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Original Article

Marginal microleakage of a resin-modified glass-ionomer restoration: Interaction effect of delayed light activation and surface pretreatment

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ABSTRACT

Background: Despite widespread clinical uses of resin-modified glass-ionomers (RMGIs), their sealing ability is still a concern. This study evaluated the effect of delayed light activation (DLA) of RMGI on marginal sealing in differently pretreated cavities.

Materials and Methods: In this *in vitro* study, two standardized Class V cavities were prepared on the buccal and lingual surfaces of 56 sound maxillary premolars at the cementoenamel junction. The cavities were randomly divided into eight equal groups. In groups 1-4 (immediate light activation [ILA]), no pretreatment (negative control [NC]) and three surface pretreatments were used, respectively as follows: Cavity conditioner, Vitremer primer, cavity conditioner plus and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). Fuji II LC (GC, Japan) was prepared and placed in the cavities and immediately light-cured according to manufacturer's instructions. In groups 5-8 (DLA), the same pretreatments were applied, respectively. After placing Fuji II LC in the cavities, the restorations were light-cured after a 3-min delay. After finishing the restorations, the specimens were placed in water for 1-week and thermocycled. Microleakage scores were determined using the dye penetration technique. Kruskal–Wallis test and Mann–Whitney U-test were used to analyze the obtained data ($\alpha = 0.05$).

Results: At the dentin margins, DLA resulted in a lower microleakage for no treatment (NC), cavity conditioner and cavity conditioner plus ACP-CPP pretreatments groups ($P \le 0.004$); however, no difference was observed for Vitremer group ($P > 0.05$). At the enamel margins, no difference was observed between DLA and ILA for all groups ($P > 0.05$); only NC group exhibited a lower microleakage in case of DLA ($P = 0.007$).

Conclusion: Delayed light activation of RMGI may lead to different effects on marginal sealing, depending on pretreatment procedures used in the cavity. It might improve dentin sealing when no treatment and conditioner alone or with CCP-ACP is used.

Key Words: Casein phosphopeptide-amorphous calcium phosphate, delayed, light activation, marginal sealing, resin-modified glass-ionomer

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INTRODUCTION

Resin-modified glass-ionomers (RMGIs) were introduced to overcome the drawbacks of conventional glass-ionomers by the addition of a small quantity of resin monomer such as 2-hydroxyethyl methacrylate (HEMA) or Bis-GMA or by modifying the polyacid with light-cured side chains. These modifications have resulted in improved mechanical, esthetic and handling properties, reduced moisture or dehydration sensitivity, decreased setting time and increased working time, while retaining the advantages of GI.[1,2]

Two setting reactions are known in RMGI: A slow, classic acid-base reaction that begins upon mixing the cement and continues up to 24 h or even 1-week, and a faster polymerization reaction of incorporated monomers that is activated by visible light exposure.[3]

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Resin-modified glass-ionomer, as a hybrid material between GI and resin composite, could bond to tooth structures through both ionic bonding of polyacrylic acid with hydroxyapatite and micro-mechanical interlocking of the monomeric component into a partly demineralized smear layer-free dentin surface.[4,5]

Although this two-fold bonding mechanism might contribute to the greater bond strength of RMGI, chemical bonding was reported to be a major factor,[5,6] explaining their excellent performance in a systematic review of clinical trials conducted by Peumans *et al.*[7] It has been demonstrated that the acid-base reaction induced chemical bonding of RMGI to dentin as a main bonding mechanism.[8]

2-hydroxyethyl methacrylate contained in RMGI which acts as a co-solvent for both the resin and acid components has a high wetting ability, enhancing bonding ability of RMGI.[4] Water serves as a reactant medium for acidic component ionization and ion transportation, and consequent acid-base reaction. However, some of water has been replaced by HEMA, resulting in a reduced/retarded acid-base reaction of RMGI.[9] As a consequence, polyacid has more opportunity for a longer time for chemical bonding.[10] On the other hand, since each mentioned setting reaction depends on reactant diffusion prior to gelation, the reactions are affected by each other. During the several-minute setting time, they compete or inhibit each other.[11] Since the chemical bonding of RMGI depends on calcium availability on tooth surfaces,[5] delayed light activation (DLA) may lead to different results when preparing tooth tissues with various pretreatment agents. Cavity conditioning plays a more important role in providing effective bonding with RMGI. Pretreatment with a diluted polyalkenoic acid conditioner is advised to remove the smear layer and partially demineralize dentin with retained smear plugs.[4] This surface conditioning could improve the bonding of RMGI through the formation of a sub-micron hybrid layer and chemical bonding with the remaining hydroxyapatite around the exposed collagen.[8]

Self-etch primers such as Vitremer primer are reported to modify the smear layer and improve the wettability of the dentin and monomer penetration into it.[12] In an attempt to maximize the chemical bonding, application of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) after cavity conditioner may be a promising procedure to provide a reactive substrate through calcium phosphate deposition.[13] So far, there is no published study regarding the effect of CPP-ACP pretreatment on marginal sealing of RMGI restorations or bonding ability of RMGI to tooth structures.

Therefore, this study was designed to compare the effect of DLA on marginal sealing of a RMGI when different types of surface pretreatments were used including cavity conditioner, acid etching, Vitremer primer and CPP-ACP.

MATERIALS AND METHODS

In this *in-vitro* study, 56 human maxillary premolars extracted following orthodontic treatment without caries, cracks or previous restorations were selected. They were stored in 0.5% chloramine solution for 2 weeks and then stored in distilled water at 4°C until use. Two standardized Class V cavities were prepared on the buccal and lingual surfaces of each teeth (3 mm width, 3 mm height, and 1.5 mm depth) with occlusal margins in the enamel and gingival margins placed approximately 1 mm below the cementoenamel junction using new straight fissure burs (#835/010, TeesKavan, Iran) for every five preparations. All dimensions of the cavities were verified with a periodontal probe. The preparations were randomly divided into eight equal groups of 14 cavities each. In the first four groups (immediate light activation [ILA]) (1-4), different surface pretreatments were used as follows:

1. Group 1 Negative control (NC) with no pretreatment.
2. Group 2 (cavity conditioner): Pretreatment with 20% polyacrylic acid (cavity conditioner, GC, Tokyo, Japan).
3. Group 3 (Vitremer primer): Pretreatment with Vitremer primer (3M ESPE, USA).
4. Group 4 (CPP-ACP): Pretreatment with cavity conditioner and then with CPP-ACP (GC, Tokyo, Japan).

The materials used and their application procedures are presented in Table 1.

Following surface pretreatment, all cavities were restored using Fuji II LC RMGI (GC, Tokyo, Japan) in powder/liquid form. Powder and liquid components were dispensed at 2.3/1 ratio by weight, mixed and placed into the cavity in one increment. The restorations were immediately light-cured according to the manufacturer's instructions.

In the second four groups (DLA) (5-8), the same surface pretreatments were applied in as groups 1-4, respectively. The RMGI was placed in the cavities, allowed to set for 3-min and light-cured according to manufacturer's instructions. The light curing was done using a halogen light-curing unit (Coltolux 75, Coltene/Whaldent AG, Alstätten, Switzerland, 500 mW/cm^2). All preparations and restorations were performed by one operator at room temperature. During procedures, the teeth were kept moist.

After water storage of the specimens at 37°C, the restorations were finished with aluminum oxide discs (Sof-Lex, 3M ESPE, USA). The restored teeth were stored at 37°C for 1-week and then thermocycled for 1000 cycles at 5°C and 55°C with a 30-s dwell time. The root apices were sealed with sticky wax (Keystone Industries GmbHD — Singen, Germany), and all the surfaces, except for the restorations and 1 mm from the margins, were coated with two layers of nail varnish. The teeth were immersed in a 0.5% methylene blue solution for 24 h. They were then rinsed, blot-dried and sectioned longitudinally through the center of the restorations from the buccal to lingual surface with a water-cooled diamond saw (Leitz 1600, Wetzlar, Germany).

The sections were blindly examined for dye penetration by two independent evaluators using a stereomicroscope (Carl Ziess Inc., Oberkochen, Germany) at ×20 magnification. The extent of the dye penetration was analyzed for both the enamel and dentin margins according to a nonparametric scale from 0 to 3 (0 = no dye penetration, 1 = dye penetration <1/2 of the cavity depth, 2 = dye penetration >1/2 of the cavity depth, 3 = dye penetration spreading along the axial wall.[14] Kruskal–Wallis test and Mann–Whitney U-test were used to analyze the obtained data (α = 0.05).

RESULTS

Dye penetration scores for the enamel and dentinal margins are presented in Tables 2 and 3. None of the groups showed complete elimination of microleakage. In order to assess the effect of DLA on microleakage of the four groups (no treatment and three pretreatments), Mann–Whitney test was performed between ILA and DLA of each treatment group for both the enamel and dentin margins. A significantly lower microleakage at the dentin margin was observed in the DLA groups for which no treatment (P = 0.004), cavity conditioner (P = 0.02) and ACP-CCP (P = 0.01) were applied. No significant difference between DLA and ILA for the primed dentin (P = 0.91) was observed. Moreover, there was no significant difference between DLA and ILA for all groups at the enamel margin (P > 0.05), except for no treatment (NC) group, revealing a lower leakage in case of DLA (P = 0.007) [Table 4].

Table 1: Materials used in this study

Material/manufacturer	Composition/batch number	Application procedure
Cavity conditioner/GC, Tokyo, Japan	Polyacrylic acid (20%), aluminum chloride (3%), distilled water/100310	Apply for 10-s, water rinse for 15-s, dry gently
Vitremer Primer/3M ESPE, St. Paul, MN, USA	HEMA, Vitrebond copolymer, ethyl alcohol/N251185	Apply for 30-s, air dry, light cure for 20-s
MI Paste/GC, Tokyo, Japan	Glycerol, 5-10% CPP-ACP pure water, zinkoxid, silicon dioxide, phosphoric acid, titanium dioxide, guar gum, sodium saccharin, ethyl-p-hydroxybanzoate, propylene glycol, butyl-p-hydroxybanzoate	Apply 0.1 mL actively in the cavity for 3-min, remove excess with absorbent paper, kept moist surface
Fuji II LC/GC, Tokyo, Japan	Powder: Flurualumino-silicate glass/1104271 Liquid: Polyacrylic acid: 2-hydroxyl ethyl methacrylate, proprietary ingredient, trimethyl hexamethylene dicarbonate/1104191	Dispense powder and liquid, mix for 10-15-s, fill the cavity, light cure for 20-s

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; HEMA: 2-hydroxyethyl methacrylate.

Table 2: Microleakage scores obtained from ILA groups

Group	Surface treatment	Enamel margins				Mean (SD)	Median	Dentin margins				Mean (SD)	Median
		0	1	2	3			0	1	2	3		
1	Negative control	1	3	4	6	2.07 (0.99)	2	1	2	4	7	2.21 (0.97)	2.5
2	Cavity conditioner	5	4	3	2	1.14 (1.09)	1	2	6	2	4	1.57 (1.08)	1
3	Vitremer primer	1	6	3	4	1.71 (0.99)	1.5	7	3	3	1	0.86 (1.02)	0.5
4	Conditioner plus CPP-ACP	4	5	2	3	1.29 (1.13)	1	2	6	3	3	1.50 (1.01)	1

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; ILA: Immediate light activation; SD: Standard deviation.

Table 3: Microleakage scores obtained from DLA groups

Group	Surface treatment	Enamel margins				Mean (SD)	Median	Dentin margins				Mean (SD)	Median
		0	1	2	3			0	1	2	3		
5	Negative control	5	6	2	1	0.93 (0.91)	1	6	4	3	1	0.93 (0.99)	1
6	Cavity conditioner	8	5	1	0	0.50 (0.65)	0	7	5	2	0	0.64 (0.74)	0.5
7	Vitremer primer	2	6	3	3	1.50 (1.01)	1	8	2	2	2	0.86 (1.16)	0
8	Conditioner+CPP-ACP	5	4	3	2	1.14 (1.09)	1	7	6	1	0	0.57 (0.64)	0.5

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; DLA: Delayed light activation; SD: Standard deviation.

Table 4: Mann–Whitney U-test results of the comparison of the effect of DLA at the enamel and dentin margins (*n* = 14)

Surface treatment	Mean rank		*P* value
	ILA	DLA	
Enemal margin			
Negative control	18.61	10.39	0.007*
Cavity conditioner	16.82	12.18	0.13
Vitremer primer	15.32	13.68	0.60
Conditioner plus CPP-ACP	15.00	14.00	0.76
Dentin margin			
Negative control	18.89	10.11	0.004*
Cavity conditioner	17.93	11.07	0.02*
Vitremer primer	14.71	14.29	0.91
Conditioner plus CPP-ACP	18.18	10.82	0.01*

*$P < 0.05$: Significant difference; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; DLA: Delayed light activation; ILA: Immediate light activation.

Furthermore, Kruskal–Wallis test was used for comparison of the four ILA groups, revealing the same significant difference at the enamel and dentin margins ($P = 0.02$). Pair-wise multiple comparisons of the four ILA groups (1-4) performed using the Mann–Whitney test showed a significantly lower microleakage at the enamel margin for cavity conditioner ($P = 0.03$) compared to no pretreatment (NC); there was no significant difference for the primer ($P = 0.35$) and CPP-ACP ($P = 0.07$) compared to NC. This test revealed no significant difference between cavity conditioner and NC ($P = 0.13$), CPP-ACP and NC ($P = 0.07$) at the dentin margin; Vitremer primer had a lower microleakage compared to NC ($P = 0.003$). However, the primer showed no significant difference with cavity conditioner and CPP-ACP groups ($P > 0.05$). Among DLA groups (5-8), there was no significant difference at both margins.

DISCUSSION

Effective marginal sealing of RMGI is an essential factor to achieve successful cervical restorations with enamel and dentin margins. Establishment of chemical bonding on the suitable tooth substrate could play an important role in marginal integrity and stability of the adhesive bond.[15]

Acid-base and photopolymerization setting reactions of RMGI occur in a series of overlapping stages with different speeds so that in competition between them, one reaction replaces a part of the other.[9,11] As the setting reactions are involved in bonding mechanism of RMGI to the tooth, the extent and speed of the reactions might influence the adhesive bond, depending on reactivity of dental substrate. In the current study, when RMGI was applied on cavity surfaces without any pretreatment, DLA resulted in an increased enamel and dentinal sealing. This finding may be related to polyacrylic acid and HEMA contained in RMGI. Polyacrylic acid acts as an ultra-mild self-etch.[16] This acid is indicated to form a nanometer sized hybrid layer[16] and also additionally creates chemical bonding with the calcium ions, which present within the smear layer.[14] Self-etching/adhesiveness of some RMGI materials is documented using interfacial ultrastructural analysis.[5] This self-adhesiveness is attributed to ionic bonding to hydroxyapatite around collagen; hence, improving hybridized dentin.[5] Furthermore, the hydrophilic property of HEMA content may contribute to mild self-conditioning characteristics of RMGI.[4] DLA may provide a sufficient time for penetrating and forming these reactions. This explanation could be supported by the results of Glasspoole *et al*. study that reported significantly higher bond strength to enamel with no pretreatment when setting of a RMGI was through self-curing reaction compared with its light curing.[17]

It seems that immediate light curing may restrict diffusion of polyacrylic acid to penetrate into the surfaces covered with the smear layer. Therefore, in this study, the positive effect of cavity conditioner pretreatment was confirmed in terms of marginal sealing, particularly at the enamel margin when the RMGI was cured immediately. This positive effect was insignificant compared with no pretreatment (NC) at the dentin margin. This result was in agreement with the findings of a recent study[18] as well as a previously conducted one.[19]

Numerous bonding/sealing studies have recommended surface pretreatment with weak acid solution such as polyalkenoic acid before RMGI application.[8,20,21] However, the lack of any beneficial effect of using cavity conditioner on dentin bonding of RMGI was reported by Inoue *et al*.[22]

The obtained results revealed that DLA on cavity conditioned-surface resulted in improved enamel and dentin marginal sealing; however, this improvement was only significant for dentin margin. Following the formation of the cross-linked polymer network by light curing, diffusion of acid-base reactants was limited.[9-11] Therefore, DLA could enhance ionic bonding of the carboxyl group of the polyacrylic acid with ion-leachable glass particles (setting reaction) or calcium from the partially demineralized tooth structures (chemical bonding at the adhesive interface).[23,24] In addition, an increased dentin permeability on cavity conditioned-dentin (with removal of smear layer) during DLA may provide an adequate water supply, facilitating more complete acid-base reaction.[25] This reaction maturation at the adhesive interface of RMGI may contribute to improved dentin marginal sealing. In a recent study, DLA decreased marginal leakage; this decrease was significant only at the enamel margin.[26] However, our results revealed a significant decreased microleakage at the dentin margin in case of DLA. DLA resulted in a lower leakage at the enamel margin of NC group. This result may be attributed to self-etching capability of RMGI on untreated enamel during the delay time before light activation. Thomas *et al*.[27] reported that DLA could produce more GI structural characteristics in the RMGI. On the other hand, this similarity was speculated to increase the chemical bonding nature,[11] resulting in a stable bonding.[28] It was reported that a relatively high (3%) polymerization shrinkage of RMGI during light curing[29] may overcome the weak early chemical bonding to the dentin, providing a pathway for microleakage at the adhesive interface.[30] It was expected that DLA may control this shrinkage to some extent, gaining the beneficial effect of chemical bonding on marginal sealing.

The other common and easy-applying surface pretreatment prior to RMGI is self-etch primers such as Vitremer primer. This light-cured primer is capable of modifying the smear layer, resulting in a closer interaction of RMGI and dentin surface.[12] When the RMGI used in this study was immediately light-cured, Vitremer primer pretreatment was beneficial in achieving significantly better sealing at the dentin margin. However, the effectiveness of this primer on enamel sealing was not significant. When the primer was used, DLA had no effect on the marginal leakage of both margins. In line with this finding, Glasspoole *et al*.[17] reported that the lack of difference between bond strength of light-cured and self-cured Vitremer RMGI to Vitremer primed-enamel may be related to a leveling effect of this light-cured primer between the two cure modes.

Casein phosphopeptide-amorphous calcium phosphate as a remineralizing and anticariogenic agent is commonly used to treat tooth sensitivity associated with or following bleaching treatment. This material has been incorporated into mouth rinses, chewing gums, sports drinks and a commercially available paste (tooth mousse [MI past]).[31] This complex creates a high concentration gradient of calcium phosphate at the tooth surface, inhibiting demineralization/promoting remineralization.[13]

The well-established anticariogenic property of RMGI is attributed to the effective role of released fluoride in enhanced remineralization process with fluorapatite through calcium phosphate precipitation along with localization of the fluoride ion at the tooth surfaces.[30] However, the presence of sufficient calcium phosphate ions is also necessary to remineralize the tooth structures with fluoroapatite.[33] This requirement may be provided by pretreatment with CPP-ACP prior to RMGI. Previously, enhanced protective and remineralizing effects of simultaneous releasing of fluoride and CPP-ACP from CPP-ACP containing GI on the damaged and adjacent dentin during acid challenge was indicated in experimental *in vitro* studies.[30,32]

The initial mild dentin demineralization was reported to facilitate remineralization induced by CPP-ACP.[13] On the other hand, depleting the dentin surface of the total mineral content by acid etching may compromise its remineralization potential.[28] Based on these findings, in the current study, CPP-ACP was applied after using cavity conditioner; this procedure revealed no effect on the microleakage at both margins when light-curing was done immediately after placement of RMGI. However, in the case of DLA, CPP-ACP pretreatment resulted in a significantly improved sealing at the dentin margin with no effect on the enamel margin.

Polyacrylic acid conditioner was suggested to be capable of bonding with remnant hydroxyapatite[35] and collagen[36] so that, after the rinsing step, some remaining polyacrylic molecules form a polymeric layer on conditioned-enamel[37] and dentin.[5] As a consequence, the demineralized dentin surface layer may afford less mineral site for bonding to carboxyl group of polyacrylic acid contained in the RMGI. Following CPP-ACP application on this surface, an increased concentration of calcium and phosphate may enhance bonding between these ions and carboxyl groups of RMGI on the treated dentin surface. In the present study, during DLA, this chemical bonding on the reactive treated surface may be established, thereby an increased marginal sealing was attained at the dentin margin.

On the other hand, Es-Souni *et al*.[37] demonstrated that the mentioned formed polymeric layer on polyacrylic acid conditioned-enamel could enhance bond strength of GI. In the current study, CPP-ACP application on the conditioned enamel resulted in deposited calcium phosphate on the polymeric layer. This deposited layer may impede the beneficial effect of the polymeric layer on bonding of RMGI. Hence, cavity conditioner plus CPP-ACP pretreatment did not alter enamel marginal sealing in this study. Further chemical analysis and scanning electron microscopy studies are required to evaluate the real interaction of this pretreatment on tooth surfaces during bonding of RMGI.

No study has been performed on CPP-ACP as an additional pretreatment step during RMGI placement in the prepared cavity. This application procedure of CPP-ACP was used only in a recent study; the resultant increased bond strength of Adper SE Plus (3M ESPE, St Paul, MN, USA) had been attributed to the increased availability of calcium phosphate on CPP-ACP treated dentin for chemical bonding to phosphoric acid ester contained in this adhesive.[38]

The outcome of the current study seems to support the important role of chemical bonding on dentin marginal sealing. Marginal sealing might be improved when light curing of the RMGI on ACP-CPP or cavity conditioner treated-dentin was delayed; in these situations, the enamel sealing was not altered. The lowest mean microleakage score at the dentin margin (0.5) was attained in the case of DLA for cavity conditioner plus ACP-CPP pretreatment and for cavity conditioner alone at the enamel margin (0.57). Therefore, RMGI restorations might benefit from the advantages of ACP-CPP pretreatment in the cavities, enhancing remineralization potential of RMGI.

In a study, DLA for 3-min and 15-s (working time based on the manufacturer's data), resulted in 15% reduction of photopolymerization reaction of a RMGI.[11] Although this reduction was speculated not to affect the material properties,[11] further studies are necessary to evaluate the effects of DLA on the other interactions of various RMGIs with tooth structures in long-term periods.

CONCLUSION

Within the limitations of the current study, it may be concluded that a 3-min delay in light activation of the RMGI might yield different outcomes on enamel and dentinal marginal sealing depending on surface conditioning and structural characteristics of the treated surfaces. It might improve dentin sealing when no treatment and cavity conditioner alone or with CCP-ACP is used and enamel sealing when no treatment is used.

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