**Short title:** Bio-active Varnish & Composite Resin Restoration

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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**

**Objective:** To evaluate the microleakage of class II composite resin restorations sealed with bioactive calcium phosphate-based fluoride varnish using the dye penetration method under a stereomicroscope at 20x magnification.Dimensional changes of composite resins are inherent due to the involved polymer technology. Hence novel clinical procedures are required to take care of the adverse consequences. One such clinical procedure is application of bioactive varnish at the Cavosurface margins of restorations. **Methods:** Twenty extracted human maxillary premolars were divided into 2 groups (n=10 each). Box-only cavities were prepared in 20 samples and restored with preheated bulk fill composite. Samples in the control group(n=10) were left intact without varnish application. Cavosurface margins of restorations in the test group (n=10) were sealed with MI varnish. Samples were immersed in 0.5% basicfuschin dye for 24hrs. They were sectioned mesiodistally and observed under a stereomicroscope at 20x for dye penetration. Cervical microleakage was recorded following the ISO score system. Results were statistically analysed with a chi-square test. **Results:**There was no significant difference in microleakage in the control and test group. But the cavosurface margins of the test group samples exhibited better performance and only 10% of the samples showed score 1 leakage. **Conclusion:** The inference of the current research is that sealing the Cavo surface margins after the composite resin restoration with bioactive varnish like MI varnish results in decreased microleakage. Decreased microleakage will enhance the long-term clinical success of the restoration as it prevents post operative sensitivity and secondary caries

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

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**INTRODUCTION:**

Dental composite resins have proven to be the materials of choice for many dentists due to the increased demand for high-quality aesthetic results in everyday dentistry [1]. However, despite the continuous evolution of these resins, dimensional change and microleakage at the interface remain as shortcomings. The resin tooth - interface can be subjected to a considerable number of stresses due to polymerization shrinkage of composite resins [2]. Because of dimensional change, micro gaps can develop, resulting in adhesive interface degradation, microleakage of saliva and bacteria, secondary caries, pulpal changes, and clinical failure of the restoration [3].

A review of literature of in vivo studies reported 62.9% and 67.4% of survival rates at 5 and 10 years respectively [4]. Many randomised clinical trials have reported better success with composite restorations but with the persisting issue of marginal integrity [5].

Research from various laboratories has 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 composite resin-tooth interface has gained significant importance.

Many surface sealants were developed to address the marginal gap associated with composite restorations. Many low-viscosity sealants containing resin monomers were developed, which could flow into the micro gaps at the resin-tooth interface and reduce the gap. Later some filled resin sealers were also developed for reinforcing the cavosurface margins. However, the real perfect seal still needs to be achieved [8]. A 12-month clinical trial evaluated the influence of surface sealants on class 1 composite restorations and reported no increase in clinical success [9].

So, the quest for newer, better sealing materials is on. Recently Bioactive materials have gained popularity in the prevention of early caries lesions. Calcium phosphate-based bioactive varnishes were evaluated to manage dental caries. A combination of fluoride and casein phospho-peptide amorphous calcium phosphate (CPP-ACP) showed improved Ca, P, and F ions release and potentially enhance the remineralising process [10].

The ideal requirements of a surface sealant are better flow and good wettability to occupy the interface. The reinforcement of the marginal interface can be sustained for a long time, probably with the natural remineralising process. Hence, the authors conceived the novel use of bioactive varnish for the sealing of microleakage space. No studies have used bioactive calcium phosphate-based varnish for reducing microleakage.

Hence this study aimed to evaluate the efficacy of bioactive calcium phosphate-based varnish application on microleakage of class II composite resin restoration.

The tested null hypothesis was that there would be no difference in the microleakage among the restorations treated with/without the varnish application.

**MATERIALS AND METHODS:**

**Study design and materials:** This in vitro study was approved by the Institutional Ethics Committee (IECVDC/2021/PG01/CE/IVT/100)

**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 sec, and air‑dried gently for 2 sec. After through rinsing with distal water for 10 seconds, the cavity was blot dried gently with adsorbent 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 sec with a gentle scrubbing motion. With a stream of air, excess adhesive was removed and polymerized 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, Stabilizers, and Pigments :<1% weight | Ivoclar Vivadent Liechtenstein, Europe | Composite resin was placed in 4 mm thick increment 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 hydrogenated rosin 1–5% silicon dioxide | GC Corporation, Tokyo, Japan | 1. Tooth surface was cleaned and softly dried using the compressed air. 2. The varnish was stirred in the unit dose container and applied as a thin and uniform layer on tooth-restoration interface with a micro-brush |

**Sample preparation:**

The sample size was estimated for microleakage scores as the primary outcome using g power 3.1 software. The calculation was based on an effect size of 1.35, an alpha level of 0.05, and a desired power of 80%. The estimated total sample size was 20. An effect size of 1.35, an alpha level of 0.05, and a target power of 80% were used in the computation. The predicted number of samples was 20. The 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.

20intact 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.

**Restorative procedure:**

On the distal side of each tooth, box-only class II cavities with the following measurements were created: 3 mm buccolingual width, 2 mm mesiodistal width, and 4 mm occlusocervical depth. Sectional matrix band and retainer were applied to the prepared cavity. The cavities were selectively etched with 37% phosphoric acid gel (N-etch, Ivoclar Vivadent products, Delhi, India) for 30 seconds on enamel and 15 seconds on dentin, rinsed with water for 10 seconds and gently dried for 2 seconds. The walls of the cavity were coated with a thin layer of Tetric N Bond, dried with gentle air and photopolymerized for 10 sec with Light Emitting Diode curing device. (Woodpecker, Guilin, China) of intensity 1,000 mW/cm2.

Bulk fill Packable nanohybrid restorative composite (Ivoclar Vivadent products, Delhi) was warmed for 5 minutes in a composite warmer (Delta Co., India)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 performed according to the directions provided by the manufacturer. After finishing and polishing, the prepared cavities were arbitrarily distributed as 10 samples in different groups using computer-generated randomisation: - ([www.randomizer.org](http://www.randomizer.org))

No sealing agent was applied along the cavo surface margins for the control group samples.

Among the samples in the test group, MI GC varnish (GC, America) -Topical fluoride varnish with calcium and phosphate was applied on the Cavo surface margins of the composite restoration using a micro brush in a uniform motion.

**Thermocycling procedure:**

Following the ISO specifications (International Organization for Standardization, ISO 11405 standard), the specimens were exposed to simulated thermal changes. Each sample was exposed to 5°C and 55°C water container for 30 seconds with transfer time of 15 seconds between the containers for a cycle. Such 500 cycles were completed for all specimens. Then specimens were stored in synthetic saliva for 30 days at simulated oral temperature. Saliva replenished for every alternate day.

**Exposure of the specimens to basic fuchsine dye:**

All the root apices were sealed with cyanoacrylate (Fevikwik Cyanoacrylate Adhesives, Pidilite Industries-India), and all teeth surfaces were coated with two layers of nail varnish (Nail trend-RELIANCE RETAIL, India) up to 1 mm from the restoration margins to prevent dye penetration into the tooth except at the resin-tooth interface. The teeth were immersed in a 0.5% basic fuchsine dye solution at room temperature for 24 h. Then the specimens were rinsed under running water and sectioned mid-sagitally in the mesiodistal plane using a diamond disc (DD0001 4A HP, To boom) mounted in a handpiece.

To prevent dye from penetrating the tooth except at the resin-tooth interface, all root apices were sealed with cyanoacrylate (Fevikwik Cyanoacrylate Adhesives, Pidilite Industries, India) and all tooth surfaces were coated with two layers of nail polish (Nail trend-RELIANCE RETAIL, India). 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 (DD0001 4A HP, To boom) mounted in a handpiece. The communications at the root end were coated with adhesive (Fevikwik Cyanoacrylate Adhesives, Pidilite Industries, India). Exposing 1mm around the resin-tooth interface, remaining tooth surface was painted with nail varnish. (Nail trend-RELIANCE RETAIL, India). The specimens were stored in basic fuchsine dye for a day in 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 (DD0001 4A HP, To boom) in the mesiodistal plane.

**Microleakage analysis:**

Sectioned restorations were observed under a Stereomicroscope. 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]

Original score system does not involve the occlusal Cavo surface hence it was included in the Scoring system by the authors.

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.

**STATISTICAL ANALYSIS:**

Statistical analyses were performed using the chi-square test.

**RESULTS:**

**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 |

Statistically significant if p<0.05

The statistical analysis has drawn the following observations.

There is no significant difference between the axial wall (p-0.586) and cervical floor (p-0.211) of both groups (Table 2). Even though there is no significant difference, only 10% of the samples showed marginal gaps in the test group, whereas 30% of the samples in the control group showed marginal gaps.

**DISCUSSION:**

The most crucial roles of dental restorations are to seal the exposed dentin and shield the pulp from the oral environment. An insufficient seal between the tooth and the restorative material may cause microleakage. Microleakage is an undetectable passage of bacteria, fluids, molecules, or ions between the cavity wall and the restorative 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 new modification may be achieved by incorporating Bis-GMA, aliphatic urethane dimethacrylates, partially aromatic urethane dimethacrylates, and highly branched methacrylates. This modification has led to 70% reduction in polymerisation shrinkage stresses [15]. In an in-vitro study, it was observed that the conventional resin composites and low-viscosity bulk-fill resin composites showed similar volumetric shrinkage of 3%. In comparison, the high-viscosity bulk-fill resin composites showed lower volumetric shrinkage ranging from 0.84 % up to 2.19% [16]. A Scientific study reported similar volumetric shrinkage of 3% for both traditional and low-viscosity bulk-fill resin composites. Comparatively, the volumetric shrinkage of the high-viscosity bulk-fill resin composites ranged from 0.84% to 2.19 %.

In this study bulk fill, resin composite was preheated to a temperature of approximately 60°C before placing and polymerising the material. Though there is improved marginal adaptation, the marginal gap of 0.7 -0.8µm in enamel and 5.5-5.2µm in dentin reported in an invitro study [17]. This indicates the requirement of new protocols to manage the marginal gap there by microleakage.

The researchers developed many surface sealants to address the marginal gap associated with composite resin restorations [18]. Many of these low viscosity sealants containing resin monomers were developed, which could flow into the micro gaps at the resin-tooth interface for restrengthening the junction. Later some filled resin sealers were also developed for reinforcing the Cavo surface margins. However, the real perfect seal was not achieved.

Hence in the test group a bioactive varnish named GC MI Varnish was used to reduce the microleakage at cavosurface margins. The specimens were suspended in unagitated artificial saliva (AS) to simulate the oral environment. Incubation of the samples was done for a month at the simulated oral environment and the saliva was replenished on alternate days.The varnish which is a combination of fluoride and casein phospho-peptide amorphous calcium phosphate (CPP-ACP) showed improved release of Ca, P, and F ions [10] and could have initiated the remineralising process at marginal gaps there by decreasing the microleakage.

The marginal adaptation of composite restorations is typically evaluated using microleakage tests. To detect microleakage, various methods have been employed, including dyes, air pressure, bacteria, radioactive isotopes, neutron activation analysis, and scanning electron microscopy. Many techniques, such as the use of dyes, radioactive isotopes, air pressure, neutron activation analysis, bacteria, and scanning electron microscope have been used to trace microleakage. Microleakage is most detected in vitro studies using dyes as tracers. For microleakage assessment, different dyes with different particle sizes are used, such as procion red dye, basic fuchsin, and methylene blue [19]. Several dyes with varying particle sizes are used for microleakage assessment, including methylene blue, basic fuchsin, and procion red dye. It is a highly feasible technique as there are no radiation hazards associated with it. Additionally, the inert dye with its distinct colour can be appreciated in contrast to the tooth and the composite restoration without reacting chemically with them [20]. 0.5%basic fuchsin dye was used in this study to measure microleakages.

Scoring of the cervical microleakage was done as per the ISO score system as mentioned above [11].

Out of the 20 samples in this study 4 samples showed the microleakage of varying degrees. In the control group out of 10,3 samples (30%) showed 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.4),9 samples (90%) showed Score 0 i.e., no dye penetration (Figure.3) was observed indicating complete marginal seal.

The statistical analysis has drawn the following observations.

There is no significant difference between the axial wall (p-0.586) and cervical floor (p-0.211) of both the groups according to Chi square test. Even though there is no significant difference, only 10% of the samples showed microleakage in the interventional group compared to conventional group where 30% of the samples showed microleakage.

The similar behaviour in terms of microleakage of both the groups could be because of prewarmed composite resins. But the cavosurface margins of the test group samples exhibited better performance and only one sample showed Score 1 leakage.

In the present study, test group cave surface margins were sealed with CPP-ACP varnish. The perfect cavo surface margins without dye penetration could be because of the deposition of calcium and phosphate leading to formation of hydroxy apatite crystals. This crystal formation might have obliterated the gap formed by the polymerization shrinkage of composite restoration. This is in accordance with the literature available with MI Varnish. The calcium, phosphate and fluoride ions required for remineralization were delivered to the tooth surface by amorphous calcium phosphate stabilized by CPP [21]. This stabilized ions are highly bioavailable as electroneutral nanoclusters in a fluorapatite like molar-relationship [22] .

Till now many research studies are carried out to evaluate the remineralising capacity of NaF plus CPP-ACP varnish. Most of these studies reported net remineralization models in the samples treated [23,24].

An in-vitro study evaluated the effect of MI dental varnish on white spot lesions. MI varnish promoted remineralisation by 41%. This was attributed to the higher level of fluoride, calcium, and phosphate ion release helping in the sub-surface lesion remineralisation [22]. The stabilization of fluoride calcium and phosphate could be because of peptide ꭤS1-CN (59-79) in the CPP-ACP forming CPP-ACPF nanocomplexes in 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 [25].

MI varnish releases a higher level of calcium, phosphate, and fluoride ions helping in preventing enamel demineralisation adjacent to orthodontic brackets. It was measured with Optical Coherence Tomography. [23]

One of the in-vitro studies with the pH cycling model proved MI varnish to be superior to silver diamine fluoride, duraphat, and clinpro varnish in preventing dentin demineralisation. The bio-availability of calcium fluoride phosphate ions from MI varnish help in fluoride uptake with an improved pattern of remineralisation along with the remineralisation of the entire body of the lesion [26].

Enamel lesions were optimally remineralised with mixture of fluoride and CPP ACP rather than only fluoride as reported in a systematic review [27]

In an invitro study it was reported that bulk-fill composite exhibited an average gap of 11.47µm from the prepared tooth surface [28]. The Control group without any surface sealant demonstrated dye penetration in 60% of the samples with scores 1 & 3. 40% of the samples did not show any dye penetration, it might be due to the sealing of the gaps with the ions in the artificial saliva.

Whereas test group with the application of MI varnish only 10% demonstrated the leakage with a score of 1. 90 % of the samples showed no dye penetration due to the efficient flow of MI varnish. It might be due to its

1) Particle size- The hydrodynamic radius of CPP-ACPF nanocomplexes is 2.12±0.26 nm which has led to better flow into the marginal gap

2) Increased bioavailability of fluoride, calcium, and phosphate might have led to the deposition of minerals

3) Because of the association of fluoride with calcium and phosphate might have led to deeper remineralisation.

The composition of MI varnish includes NaF, CPP-ACP as active ingredients along with polyvinyl acetate, hydrogenated resin, ethanol, and silicon dioxide as inactive ingredients. Polyvinyl acetate is a water-soluble polymer; hence it dissolves at a short time releasing the highest fluoride ions at a shorter time.

Polyvinyl acetate has high polarity and affinity to carbon dioxide making it highly soluble. Ethanol is another ingredient of MI varnish. This easily evaporates creating pores for the transport of water; hence the varnish takes up the water to swell, dissolve, and release ions. As the hydrodynamic radius of calcium, phosphate, and fluoride nanocomplexes is 2.12±0.26nm, they can easily penetrate the pores created to reach the deeper surfaces. This might also be the reason for fewer marginal gaps in the test group [10].

The null hypothesis was accepted because there was no statistically significant difference in the dye penetration along with the cavity interface among the 2 groups. But the cavosurface margins of the test group samples exhibited better performance and only one sample showed grade 1 leakage. Research with a larger sample is required to validate this.

**LIMITATIONS:**

* As it is an invitro study, all the oral environmental factors like pH and occlusal loading were not stimulated in the study.
* Long term effective clinical functioning of restorations can prove the success of the restoration. Hence prospective clinical trials are indicated.
* As in this study sample size was less, research with a larger sample size is indicated.

**CONCLUSION:**

* Within the limitations of this study, it can be concluded that – sealing the Cavo surface margins after the composite resin restoration with bioactive varnish like MI varnish, there is a reduction in the microleakage thereby reducing the subsequent consequences.
* The dye penetration was 30% with the composite resin restoration without surface sealants.
* Dye penetration along the Cavo surface margin was minimized i.e., only 10% by the application of MI Varnish. However, the results were not statistically significant.

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**FIGURES:**

  

Figure.1: Control group sample showing Score 1 Figure.2: Control group sample showing Score 3

  

Figure.3: Test group sample showing Score 0 Figure.4: Test group sample showing Score 1