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Efficacy of Bio-active Varnish Application on Microleakage of Class II Composite Resin Restorations: An *in vitro* Study

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Abstract

Background: Microleakage at the Cavo surface margins of Class II composite restorations remain a significant challenge, contributing to postoperative sensitivity, secondary caries, and restoration failure. Conventional composite resins undergo polymerisation shrinkage, resulting in marginal gaps and compromised seal integrity. To address these limitations, bioactive materials that can form chemical bonds with tooth structure and release therapeutic ions are being explored. Bioactive calcium phosphate-based fluoride varnishes offer potential in improving the marginal seal by reducing microleakage.

Aim: To evaluate the efficacy of bio-active varnish application on microleakage at the Cavo surface margins of restorations.

Materials and methods: Twenty extracted human maxillary premolars were divided into two groups (n = 10 each). Box-only cavities were prepared in 20 samples and restored with preheated bulk-fill composite. The samples in the control group (n = 10) were left untreated, without the application of varnish. Whereas, in the test group, the cavo surface margins of the restorations (n = 10) were sealed with MI varnish. All samples were then immersed in a 0.5% basic fuchsin dye for 24 hours. They were sectioned mesiodistally and observed under a stereomicroscope at 20x for dye penetration. Cervical microleakage was recorded following the ISO score system. The data were analysed statistically with a chi-square test.

Results: No significant difference in microleakage was observed in the control and test groups. However, the cavo surface margins of the test group samples showed improved performance, with only 10% of the samples demonstrating score-1 leakage.

Conclusion: No significant difference in microleakage was observed in the control and test groups. However, the cavo surface margins of the test group samples showed improved performance, with only 10% of the samples demonstrating score-1 leakage.

Keywords: Bulk-fill composite, Composite surface sealers, Microleakage, Stereomicroscope, Varnish.

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1. Introduction

Dental composite resins are becoming the preferred material for dentists due to the increasing aesthetic demands in their practices [1]. However, the long-term functional aesthetics of composite resin restorations remain elusive. Although these resins are continuously developing, dimensional change and microleakage at the interface remain major drawbacks. The resin-tooth interface experiences significant stresses due to polymerisation shrinkage of composite resins. [2]. Micro gaps can result from shrinkage, leading to degradation of the adhesive interface. These micro gaps can become contaminated with saliva and bacteria, leading to recurrent caries, changes in the pulp tissue, and eventual restoration failure in clinical settings [3]. A literature review of in vivo studies indicated survival rates of 62.9% at 5 years and 67.4% at 10 years [4]. Several randomised clinical trials have shown favourable results with composite restorations; however, concerns regarding marginal integrity continue to persist [5]. Various researchers suggested that prewarming the composite resin before its placement in prepared cavities may have significant clinical advantages [6]. However, a marginal gap of 0.7-5.2μm was observed with a prewarmed composite restoration [7]. So, long-term sealing of the composite resin-tooth interface has gained significant importance.

To overcome the issue of marginal gaps in composite restorations, various surface sealants have been introduced. These include low-viscosity, resin-based sealants designed to penetrate micro gaps at the resin–tooth interface and minimise discrepancies. Subsequently, filled resin sealants were also formulated to enhance the strength of cavo surface margins. Despite these advancements, achieving an ideal marginal seal remains a challenge [8]. A 12-month clinical study assessing the effect of surface sealants on Class I composite restorations found no significant improvement in clinical outcomes [9]. The pursuit of more effective sealing materials continues. In recent years, bioactive materials have garnered significant attention for their role in preventing early carious lesions. Calcium phosphate-based bioactive varnishes have been investigated for their potential in managing dental caries. Notably, the formulation of fluoride with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has shown improved release of calcium, phosphate, and fluoride ions, thereby enhancing the remineralisation process [10].

An ideal surface sealant should exhibit excellent flow and wettability to effectively penetrate and seal the tooth-restoration interface. Sustained reinforcement of the marginal seal may be supported by the natural remineralisation process over time. Building on this rationale, the authors proposed a novel approach involving the use of a bioactive varnish to seal microleakage spaces. Scanty literature is available on the effect of the application of calcium phosphate-based bioactive varnishes on the reduction of microleakage. Therefore, the present study aimed to assess the efficacy of bioactive calcium phosphate-based varnish application on microleakage of Class II composite resin restorations. The tested null hypothesis was that there would be no difference in the microleakage among the restorations treated with/without the varnish application.

2. Materials and methods

2.1 Study design and materials

This *in vitro* study was approved by the Institutional Ethics Committee (IEC-VDC/2021/PG01/CE/IVT/100), Vishnu Dental College, Bhimavaram, Andhra Pradesh, India. The materials used in the study are presented in Table 1.

2.2 Sample preparation

Sample size calculation for microleakage scores as the primary outcome was performed using G Power 3.1 software. Calculation considered an effect size of 1.35, with an alpha level of 0.05, and a target power of 80%. Based on these parameters, the required sample size was estimated to be 20. A single experienced dentist performed all sample preparations and restorations to maintain standardisation throughout the procedure. Collected teeth were cleaned of tissue fragments and any visible debris using periodontal curettes and ultrasonic scalers. They were stored in saline for not more than three months before use.

Twenty intact human maxillary premolar teeth extracted for orthodontic reasons, free of carious lesions and similar dimensions were included in the study. Carious teeth, teeth with previous restoration and previous endodontic treatment, and those with pre-existing fractures or cracks were excluded from the study.

2.3 Restorative procedure

Selected teeth were mounted in a plaster block with a healthy premolar and a molar tooth on either side to simulate the clinical scenario. Box-only Class II cavities were prepared on the distal aspect of each tooth, with the following dimensions: 3 mm buccolingual width, 2 mm mesiodistal width, and 4 mm occluso-cervical depth. A sectional matrix band and retainer were applied to the prepared cavity. The cavities were selectively etched with a 37% phosphoric acid gel, applied to enamel for 30 seconds and to dentin for 15 seconds. Following etching, the surfaces were washed with water for 10 seconds and air-dried gently for 2 seconds. The cavity walls were coated with a thin layer of Tetric N Bond, dried with gentle air, and photopolymerized for 10 seconds using a light-emitting diode (LED) curing device with an intensity of 1,000 mW/cm^2.

Bulk-fill Packable nanohybrid restorative composite was warmed for 5 minutes in a composite warmer at 60°C. The prewarmed composite resin was inserted into the cavity and light-cured for 20 seconds using the LED light source. The light-curing procedures were carried out following the manufacturer's instructions. Once the restorations were finished and polished, they were randomly assigned into two groups of 10 samples each using computer-generated randomisation (www.randomizer.org).

For the control group, no sealing material was coated on the Cavo surface margins. Among the samples in the test group, MI GC varnish -Topical fluoride varnish with calcium and phosphate was coated on the Cavo surface margins of the composite restoration using a micro-brush in a uniform motion. The MI varnish was smeared on the proximal surface, including the gingival floor, with dental floss.

Table 1. The details of the dental materials tested in the research.

S. No	Name of the product	Composition	Manufacturer	Mode of application
1.	N-etch etching gel	37 wt.% phosphoric acid in water, thickeners, and pigments.	Ivoclar Vivadent Liechtenstein, Europe	Selective etching of 30 seconds on enamel and 15 seconds on dentin was done. Rinsing was done thoroughly with water for 10 seconds, and air-dried gently for 2 seconds. After through rinsing with distal water for 10 seconds, the cavity was blotted dry gently with absorbent tissues.
2.	Tetric N-bond adhesive	Methacrylates, ethanol, water, highly dispersed silicon dioxide, and initiator.	Ivoclar Vivadent Liechtenstein, Europe	With an applicator tip, adhesive was applied for 10 seconds with a gentle scrubbing motion. With a stream of air, excess adhesive was removed and polymerised for 20 s with light curing.
3.	Tetric N-Ceram Bulk-fill	Dimethacrylates: 19-21% weight Inorganic filler: 75-77% weight, Barium glass, Prepolymer, Ytterbium trifluoride, Mixed oxides, Catalysts, Stabilisers, and Pigments:<1% weight.	Ivoclar Vivadent Liechtenstein, Europe	Composite resin was placed in 4 mm thick increments and light-cured for 20 s.
4.	MI Varnish	30–50% polyvinyl acetate, 10–30% hydrogenated rosin, 20–30% ethanol, 5% sodium fluoride, 5% CPP-ACP, 20–30% ethanol, 5% sodium fluoride, 5% CPP-ACP, and 1–5% silicon dioxide.	GC Corporation, Tokyo, Japan	1. The tooth surface was cleaned and softly dried using compressed air. 2. The varnish was stirred in the unit dose container and applied as a thin and uniform layer on the tooth-restoration interface with a micro-brush. Also, varnish was smeared on the proximal surface, including the gingival floor, with dental floss.

2.4 Thermocycling procedure

Following the ISO specifications (International Organisation for Standardisation, ISO 11405 standard), the specimens were exposed to simulated thermal changes. Each sample was exposed to water containers at temperatures of 5°C and 55°C for 30 seconds, with a transfer period of 15 seconds between the two containers during each cycle. A total of 500 cycles were completed for all specimens. Subsequently, the specimens were immersed in artificial saliva for 30 days under conditions that simulated the oral environment, with the saliva solution refreshed every other day.

2.5 Exposure of the specimens to basic fuchsine dye

To prevent dye from penetrating the tooth except at the resin-tooth interface, all root apices were sealed with cyanoacrylate, and all tooth surfaces were coated with two layers of nail polish. A 0.5% basic fuchsine dye solution was applied to the teeth and left on them for 24 hours at room temperature. Following rinsing under running water, the specimens were cut into mid-sagittal sections in the mesiodistal plane using a diamond disc mounted in a handpiece. The communications at the root end were coated with adhesive. Exposing 1mm around the resin-tooth interface, the remaining tooth surface was painted with nail varnish. The specimens were stored in basic fuchsine dye for a day at an ambient temperature. The specimens were rinsed off the dye with water, and roots were sectioned 2mm apical to the CEJ. The crowns with the restored cavities were halved with the abrasive disc in the mesiodistal plane.

2.6 Microleakage analysis

Sectioned restorations were observed under a Stereomicroscope. The tooth restoration interface at the axial wall and cervical floor was observed for dye penetration and measured at 20x magnification. ISO score system (ISO/TS 11405: 2003) was followed to score the cervical microleakage [11].

The original score system does not involve the occlusal Cavo surface; hence, it was included in the Scoring system.

Score 0 = No dye penetration.

Score 1 = Dye penetration into ½ of the cervical floor / axial wall from the occlusal Cavo surface.

Score 2 = Dye penetration more than ½ of the cervical floor without reaching the axial wall.
Score 3 = Dye penetration into the cervical and axial wall.

2.7 Statistical analysis

The obtained data were subjected to statistical analysis using the statistical package for social sciences (SPSS) version 26.0, IBM Corporation, NY, USA. Chi-square test was used to analyse the obtained data. The p value less than 0.05 was considered statistically significant.

3. Results

The percentage of microleakage of the samples is presented in Table 2. In the control group, 3 samples (30%) exhibited leakage. Out of 3, 1 sample showed a Score of 1 on the axial wall (Figure.1), 1 sample showed a Score of 1 on both axial wall and cervical floor which represents dye penetration into ½ of the axial/cervical floor, 1 sample showed a Score of 3 (Figure.2), which represents dye penetration into the cervical and axial wall. 7 samples (70%) showed a Score of 0, i.e., no dye penetration was observed. In the test group, out of 10 samples, only 1 (10%) sample showed a Score of 1 on its axial wall (Figure 3), 9 samples (90%) showed a Score of 0, i.e., no dye penetration (Figure 4) was observed, indicating complete marginal seal. However, no significant difference observed between the axial wall ($p = 0.586$) and cervical floor ($p = 0.211$) of both groups (Table 2). Although there was no significant difference, only 10% of the samples in the test group displayed marginal gaps, compared to 30% in the control group.

4. Discussion

One of the primary functions of dental restorations is to seal exposed dentin and protect the pulp from the oral environment. An inadequate seal between the tooth structure and restorative material can lead to microleakage. Microleakage is an imperceptible flow of bacteria, fluids, molecules, or ions between the cavity wall and the restoration material [12]. Restorative materials deteriorate more quickly due to microleakage around restorations, causing recurrent caries, hypersensitivity, pulpal inflammation, and pulp degradation [13].

Bulk-fill resin composite was used in the study to restore class II cavities. Bulk-fill resin composites are formulated to be used in larger increments without compromising the degree of conversion. Researchers have reported that these materials yielded promising results, primarily due to a lower polymerisation shrinkage, which is affected by the organic/inorganic matrix composition and other properties, such as viscosity and elastic modulus [14]. This improvement can be achieved by incorporating aliphatic urethane dimethacrylates, Bis-GMA, highly branched methacrylate compounds and partially aromatic urethane dimethacrylates. This modification has led to a 70% reduction in polymerisation shrinkage stresses [15]. An *in vitro* study found that both traditional and low-viscosity bulk-fill resin composites exhibited similar volumetric shrinkage, around 3%. In comparison, the high-viscosity bulk-fill resin composites showed lower volumetric shrinkage ranging from 0.84% to 2.19% [16].

In this study, the bulk-fill resin composite was preheated to approximately 60°C before being placed and polymerised. While improved marginal adaptation was observed, the marginal gap was reported to be between 0.7 to 0.8 µm in enamel and 5.2 to 5.5 µm in dentin according to an *in vitro* study [17]. This highlights the need for new protocols to address the marginal gap and reduce microleakage.

To overcome the issue of marginal gaps in composite resin restorations, various surface sealants have been introduced by researchers [18]. Several of these are low-viscosity formulations containing unfilled resin monomers, designed to penetrate micro gaps at the resin–tooth interface and reinforce the marginal junction. Later, some filled resin sealers were also developed for reinforcing the Cavo surface margins. However, the perfect seal was not achieved. Hence, in the test group, a bioactive varnish named GC MI Varnish was used to reduce the microleakage at cavo surface margins. The specimens were suspended in unagitated artificial saliva (AS) to simulate the oral environment. Samples were incubated for a month in a simulated oral environment, with saliva being replenished every other day. The varnish, which is composed of fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), demonstrated enhanced release of calcium, phosphate, and fluoride ions [10], which may have triggered remineralisation at the marginal gaps, thereby reducing microleakage.

The marginal adaptation of composite restorations is typically evaluated using microleakage tests. Various techniques, including dyes, radioactive isotopes, air pressure, neutron activation analysis, bacteria, and scanning electron microscopes, have been employed to trace microleakage [19]. Microleakage is most commonly detected *in vitro* studies using dyes as tracers. Several dyes with varying particle sizes are used for microleakage assessment, including methylene blue, basic fuchsin, and procion red dye [20]. It is a very feasible technique since there is no radiation hazards associated with it. Moreover, the inert nature and distinct colouration of the dye allow it to be visualised against both the tooth structure and the composite restoration, without undergoing any chemical interaction with them [21]. 0.5% basic fuchsin dye was used in this study to measure microleakages.

Table 2. Comparison of dye penetration between the control and test groups

Area	Group	SCORE-0	SCORE-1	SCORE-2	SCORE-3	Chi-square value	p value
Axial wall	Control group	7 (70%)	2 (20%)	0	1 (10%)	2.600	0.586
	Test group	9 (90%)	1 (10%)	0	0		
Cervical floor	Control group	7 (70%)	2 (20%)	0	1 (10%)	5.000	0.211
	Test group	10 (100%)	0	0	0		

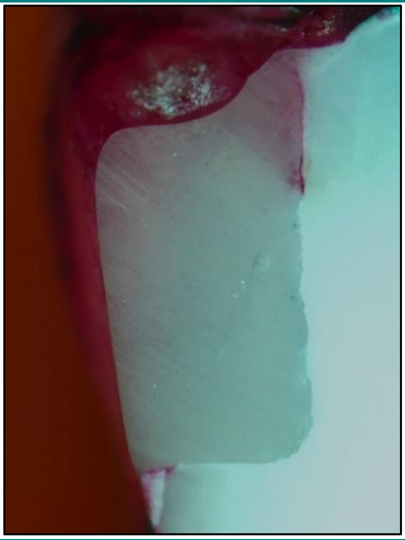

Figure 1. Control group sample showing Score 1

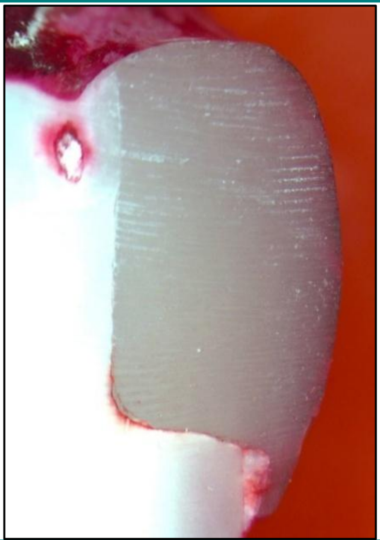

Figure 2. Control group sample showing Score 3

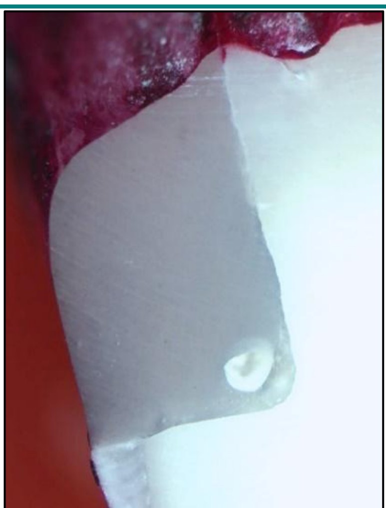

Figure 3. Test group sample showing Score 1

Figure 4. Test group sample showing Score 0

The scoring of cervical microleakage was conducted following the ISO scoring system outlined above [11]. In this study, a total of 20 samples were analysed, with 4 samples demonstrating varying degrees of microleakage. In the control group of 10 samples, 3 samples (30%) exhibited leakage. Out of 3, 1 sample showed a Score of 1 on the axial wall (Figure.1), 1 sample showed a Score of 1 on both axial wall and cervical floor which represents dye penetration into ½ of the axial/cervical floor, 1 sample showed a Score of 3 (Figure.2), which represents dye penetration into the cervical and axial wall. 7 samples (70%) showed a Score of 0, i.e., no dye penetration was observed. In the test group, out of 10 samples, only 1(10%) sample showed a Score of 1 on its axial wall (Figure 3), 9 samples (90%) showed a Score of 0, i.e., no dye penetration (Figure 4) was observed, indicating complete marginal seal. Clinically, the varnish can be applied with floss on pre-wedged teeth. The similar behaviour in terms of microleakage in both groups may be due to prewarmed composite resins. But the Cavo surface margins of the test group samples exhibited better performance, and only one sample showed Score 1 leakage.

In the current study, the cavo surface margins of the test group were sealed with a CPP-ACP varnish. The ideal cavo surface margins free from dye penetration may result from the deposition of calcium and phosphate, leading to the formation of hydroxyapatite crystals. This crystal formation may have filled the gap caused by the polymerisation shrinkage of the composite restoration, according to the literature on MI Varnish. The calcium, phosphate and fluoride ions required for remineralisation were delivered to the tooth surface by amorphous calcium phosphate stabilised by CPP [22]. These stabilised ions are highly bioavailable as electroneutral nanoclusters in a fluorapatite-like molar-relationship [23].

Numerous studies have evaluated the remineralising capacity of NaF plus CPP-ACP varnish. Most of these studies reported net remineralisation in the treated samples [24,25].
An *in vitro* investigation assessed the impact of MI dental varnish on white spot lesions and reported a 41% enhancement in remineralisation. This effect was attributed to the increased release of fluoride, calcium, and phosphate ions, which supported the remineralisation of subsurface lesions [23]. The stabilisation of fluoride, calcium, and phosphate may be due to the peptide αS1-CN (59-79) in CPP-ACP, which forms CPP-ACPF nanocomplexes within the pH range of 6-9. The hydrodynamic radius of CPP-ACPF nanocomplexes is 2.12±0.26nm, which is responsible for the better diffusion in micro- or nano-spaces [26]. An optical Coherence Tomography study reported that MI varnish releases a higher level of calcium, phosphate, and fluoride ions, helping in preventing enamel demineralisation adjacent to orthodontic brackets [24].

An *in vitro* study using a pH cycling model demonstrated that MI varnish was more effective in preventing dentin demineralisation compared to silver diamine fluoride, Duraphat, and Clinpro varnish. The enhanced bioavailability of calcium, fluoride, and phosphate ions from MI varnish contributed to greater fluoride uptake and a more uniform remineralisation pattern, including remineralisation of the entire lesion [27]. Enamel lesions were optimally remineralised with a mixture of fluoride and CPP-ACP rather than only fluoride as reported in a systematic review [28].
According to an *in vitro* study, the use of bulk-fill composite resulted in an average gap of 11.47 μm at the interface with the prepared tooth surface [29]. The control group, which did not have any surface sealant, exhibited dye penetration in 30% of the samples, receiving scores of -1 and -3. In contrast, 70% of the samples showed no signs of dye penetration. This lack of penetration may be attributed to the sealing of gaps by ions present in the artificial saliva.

In the test group treated with MI varnish, only 10% of the samples exhibited minimal microleakage (score 1), while the remaining 90% showed no dye penetration, indicating effective sealing. This outcome may be attributed to several factors. Firstly, the MI varnish contains CPP-ACPF nanocomplexes with a hydrodynamic radius of approximately 2.12 ± 0.26 nm, allowing them to flow efficiently into marginal gaps. Secondly, the formulation enhances the bioavailability of fluoride, calcium, and phosphate ions, which likely contributed to mineral deposition within the interface. Lastly, the synergistic interaction between fluoride, calcium, and phosphate may have facilitated deeper remineralization of the tooth structure.

MI varnish contains active ingredients such as NaF and CPP-ACP, along with inactive components including ethanol, polyvinyl acetate, hydrogenated resin, and silicon dioxide. The water-soluble polymer Polyvinyl acetate rapidly dissolves upon application, enabling a quick release of fluoride ions. Its high polarity and strong affinity for carbon dioxide contribute to its solubility. Ethanol, another key component, evaporates easily, forming micro-pores that facilitate water uptake. This absorption causes the varnish to swell, dissolve, and release ions. Given that the hydrodynamic radius of calcium, phosphate, and fluoride nanocomplexes is approximately 2.12 ± 0.26 nm, these particles can readily diffuse through the created pores to reach deeper regions. This mechanism may help explain the reduced marginal gaps observed in the test group [10]. The results of the present study are in agreement with a SEM study, which reported statistically significant decreased marginal micro gap by application of CPP-ACPF [30].

The null hypothesis was accepted as there was no statistically significant difference in dye penetration along the cavity interface between the two groups. However, the cavo surface margins of the test group demonstrated better sealing ability, with only one sample exhibiting Score 1 leakage. Further studies with a larger sample size are recommended to validate these findings.

This study had a few limitations that should be considered. While the dye penetration method is a commonly used and efficient technique for assessing microleakage, it may not accurately reflect clinical conditions, as it does not account for variables such as fluctuations in oral pH or the effects of occlusal forces. Additionally, the limited duration of thermocycling may not adequately simulate the long-term thermal stresses experienced in the oral environment. The true success of restorations can only be confirmed through their long-term clinical performance, highlighting the need for prospective clinical trials. Moreover, the study focused solely on maxillary premolars, which may not represent the full range of anatomical variations seen in other tooth types. Lastly, the relatively small sample size may limit the generalizability of the findings, and further research involving a larger sample is warranted.

5. Conclusion

Within the limitations of this study, it can be concluded that sealing the cavo surface margins following composite resin restoration with a bioactive varnish, such as GC MI Varnish, resulted in a reduction in microleakage and its potential consequences. In the group without surface sealants, dye penetration was observed in 30% of the samples. In contrast, the application of MI Varnish reduced dye penetration to 10%. However, this difference was not statistically significant.

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