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Original Article

Effect of Liner on the Marginal Integrity of Resin Composite Restorations after Selective Caries Excavation: An *In Vitro* Study

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Abstract

Aim: The aim of this study is to evaluate the effect of cavity liner on marginal adaptation and microleakage of resin composite restorations placed with and without selective caries excavation. **Materials and Methods:** Class II cavities were prepared in 64 extracted upper premolar teeth followed by microbiological caries induction to simulate deep carious lesions. Composite restorations were performed: Group 1A: without liner and Group 1B: with resin-modified glass ionomer liner after nonselective caries excavation; Group 2A: without liner and Group 2B: with resin-modified glass ionomer liner after selective caries excavation. Maximum gap thickness was measured using a scanning electron microscope to assess the marginal adaptation, and dye penetration method was used for microleakage assessment. **Results:** There was a statistically significant difference between the groups in maximum gap thickness based on the type of caries excavation ($P < 0.001$) but not for the placement of liner. Selective caries excavation groups had higher values at all margins. Microleakage between the groups was significantly different, both at the occlusal ($P = 0.006$) and gingival margins ($P < 0.001$), with Groups 2A and 2B showing higher microleakage. **Conclusion:** Resin composite restorations performed after selective caries excavation showed decreased marginal integrity and liner application did not contribute to any significant improvement in the same.

Keywords: Composite resin, dental caries, dental cavity lining, dental marginal adaptation

Introduction

Conventional complete caries removal approach in a deep carious lesion leads to decreased remaining dentin thickness or carious exposure of vital pulps, further leading to endodontic treatment.[1,2] In addition, the use of burs and local anesthesia causes pain and discomfort to patients.[3] Hence, based on the current understanding of dental caries, the focus of cavitated lesion therapy has shifted from complete caries removal to maintaining an adequately sealed restoration.[4] Such a restoration deprives the microbes in the demineralized dentin of substrates, reducing the number of microorganisms in the remaining carious lesion, and stimulates deposition of tertiary dentin.[1]

Thus, more conservative approaches to caries tissue removal such as selective caries excavation to soft dentin have been described. In a deep carious lesion, the peripheral enamel and dentin are excavated completely until hard dentin is reached. Caries is excavated in the remaining dentin leaving behind the soft carious dentin such that enough carious tissue has been removed to place a durable restoration, without any iatrogenic pulp exposure. Selective caries excavation is currently recommended for lesions radiographically involving the inner pulpal third or quarter of dentin or lesions with a clinically assessed risk of pulpal exposure.[5]

Although the peripheral seal is vital in the success of the treatment, resin-based composite restorations are known to lose sealing ability over time. Microleakage and marginal

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gap formation occur due to polymerization shrinkage and the stresses associated with it.[6] In addition, reduced bond strength to caries-infected dentin can affect the marginal integrity of the restoration. This can further result in secondary caries and failure of the restoration.[7,8]

The application of cavity liners before restoration has been advocated in deep carious lesions due to their remineralization and cavity disinfection effects.[9] Use of liners also decreases the bulk of the composite, thus decreasing the effects of polymerization shrinkage on the marginal integrity of the restoration.[10] Resin-modified glass ionomer is preferred as liners under resin composite restorations as they chemically bond to tooth structure and has advantages such as high early strength and less moisture sensitivity. It also bonds chemically with resin composite and has a better bond strength to dentin.[10]

The benefits of the use of liners in composite restorations carried out after selective caries excavation protocol are not conclusively established.[11] The combined effects of selective caries removal and use of liners in composite restorations need to be studied further. Hence, the purpose of this study was to evaluate the effect of cavity liner on the marginal integrity of resin composite restorations, placed after the selective and nonselective excavation of dentinal caries teeth *in vitro*, after artificial caries induction. The marginal integrity was measured in terms of marginal adaptation and microleakage. It was hypothesized that the use of resin-modified glass ionomer liners results in better marginal integrity of resin composite restorations placed after selective caries excavation.

Materials and Methods

The study with experimental *in vitro* design was initiated after obtaining clearance from the Institutional Ethics Committee (Protocol ref no. 15098). It was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Sample size

Sample size for assessing microleakage was calculated as 10 in each group, based on the formula $E = N - B - T$ where N is the assumed sample size for each group minus 1; B is the blocking component representing the environmental effects allowed for the design minus 1, and T is the treatment component minus 1. The sample size for assessing marginal adaptation was calculated as 6 in each group; keeping the power of the test as 0.25 with a predetermined Type 1 error 0.8 as per cumulative distribution function.[12] Thus, each group consisted of 16 tooth specimens, of which 10 were used for microleakage evaluation and 6 were used for assessing marginal adaptation.

Sixty-four upper premolars extracted for orthodontic reasons and which were caries free were collected and stored in formalin until the initiation of the study. The teeth were examined under a stereomicroscope (Reichert Star Stereo Zoom, San Francisco, California, USA) for various defects. Teeth with defects, erosions, microcracks, visible stains, developmental anomalies, or restorations were excluded from the study.

Specimen preparation

The contaminants and surface debris of the teeth were removed using an ultrasonic scaler. Standard Class II (mesio-proximal) cavities were prepared, and the measurement of the cavity was standardized using a calibrated periodontal probe [Figure 1]. The teeth were covered with nail varnish leaving the area of cavity preparation uncovered.

Microbiological caries induction

The specimens were sterilized using an autoclave and were transferred aseptically to McCartney bottles containing a cariogenic solution. The solution consisted of 3.7 g of brain–heart infusion (BHI) broth, 2.0 g of sucrose, 1.0 g of glucose, and 0.5 g of yeast extract (Hi-media Laboratories Private Limited, Mumbai, India) for every 100 ml of distilled water, with a pH of 4.0. This solution was autoclaved and 200 µl of 2% *Streptococcus mutans* (ATCC® 25175™, Manassas, Virginia, USA) (108 cfu/ml) was inoculated into this solution. The teeth were immersed in this acidic *S. mutans*-containing solution and incubated at 37°C in a CO_2 incubator (NuAire Inc., Plymouth, Minnesota, USA) for 6 weeks. Every 48 h, the specimens were transferred to a fresh broth culture of *S. mutans*. The viability of the organism was maintained by repeated sub-culture into a fresh BHI broth every 24 h. The *S. mutans* strain was preserved in 20% glycerol and stored at −20°C.[13] At the end of 6 weeks, caries induction was achieved. The walls of the cavity were darker and softer, as felt with a sharp explorer held without pressure.[13] The biofilms covering the teeth were removed with a swab, and the teeth were again autoclaved.

The depth of cavitation achieved after microbiological caries induction was determined by an intra-oral periapical

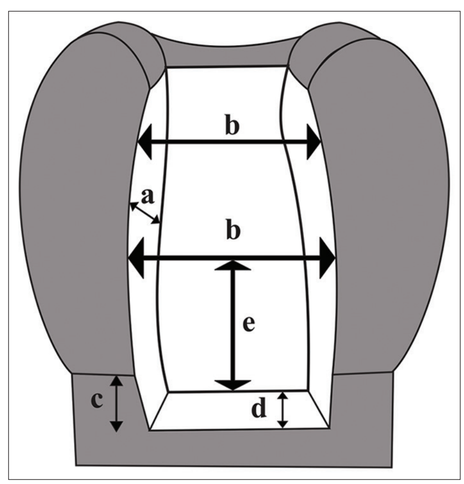

Figure 1: Schematic representation of the cavity preparation. Dimension of cavity preparation design: (a) Depth of occlusal preparation = 2.5 mm (b) Width of occlusal and proximal preparation = 3 mm (c) Location of gingival margin for mesio-proximal box preparation = 1 mm below cementoenamel junction (d) Width of gingival seat = 1.5 mm (e) Depth of axial wall = 3 mm

radiograph. Cavitation was seen extending up to the pulpal one-third or a quarter of dentin in all the teeth, thus confirming them as deep carious lesions.[11]

Caries excavation

The specimens were then randomly divided into two groups, Group 1 and 2 (*n* = 32) and then further sub-divided into two groups each: Groups 1A, 1B; 2A, 2B (*n* = 16).

In Group 1 (Complete caries removal/nonselective caries removal group), caries excavation was performed using a high-speed airotor handpiece (Pana-Air Nakanishi Inc., Kanuma, Japan) and round bur (BR-31, TR-13EF, MANI Medical India Pvt. Ltd., New Delhi, India). Caries was excavated from both enamel and dentin walls until only hard dentin was left behind. In Group 2 (selective caries removal to soft dentin group), caries was excavated from the cavity walls using the airotor and bur as in Group 1, completely on the peripheral enamel and up to hard dentin on the peripheral walls. Caries on the pulpal/axial walls was minimally removed using a spoon excavator, such that a layer of soft carious dentin was left over the pulp, to avoid exposure of the pulp chamber.[11]

The caries excavation in both the groups was carried out by a single operator and the extent of caries excavation was verified by a second experienced operator. Any tooth with exposure of the pulp chamber during caries excavation was to be discarded. However, no specimen was lost to inadvertent pulp exposure during caries excavation.

Restorations

The teeth in both the groups were further divided into two sub-groups each: Group 1A and Group 1B: comprised restorations done after complete caries excavation. In Group 1A (*n* = 16), etching (Scotch Bond, 3M ESPE, St. Paul, Minnesota, USA) and bonding (Adaper™ Single Bond 2, 3M ESPE, St. Paul, Minnesota, USA) were carried out as per the manufacturer's instructions. Universal Tofflemaire matrix with retainer was applied and nanocomposite (Filtek Z350 Universal Restorative, Shades A2B and A2E, 3M ESPE, St. Paul, Minnesota, USA) was placed in an oblique increment technique of 1.5-mm thickness with a plastic filling instrument. Each increment was light-cured (Bluedent LED Smart Express, Plovdiv, Bulgaria) for 30 s. In Group 1B (*n* = 16), 10% polyacrylic acid (GC Dentin Conditioner, GC Dental Corp, Tokyo, Japan) was applied using a cotton pellet as per the manufacturer's instructions. Resin-modified glass ionomer liner (Fuji Lining LC Paste Pak, GC Dental Corp, Tokyo, Japan) was placed (1-mm thickness) extending to the full width of pulpal floor, gingival seat, axial wall and light cured for 20 s.[14] The uniform thickness of liner was verified using a calibrated periodontal probe by subtraction method. The remaining walls were etched, the adhesive was applied to the etched surface as well as on the liner, and the nanocomposite restoration was carried out as described in Group 1A. A minimum intensity of 400 mW/cm^2 was maintained during light-curing which was verified with a radiometer (Demetron 100, Demetron Research Corp, Danbury, Connecticut, USA.), before curing of each specimen.

In Groups 2A (*n* = 16) and 2B (*n* = 16), the carious dentin was sealed under composite restorations. Restoration in these groups was carried out similarly as described in Groups 1A and 1B, respectively. Following the completion of the restoration, finishing was done using superfine diamond burs (TR-13EF, MANI Medical India Pvt. Ltd., New Delhi, India) and polished with a series of polishing disks (Sof-lex Disks, 3M ESPE, St. Paul, Minnesota, USA).

The teeth were subjected to thermocycling, to simulate oral conditions, which consisted of 500 cycles in water baths maintained at 5°C, 37°C, and 55°C with a dwell time of 15 s.[15]

Microleakage assessment

Ten teeth were randomly selected from each group, and nail varnish was removed from 1 mm wide area around the restoration margins. Then, the teeth were immersed in 0.2% basic fuchsin dye for 48 h. The specimens were rinsed with saline for 1 h and dried. Teeth were sectioned mesiodistally using low speed, water-cooled carborundum disc (Ivoclar Vivadent Marketing Pvt. Ltd., Mumbai, India). These teeth sections were observed under a compound microscope (Olympus CH20i, Tokyo, Japan) using the incident light. The extent of dye penetration was measured from both the occlusal and gingival margins. Both the sections were examined, and the highest score was considered. Two examiners, who were blinded to the group allocation, did the scoring independently. Inter-examiner reliability was calculated (Cronbach's alpha value of 0.868). The final scoring was arrived at after discussion and consensus, in case of a difference in scoring.

The scoring criteria were as follows.[16]

Occlusal margin

- Score 0 = No evidence of dye penetration
- Score 1 = Superficial penetration of dye at the margin but less than one-third of the height of the distal wall
- Score 2 = Penetration along the margin beyond one-third of the height of the distal wall, up to the pulpal floor
- Score 3 = Penetration along the pulpal floor.

Gingival margin

- Score 0 = No evidence of dye penetration
- Score 1 = Superficial penetration of dye at the margin but less than one-third of the gingival width
- Score 2 = Penetration along the margin beyond one-third of the gingival width up to the axial wall
- Score 3 = Penetration along the axial wall.

Assessment of restoration margins

Six specimens were randomly selected from each group. These teeth were sectioned mesiodistally to analyze the marginal adaptation of the composite restorations using a scanning electron microscope (SEM). The specimens were dried in a hot air oven for 15 min to make them moisture free and sputtered with pure gold using an ion sputtering unit of

10 mA (Joel JFC-1600 Autofine Coater, JEOL Datum Solution Business Operations, Tokyo, Japan) for 100 s. The stubs were loaded in a special tray and placed in a vacuum chamber of the SEM (JEOL JSM-638 OLA, JEOL Ltd, Tokyo, Japan). The surface was scanned and observed under different magnifications (×200 and ×500). The marginal adaptation of the restorations was assessed by analyzing the gaps in the Class II restorations along the following margins:

A. Internal adaptation along the axial wall (dentin and composite/liner interface) (axial margin)
B. Marginal adaptation with cervical dentin (dentin and composite/liner interface) (gingival margin)
C. Marginal adaptation with proximal enamel (enamel and composite interface) (proximal margin)
D. Marginal adaptation with occlusal enamel (enamel and composite interface) (occlusal margin).

Illustrative images of SEM images at various margins or interfaces are shown in Figure 2. The marginal adaptation was classified as a "gapped margin" if the interface had gaps more than 2 µm wide.[6] The maximum gap thickness for each margin was noted. The length of each gap was noted and a sum of the total gapped margin length was obtained. Further, for each margin, the total length of the margin was measured using an image software (Image J software, NIH, USA). The extent of gapped margin for each margin of restoration was expressed as percentage length of gapped margin in relation to the total margin length, similar to the assessment of percentage length of continuous margin by Campos *et al*.[17] All the readings were taken by a single operator who was blinded to the group allocation. Both the sections were used in the SEM analysis, and an average was taken.

Statistical analysis

Data were analyzed using Statistical Package for Social Science, Version 17 (SPSS Inc., Chicago IL, USA). Descriptive statistics were obtained. The Chi-square test was applied to check the difference in microleakage between the groups. One-way ANOVA and *post hoc* (Tukey's) tests were applied for intergroup comparison of maximum gap thickness and percentage length of gapped margin at the four margins. Repeated measures of ANOVA were used to compare the marginal adaptation between the groups taking into account three factors as follows: type of caries excavation, application of resin-modified glass ionomer liner, and type of margin at a time. The level of significance was $P < 0.05$.

Results

Microleakage assessment

The difference in microleakage between the groups was statistically significant both at the occlusal and gingival margin [Table 1]. At the occlusal margin, most specimens belonging to restorations performed after selective caries excavation (Groups 2A and 2B) exhibited microleakage along the pulpal floor or along the axial wall, whereas no specimen from Groups 1A and 1B (restorations performed after complete caries excavation) exhibited a score of three (microleakage along the pulpal floor). At the gingival margin also, all specimens in Group 2A and 2B showed microleakage extending beyond one-third of the gingival width and along the axial wall, whereas specimens in Groups 1A and 1B had lower scores of microleakage.

Marginal adaptation

Table 2 represents the mean maximum gap thickness and percentage length of gapped margin measured at the four margins. One-way ANOVA test revealed a statistically significant difference between the groups in terms of maximum gap thickness and percentage length of gapped margins with groups where selective caries excavation was

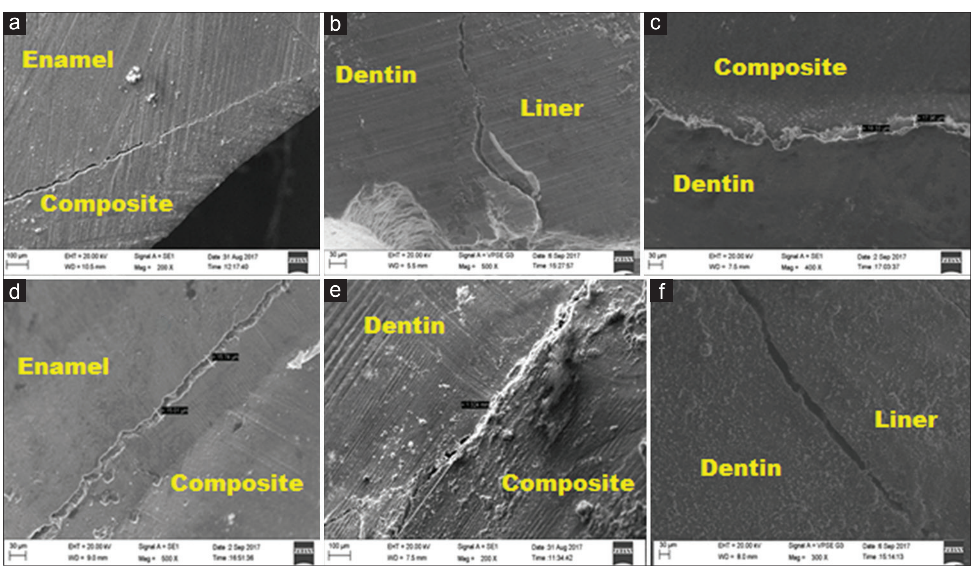

Figure 2: Representative scanning electron microscope image at × 200. (a-c) Marginal adaptation in restorations performed after complete caries excavation; (d-f) Marginal adaptation in restorations after selective caries excavation at the following interfaces: (a) Enamel and composite. (b) Dentin and liner. (c) Composite and dentin. (d) Enamel and composite. (e) Dentin and composite. (f) Dentin and liner

Table 1: Comparison of the frequency distribution of microleakage scores between the groups

Margin	Dye penetration score	Group 1A (%)	Group 2A (%)	Group 1B (%)	Group 2B (%)	χ^2	*P*
Occlusal margin	Score 1	4 (40)	0	4 (40)	0	18.18	0.006
	Score 2	6 (60)	5 (50)	6 (60)	5 (50)		
	Score 3	0	5 (50)	0	5 (50)		
	Total	10 (100)	10 (100)	10 (100)	10 (100)		
Gingival margin	Score 1	5 (50)	0	0	0	24.70	<0.001
	Score 2	3 (30)	5 (50)	10 (100)	6 (60)		
	Score 3	2 (20)	5 (50)	0	4 (40)		
	Total	10 (100)	10 (100)	10 (100)	10 (100)		

Table 2: Comparison of marginal adaptation at various margins (one-way ANOVA)

	Group	Mean±SD			
		Axial	Gingival	Occlusal	Proximal
Maximum gap thickness (µm)	1A	12.63±0.89	11.78±0.69	6.15±1.24	11.80±1.48
	2A	26.36±0.88	30.41±2.44	20.97±3.25	15.00±2.23
	1B	16.83±1.94	16.61±2.57	26.37±7.03	10.34±0.82
	2B	21.18±3.26	21.06±2.99	14.20±2.54	18.31±3.23
	P	<0.001	<0.001	<0.001	<0.001
Percentage length of gapped margin (%)	1A	18.46±2.78	24.00±5.04	23.66±1.93	23.32±1.13
	2A	50.07±5.61	52.64±3.63	48.78±3.42	48.16±4.02
	1B	15.20±1.60	17.48±2.48	22.41±2.90	16.08±1.34
	2B	50.38±4.06	51.69±4.87	48.68±1.73	46.16±4.09
	P	<0.001	<0.001	<0.001	<0.001

SD – Standard deviation

performed (Group 2A and 2B) showing higher values at all margins.

Further, the specimens of Group 1A restored without resin-modified glass ionomer liner, after complete caries excavation, were found to have the lowest values for maximum gap thickness [Table 2]. Least values were seen along the proximal margin, and higher values were seen along the gingival and axial margins for all the groups. *Post hoc* test (Tukey's) was applied for intergroup comparison of maximum gap thickness. The difference in the maximum gap thickness measured at the four margins was statistically significant between all the four groups except at the occlusal and proximal margins [Table 3].

Repeated measures of ANOVA revealed a statistically significant difference between the groups in terms of maximum gap thickness when the type of caries excavation ($P < 0.001$, $F = 10.20$) and the type of margin ($P < 0.001$, $F = 0.76$) were taken into account. There was no statistically significant difference between the groups irrespective of liner placement during composite restoration ($F = 0.31.44$, $P = 0.386$).

Groups 1A and 1B with complete caries excavation had lower values of percentage length of gapped margins with least values in specimens restored using resin-modified glass ionomer liner and the difference was significant. *Post hoc* test (Tukey's) revealed that the difference in the percentage length of gaps at the four margins was statistically significant, except between

Table 3: Intergroup comparison of marginal adaptation at various margins (*post hoc* Tukey test)

Variable	Margin	Significance
Maximum gap thickness	Axial	1A<1B<2B<2A
	Gingival	1A<1B<2B<2A
	Occlusal	1A<2B<2A=1B
	Proximal	1B<2A; 1A=2A; 1A<2B; 2A=2B; 1B<2B; 1B=1A
Percentage length of gapped margin	Axial	1B=1A<2A=2B
	Gingival	1B=1A<2B=2A
	Occlusal	1B=1A<2B=2A
	Proximal	1B<1A<2B=2A

Groups 1A and 1B, 2A and 2B at the axial, gingival, and occlusal margins [Table 3].

Repeated measures of ANOVA showed that there was a statistically significant difference between the percentage length of the gapped margin of the groups when the type of caries excavation ($P < 0.001$, $F = 199.36$) was considered.

Discussion

In this study, a microbiological caries induction protocol was followed to induce carious lesions in the extracted teeth, which results in softening of dentin and collagen degradation similar to that of natural caries lesions. The lesions are similar to natural dentinal caries in form, color, and texture.[18] The soft carious dentin so obtained can be reliably utilized to study the bonding characteristics of restorative materials after selective excavation protocol.[13] Previous *in vitro* models used chemical methods like pH-cycling model, which cause only superficial dentin demineralization.[18] In the current study, microbiological caries induction was done after standard cavity preparation, which resulted in the formation of dentinal lesions extending to pulpal one-third of dentin, where selective caries excavation is indicated to reduce the risk of pulp exposure.[11]

The marginal adaptation of the restorations was assessed quantitatively, using SEM in this study. Another method involves taking impressions and fabricating replicas for SEM analysis. However, the deterioration of silicone replicas can cause defects in the impression leading to errors in SEM images.[19] The maximum thickness of the gaps and percentage gap length were measured as a percentage of margins with gaps itself may not be a good indicator of susceptibility to marginal caries.[20] An acceptable correlation between microleakage assessment by dye penetration method and marginal adaptation evaluation under SEM has been found, and hence, both were used to enhance validity.[21] The extent of dye penetration is a surrogate marker for the extent of ingress of bacteria and oral fluids at the margins.[20]

A significant difference in the marginal integrity was noted between the complete caries excavation and selective caries excavation groups. This is in contrast to a previous study,[7] where the placement of restoration over demineralized dentin did not affect the marginal integrity of the restoration. This could be because the study utilized a pH-cycling model to induce a more superficial demineralization of dentin. However, enamel margins showed better adaptation compared to dentinal margins,[7] which are similar to the results of this study. Earlier studies reported inferior bond strength of adhesives to carious dentin. Bonding to soft carious dentin results in low bond strength due to its low tensile strength, low modulus of elasticity along with its inherently wet nature.[22,23] Formation of an "altered hybrid layer" between composite and carious dentin contributes to the difference in bonding to carious dentin when compared to hard dentin.[24] The carious dentin allows only superficial monomer penetration so that tag formation does not occur in many dentinal tubules, resulting in shorter resin tags, cracks, and pores in the hybrid layer.[24,25] This explains the inferior marginal adaptation in selective caries excavation groups.

No significant difference was noted among the groups based on the type of liner used. This is in contrast to the previous studies[6,26] but in agreement with the study by Vilkinis *et al*.[27] In Class II cavity, gap formations occur in the axial wall and gingival wall due to the inadequate adaptation of the liner using hand instruments. In spite of the excellent bond of glass ionomer to dentin, the polymerization shrinkage in the overlying composite induces stresses leading to crack development in the liner cement and dentin.[28] The bonding of glass ionomer cement to carious dentin is affected by the difference in permeability and water content when compared with normal dentin. The demineralized dentin has lower mineral content resulting in lower calcium and phosphate ions, thus leading to the weaker bond of glass ionomer with dentin.[29]

An *in vitro* study design enables the clinician to understand the properties of dental materials under controlled conditions with low risk of bias, thus complimenting clinical research.[8,30] However, there are limitations owing to the *in vitro* study design. Microleakage analysis by dye penetration may over-estimate the lack of marginal integrity.[21] There is only moderate evidence of correlation of clinical parameters such as marginal staining and secondary caries with marginal adaption using SEM.[20,21] Hence, the study results should be generalized to clinical situations with caution. However, considering the findings of the present study and the lack of decisive clinical evidence favoring better prognosis of composite restorations after selective caries excavation over the long term,[1] the decision to use selective caries excavation in deep carious lesion requires further evidence.

Conclusion

Within the limitations of the present study, it can be concluded that:

1. Resin composite restoration performed after selective caries excavation results in decreased marginal integrity when compared to restoration after nonselective removal to hard dentin
2. Resin-modified glass ionomer liner application may not contribute to any significant improvement in the marginal integrity of restorations after selective caries excavation.

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Conflicts of interest

There are no conflicts of interest.

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