

Assessment of Microleakage Using Dye Penetration Method in Primary Teeth Restored with Tooth-colored Materials: An *In Vitro* Study

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ABSTRACT

Aim: The present study aimed to assess the microleakage in primary teeth restored with tooth-colored materials using the dye penetration method.

Materials and methods: A total of 60 healthy primary molar teeth were included in this study and standardized class II mesio-occlusal cavities were prepared on the samples. Consequently, these teeth were randomly divided into three experimental groups ($n = 20$ each group) such as group I: nano-filled resin-modified glass-ionomer, group II: nanocomposite resin, and group III: Cention N. After completing all the restorations, the restored teeth were subjected to 100 cycles of thermocycling. Next, all the surfaces of the tooth, except the restoration and a 1-mm zone adjacent to the restoration's margins, were covered with two coats of nail varnish. The coated teeth were then submerged in a 0.5% basic fuchsin dye solution. The teeth were then sectioned along the center of each restoration mesiodistally. Each part was visualized under a stereomicroscope at $\times 40$ magnifications to assess microleakage.

Results: Out of all the included restorative materials, the least microleakage was demonstrated by teeth restored by the nano-filled resin-modified glass-ionomer (RMGI) group (1.05 ± 0.21) followed by the Cention N group (1.84 ± 0.14) and the nanocomposite resin group (3.10 ± 0.03). A statistical method involving the analysis of variance revealed a statistically significant difference among the different restorative materials. Multiple comparisons among the restorative materials showed a statistically significant difference between groups I and II and groups II and III restorative materials ($p < 0.05$). The dye penetration score 1 was more [11 (55%)] for the nano-filled RMGI group, score 3 was more [12 (60%)] for the nanocomposite resin group, and score 2 was more [9 (45%)] for the Cention N group.

Conclusion: The present study showed significantly less microleakage associated with the nano-filled resin-modified glass ionomer group than nanocomposite resin and Cention N groups.

Clinical significance: Since many years, dentists have encountered a challenging problem with cervical lesions. Thus, an interdisciplinary treatment approach is the appropriate option in the management of carious teeth that involve gingival recession and cervical extension.

Keywords: Class II cavity preparation, Microleakage, Primary molars, Thermocycling.

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INTRODUCTION

The steps involved in the restoration of a carious tooth are preparation of a cavity, debridement of carious tissue and causative microorganisms, and restoration of the ensuing cavity with a suitable restorative material. The objective of tooth restoration is to reestablish the esthetics of the tooth and mastication, and to avoid the return of caries,¹ while preserving the biologic integrity of the teeth in harmony with the conditions of the oral cavity.² The most essential factor that decides the durability of the restoration is its ability to conform to the cut tooth surface and to seal the walls of the cavity. Ideally, a firm bond between the restorative material and tooth surface should result in a snug, concealed marginal seal.¹ Despite the stupendous technological improvement, no restorative material impeccably adheres to the tooth surface. This results in a breach along the margins of the cavity and the restorative material, thus, leading to microleakage.²

Microleakage includes the movement of microorganisms, fluid, and chemical compounds along with the tooth-restoration interface. The consequences of such microleakage are staining or discoloration of the restoration, sensitivity of tooth, relapse of caries, and, ultimately, failure of the restoration.³ Based on the above-mentioned consequences, the amount of microleakage becomes an important criterion for the selection of a restorative material.

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The available microleakage assays offer valuable information on the functioning of restorative materials. Various recognized methods for evaluating microleakage have been used. These include use of dyes, air pressure, radioactive isotopes, scanning electron microscope, micro-computed tomography (μ CT), neutron activation analysis, and bacterial activity analysis, each having both merits and demerits.⁴ Due to the lack of demonstration of the real nature of microleakage, few older methods are no longer used.⁵ Thus, this trial was carried out to compare and assess the magnitude of microleakage using the dye penetration method in primary teeth that were restored with tooth-colored materials.

MATERIALS AND METHODS

This *in vitro* research was carried out at the Department of Pediatric and Preventive Dentistry, Sree Anjaneya Institute of Dental Sciences, Kerala. A total of 60 healthy primary molars (Fig. 1) teeth that were extracted for various therapeutic reasons at the Department of Pediatric Dentistry were used to evaluate the extent of microleakage. All teeth were debrided and stored in distilled water at room temperature during the entire study period.

The included teeth were healthy, non-restored primary molars. The carious, fractured, or previously restored teeth were excluded from the study.

Preparation of Class II Cavities

Class II mesio-occlusal cavities were made uniformly on the samples with the following dimensions: 2 mm wide buccolingually, 2 mm deep pulpally, and 1.5 mm wide gingival seat of the proximal box placed 1 mm above cement enamel junction. The cervical margin in the proximal box had to rest on enamel. The axiopulpal line angle was rounded and all the cavosurface line angles were butt-jointed.

The teeth were further randomly divided into three investigational groups ($n = 20$, each group) and filled per the manufacturers' instructions.

Group I: Nano-filled Resin-modified Glass-ionomer

The cavity was initially treated with a thin smear of nano-ionomer primer for 15 seconds and a stream of dry air was used for 10 seconds to dry it thoroughly. With a visible light curing device, the smear

was light-cured for 20 seconds. After this, two pastes (Ketac N100, 3M-ESPE) of an equal amount were dispensed and mixed with a plastic spatula for 20 seconds. This mixture was then used to fill the cavity and was light-cured for 20 seconds.

Group II: Nanocomposite Resin

The cavities in the teeth belonging to this group were rinsed with water and compressed air was used to dry it thoroughly, 37% phosphoric acid gel was used for 60 seconds for acid etching, and, later, the cavity was again rinsed with water thoroughly for 30 seconds. Soon after this, compressed air was used for 15 seconds to dry the cavity completely, after which dentin primer bonding agent was coated; the bonding agent was coated onto the cavity over a duration of 60 seconds. Next, the procedure of light-curing the bonded cavity was performed for 20 seconds. Matrix bands that are translucent and supported by a retainer were used for isolation of the tooth. Nanocomposite resin (Z350, 3M ESPE Filtek™ Universal Restorative, USA) was added on to the cavity and cured increment-wise. After 15 minutes, the cured restoration was finished and polished.

Group III: Cention N

The class II cavity prepared in teeth belonging to this group was rinsed thoroughly with water and dried with compressed air. The quantity of powder that was taken accounts for one measuring scoop and the amount of liquid taken was one drop (this matches to a ratio by weight of 4.6:1) [Ivoclar Vivadent] was taken. The powder was separated into two similar considerable parts using a plastic spatula. The fluid was spread to expand the surface. The main part of powder was mixed thoroughly with the whole fluid administered on the blending cushion. After each one of the segments has been mixed together, the remaining powder was involved and mixed again until (45–60 seconds) a uniform consistency was achieved. The working time was 3 minutes from the beginning of mixing. The material was applied to the cavity, adapted, and condensed carefully. After placement, the restoration was light-cured for 40 seconds followed by finishing, polishing, and checked for occlusal high points.

After restoring all the teeth, the restored teeth were subjected to thermocycling (100 cycles). The temperature and the duration were as follows: 5°C, 30 seconds; 19°C, 20 seconds; 55°C,



Fig. 1: Primary molars used in the study

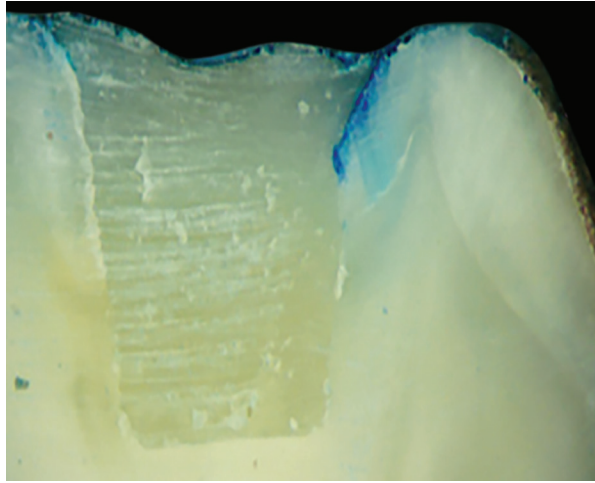


Fig. 2: Microleakage assessment using a stereomicroscope

30 seconds. Subsequent to thermocycling, two coats of nail varnish were applied onto the entire tooth surface except for the restoration and a 1-mm area around the margins of restoration. The green compound was used to mask the root apices. The teeth that were coated were later submerged for a period of 1 day at 37°C in the basic fuchsin dye solution (0.5%) (Basic Fuchsin Solution, 0.5 AQ, Rowley Biochemical, Danvers, MA). After removal from the dye, the teeth were thoroughly rinsed with water, desiccated, and then fixed in resin in advance to cutting. The teeth were cut along the mesiodistal extent over the middle of all the restorations. Each tooth part was seen under a stereomicroscope (Fig. 2) at x40 magnification to assess microleakage. The linear diffusion of the dye from the external margin of the cement was scored according to the criteria given by Popoff et al.,⁶ which is as follows:

- Score 0: No microleakage
- Score 1: Dye penetration up to one-third of the axial wall
- Score 2: Dye penetration up to two-thirds of the axial wall
- Score 3: Dye penetration onto the entire axial wall
- Score 4: Dye penetration onto the pulpal wall.

Statistical Analysis

A SPSS version 20 was adopted in this study. Statistical analysis was performed using the analysis of variance (ANOVA) test. The differences between each group were established using the Tukeys *post hoc* test. The results were inferred to be statistically significant if a *p* value of <0.05 was obtained.

RESULTS

Table 1 and Figure 3 demonstrate the mean as well as the standard deviation of three restorative materials. The mean value for the nano-filled RMGI group was 1.05 ± 0.21, the nanocomposite resin group was 3.10 ± 0.03, and the Cention N group was 1.84 ± 0.14.

The mean microleakage of different restorative materials is given in Table 2. Among the included restorative materials, the

Table 1: Mean and standard deviation of three materials used in the study

Groups	n	Mean ± Std. deviation
Group I—nano-filled RMGI	20	1.05 ± 0.21
Group II—nanocomposite resin	20	3.10 ± 0.03
Group III—Cention N	20	1.84 ± 0.14

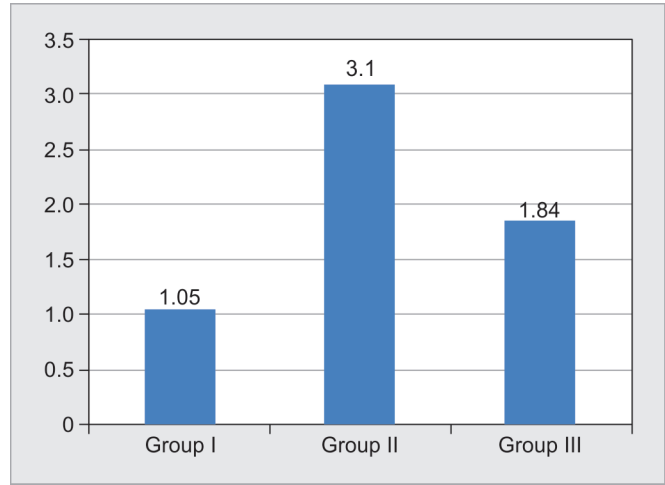


Fig. 3: Mean microleakage of three materials used in the study

Table 2: Mean microleakage of different restorative materials

Groups	Mean ± SD	F value	p value	Significance
Group I—nano-filled RMGI	1.05 ± 0.21	21.283	0.001	HS
Group II—nanocomposite resin	3.10 ± 0.03			
Group III—Cention N	1.84 ± 0.14			

p < 0.05; HS, highly significant

Table 3: Multiple comparisons Tukey HSD

Group	Compared with	Mean difference (I-J)	Sig.
Group I	Group II	-2.05*	0.001
	Group III	-0.79	0.08
Group II	Group I	2.05*	0.001
	Group III	1.26*	0.001
Group III	Group I	0.79	0.06
	Group II	-1.26*	0.001

*Significant, *p* < 0.05

lowest microleakage was found to be associated with teeth restored by the nano-filled RMGI group (1.05 ± 0.21), followed by the Cention N group (1.84 ± 0.14) and the nanocomposite resin group (3.10 ± 0.03). An analysis of variance revealed a statistically significant difference between the different restorative materials.

The multiple comparisons among the restorative materials are shown in Table 3. A statistically significant difference among group I vs group II and group II vs group III restorative material (*p* < 0.05) can be seen. However, no statistically significant difference was seen between groups I and III (*p* > 0.05).

Table 4 shows the distribution of microleakage scores. Score 1 was more [11(55%)] among the nano-filled RMGI group, score 3 was more [12(60%)] among the nanocomposite resin group, and score 2 was more [9(45%)] among the Cention N group.

DISCUSSION

There has been an endless quest for an appropriate restorative material and restoration technique that warrants firm adherence to the tooth surface with the purpose of reducing the likelihood of microleakage. It is of utmost importance to maintain the marginal seal over an extended period so as to minimize or at



Table 4: Distribution of microleakage scores

Groups	Microleakage scores of the specimens				
	Score 0	Score 1	Score 2	Score 3	Score 4
Group I— nano-filled RMGI	8 (40%)	11 (55%)	1 (5%)	0	0
Group II— nanocomposite resin	2 (10%)	1 (5%)	5 (25%)	12 (60%)	0
Group III— Cention N	2 (10%)	8 (40%)	9 (45%)	1 (5%)	0

least stop potential problems that are encountered clinically such as the marginal discoloration and secondary caries resulting from microleakage.⁷

Microleakage that occurs along the boundary of tooth and restoration is the most challenging problem confronted with posterior resin restorations. Several different methods of restoration placement are being tried to minimize the shrinkage associated with polymerization of resins, with an objective to reduce the volume-based shrinkage and, ultimately, the bonded to unbonded restoration surface ratio.⁸ The deformation of the plastic or resin flow that occurs during the polymerization process may moderately compensate for the shrinkage stress that has been created. The plastic deformation that is permanent mostly occurs during the early stages of the process of setting of resin material. With the ensuing process of setting, there is a steady decrease in the contraction and resin flow, resulting in increased stiffness. This kind of low-related compensation is affected by the “C-Factor,” a restoration configuration.⁹

In this study, the maximum microleakage was demonstrated by nanocomposite resin compared to other tooth-colored materials. The results of our study link with the results of studies done in the past such as Derhami et al.,¹⁰ Hilton et al.,¹¹ and Demarco et al.,¹² which established the margins along the gingival surface of a composite-restored class II to be a potentially greater source of microleakage compared to occlusal margins. This could be attributed to the reduced thickness of enamel along the cavosurface margin of the proximal aspect which requires the bonding of restorative materials to a greater amount of dentin; a complex, unreliable substance than enamel. Yet another cause for increased microleakage associated with the gingival margins is the distance of the light source from the restorative material at the base of the proximal box as compared to that at occlusal surfaces.

The property of the restorative material to firmly seal the cavity margins decides its durability.¹³ Thus, the ability of restorative material to reduce the amount of microleakage along the boundary of tooth and restoration is vital in determining its clinical success. Several methods have been used to assess the degree of microleakage and the reliability of restorations alongside the margins. The dye diffusion method is one of the most frequently used methods. Various methods that measure the extent of microleakage have been adapted by studies that attempt to evaluate seal along the margins. These include the use of different dyes, chemical markers, radioactive isotopes, air pressure, bacteria, and electrochemical method. Some of the commonly used dyes are methylene blue, aniline blue, fluorescein, eosin, erythrosine, and Indian ink.¹⁴

Fahmy and Farrag¹⁵ used silorane or methacrylate nano-filled composite to restore primary molars that had class II cavities

in their recent study that aimed to assess the extent amount of microleakage. The methods adapted were an open sandwich, closed sandwich, and total bonding. The total bonding method demonstrated superior marginal sealing when compared with two sandwich techniques. Beznos¹⁶ demonstrated RMGI to not prevent the widespread microleakage along the margins of cervical regions of open sandwich restorations.

Nano-filled RMGI is an innovative development that scientifically combines the profits of a light-cured resin-modified glass ionomer and nano-filler bond technology. Similar to other RMGIs, nano-filled RMGI which is a true RMGI goes through the reactions of both glass ionomer and free radical, and this has been clearly demonstrated by infrared (IR) analysis.¹⁷

This study demonstrated a nano-filled resin-modified glass ionomer to be associated with less microleakage compared to other materials. This finding is the same as that obtained by Abd El Halim and Zaki,¹⁸ who reported the bond along the tooth–restoration interface to be of a higher magnification of the nano-filled RMGI, and they showed an unclear boundary between the margin of the tooth structure and the restoration, proposing the formation of a chemical bond between the tooth and glass-ionomer cement (GIC). The most probable reason for the association of increased microleakage with GIC and RMGIC could be the nonuse of primer with these types of glass ionomers, while nano-filled RMGI has the advantage of the use of primer that has an acidic nature. The primer functions to transform the smear layer and wet the tooth surface sufficiently so as to enable firm bonding between the restorative material and hard tissue.

The lowest microleakage was found to be associated with nano ionomer and was more reliable than the other materials, and adapted significantly well with the tooth structure. When used to restore class V cavities, it demonstrated an improved performance than the other two materials. Nano-ionomer cements have been recommended by Wadenya et al.¹⁹ to be used for regular dental procedures and atraumatic restorative techniques. Upadhyay and Rao²⁰ also reported the lowest microleakage associated with nano-filled resin-modified glass ionomer. The use of nano-filled resin-modified glass ionomer has been recommended for all types of restorations in the primary tooth and been called as “tissue-specific direct tooth repair.” The smaller particle size of nano-ionomer may have provided increased surface area and improved material flow, resulting in enhanced adaptation with tooth interface.

In the current study, Cention N demonstrated less microleakage than nanocomposite resin. Cention N is a direct, tooth-colored restorative material that is used as a basic filling material. The powder contains different glass fillers, initiators, and pigments, while the liquid consists of dimethacrylates and initiators. Cention N displays a superior polymer network density and extent of polymerization over the entire depth of the restoration and this is mainly because of the unique combination of cross-linking methacrylate monomers with a self-cure initiator that is stable and efficient.²¹

In the current study, Cention N demonstrated less microleakage than nanocomposite resin. This result is similar to the study done by Samanta et al.²² stating that Cention N shows a high polymer network density and degree of polymerization over the complete depth of the restoration. It also contains a special isofiller which behaves as a shrinkage stress reliever and reduces the shrinkage force.

The least leakage at the dentin–restoration junction was found with teeth restored with Cention N. This is because of the

tooth–restoration interface that is largely sealed with an acid-resistant, resin–dentin interdiffusion zone, i.e., a hybrid layer. The present study findings are in accordance with that of the study conducted by Lopes²³ and Meshram and Meshram²⁴. The liquid contains four different dimethacrylates, other additives, and initiators. A combination of urethane dimethacrylate (UDMA), an aromatic–aliphatic–UDMA, tricyclodecane–dimethanol dimethacrylate (DCP), and PEG-400DMA cross-links during polymerization to form good long-term stability and strong mechanical properties.

However, as the present study was an *in vitro* study, other *in vivo* studies are required in the future to substantiate these results. The crucial properties such as durability of restoration, strength, and marginal adaptability should be clinically assessed.

CONCLUSION

This study concludes that nano-filled resin-modified glass ionomer showed significantly less microleakage compared to nanocomposite resin and Cention N. In due course, it is important to design *in vivo* studies that would assess microleakage under the factual conditions of the oral environment.

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