

Original Article

The effect of mechanical load cycling and polishing time on microleakage of class V glass-ionomer and composite restorations: A scanning electron microscopy evaluation

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ABSTRACT

Background: Microleakage is one of the challenging concerns in direct filling restorations. Understanding of its related factors is important in clinical practice. The aim of this study was scanning electron microscopy (SEM) evaluation of marginal integrity in three types of tooth-colored restorative materials in class V cavity preparations and the effect of load cycling and polishing time on the microleakage.

Materials and Methods: In this *in vitro* experimental study, class V cavity preparations were prepared on the buccal and lingual surfaces of 60 bovine incisors. The specimens were divided into three groups each containing 20 teeth: group 1: Filtek Z350, Group 2: Fuji IX/G Coat Plus, Group 3: Fuji II LC/GC varnish. In each group, 2 subgroups ($n = 20$) were established based on finishing time (immediate or delayed by 24 h). All specimens were thermocycled ($\times 2,000, 5-50^{\circ}\text{C}$). In each subgroup, half of the teeth were load cycled. Epoxy resin replicas of 24 specimens were evaluated under field emission-SEM and interfacial gaps were measured. All teeth were then immersed in 0.5% basic fuchsin dye for 24 h, sectioned and observed under stereomicroscope. Data were analyzed with Kruskal-Wallis' test and Mann-Whitney *U* test and a comparison between incisal and cervical microleakage was made with Wilcoxon test. $P < 0.05$ was considered as significant.

Results: Load cycling and filling material had a significant effect on microleakage, but polishing time did not. Cervical microleakage in Z350/load cycle/immediate polish and Fuji IX/load cycle/immediate or delayed polish and Fuji IX/no load cycle/immediate polish were significantly higher than incisal microleakage.

Conclusion: It was concluded that the cervical sealing ability of Fuji IX under load cycling was better than Fuji II LC. Under load cycling and immediate polishing Z350 showed better marginal integrity than both Fuji II LC and Fuji IX. The immediate polishing didn't cause a statistically significant increase in microleakage of evaluated tooth-colored class V restorations.

Key Words: Composite resins, dental leakage, dental polishing, glass ionomer cements

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INTRODUCTION

Microleakage is the most important factor in cervical restorations.^[1] Microleakage at the tooth/restoration interface may result in the breakdown of the margins of

the restorations, the development of secondary caries at the tooth/restoration interface or even post-operative sensitivity and pulp pathology.^[2] The oral environment (including occlusal forces and temperature variation) and differences between the physical properties of teeth and restorative materials can contribute to microleakage.^[3] Restorations with margins in gingival region and especially located below the cement-enamel junction exhibit still difficulties in achieving properly sealed restorative margins.^[4,5]

Glass-ionomer cement (GIC) has been recommended to improve dentine sealing performance because of its

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ability to form a chemical bond to both enamel and dentine.^[6,7]

Recently a new concept of restorative system, which combines a GIC (Fuji IX) and a post coating material (G coat plus) was proposed. It has claims about improved physical properties, faster finishing and reduced microleakage after nanofilled varnish application.^[8] Literature have shown that occlusal stress concentrates at the cervical region.^[9,10] Lee and Eakle recommended that the tensile stresses induced in teeth subjected to a masticatory load may partly be responsible for the development of cervical lesions.^[11] Although many authors have investigated the mechanical forces that influence in class V microleakage,^[12-14] the load cycling influence in marginal integrity remains unclear. The polishing period is another factor that may influence seal ability around a cervical restoration. In previous studies, polishing after storage in water for 1 day were proposed to improve gap formation for cervical restorations of a resin modified (RM)-GIC or a conventional GIC.^[15-18] Although, Magni *et al.* reported immediate polishing did not increase the gaps of coated Fuji IX restorations.^[8] Irie *et al.* mentioned that after 1 day storage, Fuji IX performed better than its conventional GIC (Fuji II).^[19]

The aim of this study was scanning electron microscopy (SEM) evaluation of marginal integrity in three types of tooth-colored restorative materials (Z350, Fuji IX, Fuji II LC) in class V cavity preparations and the effect of load cycling and polishing time on the microleakage.

MATERIALS AND METHODS

In this *in vitro* experimental study, 60 intact extracted bovine incisors were used. The teeth were cleaned, disinfected by soaking in 0.5% chloramine T for 1 week and stored in saline at 4°C until use. Class V cavities were prepared in the buccal and lingual surfaces of all teeth with the gingival margin 1 mm below the cemento-enamel junction. The preparations were standardized at 3 mm long, 3 mm wide and 1 mm deep in dentin with butt-joint margins. A high-speed hand piece with water spray was used with a diamond round bur (DIAMIR, Italy, FGS 001018) to prepare cavities.

The occlusal margins were placed in enamel and the gingival margins in dentin/cementum. The specimens

were kept hydrated in distilled water throughout all the steps. Cavities were randomly divided into three groups of 40 specimens each. In Group 1, 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, Dental Products, USA) was applied to the enamel surface for 30 s and to the dentin surface for 15 s and then rinsed with water spray for 20 s and excess water removed with a light air stream so as to avoid desiccation. Two layer of Adper Single Bond (3M ESPE, Products, St Paul, MN, USA) was applied according to manufacturer's instructions and cured with halogen light (Optilux 501, QTH, Kerr, USA) for 10 s. Then the cavities were restored with Z350 (3MESPE, Dental Products, USA) in three increments and each increment cured for 40 s.

In Group 2, the cavity conditioner (GC Corp, Tokyo, Japan) was applied for 20 s and then rinsed with water. The cavities were restored with GIC (Fuji IX, GC Corp, Tokyo, Japan). The restored teeth were then coated with a coating (G Coat Plus, GC Corp, Tokyo, Japan) and light cured for 20 s.

In Group 3, the cavity conditioner (GC Corp, Tokyo, Japan) was applied for 20 s and then rinsed with water. The cavities were restored with RM-GI (Fuji II LC, GC Corp, Tokyo, Japan) and then the restorations were light-cured for 20 s. The restored teeth were then coated with a varnish (Fuji Varnish, GC Corp, Tokyo, Japan).

All specimens were stored in an incubator (Malek teb, Iran) at 37°C and 100% relative humidity.

In each group, half of the specimens ($n = 20$) were finished immediately using super-fine grit diamond bur (DIAMIR, FGSF 273012, Italy) (15 s in each margin) and polished using abrasive points (Silicone Mide, Shofu, Kyoto, Japan) (15 s in each margin) and the rest were finished/polished after 24 h.

All specimens were thermocycled ($\times 2,000$, 5-50°C) with a dwell time of 30 s in each bath and a transfer time of 10 s between each bath. In each sub group, half of the teeth were load cycled. Load cycling was performed by chewing stimulator (SD Mekanotronic, Germany) equipped with an oral environmental chamber (artificial mouth) [Figure 1]. This system is capable of simulating the 3-dimensional motion of the mandible during chewing with a 2-dimensional movement and has been previously described.^[20,21] Chewing stroke details were as follow: stroke length, 0.8 cm; frequency, 2 Hz; programmed load for the load control part of the stroke, 100 N. Total load

cycling time was approximately 7 h, with a rate of 2 Hz (50,000 cycles). Wet paper towels were placed around the samples and moistened every 30 min to keep tooth tissues from dehydrating.

Chewing strokes were applied in 2 stages

Through the buccal incline of the tooth, 25,000 cycles were first performed in the lingual-buccal direction, then the tooth was rotated through 180° and 25,000 more chewing cycles were again applied through the lingual incline in the buccolingual direction [Figures 2 and 3].

Field emission (FE)-SEM replicas preparation

An impression (Permadyne Garant; 3M ESPE) was taken of 24 specimens (2 randomly selected specimens of each subgroup) and Epoxy resin replicas (Epothin, Buehler Ltd., Lake Bluff, IL) were obtained.

Each replica was then mounted on a metallic stub, gold-sputtered (Polaron Range SC7620; Quorum Technology, Newhaven, UK), and observed under a FE-SEM (Hitachi s-4160, Japan) at $\times 1,0000$ and interfacial gaps were measured [Figure 4]. The total length of the interfacial gaps was expressed as a percentage of the length of the whole restoration margins.

Microleakage test

To prevent dye penetration in areas other than the exposed margins, the teeth were sealed with 3 layer of nail varnish within 1 mm of the restoration margins. The teeth apices were sealed with a light-cured composite. Finally, the teeth were immersed in 0.5% basic fuchsin dye (Labtron, Tehran, Iran) for 24 h.

Before sectioning, the teeth were thoroughly washed with water to remove the superficial dye. After embedding in acrylic resin, three bucco-lingual sections



Figure 1: Load cycling was performed by chewing stimulator (SD Mekanotronik, Germany) equipped with an oral environmental chamber

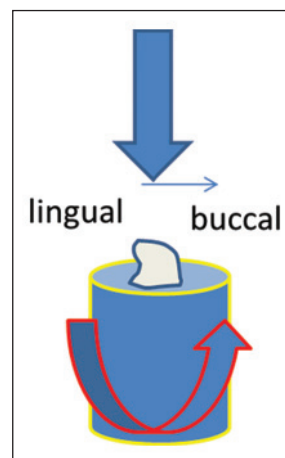


Figure 2: 25,000 cycles were performed in lingual-buccal direction of the tooth, then the tooth was rotated through 180°

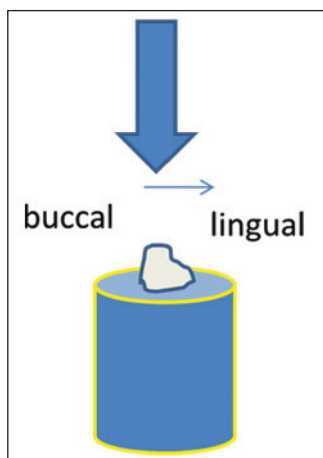


Figure 3: 25,000 more chewing cycles were again applied in the buccolingual direction

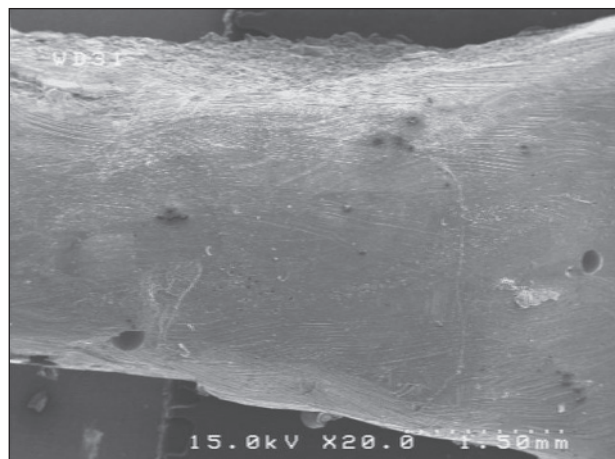


Figure 4a: Field emission scanning electron microscopy micrograph of a replica ($\times 20$)

(mesial, middle and distal) were obtained from each specimen through cutting in a bucco-lingual direction with a diamond saw mounted in a cutting machine (Presi, Mecatome, T 201 A, France) under water cooling.

Each section was then inspected under a stereomicroscope (Nikon 800, Tokyo, Japan) at $\times 10$, $\times 40$ magnifications.

The staining along the tooth restoration interface was recorded according to the following criteria:

- 0 – No evidence of dye penetration at tooth/restoration interface.
- 1 – Dye penetration along the interface half the depth of the cavity.
- 2 – Dye penetration to full depth of the cavity but not involving the axial wall.
- 3 – Dye penetration along the axial wall [Figure 5].

Statistical analysis

The software Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data were analyzed using Kruskal-Wallis' test and Mann-Whitney *U* test. Wilcoxon matched pair test was used to compare the occlusal and cervical leakage of each specimen. $P < 0.05$ was considered as significant.

RESULTS

Polishing time effect

Occlusal and cervical microleakage was not affected by polishing time in none of the three restorative materials apart from loading status [Table 1].

Mechanical load cycling effect

The mechanical load cycling caused a statistically significant increase in cervical microleakage of Fuji

IX/immediate polish and Fuji II LC/immediate or delayed polish and in occlusal microleakage of Fuji II LC/immediate polish [Table 2].

Margin effect

Cervical microleakage in Z350/load cycle/immediate polish and Fuji IX/load cycle/immediate or delayed polish and Fuji IX/no load cycle/immediate polish were significantly higher than occlusal microleakage [Table 3].

Filling material effect

Occlusal microleakage among these materials under load cycling and immediate polish was significantly different. (Fuji II LC > Fuji IX > Z350) [Table 4].

Cervical microleakage among these materials under load cycling and immediate or delayed polish was significantly different. (Fuji II LC > Fuji IX > Z350) [Table 4].

Table 5 summarizes the interfacial gap formation observed by FE-SEM. Due to the limited sample size ($n = 24$), the statistical analysis of the data was not performed.

DISCUSSION

Bovine teeth were used in the present study because the studies in the literature have disclosed comparable results mainly to the human teeth.^[22,23] Moreover, Kubo *et al.* offered that seems to be no differences in stress distribution between bovine and human incisors under load cycling.^[24]

Kubo *et al.* used repeated bucco-lingual loading and suggested that bucco-lingual loading could possibly generate greater tensile stress at the adhesive interface than axial loading; which may result in bond degradation. The authors attributed this fact to a flexural load incidence to the teeth.^[24] However, in the present study, since the teeth were fixed to a rigid

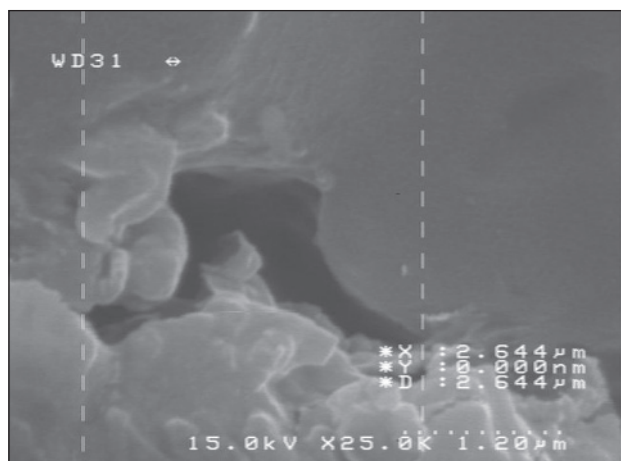


Figure 4b: Field emission scanning electron microscopy micrograph of the same replica ($\times 10,000$)

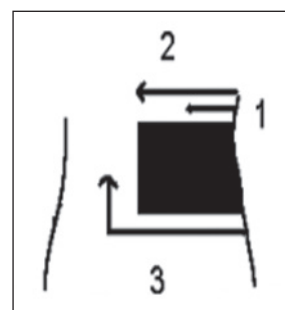


Figure 5: The dye penetration depth along the occlusal and cervical cavity wall was scored from 0 to 3

Table 1: Effect of polishing time on microleakage levels of occlusal and cervical margins (Mann-Whitney *U* test)

Margin	Filling materials	Loading status	Polishing time	<i>n</i>	Mean rank	<i>P</i> value
Occlusal margin	Z350	Cycle	Immediate polish	20	19.30	0.529
			Delayed polish	20	21.70	
	Fuji IX		Immediate polish	20	20.62	0.947
			Delayed polish	20	20.25	
	Fuji II LC		Immediate polish	20	21.65	0.547
			Delayed polish	20	19.35	
	Z350	No cycle	Immediate polish	20	20.45	0.989
			Delayed polish	20	20.55	
	Fuji IX		Immediate polish	20	20.62	0.947
			Delayed polish	20	20.38	
	Fuji II IC		Immediate polish	20	20.70	0.925
			Delayed polish	20	20.30	
Cervical margin	Z350	Cycle	Immediate polish	20	20.95	0.82
			Delayed polish	20	20.05	
	Fuji IX		Immediate polish	20	20.75	0.904
			Delayed polish	20	20.25	
	Fuji II IC		Immediate polish	20	20.50	1
			Delayed polish	20	20.50	
	Z350	No cycle	Immediate polish	20	20.08	0.82
			Delayed polish	20	20.92	
	Fuji IX		Immediate polish	20	21.10	0.758
			Delayed polish	20	19.90	
	Fuji II IC		Immediate polish	20	20.68	0.925
			Delayed polish	20	20.32	

Table 2: Effect of loading status on microleakage levels of occlusal and cervical margins (Mann-Whitney *U* test)

Margin	Filling materials	Polishing time	Loading status	<i>n</i>	Mean rank	<i>P</i> value
Occlusal margin	Z350	Immediate polish	Cycle	20	19.70	0.678
			Non-cycle	20	21.30	
	Fuji IX	Immediate polish	Cycle	20	21.85	0.478
			Non-cycle	20	19.15	
	Fuji II LC	Immediate polish	Cycle	20	24.45	0.033 ^a
			Non-cycle	20	16.55	
	Z350	Delayed polish	Cycle	20	20.80	0.883
			Non-cycle	20	20.20	
	Fuji IX	Delayed polish	Cycle	20	21.88	0.461
			Non-cycle	20	19.12	
	Fuji II IC	Delayed polish	Cycle	20	23.30	0.134
			Non-cycle	20	17.70	
Cervical margin	Z350	Immediate polish	Cycle	20	21.35	0.659
			Non-cycle	20	19.65	
	Fuji IX	Immediate polish	Cycle	20	24.35	0.038 ^b
			Non-cycle	20	16.65	
	Fuji II IC	Immediate polish	Cycle	20	24.82	0.018 ^c
			Non-cycle	20	16.18	
	Z350	Delayed polish	Cycle	20	20.35	0.947
			Non-cycle	20	20.65	
	Fuji IX	Delayed polish	Cycle	20	23.75	0.081
			Non-cycle	20	17.25	
	Fuji II IC	Delayed polish	Cycle	20	24.72	0.021 ^d
			Non-cycle	20	16.28	

^{a,b,c,d}Were significantly different (*P* < 0.05)

Table 3: Wilcoxon test results for comparing microleakage levels of occlusal and cervical margins

Filling materials	Polishing time	Loading status	Margin	n	P value
Z350	Immediate polish	Cycle	Occlusal	20	0.02 ^a
			Cervical	20	
	Immediate polish	Non-cycle	Occlusal	20	0.557
			Cervical	20	
Delayed polish	Cycle	Occlusal	20	0.916	
		Cervical	20		
Delayed polish	Non-cycle	Occlusal	20	0.13	
		Cervical	20		
Fuji IX	Immediate polish	Cycle	Occlusal	20	0.003 ^b
			Cervical	20	
	Immediate polish	Non-cycle	Occlusal	20	0.025 ^c
			Cervical	20	
Delayed polish	Cycle	Occlusal	20	0.001 ^b	
		Cervical	20		
Delayed polish	Non-cycle	Occlusal	20	0.271	
		Cervical	20		
Fuji II LC	Immediate polish	Cycle	Occlusal	20	0.342
			Cervical	20	
	Immediate polish	Non-cycle	Occlusal	20	0.66
			Cervical	20	
Delayed polish	Cycle	Occlusal	20	0.039 ^d	
		Cervical	20		
Delayed polish	Non-cycle	Occlusal	20	0.714	
		Cervical	20		

^{a,b,c,d}Were significantly different ($P < 0.05$)

base, during the compressive load, the teeth were indirectly submitted to a flexural load.^[25]

Mechanical load cycling effect

In the present study, occlusal and cervical microleakage of Z350/single bond was not affected by mechanical load cycling in none of the polishing time. These results are in agreement with other studies,^[25,26] although some reports showed increased microleakage of resin composite restorations under load cycling.^[13,27] These differences found in *in vitro* studies, can be related to differences in tested materials, load magnitude and its application method, cavity preparation and/or evaluation technique. Z350 is formulated using both nanoparticle and nanocluster fillers (zirconia/silica fillers). The “nanoclusters” provided a distinct reinforcing mechanism compared with the microhybrid, microfill systems resulting in significant improvements to the strength and reliability. Silane infiltration within interstices of the nanoclusters may modify the response to loading induced stress, thereby enhancing damage tolerance and providing the potential for improved clinical performance.^[28]

Table 4: Effect of filling material on microleakage levels of occlusal and cervical margins (Kruskal-Wallis test)

Margin	Polishing time	Loading status	Filling materials	n	Mean rank	P value	
Occlusal margin	Immediate polish	Cycle	Z350	20	20.60	0.001 ^a	
			Fuji IX	20	30.95		
			Fuji II LC	20	39.95		
		No cycle	Z350	20	28.05		0.578
			Fuji IX	20	30.58		
			Fuji II LC	20	32.88		
	Delayed polish	Cycle	Z350	20	24.65	0.059	
			Fuji IX	20	30.12		
			Fuji II LC	20	36.72		
		No cycle	Z350	20	28.15		0.595
			Fuji IX	20	30.50		
			Fuji II LC	20	32.85		
Cervical margin	Immediate polish	Cycle	Z350	20	19.78	0.002 ^b	
			Fuji IX	20	35.52		
			Fuji II LC	20	36.20		
		No cycle	Z350	20	26.35		0.341
			Fuji IX	20	33.40		
			Fuji II LC	20	31.75		
	Delayed polish	Cycle	Z350	20	20.40	0.004 ^c	
			Fuji IX	20	34.72		
			Fuji II LC	20	36.38		
		No cycle	Z350	20	28.18		0.709
			Fuji IX	20	31.25		
			Fuji II LC	20	32.08		

^{a,b,c}Were significantly different ($P < 0.05$)

Table 5: Results of interfacial gap formation observed by FESEM

Filling materials	Polishing time	Loading status	Interfacial gaps of two specimens of each subgroups	
Z350	Immediate polish	Cycle	6.861	1.534
Z350	Immediate polish	No cycle	0	0
Z350	Delayed polish	Cycle	1.351	2.67
Z350	Delayed polish	No cycle	0.281	0
Fuji IX	Immediate polish	Cycle	1.647	8.167
Fuji IX	Immediate polish	No cycle	0	0.71
Fuji IX	Delayed polish	Cycle	0.84	0
Fuji IX	Delayed polish	No cycle	0	0.371
Fuji II LC	Immediate polish	Cycle	0.01	1.712
Fuji II LC	Immediate polish	No cycle	0.02	0.55
Fuji II LC	Delayed polish	Cycle	1.579	2.718
Fuji II LC	Delayed polish	No cycle	0	0.37

FESEM: Field emission scanning electron microscopy

In our study, the mechanical load cycling caused a statistically significant increase in cervical microleakage of Fuji IX when polished immediately, which is in conflict with an earlier study.^[29] This result is probably due to higher mineral content of enamel

than dentin, which explain better sealing ability of Fuji IX in enamel than in dentin. Also, conventional GICs develop adhesion to dentin over time. Ichim *et al.* revealed that the cervical margin concentrates higher tensile stresses and hence this area is the most likely potential failure site of the filling.^[30]

The mechanical load cycling caused a statistically significant increase in cervical microleakage of Fuji II LC when polished immediately or after storage in water for 1 day. Also, the load cycling caused a significant increase in occlusal microleakage of Fuji II LC when polished immediately. These results are in agreement with clinical studies.^[14,31] Under highly oblique forces the tensile stresses on the occlusal margin of the restoration are lower than those on the cervical interface and hence a crack has a lower probability to initiate at this point. However, crazing enamel can be associated with the use of RM-GIC.

Margin effect

Cervical microleakage in Z350 under load cycling when polished immediately was significantly higher than occlusal microleakage. These results were in agreement with earlier studies.^[18,32] Other reports did not show a significant difference between cervical and occlusal microleakage of resin composites.^[33,34] These differences in studies can be related to differences in tested resin composites and load direction.

In our study, there were no statistically significant differences between the occlusal and cervical microleakage in unloaded resin composite restorations. This result is probably due to using the oblique incremental technique which was applied to decrease the tension generated by c-factor through the reduction of composite volume.

Comparing the infiltration degree in enamel and cementum margins, in the same sample, the staining solution penetration was higher in cementum margins, for Fuji IX without load cycling when polished immediately and for Fuji IX under load cycling and Fuji II LC under load cycling and delayed polishing. These findings also confirm the observations by Ichim *et al.* that revealed under the action of para-functional loadings, GIC restorations undergoes strain softening in the cervical region prior to fracture.^[30]

Cervical microleakage in Fuji II LC under load cycling and immediate polishing was not significantly higher than occlusal microleakage. Possible explanation is the ability of RM-GI to adhere immediately to dentin.^[35]

Filling material effect

Occlusal microleakage among these materials under load cycling and immediate polish were significantly different, which Fuji II LC showed highest microleakage and Z350 showed lowest microleakage (Fuji II LC > Fuji IX > Z350).

Cervical microleakage among these materials under load cycling and immediate or delayed polish were significantly different, which Fuji II LC showed highest microleakage and Z350 showed lowest microleakage (Fuji II LC > Fuji IX > Z350).

Fuji IX behaved similarly to the resin composite (Z350) and to the RM-GI (Fuji II LC) without loading (occlusal and cervical microleakage). These results were in agreement with earlier studies.^[36,37] This agreement can be related to similar cavity shape and evaluation technique.

Polishing time effect

In our study, occlusal and cervical microleakage were not affected by polishing time in none of the three restorative materials apart from loading status. These results were in agreement with earlier studies,^[13,27] but did not confirm the observations by Irie *et al.* that mentioned polishing should be delayed to a later time to prevent interfacial gap-formation between the GI material and the class I cavity.^[19] These differences can be related to different polishing instruments, using bovine teeth and applying G coat plus in our study which can improve interfacial gap formation in Fuji IX when polished immediately.^[8]

These findings are also confirmed by interfacial gap formation measured by FE-SEM because interfacial gaps were under 0.71% of the whole restoration margins in all unloaded restorations and in these groups, samples under immediate and delayed polishing, had similar interfacial gaps. However, due to the limited sample size, the statistical analysis of the data was not performed.

CONCLUSION

Within the limitation of this study the following conclusions are drawn:

1. This study has shown that the cervical sealing ability of Fuji IX under load cycling was better than Fuji II LC. Under load cycling and immediate polishing Z350 showed better marginal integrity than both Fuji II LC and Fuji IX.
2. Immediate polishing in evaluated tooth-colored class V restorations is recommended.

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