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Effect of Two Different Haemostatic Agents on Microleakage in Subgingival Class II Restorations Restored with Flow Bulk-Fill Composite and Universal Restorative Composite by Co-curing Technique: An *in vitro* Study

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Abstract

Background: Microleakage at subgingival margins is a persistent challenge in composite restorations, particularly when gingival fluid and blood are contaminated. Haemostatic agents such as ferric sulfate and aluminium chloride are frequently used to control bleeding; however, their effects on marginal integrity and bonding remain controversial.

Aim: To evaluate the effect of two different haemostatic agents—aluminium chloride and ferric sulfate—on microleakage in subgingival Class II restorations restored with flow bulk-fill and universal restorative composites using the co-curing technique.

Materials and methods: Forty-five extracted human molars were divided into three groups (n=15 each): Group I: No haemostatic agent (control), Group II: Aluminium chloride (Ultradent Viscostat Clear), and Group III: Ferric sulfate (Ultradent Viscostat). Standardized Class II cavities were prepared 1.5 mm below the CEJ. After applying the respective agents, cavities were restored using a universal adhesive (3M ESPE Single Bond), flow bulk-fill composite (3M ESPE Filtek), and nano-hybrid composite (3M ESPE Filtek Z350 XT) via the co-curing technique. Samples were thermocycled, immersed in 2% methylene blue dye, and evaluated under a stereomicroscope (10×). Data were analysed using the Kruskal–Wallis test and the Mann–Whitney U test.

Results: The control group exhibited the least microleakage, followed by the aluminium chloride group and ferric Sulfate group. Statistically significant differences ($p < 0.05$) were observed among the groups.

Conclusion: Ferric sulfate significantly increased microleakage compared to aluminium chloride and control. Aluminium chloride demonstrated better compatibility with adhesive procedures. Thorough rinsing and drying of haemostatic residues are critical to achieve optimal bonding and reduce restoration failure.

Keywords: Aluminium chloride, co-curing technique, Composite resin, Ferric sulfate, Haemostatic agents, Microleakage.

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1. Introduction

Composite resins are widely used for posterior restorations due to their aesthetics, minimal invasiveness, and strong adhesive properties. However, achieving durable bonding in Class II restorations extending below the cemento-enamel junction (CEJ) remains a challenge due to the less mineralized nature and tubular composition of dentin [1]. This condition predisposes restorations to polymerization shrinkage, gap formation, and microleakage.

Microleakage, defined as the penetration of fluids, bacteria, and ions between the cavity wall and restorative material, contributes to marginal staining, recurrent caries, and postoperative sensitivity [2,3]. Flowable bulk-fill composites act as stress-relieving liners that improve marginal adaptation [4,5]. The co-curing technique, involving simultaneous polymerization of Flowable and conventional composites, enhances adaptation and reduces polymerization stress in deep cavities [6,7].

Subgingival margins are frequently exposed to blood contamination during restorative procedures. Haemostatic agents such as ferric sulfate and aluminium chloride are commonly employed to control bleeding but may adversely affect adhesion by modifying dentin surface chemistry and interfering with resin infiltration [8,9].

Ferric sulfate ($Fe_2(SO_4)_3$), a strong acidic agent (pH ≈ 1), can cause surface precipitation and inhibit adhesive penetration, resulting in increased microleakage [10]. In contrast, aluminium chloride ($AlCl_3$), with milder acidity (pH ≈ 4–5), causes minimal surface alteration and has been associated with improved bonding performance [11]. Although several studies have investigated the influence of haemostatic agents on dentin bonding, limited evaluation exists regarding their effect when using newer restorative strategies such as the co-curing technique with flow bulk-fill composites. This lack of consensus and insufficient evidence highlights the need to compare the effects of aluminium chloride and ferric sulfate on microleakage in subgingival Class II restorations. The present study evaluates the effect of aluminium chloride and ferric sulfate on microleakage in subgingival Class II restorations restored by the co-curing technique. The null hypothesis of this study was that there would be no significant difference in microleakage among restorations treated with aluminium chloride, ferric sulfate, or no haemostatic agent.

2. Materials and methods

Materials used in the study are presented in Table 1. This *in vitro* study was carried out in the Department of Conservative Dentistry and Endodontics, G. Pulla Reddy Dental College and Hospital, Kurnool, India. Ethical clearance was obtained from the Institutional Ethics Committee (IEC) and Research Board (IEC Reference No: GPR/23/04/RC/CONS/23M004/IEC).

Table 1. Materials used in the study.

Product name	Manufacturers' details
Aluminium chloride haemostatic agent	Viscostat Clear, Ultradent Products Inc., USA
Ferric sulphate haemostatic agent	Viscostat, Ultradent Products Inc., USA
Universal adhesive	3M ESPE, Seefeld, Bavaria, Germany
Flow bulk-fill composite	3M ESPE, Seefeld, Bavaria, Germany
Nano-hybrid universal composite	3M ESPE, Seefeld, Bavaria, Germany

2.1 Sample collection

Forty-five freshly extracted human molars extracted for orthodontic and periodontal purposes, not due to carious involvement, were collected for this study. The teeth were cleaned of debris and stored in distilled water until use.

Inclusion criteria comprised sound, freshly extracted human molars free from caries, restorations, cracks, or structural defects, with intact proximal surfaces suitable for standardized Class II cavity preparation.

Exclusion criteria included teeth with carious lesions, previous restorations, visible cracks, hypoplastic defects, root resorption, incomplete root formation, or any structural compromise that could interfere with cavity preparation or bonding procedures.

2.2 Cavity preparation

Standardized Class II cavities were prepared on the proximal surfaces of all selected molars using diamond burs under constant water cooling to avoid thermal damage. The gingival margins were extended 1.5 mm below the cementoenamel junction (CEJ) to simulate subgingival margins. Care was taken to maintain uniform cavity dimensions across all samples to ensure consistency and comparability of results. Following preparation, the teeth were thoroughly rinsed and stored in distilled water until further procedures.

2.3 Contamination of the cavity with blood followed by the application of haemostatic agents

Each prepared cavity was contaminated with a drop of freshly obtained human blood for 15 seconds to simulate clinical conditions encountered during subgingival restorative procedures, where gingival bleeding commonly interferes with bonding. The contamination time of 15 seconds was standardized to reproduce a clinically relevant exposure period sufficient to allow blood components to interact with the dentin surface without excessive clot formation, as adopted in previous *in vitro* contamination studies.

Following blood contamination, the respective haemostatic agents were applied in Groups II and III for 2 minutes in accordance with the manufacturers' instructions and previous literature to achieve effective haemostasis. Aluminium chloride (Viscostat Clear, Ultradent Products Inc., USA) and ferric sulfate (Viscostat, Ultradent Products Inc., USA) were actively applied using a micro brush tip to ensure uniform surface contact, in groups 2 and 3, respectively. After the application period, the cavities were thoroughly rinsed with water spray for 30 seconds to remove residual haemostatic agent and blood contaminants, followed by gentle air drying to avoid desiccation of dentin before adhesive application.

2.4 Restoration and grouping of samples

The forty-five teeth were randomly divided into three groups (n = 15 each):

- Group I (Control): Contamination with blood only, and no haemostatic agent applied.
- Group II: Blood contamination followed by aluminium chloride application (Viscostat Clear, Ultradent, South Jordan, Utah, USA).
- Group III: Blood contamination followed by ferric sulfate application (Viscostat, Ultradent, South Jordan, Utah, USA).

After rinsing and drying, all cavities were restored using a universal adhesive (3M ESPE Single Bond Universal, Seefeld, Bavaria, Germany). A flow bulk-fill composite was placed on the gingival seat, and a nanohybrid composite (Filtek Z350 XT, Seefeld, Bavaria, Germany) was layered above. Both materials were co-cured simultaneously using an LED curing unit (Woodpecker, Guilin, Guangxi, China) to achieve optimal polymerization and marginal adaptation.

2.5 Microleakage evaluation

Specimens were immersed in 2% methylene blue dye for 24 hours. The teeth were then sectioned longitudinally and examined under a stereomicroscope (Olympus SZ61, Olympus Corporation, Tokyo, Japan) at 10× magnification. Dye penetration along the cavity wall was evaluated using a standardized ordinal scoring system. Dye penetration along the gingival margin was scored as:

- Score 0: No dye penetration.
- Score 1: Dye penetration up to "one-third" of the cavity depth.
- Score 2: Dye penetration up to "two-thirds" of the cavity depth.
- Score 3: Dye penetration extending to the full depth of the cavity wall.

2.6 Statistical Analysis

Data were analysed using IBM SPSS Statistics Version 25.0. Since microleakage scores were ordinal in nature, non-parametric statistical tests were applied. The Kruskal–Wallis test was used to compare microleakage among the three groups. Pairwise comparisons were performed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

3. Results

The overall comparison of microleakage scores among the three groups using the Kruskal–Wallis test is presented in Table 2. The samples contaminated with blood followed by ferric oxalate application (Group 3) demonstrated more microleakage (2.47 ± 0.64) than the samples contaminated with blood followed by aluminum chloride application, Group 2 (1.67 ± 0.98) and the samples contaminated with blood only (Group 1) showed the least microleakage among the groups (0.73 ± 0.80). A statistically significant difference ($p < 0.001$) was observed among groups; therefore, the null hypothesis was rejected. Pairwise comparisons using the Mann–Whitney U test are shown in Table 3. Significant differences were observed between all groups ($p < 0.05$).

Table 2. Comparison of mean microleakage scores among the three experimental groups (Kruskal–Wallis test)

Groups	Mean ± SD	Kruskal-Wallis test value	P-value
Group 1	0.73 ± 0.80	19.73	< 0.001*
Group 2	1.67 ± 0.98		
Group 3	2.47 ± 0.64		

*Statistically significant

Table 3. Pair-wise comparison of microleakage scores between the three groups.

Groups		Mann-Whitney U-value	P-value
Group 1	Group 2	54	0.012*
	Group 3	14.5	< 0.001*
Group 2	Group 3	59	0.019*

*Statistically significant

4. Discussion

Microleakage remains a major factor influencing the long-term clinical success of composite restorations, especially when gingival margins extend below the cementoenamel junction. Subgingival Class II restorations are particularly challenging due to difficult isolation, increased moisture contamination, and frequent need for haemostatic control. The present *in vitro* study evaluated the influence of two haemostatic agents—aluminium chloride and ferric sulfate—on microleakage of composite restorations placed using a flow bulk-fill liner and restored with a universal composite employing the co-curing technique.

The control group demonstrated the least microleakage, indicating that the absence of chemical contamination allows optimal interaction between adhesive systems and dentin substrate. Previous studies have emphasized that dentin contamination significantly compromises hybrid layer integrity and increases marginal leakage [12,13]. When uncontaminated dentin is bonded under proper adhesive protocol, improved marginal adaptation and reduced dye penetration are observed.

The aluminium chloride group showed slightly increased microleakage compared to the control, but significantly lower leakage than the ferric sulfate group. Aluminium chloride is a moderately acidic haemostatic agent that produces haemostasis primarily through protein coagulation and vasoconstriction. Studies evaluating dentin surface morphology have demonstrated that aluminium chloride does not produce dense, insoluble precipitates that permanently occlude dentinal tubules when properly rinsed [14]. Consequently, adhesive penetration into dentin is less adversely affected. Investigations assessing marginal integrity have similarly reported that aluminium chloride contamination results in comparatively lower microleakage than ferric sulfate [14,15].

However, some authors have reported a reduction in bond strength when cleansing protocols were inadequate or when primary teeth substrates were used [13]. These discrepancies may be attributed to differences in dentin structure, adhesive strategy, or duration of haemostatic exposure. Nonetheless, when appropriate rinsing and drying are performed, aluminium chloride appears to be relatively compatible with adhesive restorative procedures.

In contrast, ferric sulfate exhibited the highest microleakage values in the present study. Ferric sulfate is highly acidic and achieves haemostasis by forming ferric ion–protein complexes. Surface analytical studies have demonstrated that ferric sulfate may leave iron-rich residues and insoluble precipitates on dentin surfaces even after rinsing [15]. Such deposits can interfere with adhesive infiltration and compromise hybrid layer formation. Additionally, excessive demineralization and possible collagen collapse may further reduce effective bonding, thereby increasing marginal leakage. These findings are consistent with previous investigations that reported compromised bond strength and sealing ability following ferric sulfate contamination [16].

The restorative protocol used in this study may also have influenced overall marginal integrity. The flow bulk-fill composite employed as a liner has a lower elastic modulus and greater adaptability to cavity walls, allowing it to function as a stress-absorbing layer during polymerization shrinkage. Mechanical evaluations of bulk-fill materials have demonstrated adequate depth of cure and favourable stress distribution in deep proximal boxes [17,18]. When the Flowable liner and overlying conventional composite are polymerized simultaneously using the co-curing technique, cohesive integration between layers may occur. Simultaneous curing reduces the likelihood of interlayer void formation and minimizes interface disruption that could arise during incremental curing. Experimental studies evaluating bulk-fill restorative strategies have suggested improved marginal adaptation and reduced polymerization stress concentration when stress-modulating liners are incorporated [6,7]. This may explain the relatively controlled microleakage observed even in contaminated groups within the present study.

Clinically, haemostatic control is often unavoidable in subgingival restorative procedures. While ferric sulfate provides rapid haemostasis, its potential to compromise adhesive performance warrants cautious use. Aluminium chloride appears to produce comparatively fewer adverse effects on marginal sealing when proper rinsing and surface management are performed. Incorporating stress-relieving liners and employing techniques that enhance interfacial integrity, such as co-curing, may further improve clinical outcomes in deep Class II restorations.

The limitations of this study include its *in vitro* design and the inability to simulate pulpal pressure, long-term thermomechanical loading, and oral environmental variables. Further *in vivo* studies are recommended to evaluate long-term clinical performance.

5. Conclusion

Ferric sulfate significantly increased microleakage compared to aluminium chloride and control. Aluminium chloride demonstrated better compatibility with adhesive procedures. Proper rinsing of haemostatic agents remains essential to optimize bonding outcomes.

Conflicts of interest: The authors declared no conflicts of interest.
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