Effect of Two Traditional Polyacrylic Acid Conditioners and 2% Chlorhexidine Digluconate on Cavosurface Microleakage of Glass Ionomer Restorations

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Objectives: *A lack of appropriate adhesiveness is one of the biggest problems in restorative dentistry today and the main cause of microleakage. This is especially true in pediatric dentistry where moisture control is more difficult to achieve. Glass ionomer restorative materials increase adhesion and decrease microleakage given their chemical adhesion to the remaining tooth substance. Pretreatment improves the adhesion quality. The aim of this study was to assess the microleakage of Glass ionomer restorative materials following application of 20% polyacrylic acid, 10% polyacrylic acid or 2% chlorhexidine digluconate in Class V cavities.* ***Study design:*** *Two Class V preparations were prepared on the buccal and lingual surfaces of 24 extracted human molars. The gingival wall was set below or above the CEJ. The teeth were divided into 2 groups. Group 1 was treated with 20% polyacrylic acid or 10% polyacrylic acid. Group 2 was treated with 10% polyacrylic acid or 2% chlorhexidine digluconate. Microleakage was evaluated using a light–reflecting stereomicroscope and stain penetration test.* ***Results:*** *Two percent chlorhexidine digluconate was as efficient as the other conditioners. No statistically significant differences were found among the three types of conditioners. Dye penetration was significantly greater into dentin than into enamel among all three conditioners in both groups (P<0.001).* ***Conclusion:*** *Two percent chlorhexidine digluconate, with its known added advantages, can be used as a pretreatment conditioner in GI restorations.*

Key words: GI restorations, microleakage, polyacrylic acid conditioner, 2% chlorhexidine digluconate.

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INTRODUCTION

One of the most important requirements in restorations is the ability of the restoration material to bond well to the tooth substance and prevent cavosurface microleakage. This property results in optimal effectiveness of the restorative material[1]. Restoration of young carious molars is still a major concern when treating children, particularly in class II restorations where gingival margins extend below the cemento-enamel junction (CEJ) onto the dentin [2,3]. Currently, adhesive restorative materials lack an adequate marginal seal and therefore, are unable to prevent microleakage[2,3]. Several studies using various techniques, adhesive methods and restorative materials have attempted to determine the ideal material for restoration[4-9].

Glass ionomer (GI) is a tooth-colored adhesive restorative material that is increasingly used in the field of operative dentistry and is a commonly used material in pediatric dentistry. GI technology has undergone tremendous improvement since its introduction and has been able to overcome most of the disadvantages of other restorative materials for children, given its high strength, wear resistance, chemical adhesion to the tooth structure under the moist conditions of the mouth [10,11,] fluoride release and radio opacity. GI is also reportedly less technique sensitive. In fact, GI cements have the ability to form both a chemical and a micromechanical bond with the tooth surface8, which increases adhesion to the tooth substance, increases retention, and as a result, reduces cavosurface microleakage 8.

For good adhesion, close contact must exist between the two substances being joined, and a smear layer, which can be created during the preparation of the tooth, can interrupt this intimate contact and damage the adhesion quality [8,10,12]. Several studies have suggested using a pretreatment on the tooth surface before placing the GI. These studies have recommended using various conditioners that have weak mineral etchants or milder organic acids such as nitric acid, (2.5%), citric acid (10%), pyruvic acid (10%), polyacrylic acid (20%), ferric chloride, aluminum chloride and oxalic acid (1.5 to 3.5%)[10,5].

In the literature, there are differences in opinion regarding the most efficient type of conditioner, as well as the concentration and duration of surface application of the conditioner. There is also significant controversy as to whether the use of a conditioner is needed at all[13,14,15]. For example, Tanumiharja *et al* [16] examined the influence of four different conditioners on the quality of GI adhesion to dentin. The conditioners were applied to the dentin according to the manufacturer's instructions. The authors concluded that there was no significant difference between the samples that received conditioning and the samples that did not. Hassan and Badr[17] also concluded that applying polyacrylic acid on dentin did not enhance the bond between the GI and dentin. Conversely, Charlton *et al* [18] compared untreated dentin to dentin pretreated with 10% polyacrylic acid and found that the use of conditioner significantly increased the bonding quality to dentin. Inoue *et al* [19] also recommended using a polyacrylic acid conditioner to improve the bonding effectiveness of GI to dentin. In comparison to previous studies investigating the bond strength of GI to dentin, Glasspoole *et al* [9] evaluated the bond strength of GI to enamel. The authors used three different surface treatments, 10% polyacrylic acid for 20 sec, 35% phosphoric acid for 15 sec and Vitremer primer (3M ESPE, St. Paul, MN, USA), and concluded that the two polyacrylic acid conditioners significantly improved the bond to the enamel compared to surfaces that did not undergo pretreatment.

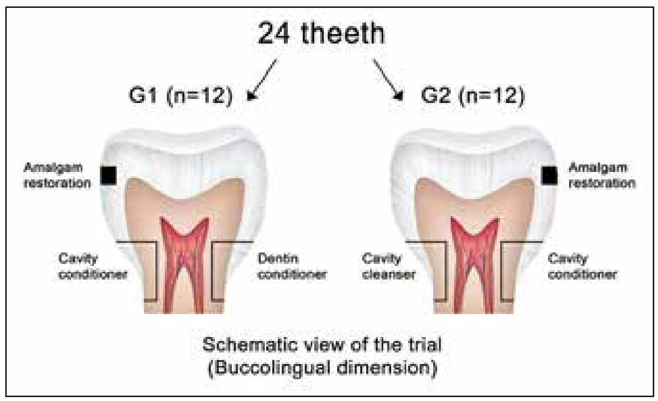
Due to controversial opinions regarding the type, concentration and duration of conditioner application, studies have continued to assess the cavosurface quality between GI and teeth. In contrast to the majority of studies that have investigated microleakage in the enamel or dentin only, in our study, we investigated microleakage in both the enamel and dentin in the same tooth using different conditioners. To date, no studies have evaluated the effect of 2% chlorhexidine digluconate as a dentin-glass ionomer restoration conditioner. Given that 2% chlorhexidine digluconate removes debris and the smear layer in addition to its other properties, we expected that it could be used as a conditioner with similar results as traditional polyacrylic acid conditioners.

The aim of the present in vitro study was to use staining methods to evaluate microleakage using a new glass ionomer restoration material combined with 10% polyacrylic acid, 20% polyacrylic acid or 2% chlorhexidine digluconate in class V cavities prepared using a high-speed bur.

MATERIALS AND METHOD

A total of 24 sound human third molars with no structural abnormalities were selected. The study was approved by the Institutional Review Board, Helsinki Committee. The extracted teeth were cleaned using an ultrasonic device and stored in distilled water at 4°C. Two Class V preparations were made for each tooth using high-speed 330 tungsten-carbide burs (SSW, London, U.K.) with water cooling (8000B GENTLEsilence LUX, KaVo Dental GmbH, Biberach, Germany). The preparations were performed on the buccal and lingual surfaces. The occlusal margins for all of the preparations were in the enamel, and the gingival margins were located 1 mm apical to the cemento-enamel junction (CEJ). The cavity dimensions were standardized (3 mm in width, 3.5 mm in height and 1.8 mm in axial depth) (Fig. 1). All prepared teeth were randomly divided into 2 groups of 12 teeth each, according to the conditioner material used. The three conditioners we examined included dentin conditioner (polyacrylic acid 10%, distilled water 90%, GC Dental Industrial Corp., Tokyo, Japan) for 20 sec, cavity conditioner (polyacrylic acid 20%, aluminum chloride hydrate 3%, distilled water 77%, GC Dental Industrial Corp., Tokyo, Japan) for 10 sec and cavity cleanser (chlorhexidine digluconate 2%, Bisco, Inc., Schaumburg, IL, USA) for 20 sec.

Figure 1. Schematic presentation of the cavity preparation and location.



In both groups, the cavity conditioner was used as the control and was randomly applied to either the buccal or lingual surface. To differentiate between the two sides of the same tooth, a small amalgam restoration (2 mm/2 mm/2 mm) was made on the surface that was treated with the cavity conditioner (Fig. 1). All samples were restored with GI, EQUIA™ (Powder: fluoroaluminosilicate glass 90-100%, polyacrylic acid 5-10%; Liquid: polyacrylic acid 30-40%, proprietary ingredients 5-15%; GC Dental Industrial Corp., Tokyo, Japan) according to the manufacturer's instructions.

Visible overhangs were removed using a No. 15 scalpel blade (Swann-Morton, UK).

All samples were stored in distilled water at 37°C for 7 days and were then subjected to thermo-cycling (i.e., thermal stress) for 2000 cycles at 5°C and 55°C followed by a water bath for 5 sec and a dwell time of 5 sec between each bath using a TC2000 automatic device (Y. Manes, Tel-Aviv, Israel).

The root apices were sealed with Duralay (Reliance Dental Mfg. Co., IL, USA). The exposed crown and root structures were covered with two coats of nail varnish, except for the restoration and 1 mm around the cavosurface margins. The teeth were then immersed in

0.5% basic fuchsine solution for 48 h at 37°C to produce a visible stain. All samples were carefully rinsed with tap water for 1 h and were embedded in an epoxy resin and sectioned longitudinally through the restoration in a buccolingual direction using an ISOMET plus Low Speed Saw (Buehler Ltd., IL, USA) to obtain 3 sections of each restoration. Dye penetration was used to determine the micro-leakage at the cavosurface margins. The cut sections were examined under a light–reflecting stereomicroscope at 18x magnification by two different examiners using the scoring criteria shown in Table 1.

Table 1. Scoring criteria for dye penetration.

Score	Content
0	No tracer penetration.
1	Penetration < half the wall length.
2	Penetration > half the wall length.
3	Penetration to axial/ pulpal floor.
4	Penetration along the axial wall

The Wilcoxon Signed-Rank statistical test was used to analyze the collected data.

RESULTS

The mean and standard deviation of the amount of dye penetration were calculated for the different conditioners in the different groups (Table 2 and 3).

The Wilcoxon Signed-Rank test was performed to examine whether there were differences in dye penetration between the cavosurface of the enamel and the dentin for each of the conditioners used in the study and whether there were differences between the different conditioners in the amount of dye penetration into the enamel and dentin. We found that for all three conditioners used in the study, dye penetration at the dentin cavosurface was greater than that at the enamel cavosurface (Tables 2 and 3). Dye penetration at the dentin was significantly greater than dye penetration at the enamel for the two conditioners in group 1 (Table 2, cavity conditioner: p<0.001, dentin conditioner: p<0.007). For the cavity conditioner, the mean dye penetration at the enamel was 0.8 ± 1.1, whereas the mean dye penetration at the dentin was 2.2 ± 1.6. For the dentin conditioner, the mean dye penetration at the enamel was 1 ± 1.4, where it was 2.04 ± 0.9 at the dentin.

Similar results were found in group 2 (Table 3). Dye penetration at the dentin was significantly greater than dye penetration at the enamel for the two different conditioners (cavity conditioner: p<0.001, cavity cleanser: p<0.001). For the cavity conditioner, the mean dye penetration at the enamel was 0.17 ± 0.6, whereas the mean dye penetration was 2.04 ± 1.6 at the dentin. For the cavity cleanser, the mean dye penetration at the enamel was 0.04 ± 0.2, whereas the dye penetration was 1.7 ±1.4 at the dentin.

No significant differences in dye penetration were found between the different conditioners at the enamel or dentin (Table 4). In group 1, the dye penetration was similar between the cavity conditioner and dentin conditioner at the dentin (p=0.596) and the enamel (p=0.806). Similar results were found in group 2 (Table 5). No significant differences were found between the two different conditioners at the dentin (p=0.426) or enamel (p=0.18).

Table 2: Dye penetration in group 1.

	Dentin conditioner		Cavity conditioner	
	St dev	Mean	St dev	Mean
Enamel	1.4±	1	1.1±	0.8
Dentin	0.9±	2.04	1.6±	2.2
P value	P<0.007		P<0.001	

*Comparison between the mean dye penetration of the cavity conditioner and the dentin conditioner at the enamel and dentin.

Table 3: Dye penetration in group 2.

	Cavity cleanser		Cavity conditioner	
	St dev	Mean	St dev	Mean
Enamel	0.2±	0.04	0.6±	0.17
Dentin	1.4±	1.7	1.6±	2.4
P value	P<0.001		P<0.001	

*Mean dye penetration at the enamel and dentin for the cavity conditioner and the cavity cleanser.

Table 4: Comparison between the different conditioners in group 1.

	Dentin		Enamel	
	St dev	Mean	St dev	Mean
Cavity conditioner	1.6±	2.1	1.1±	0.8
Dentin conditioner	1.5±	2	0.9±	1
P value	P=0.596		P=0.806	

*Comparison between the mean dye penetration of the cavity conditioner and the dentin conditioner at the enamel and dentin for both sides of the tooth.

Table 5: Comparison between the different conditioners in group 2.

	Dentin		Enamel	
	St dev	Mean	St dev	Mean
Cavity conditioner	1.6±	2	0.6±	0.17
Cavity cleanser	1.4±	1.7	0.9±	0.04
P value	P=0.426		P=0.18	

*Comparison between the mean dye penetration of the cavity conditioner and the dentin conditioner at the enamel and dentin for both sides of the tooth.

DISCUSSION

The marginal seal obtained by restorative materials is important for restoration longevity[1]. GI is the only restorative material known for its ability to form both a chemical and micromechanical bond to the tooth substance[8,11]. Chemical bonding is obtained by the ionic interaction of the carboxyl groups of polyalkenoic acid with the calcium ions of hydroxyapatite that remain attached to the collagen fibrils[8]. The micromechanical bond is achieved through adsorption, diffusion and ionic exchange between the mineral components of the tooth structure and the organic components of the GI[20,21]. The initial stage of bonding is a weak hydrogen bond due to polar attraction between the tooth and the freshly placed GI. At this stage, the acidity of the GI allows it to act as a self-demineralizing agent on the tooth smear layer[22], which was confirmed by Sennou and Shimada[23].

As the GI ages, the hydrogen bonds are replaced by a stronger chemical bond. Therefore, it has been suggested that conditioning the tooth surface prior to GI placement is not a necessary step to achieve adequate bonding.

In contrast to these findings, many studies have recommended that pretreatment of the cavity surface is necessary before GI placement to obtain a better adhesion between the restorative material and the remaining tooth structure[,18,19,13]. These studies have recommended that the smear layer, which fills the orifices of the dentin tubules and decreases permeability by up to 86%, should be removed[24]. Usually, the smear layer is also contaminated with microorganisms, which is another reason why it should be removed before placing the GI restoration. Removing the smear layer can be achieved by applying an altering solution prior to the GI filling[10]. Several acid conditioners have been recommended for use on the cavity walls to improve the interaction between the GI restoration and the tooth surface[10]. These different conditioners can cause different effects on the smear layer, from total removal to partial removal. Several studies have tried to assess the most effective conditioner material[14,13,9]. Powis *et al* [14] evaluated the effects of 15 conditioner materials on the adhesion of glass ionomer to dentin and enamel. They found that the high molecular weight conditioners, such as polyacrylic acid, were more effective compared with the low molecular weight acid conditioners.

Polyacrylic acid in various concentrations has been suggested to be the ideal conditioner[19]. Polyacrylic acid has the ability to maximize bond strength by removing the smear layer, increasing the wettability of the dentin and improving ion exchange with the GI restorative material[25]; however, the suggested concentrations and application times vary among different studies. Bishara *et al*.[15] evaluated bond strength using 10% polyacrylic acid and 20% polyacrylic acid applied to the enamel for 20 sec. The authors concluded that increasing the concentration from 10% to 20% significantly increased the bond strength to the enamel. Hajizadeh *et al* [26] found that 10% polyacrylic acid applied to dentin for 20 sec yielded the highest bond strength to dentin compared with the bond strength of two other conditioning agents, APF (1.1% acidulated phosphate fluoride) applied for 1 min and 37% phosphoric acid applied for 30 sec. However, Hajizadeh did not examine 20% polyacrylic acid in their study; therefore, it is difficult to make a comparison between the results found in the two different studies. In addition, the differences in the results of these two trials might be explained by the variability in the type of conditioner, the concentration, the duration of application and the tooth hard tissues (enamel or dentin) investigated. In our study, we assessed cavosurface microleakage of class V GI restorations that were pretreated with 10% polyacrylic acid for 20 sec or 20% polyacrylic acid for 10 sec, according to the manufacturer's instructions. We evaluated both the dentin and the enamel and observed that there were no significant differences in the amount of microleakage between the different concentrations. These findings are in agreement with the findings of Tanumiharja *et al*.[16], who also found no differences in bond quality between these two conditioners when applied according to the manufacturer's instructions. In contrast, we found significant differences in microleakage for the different tooth structures. Dye penetration was significantly greater in the dentin than in the enamel for both conditioners.

To enhance the adhesion quality of GI to dentin, previous studies have tried to find a substitute for the traditional polyacrylic acid[13,27,28]. Hamama *et al* [28] investigated the use of 37% phosphoric acid applied for only 5 sec on dentin and compared it to 25-30% polyacrylic acid applied for 10 sec (PAA), 20% polyacrylic acid applied for 10 sec (cavity cleanser) and Ketac Nano priming agent applied for 15 sec and found there were no differences in bond strength between the polyacrylic acid and the phosphoric acid that was applied for a reduced time. Shashirekha *et al* [13] compared the shear bond strength of GI surface pretreatment using 10% polyacrylic acid for 30 sec, 10% maleic acid for 30 sec and 5.25% sodium hypochloride for 30 sec. The authors found that, unexpectedly, 10% maleic acid had the best results in terms of bond strength, that maleic acid removed the smear layer but not the smear plugs and that maleic acid did not demineralize the dentin to its depth[29].

In our study, in addition to investigating the effects of different polyacrylic acid concentrations (10% and 20%) on the microleakage of a new glass ionomer restoration (GC EQUIA forte™, GC EUROPE, Leuven, Belgium) in both the enamel and dentin of the same tooth, we also investigated the use of chlorhexidine digluconate 2% as a surface conditioner.

Chlorhexidine digluconate 2% has three modes of action: antibacterial activity, matrix metalloproteinase (MMPs) inhibition and debris removal[30]. The antibacterial activity of chlorhexidine is thought to involve cationic chlorhexidine molecules that are rapidly attracted to the negatively charged bacterial cell surface. This alters the integrity of the bacterial cell membrane and causes leakage of low molecular weight cytoplasmic components[30]. Several studies have shown hydrolytic degradation of collagen matrices in aged dentin-resin bonds[31,32], even in the absence of bacterial enzymes[33], which occurs through host-derived MMPs. MMPs are a class of zinc- and calcium-dependent endopeptidases that are responsible for degrading nearly all extracellular matrix components of connective tissues[33]. Human dentin contains collagenase (MMP-8), gelatinases (MMP-2 and MMP-9) and enamelysin (MMP-20) [34]. Previous studies have revealed that chlorhexidine may function as a potent MMP inhibitor. With etch-and-rinse adhesive systems, pretreating the cavity with chlorhexidine after phosphoric acid etching may prevent or delay the interfacial degradation of the dentin-resin bond[33,35].

Because of its actions, chlorhexidine digluconate 2% is recommended for use in restorative dentistry after tooth preparation or etching with phosphoric acid and prior to sealing the dentinal tubules. The role of 2% chlorhexidine digluconate as an acid conditioner prior to the placement of GI restoration is unknown. Powis *et al* [14] investigated the effects of 15 conditioners, including 0.1% chlorhexidine gluconate applied for 60 sec, on the adhesion quality between GI and dentin or enamel. They found that pretreatment with 0.1% chlorhexidine digluconate on the tooth surface resulted in a smooth surface, but the bond strength of GI to the tooth structure was lower than in teeth that had not undergone pretreatment conditioning.

The results of our study showed that 2% chlorhexidine digluconate applied for 20 sec had the same effect on the marginal seal as did the polyacrylic acid and that there were no significant differences in dye penetration between the different conditioners at the enamel and dentin. This suggests that chlorhexidine digluconate had no advantage over the other conditioners in preventing dye penetration in dentin.

In the present study, greater microleakage was detected in the dentin than in the enamel for all three conditioners These findings may be due to the histological composition of the enamel and dentin. Although enamel is almost completely mineralized, dentin has a lower mineral content and an organic matrix that has a moist surface, which impairs the bonding mechanism[1]. Therefore, the bond strength to enamel is typically stronger and more stable than that to dentin, and microleakage along the enamel restoration interface is reduced or completely prevented in enamel. The present findings suggest that clinicians may use 2% chlorhexidine digluconate as a pretreatment conditioner prior to the placement of GI. This could be useful when treating children where optimal conditions for tooth-colored restorations are often compromised by the child's behavior.

CONCLUSION

1. No significant differences in the marginal seal quality were found among the three types of conditioning materials used for the various tooth surfaces.
2. The marginal seal at the enamel was better compared to that at the dentin for the three conditioning materials used in the study.

Clinical significance**:** The present study showed that 2% chlorhexidine digluconate can be used as a surface conditioner before GI placement in the prepared cavity.

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