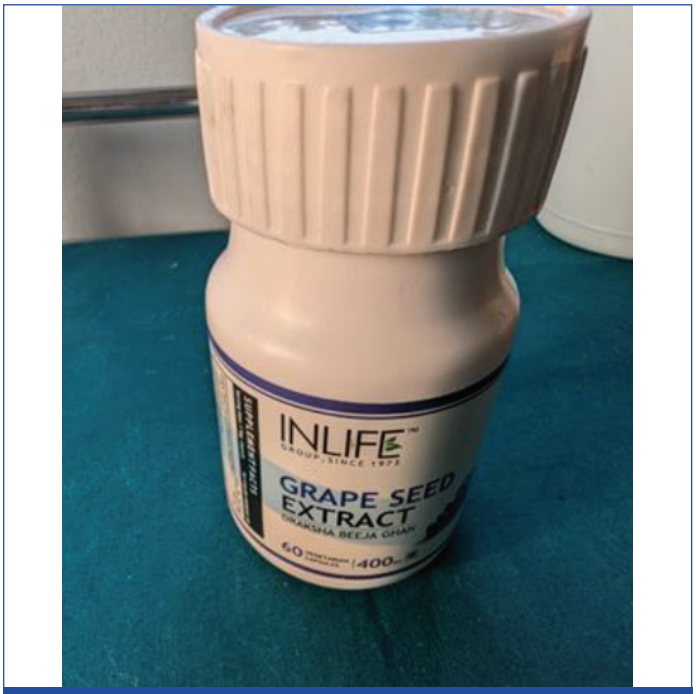
[Table/Fig-2]: Class V cavity preparation.



[Table/Fig-3]: Etching of cavity walls with 37% phosphoric acid.

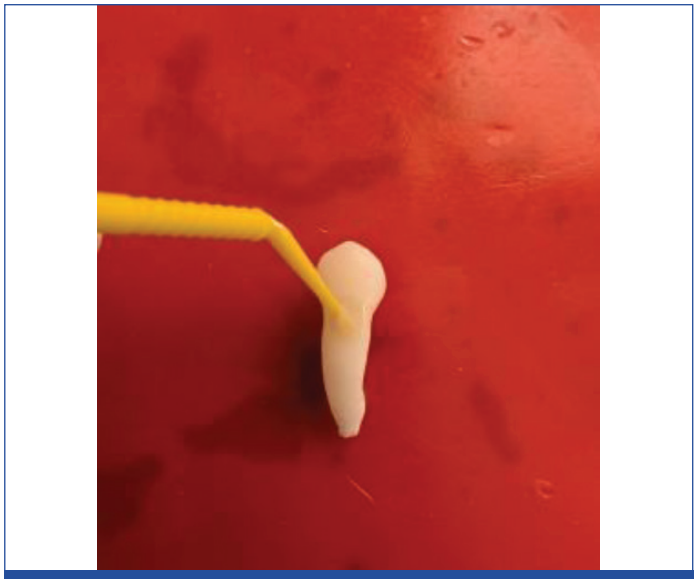


[Table/Fig-4]: Application of Chlorhexidine (CHX) with micro applicator tip.

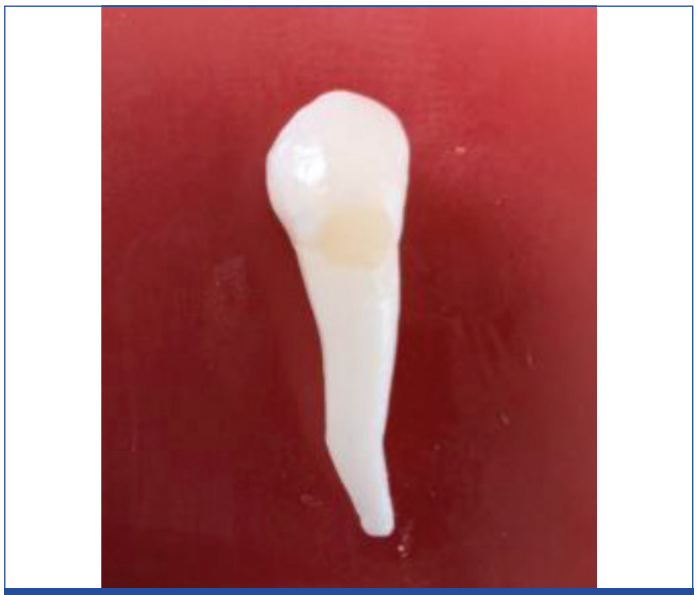


[Table/Fig-5]: Grape Seed Extract (GSE) capsules (Inlife GSE).

[Table/Fig-6]: Grape Seed Extract (GSE) solution.



[Table/Fig-7]: Application of 6.5% GSE solution.



[Table/Fig-8]: Composite restoration finished using Shofu polishing discs.

To simulate the thermal stresses experienced by dental restorations in the oral cavity due to the ingestion of hot and cold foods and beverages. The restored specimens were thermocycled up to 1500 cycles between 5° and 55° with 30 seconds dwell time [15].

Dye penetration technique: After thermocycling, the samples were immersed in 2% methylene blue dye for 24 hours, then removed and rinsed thoroughly. Thereafter, they were sectioned longitudinally through the centre buccally to pass through the restoration. The marginal permeation of the dye was assessed using a stereomicroscope and scored.

The microleakage scoring of the sectioned specimens was performed by a single, blinded examiner. To ensure the reliability of the measurements, the examiner was subjected to a calibration exercise. To assess intra-examiner reliability, 20% of the samples were re-evaluated after a one-week interval. Cohen's kappa analysis revealed a value of κ=0.84, indicating strong agreement.

Microleakage scoring was done using Owens and Johnson (2005) criteria [16].

0=No dye penetration

1=Penetration <1/2 of the cavity wall depth.

2=Penetration up to 1/2 of the cavity wall depth.

3=Penetration >1/2 of the cavity wall depth, but not including the pulpal floor.

4=Penetration including the pulpal floor/axial wall

STATISTICAL ANALYSIS

All statistical procedures were performed using SPSS 20.0. Calculations for the power (80%) of the study were performed before the commencement of the study. All quantitative variables expressed in mean and standard deviation. Qualitative variables were expressed in percentages. An Independent t-test was used for the association between quantitative variables. Probability value $p<0.05$ was considered statistically significant.

RESULTS

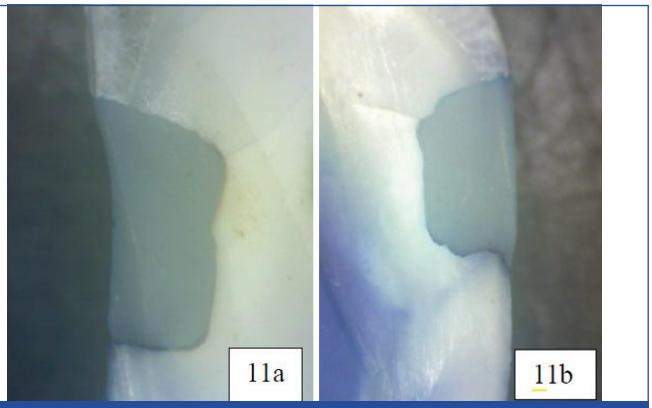
Both the experimental groups exhibited microleakage in varying degrees with scores ranging from 0 to 4 [Table/Fig-9]. Though the GSE group had three samples with no leakage as opposed to 1 in the CHX group, these differences were not statistically significant [Table/Fig-10]. Representative stereomicroscopic images of Group 1 and Group 2 are shown in [Table/Fig-11a,b,12a,b], respectively.

Scoring	0	1	2	3	4
Group 1 Chlorhexidine (CHX)	1	3	3	2	3
Group 2 Grape Seed Extract (GSE)	3	1	4	1	3

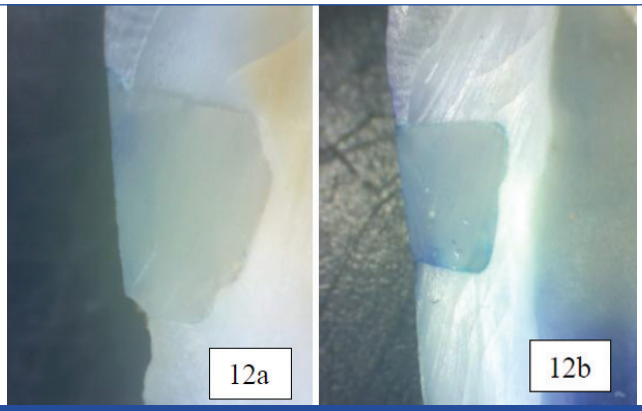
[Table/Fig-9]: Data are presented as frequency counts for each microleakage score. Scores were assigned based on Owens and Johnson's (2005) criteria.
CHX: Chlorhexidine; GSE: Grape seed extract.

Groups	N	Mean±SD	p-value	t-value
Group 1 Chlorhexidine (CHX)	12	2.66±1.302	0.13 (Independent t-test)	t=1.54
Group 2 Grape Seed Extract (GSE)	12	1.83±1.33		

[Table/Fig-10]: Intergroup comparison between the two groups.



[Table/Fig-11]: Stereomicroscopic image of Group 1, 11a scored as 3 and 11b scored as 4.



[Table/Fig-12]: Stereomicroscopic image of Group 2, 12a scored as 0 and 12b scored as 4.

DISCUSSION

The limitations of dentin as a bonding surface stem from its complex structure and its affinity for water. Dentin constitutes the bulk of the tooth with a mineral content of 70% dry weight, 20% organic matrix, and 10% water [17,18]. The stability, tensile strength, and viscoelastic properties of collagen fibrils are largely attributed to covalent cross-links formed within and between molecules of the fibrils. A range of natural and synthetic agents are reported to enhance these collagen cross-links in biological tissues [7,19,20].

Bedran-Russo AK et al., reported that CHX acts both as a cross-linking agent and an inhibitor of MMPs in dentin, enhancing the longevity of resin-dentin adhesion. It is thought to work by binding to the active sites of MMP enzymes responsible for breaking down collagen, the main structural component of dentin. Through this inhibition, CHX helps maintain the collagen framework within the hybrid layer created during bonding, contributing to a stronger and longer-lasting bond [7].

The PAs are naturally occurring bioflavonoids predominantly present in sources such as grape seed, pine bark, cranberries, lemon tree bark, and leaves of the hazelnut tree [8]. In-vitro studies have demonstrated that grape seed-derived PAs can effectively cross-link collagen and improve bond strength following dentin pretreatment in the total etch technique [9,10].

The present study was conducted to evaluate whether the pretreatment agents, namely CHX and GSE, could eliminate microleakage. Based on the findings, the null hypothesis was accepted. However, pretreatment of etched dentin with CHX or GSE did not effectively control microleakage, despite reports indicating improved bond strength. Similar findings with GSE were also reported by Wardojo SP et al., [14].

Although the GSE group exhibited a lower mean microleakage score than the CHX group, this difference did not reach statistical significance. This finding can be attributed to GSE's unique dual mechanism of action: its proanthocyanidins (PAs) actively cross-link dentin collagen [10], thereby stabilizing the demineralized matrix and promoting the formation of a tight adhesive interface. In contrast, CHX performed favorably due to its well-established role as an MMP inhibitor; however, its primary effect is to prevent collagen degradation over time rather than to provide the immediate mechanical stabilization afforded by GSE.

While a separate untreated control group was not included in the present study—because 2% CHX is an established benchmark and was used as the positive control—the absence of an untreated baseline should nonetheless be taken into account when interpreting absolute microleakage values. These findings suggest that GSE may be considered a potential natural alternative; however, further studies are required to confirm its clinical effectiveness.

In the present study, microleakage was assessed using the dye penetration method with 2% methylene blue, chosen for its simplicity, cost-effectiveness, and ability to offer clear visualisation of the cavity in digital images due to its strong contrast with surrounding tissues [19,20]. Microleakage was evaluated using a stereomicroscope, a straightforward and efficient tool that allows three-dimensional visualisation of specimens. It also facilitates real-time observation on monitors and offers a wide range of resolution and magnification settings, along with improved visibility through various illumination features [21].

In the present study, a separate untreated control group was not included because 2% CHX is one of the most extensively investigated MMP inhibitors for preservation of dentin bonding and the most widely accepted method for inhibiting dentinal MMPs and preserving hybrid layer integrity. It therefore served as the positive control in this study design [22,23].

The primary objective of the research was to compare the effect of GSE with that of this established benchmark (CHX) on the reduction of microleakage in composite restorations. Including an additional untreated (no-pretreatment) control group would not have directly contributed to this comparative evaluation, since the focus was on determining whether GSE could perform as well as or better than CHX.

Moreover, several earlier studies have already documented the baseline level of microleakage in acid-etched, non pretreated dentin, and consistently reported greater leakage compared to CHX-treated dentin [24]. Hence, to avoid unnecessary sample expansion and to adhere to the principles of ethical use of biological samples, the study design was limited to two treatment groups.

Limitation(s)

Despite the promising findings, the results of this study must be interpreted within the constraints of its in vitro design. Although essential for isolating variables, this model inherently lacks the complex biological and mechanical factors of the oral environment—such as pulpal pressure, continuous salivary flow, and cyclic masticatory forces—that can significantly influence the long-term integrity of the dentin–restoration interface. The evaluation method, dye penetration assessment, is also a limitation, as it provides only a two-dimensional, semi-quantitative measure of microleakage rather than a comprehensive volumetric analysis of the three-dimensional gap. Finally, although thermocycling was used, this procedure represents only a short-term ageing model and does not fully reproduce the hydrolytic and enzymatic degradation that occurs over years of clinical service, underscoring the need for future longitudinal in vivo studies to confirm the long-term preservation benefits of these novel conditioners.

CONCLUSION(S)

Within the limitations of the present study, it was concluded that pretreatment of etched dentin with CHX or GSE does not eliminate microleakage, despite previous reports of improved bond strength. Although a few specimens pretreated with GSE exhibited zero leakage, this finding was statistically insignificant. Further studies with a larger sample size are required to validate these results. Additional research is also necessary to identify techniques that can simultaneously improve bond strength and eliminate microleakage, which are essential for achieving a stable, long-lasting composite restoration.

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