RESEARCH PAPER

Comparative Evaluation of Coronal Microleakage in Class-I Cavities Restored with Dental Composite Resin Using Three Different Cavity Liners: An In-Vitro Confocal Laser Scanning Microscope Study

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ABSTRACT

Aim:
In-vitro comparative evaluation of microleakage in Class-I composite restorations with three different cavity liners using CLSM analysis.

Materials and Method:

Forty extracted human permanent maxillary first and second premolars were collected. Standardized Class-I cavities were prepared. The specimens were randomly divided into four groups (n = 10) and restored with: Group A: Bulk Fill composite without a liner; Group B: Resin Modified Glass Ionomer Cement (RM-GIC) as liner with Bulk Fill composite; Group C: Flowable composite as liner with Bulk Fill composite; and Group D: Calcium hydroxide as liner along with GIC with Bulk Fill composite as final restorations. All specimens were then subjected to thermocycling, immersed in Rhodamine B dye, sectioned longitudinally and evaluated using CLSM to assess depth of dye penetration in Class-I composite restorations with three different cavity liners.

Statistical Analysis:

One-way ANOVA and Games-Howell post-hoc tests were used; $P<0.05$ was considered statistically significant.

Results:
Group B (Resin-modified GIC as cavity liner followed by Bulk-fill composite) restoration showed the least dye penetration followed by Group C and Group D. Group A (Bulk-Fill composite placed without the use of cavity liner) exhibited maximum dye penetration.

Conclusion:
Placing Resin-modified GIC as cavity liner followed by Bulk-fill composite restoration showed the least coronal microleakage in the dye penetration test compared to the other liners used in our study. Whereas, Bulk-Fill composite placed without the use of cavity liner showed the maximum microleakage.

Keywords: Composite resin, Cavity liner, Dye penetration, Microleakage

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Introduction:

The longevity of dental restorations is dependent on the interface between the restorative material and tooth structure.[1] The oral cavity, with its associated temperature changes, masticatory load and chemical attacks by acids and enzyme in the food, creates a challenge for tooth-composite bond resulting in degradation of bonding at the tooth-restoration interface and the formation of gaps can result in the passage of bacteria, fluids, or ions between the cavity wall and the resin composite, a process known as Microleakage.[2] Marginal microleakage is commonly observed with various restorative materials especially resin based restorations. Marginal microleakage can lead to discolouration and secondary caries and if not treated in time, it can cause pulpal infection. Controlling microleakage has always been an important goal of Restorative dentistry.

Marginal microleakage in Dental composites occurs because of polymerization shrinkage of the resin. During the setting process, polymerization shrinkage of a resin composite can create forces that disrupt the bond to cavity walls of tooth structure.[3] The contracting forces built up in the polymerizing resin and the bond of adhesive resins to the wall of the tooth structure is one of the main causes of marginal failure and subsequent microleakage.[3,4]

Beautifull Bulk restorative (Shofu Inc., Kyoto, Japan) is a bulk fill composite based on Surface Pre-Reacted Glass (S-PRG) fillers technology which imparts sustained fluoride release with an anti-plaque effect, this makes it beneficial for cases with high caries risk due to its ability to minimize secondary caries formation.[5] Xtracem LC (Mediclus Co., Ltd., Cheongju, South Korea) is Resin-modified Glass Ionomer Cement with an average particle size of less than 5 microns. This ensures a very low film thickness. It has excellent strength and bicompatible to pulpal tissue with high wear and erosion resistance.[6] Filtek flowable Composite (3M Oral Care, USA) is a nano-flowable resin composite widely available for many clinical applications with excellent physical and mechanical properties. Their low viscosity and high flowability offer better adaptation to the internal cavity walls, accessibility to the difficult-to-reach areas of cavity preparation with relative ease of placement.[7]

A Cavity liner (Coltene, Altstatten, Switzerland) is a self-cure, rigid and radiopaque compound based on calcium hydroxide, indicated for pulp and protective lining under restorative materials. It does not inhibit the polymerisation of composite resins. It has high resistance to dissolution by phosphoric acid, conditioning technique and optimum solubility upon contact with water.[8]

Microleakage is commonly assessed in-vitro using dye penetration test at the tooth-restoration interface. Confocal Laser Scanning Microscope (CLSM) is a non-destructive technique for visualizing sub-surface tissue features. One of its advantages is the clear indication of leakage limits due to a lens focus that can occur some microns beneath the observed surface. This eliminates the stain spread caused by specimen sectioning and also avoids polishing artifacts that exaggerate dye penetration.[9]

The aim of our in-vitro study was comparative evaluation of coronal microleakage in Class-I cavities restored with Dental Composite resin using three different cavity liners with Confocal Laser Scanning Microscope analysis.

Materials and method:

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Triveni Institute of Dental Sciences, Hospital and Research Centre, Bodri, Bilaspur. India. After obtaining the Institutional Ethical Committee clearance; TIDSHRC/IEC/TEC No.2026/S-08. Forty freshly extracted human permanent maxillary first and second premolar teeth were obtained from the Dept. of Oral and Maxillofacial Surgery in our institution and stored in 0.9% Normal saline (Nivy Remedies Pvt. Ltd Hatbaria, West Bengal) until use.

Inclusion criteria: Teeth without any previous Caries or non-caries lesions, fractured, restored, endodontically treated, cracked and teeth extracted only for orthodontic purpose or periodontally compromised were only included. Exclusion criteria: Teeth with previous: Caries or non-caries lesions, restored, fractured, cracked, endodontically treated and also teeth with any developmental defects or anomalies.

The collected teeth were all cleaned of superficial debris, calculus and residual tissue tags using ultrasonic instruments. Occupational Safety and Health Administration, Centre for Disease Control and Prevention recommendations and guidelines were strictly followed during the collection, sterilisation and handling of extracted teeth to prevent any biohazard transmission.

All specimens (n=40) were then mounted in self-cure acrylic resin (DPI-RR Cold Cure, Dental Products of India, Mumbai) blocks upto the level of cemento-enamel junction (CEJ) for ease of handling of specimens during cavity preparation and restorative procedure. Greene Vardiman (G.V.) Black's Class-I cavities were prepared on the occlusal surfaces of all teeth along their central groove using high-speed contra-angled air turbine hand piece (NSK, Pana Air, Japan) with No. 245 Tungsten carbide bur (Mani, Japan). The dimensions of prepared cavities in all specimens were standardized with the cavity depth of 2mm, bucco-lingual width of 3mm and mesio-distal width of 5mm. A William's graduated periodontal probe (GDC, Mumbai, India) metallic scale and divider were used to measure the dimensions accurately in all prepared cavities.

All specimens (n=40) were randomly divided into four groups, 10 specimens each based upon the type of cavity liners used. Group 1: Bulk- fill composite resin without liner, Group 2: Resin–Modified Glass Ionomer Cement (RM-GIC) as cavity liner and Bulk-fill composite resin as restorative material, Group 3: Flowable composite as cavity liner and Bulk-fill composite resin as restorative material, Group 4: Calcium Hydroxide as cavity liner and Type-II GIC followed by Bulk-fill composite resin as restorative material (Figure no.1).



Figure no.1: A. Bulk-Fill composite, B. RM-GIC liner, C. Flowable Composite, D. Calcium Hydroxide cement

Group A (n=10): All the prepared cavities were first acid-etched with 37% phosphoric acid (Prime Dental Products, Thane, Maharashtra, India) for 15 seconds, rinsed with water for 10 seconds to completely remove the etchant from the tooth surface and were gently air dried. Tetric N-Bond Dental adhesive (Ivoclar Vivadent, Schaan) was placed on all the prepared walls of the cavity using a microbrush following manufacturer instructions, light cured for 10 seconds. Cavities were restored entirely with Bulk fill composite resin (Shofu Inc., Kyoto, Japan) using a Teflon coated composite carrier instrument (Hu-Freidy, Chicago, USA) and light cured (Saab, China) for 20 seconds.

Group B (n=10): 0.8mm thickness of RM-GIC as cavity liner (XtraCem-LC, Mediclus Co., Ltd., Cheongju, South Korea) was first placed on the pulpal floor of the prepared cavity. All the walls of prepared cavities were then acid-etched with 37% phosphoric acid for 15 seconds, rinsed with water for 10 seconds to completely remove the etchant from the tooth surface and were gently air dried. Tetric N-Bond Dental adhesive was placed on all the prepared walls of the cavity using a microbrush following manufacturer instructions, light cured for 10 seconds. Cavities were restored entirely with Bulk fill composite resin using Teflon coated composite carrier instrument and light cured for 20 seconds. .

Group C (n=10): 0.8mm thickness of Flowable composite resin as cavity liner (Filtek, 3M Oral Care, USA) was first placed on the pulpal floor of the prepared cavity. All the walls of prepared cavities were then acid-etched with 37% phosphoric acid for 15 seconds, rinsed with water for 10 seconds to completely remove the etchant from the tooth surface and were gently air dried. Tetric N-Bond Dental adhesive was placed on all the prepared walls of the cavity using a microbrush following manufacturer instructions, light cured for 10 seconds. Cavities were restored entirely with Bulk fill composite resin using Teflon coated composite carrier instrument and light cured for 20 seconds.

Group D (n=10): 0.8mm thickness of Calcium hydroxide cement(Coltene, Altstatten, Switzerland) as cavity liner was first placed followed by Type-II GIC (Shofu Inc, Japan) placed over it on the pulpal floor of the prepared cavity. All the walls of prepared cavities were then acid-etched with 37% phosphoric acid for 15 seconds, rinsed with water for 10 seconds to completely remove the etchant from the tooth surface and were gently air dried. Tetric N-Bond Dental adhesive was placed on all the prepared walls of the cavity using a microbrush following manufacturer instructions, light cured for 10 seconds and were then restored with Bulk fill composite resin using a Teflon coated composite carrier instrument and light cured for 20 seconds.

All the composite restorations were then finished & polished using Finishing burs and Sof-Lex polishing discs (3M ESPE, Dental products, St. Paul, USA). All the specimens were then carefully removed from the acrylic resin blocks then subjected to Thermocycling at 4º ± 2º C and 56º ± 2º C for 500, 2-minute cycles with a dwell time of 30 seconds as per ISO 11405.[10] To prevent any dye leakage from root apex, the apical foramen of all specimens were sealed with cyanoacrylate glue (Benson Polymer Limited, India). All the specimens were then double coated with nail varnish (Elle 18, Hindustan Unilever, India) except over the composite resin restorations and 1mm along the tooth-restoration interface. All the specimens were then immersed in Rhodamine B dye solution for 48 hours for the dye penetration test at 100% relative humidity at room temperature (Figure no.2).

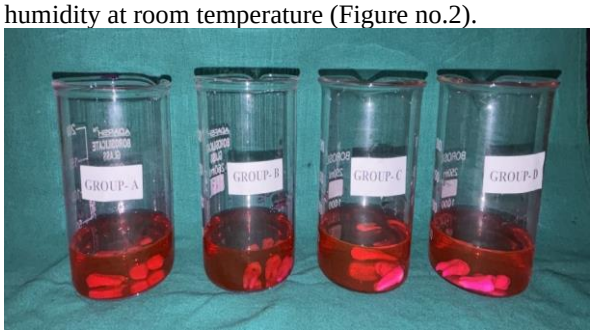


Figure no.2: Specimens immersed in Rhodamine B dye solution

All specimens were then removed from the dye, thoroughly rinsed under running tap water for 5 minutes and air-dried. Nail varnish and Cyanoacrylate glue were removed with scalpel. All the specimens were then longitudinally sectioned along mesio-distally using a diamond disc (DFS, Germany) attached to low-speed micro-motor straight hand piece (NSK, Japan) along with the use of a fine hand chisel (GDC, India), carefully splitting into two equal halves, buccal and palatal halves. From each specimen, the most representative half of the entire crown was selected and the other halve was discarded. Each selected samples were then examined for the extent of coronal dye penetration under Confocal Laser Scanning Microscope [Carl Zeiss Micro-imaging, GmbH, Jena, Germany] (Figure no.3) with DPSS (Diode Pumped Solid State) 561 nanometers LASER (Light Amplification by Stimulated Emission of Radiation) at 10X in the fluorescence mode for the presence, absence and extent of dye penetration/microleakage (Figure no.4). The depth of dye penetration was measured in micrometers (µm) using Image J (Java based image processing program) software. The obtained data was recorded and tabulated.

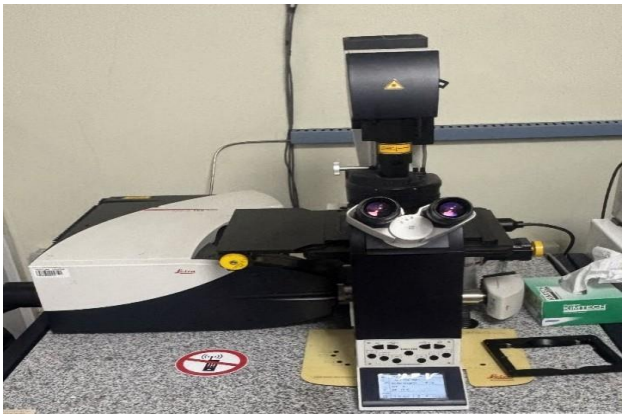


Figure no.3: Confocal Laser Scanning Microscope used in our study.

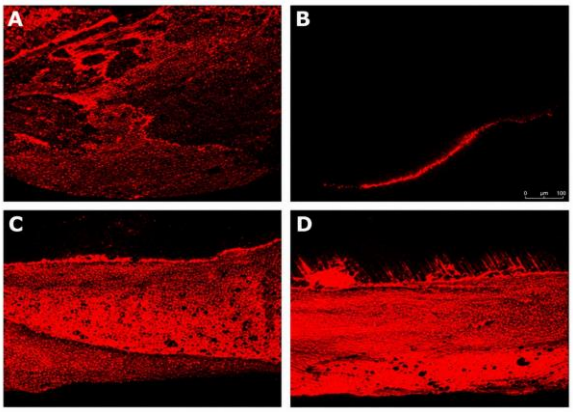


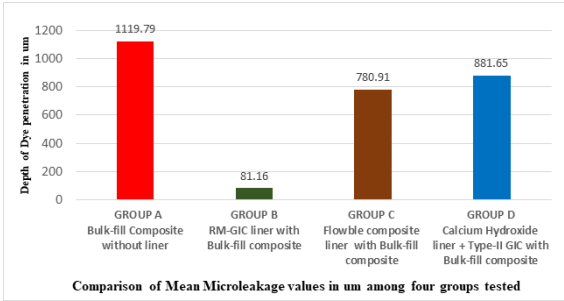
Figure no.4: Confocal Laser Scanning Microscope images; A. Bulk fill composite without cavity liner B. RM-GIC with Bulk fill composite C. Flowable composite with Bulk fill composite D. Calcium hydroxide as cavity liner + Type II GIC and Bulk fill composite resin.

Results:

Data was analyzed using SPSS software (IBM Corp., Armonk, NY). Descriptive statistics was calculated for all groups. One-way analysis of variance (ANOVA) test was used to determine intra-group differences in the depth of dye penetration depth among the restorative techniques used in our study (Table no.1), followed by Games–Howell post-hoc test used for pair-wise comparison. P<0.05 was considered statistically significant.

Groups	No. of specimens used	Mean±SD	P-value
Group A:- Bulk-fill composite without cavity liner	10	119.79±86.98	0.523
Group B:- RM-GIC cavity liner + Bulk-fill composite	10	72.77±86.13	0.569
Group C:- Flowable composite cavity liner + Bulk-fill composite	10	780.91±10.43	0.886
Group D:- Calcium hydroxide cavity liner + Type-II GIC + Bulk-fill composite	10	881.65±16.19	0.146

Table no.1: One-Way Analysis of Variance: Intra-group comparison in the extent of Coronal Dye penetration (CLSM images) among the four groups tested.



Graph no. 1: Vertical Bar graph - Coronal Dye penetration in micrometers (µm) among four groups tested.

According to One Way ANOVA test between the four groups tested; Group B; in which the prepared Class-I cavities were restored with RM-GIC as cavity liner followed by Bulk fill composite resin showed the least dye penetration at the tooth-restoration interface with the mean of 81.16um followed by Group C; prepared cavities restored with Flowable composite as cavity liner followed by Bulk fill composite resin with the mean of 780.91µm, followed by Group D; with the mean of 881µm. The maximum dye penetration was

seen in Group A, prepared cavities restored with Bulk fill composite resin without the use of cavity liner with the mean of 1119.79 µm.

Pairwise comparision (Microleakage)	Mean Difference	P value
Group A Vs Group B	1038.63	P<0.001 HS
Group B Vs Group C	699.75	P<0.001 HS
Group B Vs Group D	800.49	P<0.001 HS
Group A Vs Group C	338.88	P<0.001 HS
Group A Vs Group D	238.14	P<0.001 HS
Group C Vs Group D	100.74	P>0.05 NS

Table no. 2: Games-Howell post-hoc test: Inter-group comparison of dye penetration among the four groups tested.

P: Probability **HS:** Highly Significant **NS:** Non Significant

Games–Howell post-Hoc test showed that all inter-group comparisons were statistically highly significant as P<0.001, except; Group C in which the prepared cavities were restored with Flowable composite with Bulk fill composite resin and Group D; the prepared cavities were restored with calcium hydroxide as cavity liner and Type II GIC with Bulk fill composite resin, as P>0.05, stastically non-significant.

Discussion:

Microleakage at the tooth-restoration interface is regarded as a major determinant of restoration longevity. It is defined as the clinically undetectable passage of bacteria, fluids, molecules and ions between the cavity wall and the restorative material.[11,12] Persistent marginal gaps predispose to secondary caries, the most frequent reason for replacement of restorations, and to postoperative sensitivity, marginal discoloration and pulpal injury.[13,14] Conventional composite resins do not provide a hermetic seal and the present study was designed to compare how three liners influence marginal dye penetration beneath a bulk-fill composite in Class I cavities, assessed by Confocal Laser Scanning Microscope (CLSM).[14,15]

The principal finding of our study was that all three cavity liners used reduced the dye penetration compared to the unlined bulk-fill composite. The RMGIC liner reduced dye penetration by approximately 93% compared to no cavity liner used, whereas the flowable and calcium hydroxide/GIC cavity liners reduced it by approximately 30% and 21%, respectively.

The highest microleakage in Group A is consistent with the mechanics of polymerization shrinkage. When a composite resin is cured as bonded to rigid cavity walls of tooth structure, contraction cannot occur freely and stress accumulates at the interface. This stress is governed by the configuration factor of the cavity, together with the shrinkage, elastic modulus and degree of conversion of the material.[16,17] A Class-I preparation has a high configuration factor, and a bulk-fill increment of upto 4 mm thickness concentrates the shrinkage in a single mass. Bulk-fill composites achieve adequate depth of cure through increased translucency and modified photoinitiator systems, but their marginal and internal adaptation has not been shown to be consistently superior to incremental placement.[18,19,20] Mundaragi VAC et al,[21] likewise reported the highest microleakage in bulk-fill composites placed without an liner, and restorative technique has been shown to influence leakage in other cavity designs. When stress exceeds the bond strength of the adhesive, microgaps form and allow microleakage.[22]

The superior sealing ability of the RM-GIC as cavity liner in Group B can be attributed to its adhesion mechanism and mechanical behaviour. Resin-modified glass ionomers bond to tooth structure through ionic interaction with calcium of hydroxyapatite in combination with micromechanical retention provided by the resin component.[23] They also have a lower elastic modulus than filled composites, so the liner can absorb part of the contraction stress developed in the overlying composite. Our result agrees with Arora R et al[24] reported that RMGIC liner significantly reduced gingival microleakage in Class II cavities, and with Ozcan S et al[25] reported less leakage with RMGIC than with other liners after thermocycling and occlusal loading. Arora R et al[24] observed no difference between liners at enamel margins and the least leakage with a flowable liner at dentin margins, whereas RMGIC was clearly superior in the present Class I model. Differences in cavity design, margin location, composite type and the scoring method (ordinal scores versus a continuous measurement in micrometres) may account for this.

The Flowable composite liner used in Group C reduced leakage, but less than RMGIC. The low filler content of flowable composites gives them a low elastic modulus and good adaptation to the cavity floor, and the resulting elastic layer is thought to relieve part of the polymerization stress.[26] The magnitude of this benefit depends on the composite and layering technique used, which may explain the intermediate performance of flowable liners.[24]

Group D, in which Calcium hydroxide cement as cavity liner and Type-II GIC as base followed by Bulk-fill composite restoration, showed the highest dye penetration among all the four groups. Calcium hydroxide liners were developed to protect the pulp and stimulate reparative dentin deposition rather than to provide a durable seal, they are soluble, mechanically weak and do not bond to compsite resin.[27] A conventional glass ionomer cement (Type-II GIC) is brittle, sensitive to early moisture exposure and has a higher modulus than RM-GIC, which limits its ability to compensate for polymerization shrinkage. Either way, both materials were clearly inferior to the RM-GIC as cavity liner. Calcium hydroxide cement as cavity liner

has low elastic modulus and high solubility hindering the restoration's ability to provide a perfect seal and Munoz-Sandoval et al[28] likewise observed a trend towards higher microleakage with calcium hydroxide cement as cavity liner, and cautioned that it reduce restoration longevity.[29,30]

Dye penetration tests were used earlier to assess microleakage and this test remains as the most commonly used method because of its simplicity.[28,29] Rhodamine-B is a fluorescent dye and it has smaller molecular size, thus helps to penetrate deeper at the tooth-restoration interface. CLSM images provide optical sectioning with a sharper dye front compared to conventional light microscope, thus allowing greater penetration depth and can be measured in micrometres. Thermocycling is a standard method of ageing tooth specimens; the 500 cycles used in our study followed the minimum recommended thermocycling cycles as stated in the ISO standard.[10]

Conclusion:

Within the limitations of our in-vitro study, RM-GIC as cavity liner followed by Bulk-fill composite restoration showed the lowest microleakge followed by Flowable composite as cavity liner followed by Bulk-fill composite restoration. whereas, Calcium hydroxide cement as cavity liner followed by Type-II GI and Bulk fill composite for restoration showed the maximum microleakage in the dye penetration test as examined using CLSM analysis. However, none of the Cavity liners used in our study were able to completely prevent microleakage under composite resin restorations.

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Source of Funding: Nil

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