Original Article

Deep margin elevation in class II cavities: A comparative evaluation of microleakage and interface integrity using confocal laser microscopy and scanning electron microscopy

K. Hanisha Reddy, B. Devi Priya, D.L Malini, T. Murali Mohan, Swetha Bollineni, Hari Chandana Gandhodi

Department of Conservative Dentistry and Endodontics, Government Dental College and Hospital, Vijayawada, Andhra Pradesh, India

Abstract

Aim: This study aims to evaluate the microleakage between the gingival seat and base material and to assess the interface integrity between the base material and overlying composite in class II cavities restored using deep margin elevation.

Materials and Methods: Thirty maxillary molars (n = 30) were taken, and class II cavities were prepared with a gingival seat extending below the cementoenamel junction. These teeth were divided into three groups for subgingival margin elevation using different materials: Group A (n = 10) – flowable composite, Group B (n = 10) – glass ionomer cement (GIC), and Group C (n = 10) – GIC with nanohydroxyapatite (GIC n-HAp). The remaining cavities were restored with bulk-fill composite. After undergoing 1000 thermocycling cycles, half of the samples were examined for microleakage using confocal laser microscopy, and the other half were assessed for interface integrity using scanning electron microscopy. Microleakage was statistically analyzed by one-way ANOVA, and interface integrity was analyzed by Kruskal–Wallis tests.

Results: The study found that GIC n-HAp exhibited significantly lower microleakage between the base material and gingival seat than flowable composite and GIC. However, regarding interface integrity between the base material and bulk-fill composite, flowable composite, and GIC outperformed GIC n-HAp.

Conclusions: Incorporating n-HAp into GIC effectively reduced microleakage at the dentin-base material interface. However, the interface integrity between GIC n-HAp and the composite poses a challenge.

Keywords: Deep margin elevation; interface integrity; microleakage; scanning electron microscopy

INTRODUCTION

Preserving tooth structure while achieving functional and esthetic outcomes are critical goals in restorative dentistry. Achieving these goals, especially in proximal margins located subgingivally, is complicated due to issues such as achieving proper isolation, taking impressions, and choosing suitable materials to restore. The precise management of these margins is essential for the restoration's structural integrity and for maintaining periodontal health.

The conventional strategies for addressing subgingival margins, including orthodontic extrusion and surgical crown lengthening, aim to improve access to these

Address for correspondence:
Dr. K. Hanisha Reddy,
Department of Conservative Dentistry and Endodontics,
Government Dental College and Hospital, NTR Colony, Gunadala,
Vijayawada - 520 004, Andhra Pradesh, India.
E-mail: gandhodichandana067@gmail.com

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problematic areas by apically repositioning the supporting tissue. While effective, these methods can be invasive, time-consuming, and may lead to undesirable outcomes such as periodontal recession or prolonged treatment durations, thus necessitating the exploration of less invasive alternatives.[1]

The deep margin elevation (DME) technique has emerged as a promising, less invasive alternative. The DME technique involves coronal elevating the gingival seat by applying a base material directly, thereby obviating the need for more aggressive surgical interventions.[2] This innovative approach has gathered attention for its utility in direct and indirect restorations.

The material selection for elevating and adapting the gingival seat to the dentin is crucial. Several materials, such as glass ionomer cement (GIC), resin-modified GIC, and flowable composites,[3] have been used for DME elevation. Despite many advancements, the literature continues to debate the optimal choice of restorative material for repositioning deep proximal cervical margins.

This study aims to bridge this knowledge gap by focusing on two primary objectives: (i) to assess the microleakage occurring between the gingival seat and the applied base material and (ii) to evaluate the interface integrity between the base material and the overlying composite in DME restorations.

The null hypotheses tested were that: (1) there was no significant difference in microleakage between the base material and gingival seat among the tested materials (flowable composite, GIC, and GIC with nanohydroxyapatite [n-Hap]). (2) There was no significant difference in interface integrity between the base material and overlying composite among the tested materials.

MATERIALS AND METHODS

Sample selection and preparation

This study used 30 human maxillary molars ($n = 30$) recently extracted for periodontal reasons without caries or fractures. Each tooth was cleaned and stored in a 0.9% saline solution at four degrees until the commencement of the experiment. The teeth were prepared with standardized class II cavities using a high-speed handpiece under water cooling. The cavities were designed with specific dimensions: 2.5 mm in buccolingual width, 1.5 mm in mesiodistal width at the gingival seat, and 3.0 mm depth, extending 1.0 mm beneath the cementoenamel junction to simulate subgingival margin conditions.

The prepared molars were randomly assigned to three groups comprising 10 teeth.

- Group A (flowable composite group): The cavities were etched with 37% phosphoric acid for 15 s, rinsed thoroughly, and dried. A bonding agent (Adper Single Bond 2, 3M ESPE) was applied and light-cured for 20 s. A 1 mm thick layer of a flowable composite (GrandioSO Heavy Flow, VOCO) was applied as the base material, followed by the restoration of the remaining cavity space with a bulk-fill composite (3M™ Filtek™ One Bulk Fill Restorative) and cured for 40 s using an LED curing light
- Group B (GIC group): A 1 mm thick layer of GIC (GC Gold Label) was placed directly onto the gingival seat. The cavity preparation underwent etching, bonding, and restoration processes identical to those described for Group A, with the bulk-fill composite serving as the restorative material
- Group C (GIC n-HAp Group): The base layer for this group consisted of a 1 mm thick layer of GIC n-HAp (manufactured by Nanowings by sol–gel method, Pvt. Ltd, HYD). The subsequent steps of etching, bonding, and restoration of the cavity with bulk-fill composite were performed in the same manner as in the other groups.

Following the restoration process, all specimens were polished using diamond finishing burs and Shofu polishing discs to achieve a smooth surface.

To simulate the thermal stresses, restorations underwent thermocycling for 1000 cycles between 5°C and 55°C, with a dwell time of 30 s in each bath and a transfer time of 10 s between baths, using a thermocycler (Model: HO-THC-01).

Microleakage evaluation

This study component assesses microleakage between the dentin surface at the gingival seat and the applied base material.

- Testing methodology: Half of the samples ($n = 5$) from each group were selected for microleakage analysis. To prevent dye infiltration into areas other than the intended testing sites, teeth were covered with nail varnish, leaving a minimal gap around the restoration edges. This coating left a marginal gap of approximately 1 mm around the restoration edges to isolate the test area. The samples were subsequently immersed in a 0.5% rhodamine B fluorescent solution for 2 days. Following immersion, each tooth was thoroughly rinsed in distilled water to remove any excess dye
- The teeth were then sectioned longitudinally in a mesiodistal direction using a precision Baincut LSS microtome with a diamond blade. The sections were examined using a Stellaris 5 Confocal Scanning System, with a single observer conducting all evaluations to maintain consistency. Each sample was scanned over a standardized area of 273 µm × 273 µm. For a comprehensive analysis, two images of each sample

were captured at predefined depths of 211 µm and 273 µm, ensuring a thorough assessment of dye penetration.

Interface integrity evaluation

This part of the study examines the interface between the base material and the bulk-fill composite.

- Scanning electron microscopy (SEM) examination: The remaining 15 specimens were examined for the interface integrity between the base material and the bulk-fill composite. Each specimen was mounted on aluminum stubs and subjected to a gold sputter coating to enhance and improve image quality under SEM
- The samples were then examined using a high-resolution JSM–IT800 NANO SEM, focusing on the critical interface of the base material and the composite. Observations were made at varying magnifications from ×25 to ×500 to evaluate potential gaps or flaws at the interface meticulously.

Statistical approach

To ensure the integrity of the data analysis, the Shapiro–Wilk test was employed to confirm the normal distribution of the dataset. A significance level was set at $P < 0.05$ for all statistical tests. Microleakage data, adhering to a normal distribution, were analyzed using a one-way ANOVA test to determine statistically significant differences between groups. The interface integrity data, which did not follow a normal distribution, were assessed using the Kruskal–Walli's test.

Scoring for microleakage at the cervical wall

The degree of dye penetration was assessed using a scale ranging from 0 to 3, as described by Benny *et al.*[4] and categorized as follows:

- Score 0: No evidence of dye penetration
- Score 1: Dye penetration extending up to half the length of the cervical wall
- Score 2: Dye penetration beyond half but not exceeding the entire length of the cervical wall
- Score 3: Dye extending from the cervical to axial walls, potentially progressing toward the pulp.

RESULTS

The outcomes of this investigation are divided into two principal evaluations: microleakage evaluation and interface integrity assessment.

Microleakage evaluation

Microleakage was assessed to ascertain the extent of dye penetration at the interface between the dentin surface at the gingival seat and the applied base materials. The results are shown in Figure 1 and summarized in Table 1.

The GIC-n-HAp group exhibited significantly lower dye penetration than the flowable composite and the conventional GIC groups, indicating reduced microleakage. The statistical analysis revealed a significant difference in mean microleakage scores, with the GIC-n-HAp group demonstrating a mean score of 1.4, which was notably lower than those of the flowable composite (2.2) and GIC (2.8) groups.

Interface integrity assessment

This part of the study evaluated the interface integrity between the base material and the bulk-fill composite, mainly focusing on the mean gap distance as an indicator of potential voids or weaknesses at the interface. The findings are shown in Figure 2 and are presented in Table 2.

Interestingly, while the GIC-n-HAp group showed improved performance in microleakage, it demonstrated a higher mean gap distance (17.384 µm) compared to the flowable composite (11.276 µm) and GIC (12.116 µm) groups.

DISCUSSION

The long-term durability of restoration depends on the integrity of the tooth-restorative material interface. Particularly for restorations that extend subgingivally, selecting an appropriate base material is crucial, as it must bond effectively to the gingival seat and the overlying restorative material.

Our study investigated the microleakage between the gingival seat and three different base materials: GIC, flowable composites, and GIC n-HAp. Another parameter is the interface integrity between the base and overlying restorative materials.

Due to its viscosity and lower elastic modulus, flowable composite has been used as a potential material for deep-margin elevation. This study compares microleakage

Table 1: Summary of microleakage scores and statistical analysis

Groups	Score				Mean	SD
	0	1	2	3		
Flowable	0	1	2	2	2.2	0.748331
GIC	0	0	1	4	2.8	0.4
GIC-n-HAp	0	3	2	0	1.4*	0.489898

*The statistical difference with $P<0.05$. GIC: Glass ionomer cement, SD: Standard deviation, n-HAp: Nanohydroxyapatite

Table 2: Interface integrity assessment summary

Groups	Count	Sum	Average	Variance
Flowable	5	56.38	11.276	0.68953
GIC	5	60.58	12.116	1.02448
GIC-n-HAp	5	86.92	17.384*	3.75733

*The statistical difference with $P<0.05$. GIC: Glass ionomer cement, n-HAp: Nanohydroxyapatite

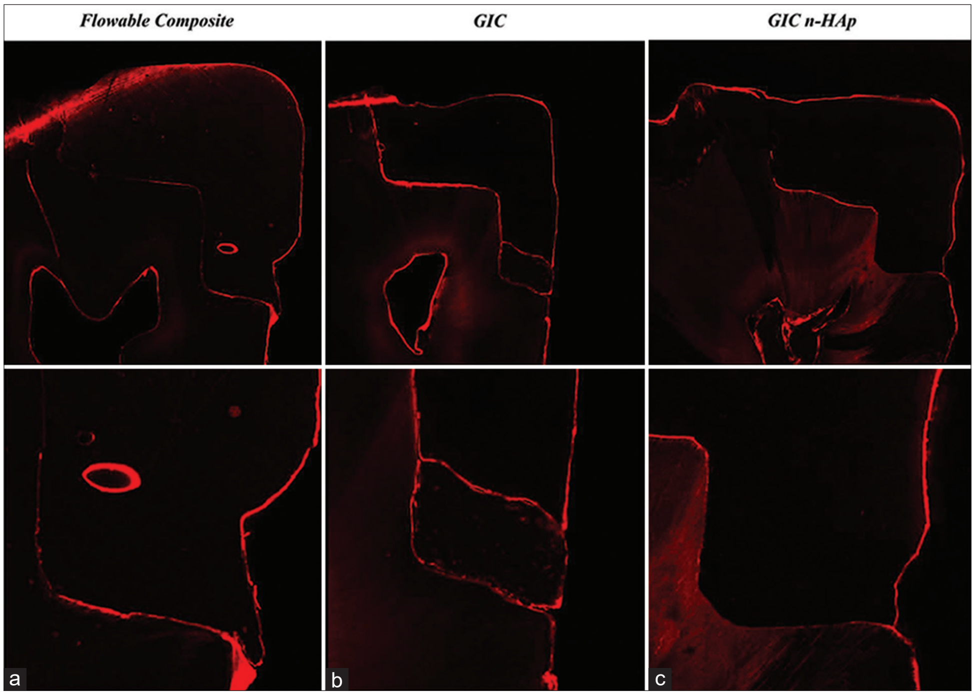

Figure 1: Confocal images showing microleakage between the gingival seat and base material. (a) Flowable composite, (b) Glass ionomer cement (GIC), and (c) GIC with nano-hydroxyapatite. GIC: Glass ionomer cement, GIC n-Hap: GIC with nanohydroxyapatite

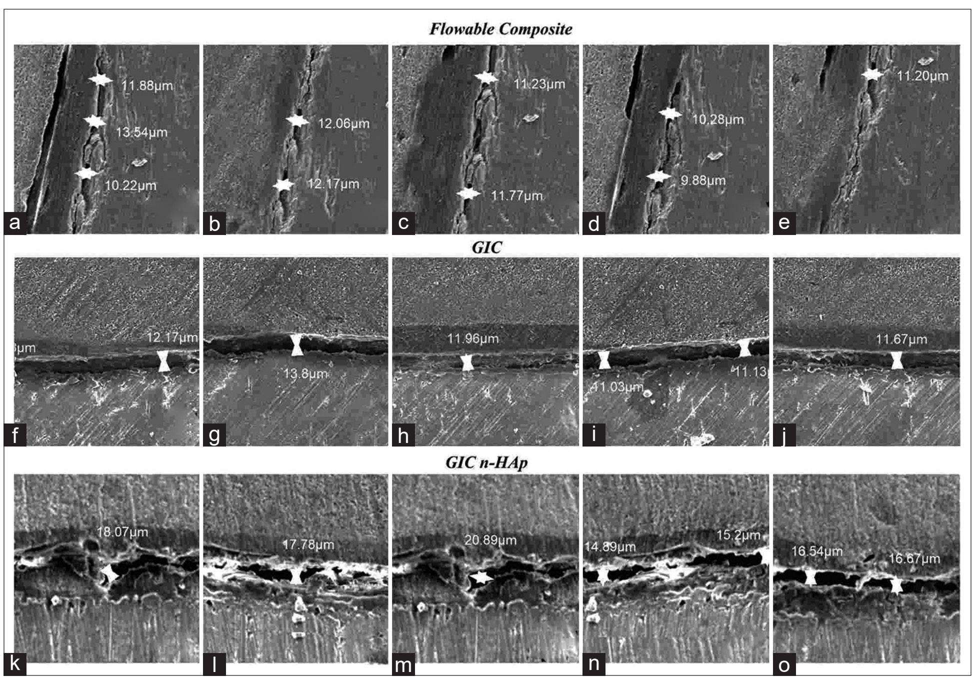

Figure 2: Scanning electron microscopy showing the mean distance between the base material and bulk-fill composite. (a-e) Flowable composite, (f-j) Glass ionomer cement (GIC), and (k-o) GIC with nanohydroxyapatite. GIC: Glass ionomer cement, GIC n-Hap: GIC with nanohydroxyapatite

between the gingival seat and flowable composite, GIC, and GIC n-HAp as base materials.

The physical properties of flowable composites are attributed to their filler content, so among flowable composites, we chose GrandioSO heavy flow by VOCO because of its high filler content (83%).

GIC, due to its hydrophilic nature, coupled with its ability to chemically bond to dentin, flexibility, and the improved mechanical and wear resistance of newer formulations, compatibility with the biological system, similar coefficient of thermal expansion to dentin, no polymerization shrinkage renders it, especially suitable for deep dentin or cementum margins.

Recent advances in GIC have significantly enhanced fracture toughness and sustained dentin adhesion over the long term, as evidenced by Lucas's research.[5]

The choice of GC Gold Label I was driven by its exceptional properties, including its self-curing luting, sustained fluoride release, and lining capabilities.

In our study, GIC was augmented with n-HAp, supplied by Nanowings Pvt. Ltd, Hyderabad, prepared by sol–gel method.[6] This incorporation was predicated on the premise that n-HAp could significantly reinforce the mechanical and biological properties of GIC.[7]

The addition of n-HAp to GIC not only reduces cytotoxicity and the potential for microleakage but also enhances fluoride release and antibacterial efficacy. This is attributed to n-HAp's biocompatibility and its structural similarity to bone and dental enamel, which promotes a more seamless integration with the natural tooth structure and improves the longevity and functionality of restorations.[7,8]

The current choice for posterior restoration is bulk-fill composite because of its less postgel shrinkage, increased translucency, better adaptability to the cavity preparation walls, lower wear resistance, less postoperative sensitivity, fewer secondary caries, and increased depth of cure. It is selected as the overlying restoration.[9]

Thermocycling was employed as a critical stress test to simulate the thermal dynamics encountered within the oral environment, a methodology supported by existing studies for its efficacy in evaluating restorative material performance.[10] Confocal laser scanning microscopy allowed for an unprecedented detailed examination of the microleakage phenomenon, which enabled visualization of subsurface interactions between the dentin and base materials, providing valuable insights into the sealing effectiveness of dental restorations.[11]

Similarly, the marginal gap between the base material and bulk-fill composite is assessed under the SEM for interface integrity.

Microleakage evaluation between the base material and gingival seat

Microleakage is the passage of bacteria, liquids, molecules, and ions through the cavity wall and restorative material, which is not clinically detectable. Microleakage negatively affects the restoration, and in deep class II restorations with no enamel surface for bonding, the cementum-dentin gives a less stable interface for the restoration.[12]

The results showed that while all materials exhibited some degree of marginal microleakage, there were notable differences in performance among them.

Flowable composites, known for their lower viscosity,[13] demonstrated reduced microleakage compared to GIC but exhibited more leakage than the GIC enhanced with n-HAp. The study observed that GIC combined with n-HAp showed significantly lower microleakage, likely due to the formation of new n-HAp crystals within a mineralizing zone, a phenomenon not seen in samples with GIC alone.[14]

Furthermore, hydroxyapatite's high solubility contributes to its effectiveness in filling micropores within the dentin, as it releases inorganic ions that aid in sealing these gaps.

Interface integrity between base materials and bulk-fill composite

Interface integrity between base material and bulk-fill composite, flowable composite, and GIC is better than that of GIC n-HAp.

The study's analysis of the interface integrity concentrated on detecting any discontinuities or gaps at the junction between the base material and bulk-fill composite, which is crucial for the restoration's structural soundness.

Jordan's research highlights that the ionomer-composite combination in dental restorations forms a reliable chemical bond with dentin, primarily through micromechanical adhesion.[15] The superior interface observed between the flowable composite and bulk-fill composite can be linked to the cohesive interaction between their organic matrices and filler particles, enhanced by coupling agents. This compatibility ensures a uniform and strong bond, which is crucial for the restoration's durability.

A key observation was the comparatively weaker bond between GIC with nanohydroxyapatite and bulk-fill composite, in contrast to the more robust interfaces formed with GIC and flowable composite. Several factors might explain these results: there is a compositional disparity between the composite and the GIC-n-HAp mix, and due to inadequate wetting of n-HAp particles within the resin matrix, leading to particle agglomeration and uneven nanoparticle distribution and challenges related to surface modification in GIC after incorporating hydroxyapatite.[16]

These findings underscore the importance of selecting an appropriate restorative material to complement the base material in subgingival restorations. Despite the emerging popularity of materials like GIC n-HAp, their bonding efficacy with composite materials requires further evaluation.

The observed limitations in the interface integrity between GIC-n-HAp and bulk-fill composites highlight the necessity for further research. Future studies could explore the modification of n-HAp, such as through silanization, to enhance its compatibility with composite matrices. In addition, developing new composite formulations specifically designed to complement the properties of GIC-n-HAp could offer promising avenues for improving the overall performance of subgingival restorations.

CONCLUSIONS

The incorporation of n-HAp into GIC represents a significant advancement in the quest to minimize microleakage in subgingival restorations. Our study confirms the significant potential of GIC n-HAp to reduce microleakage at the dentin-base material interface compared to conventional GIC and flowable composites. However, despite these improvements, concerns persist regarding the interface integrity between the GIC n-HAp and the overlying bulk-fill composite material. This highlights the importance of further research and development in material science to address this crucial aspect of clinical adaptation.

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Conflicts of interest
There are no conflicts of interest.

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