Original Article

Microleakage of Two Self-Adhesive Cements in the Enamel and Dentin After 24 Hours and Two Months

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Abstract
Objective: Microleakage is a main cause of restorative treatment failure. In this study, we compared occlusal and cervical microleakage of two self-adhesive cements after 24 hours and two months.
Materials and Methods: In this *in-vitro* experimental study, class II inlay cavities were prepared on 60 sound human third molars. Composite inlays were fabricated with Z100 composite resin. The teeth were randomly assigned to six groups. RelyX-Arc (control), RelyX-Unicem and Maxcem were used for the first three groups and specimens were stored in distilled water at 37°C for 24 hours. The same cements were used for the remaining three groups, but the specimens were stored for 2 months. The teeth were subjected to 500 thermal cycles (5°C and 55°C) and immersed in 0.5% basic fuchsin for 24 hours and then sectioned mesiodistally and dye penetration was evaluated in a class II cavity with occlusal and cervical margins using X20 magnification stereomicroscope. Data were analyzed using Kruskal Wallis and Mann-Whitney U tests.
Results: After 24 hours, cements had significant differences only in cervical margin microleakage (P=0.0001) and microleakage of RelyX-Unicem and Maxcem was significantly more than that of RelyX-Arc (both P=0.0001). Cervical microleakage in RelyX-Unicem and Maxcem was greater than occlusal (P=0.0001 and P=0.001, respectively). Microleakage was not significantly different between the occlusal and cervical margins after 2 months.
Conclusion: Cervical microleakage was greater than occlusal in RelyX-Unicem and Maxcem after 24h. The greatest microleakage was reported for the cervical margin of RelyX-Unicem after 24 hours.
Key Words: Cervical; Occlusal; Leakage; Self-Adhesive; Cement

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INTRODUCTION

Preserving pulpal health is among the most important goals of dental treatments. The most important factor irritating dental pulp is the microleakage [1].

Microleakage is a dynamic phenomenon allowing bacteria, fluids, molecules and ions pass through the interface of the restoration and cavity walls; however, in some cases, it is not clinically obvious [2].

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Presence and continuation of microleakage can cause secondary caries, discoloration of restoration margins, hypersensitivity of the tooth and pulpal injury [3].

The most important cause of microleakage in indirect composite resin restorations is polymerization shrinkage and subsequent mechanical and thermal tensions. Several studies have mentioned the benefits of dentin adhesive systems in obtaining a strong and durable adhesion between the composite resin and tooth structure [4, 5]. Prevention of microleakage greatly depends on establishing and maintaining a good seal at the interface of restorative material and tooth structure. In the new generation of dentin bonding agents, the bond strength and marginal integrity have significantly improved [6, 7].

At present, use of restorations that chemically bond to the tooth structure especially composite inlays has increased. This raise is indebted to the development of bonding agents that help in achieving conservative treatments with high esthetic qualities [8]. Long-term success of indirect composite resin restorations depends on the preparation design, cementation technique and finishing procedures; which are now considered the key factors in this respect [9]. Quality of the margins of composite tooth-colored bonding inlays depends on several factors such as the bonding system and the type of composite resin material [10].

Resin-based adhesives are used for cementation of inlays, onlays, crowns, posts and veneers. In the past, all resin cements were based on etch and rinse or were self-etch and used with low viscosity composite resins. These multi-phase methods are complicated and technique sensitive and may compromise the success of bonding [11].

In 2002, self adhesive resin cement was introduced to the market that made simultaneous application of adhesive and cement feasible and on the other hand, eliminated the preparation and conditioning phase of tooth and restoration before bonding. Properties of this adhesive cement are based on acidic monomers that demineralize tooth structure and bond the two materials [12]. This cement contains an organic matrix including multi-purpose methacrylate phosphoric acid that reacts with the organic material (72% of the weight) present in hydroxyapatite of enamel and dentin [13]. This cement has been recommended for cementation of all metallic-base materials, ceramic crowns and indirect composite restorations [14, 15].

Self-adhesive resin cement systems etch the enamel and dentin and penetrate into them at the same time. In this way, resin monomers are capable of penetrating through the smear layer of the underlying dentin without the need for separate etching, rinsing and air-drying [16]. Combination of these two phases into one simplifies and expedites the work, obviates the need for rinsing of the acidic gel, and eliminates the risk of over-etching and over-drying [17].

Additionally, self-adhesive systems result in low but uniform penetration of resin into dentin and are less sensitive to moisture/salivary contamination when compared with total etch systems [5, 18, 19].

There has been controversy regarding the durability of the various generations of self-adhesive resin cements. Some studies indicate that microleakage increases in time [5], but other studies showed that time did not have a significant effect on microleakage and some studies have even mentioned that restoring the specimens in artificial saliva for 4 months decreased microleakage [20]. In general, recent studies have demonstrated that bond strength immediately after bonding is not necessarily similar to its rate after a long period of time [21-23].

This study sought to assess the microleakage of RelyX-Unicem and Maxcem self-adhesive cements and to compare it with that of RelyX-Arc (control group). The effect of time on the microleakage score in the mentioned systems was also evaluated.

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MATERIALS AND METHODS

This in-vitro randomized controlled experimental study was performed on 60 intact human third molars. The teeth were disinfected with 1% chloramine T solution and randomly divided into six groups of ten, each based on the type of adhesive used and storage time. Class II inlay boxes were prepared on the mesial or distal surface of each tooth using high speed hand piece (NSK, Japan) and 008 bur (D & Z, Germany) along with air and water spray. Dimensions of the boxes were 2 mm mesiodistally and 4 mm buccolingually and the occlusal margins of the boxes were in the enamel and gingival margin 1 mm below the CEJ (Figure 1) [24]. A new bur was used for each of the five boxes. Following cavity preparation and application of separating medium, oblique increments of composite resin (Filtek Z100, 3M-ESPE, USA) were inserted in the cavity and were initially cured (40 seconds) using halogen light curing unit (Arialux Blue Point, Apadanatak, Tehran, Iran) with an intensity of 680-720 mW/cm2. Then, the composite inlay was removed from the tooth and post curing was carried out at 100˚C for 15 minutes [25]. All bonding surfaces of inlays were sandblasted using intraoral microetcher device (Microetcher, USA) and 50 micron Al_2O_3 powder (Ortho Technology) for 5 seconds from 5 mm distance and 60 PSI pressure.

The sandblasted surfaces were irrigated with water and air-dried. In groups 1 and 4, the tooth surfaces were etched with 37% phosphoric acid gel (Ivoclar, Vivadent, Liechtenstein) for 15 seconds and rinsed for 10 seconds. After using two layers of Adper Single Bond Plus bonding (3M, USA), RelyX-Arc cement (3M, USA) was applied to the bonding tooth surfaces. The sandblasted inlays were then placed in their respective locations and fixed by maximum hand pressure required to seat inlay in its correct position. After 3-5 minutes, the extra cements were removed and each margin was light cured for 40 seconds.

Fig 1. Occlusal margin of the class II box in the enamel and gingival margin one mm below the CEJ

In groups 2 and 5, RelyX-Unicem (Maxicap, 3M-ESPE, USA) was used for cementation of the inlays according to the manufacturer's instructions. After 2 minutes, the extra cements were removed and each margin was light cured. In groups 3 and 6, inlays were cemented using Maxcem cement through similar phases. Restorations were finished and polished using Gold composite polishing bur (D &Z, Germany) and Soflex discs (3M, USA). Next, groups 1 to 3 and groups 4 to 6 were stored for 24 hours and 2 months, respectively in distilled water at 37°C. Therefore, of each cement, one group was restored for 24 hours and another one for 2 months. After 500 thermal cycling at 5°C and 55°C (the ISO TR 11450 standard -1994)[26], the teeth apices were sealed with flowable composite resin and all tooth surfaces except for the restored area and about 1 mm around the restoration margins were covered with 2 layers of nail varnish.

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In the next phase, all samples were placed in 0.5% basic fuchsin for 24 hours. After dye penetration and irrigation of the teeth, samples were cut mesiodistally using the cutting machine. Microleakage score in occlusal and cervical margins of the boxes was measured quantitatively using a stereomicroscope (Olympus, Japan) with X20 magnification and microleakage was evaluated on a scale of 0 to 3 (Table 1) [27]. Classification of microleakage in occlusal and cervical margins is demonstrated in Table 1. Data were analyzed using SPSS for Windows version 15 (SPSS Inc., Chiago, Ill, USA). Kruskal Wallis test was used for general comparison of microleakage in the three cement groups. Dunn test was used for paired comparisons. P<0.05 was considered statistically significant.

RESULTS

After 24 hours:

In the RelyX-Arc samples (control group), the mean rank of microleakage was 9.50 on the occlusal surface and 11.50 on the cervical surface. Although the mean rank of microleakage was higher on the cervical surface, this difference between the two surfaces for RelyX-Arc cement was not statistically significant (P=0.48).

For RelyX-Unicem cement, the mean rank of microleakage was 5.50 on the occlusal surface and 15.50 on the cervical surface. Dunn test showed a significantly lower microleakage on the occlusal surface compared with the cervical surface (P=0.0001).

In the Maxcem group, the mean rank of microleakage was 6.45 on the occlusal surface and 14.55 on the cervical surface. The difference between the two surfaces for this cement was statistically significant (P=0.001).

After 2 months:

Dunn test demonstrated that in the RelyX-Arc control cement microleakage was not significantly greater on the cervical surface compared with the occlusal surface (mean rank of 13.20 versus 7.80, P=0.11).

No significant difference was detected in the microleakage between occlusal (mean rank of 8.95) and cervical (mean rank of 12.05) surfaces in the RelyX-Unicem specimens (P=0.75).

The difference in this respect in Maxcem was not statistically significant either (mean rank of 8.20 in the occlusal surface and mean rank of 12.80 in the cervical surface, P=0.26).

Table 1. Classification of microleakage at the occlusal (A) and cervical (B) margins of cavities [26]

A: Occlusal margin

0	No microleakage
1	Microleakage not reaching the DEJ
2	Microleakage penetration over the DEJ
3	Microleakage into the dentin tubules and towards the pulp

B: Cervical margins

0	No microleakage
1	Microleakage less than half the cervical wall of the cavity
2	Microleakage penetration through all the cervical wall of cavity
3	Microleakage along the cervical or axial walls, into the dentin tubules and towards the pulp

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Comparison of 24 hours and 2 months:

In RelyX-Arc control cement, the difference in the mean rank of microleakage on the occlusal surface after 24 hours (10) and 2 months (11) was not statistically significant (P=0.74). On the cervical surface, although the cervical microleakage was relatively greater after 2 months (mean rank of 12.80) compared with 24 hours (8.2), this difference was not statistically meaningful (P=0.26).

For RelyX-Unicem, the difference in the mean rank of microleakage on the occlusal surface after 24 hours (12.50) and 2 months (8.5) was not significant (P=0.41). The mean rank of microleakage on the cervical surface after 24 h was significantly greater than the rank after 2 months (mean rank of 15.45 versus 5.55, P=0.0001). For Maxcem specimens, the difference in the mean rank of microleakage on the occlusal surface after 24 h (11.65) and 2 months (9.35) was not significant (P=0.39). On the cervical surface, the difference in microleakage after 24 h (13.35) and 2 months (7.65) was not statistically significant either (P= 0.085). Table 2 demonstrates the rank of microleakage on the occlusal and cervical surfaces of the three samples of three cements after 24 h and 2 months.

Dunn test was used for comparison of microleakage on the cervical and occlusal surfaces of samples of three cements after 24 hours and 2 months. The only significant difference was detected in the microleakage on the cervical surface after 24 hours in the three groups (P=0.0001). Occlusal microleakage in the three groups after 24 hours (P=0.11), and after 2 months (P=0.55) and cervical microleakage after 2 months (P=0.3) in the three cements were not significantly different.

Paired comparison of groups:

Since the difference in cervical microleakage after 24 hours was statistically significant between the three groups (P=0.0001), Dunn test was used for pairwise comparison of groups and the results revealed that the difference in microleakage between RelyX-Arc and RelyX-Unicem (P=0.0001) and RelyX-Arc and Maxcem (P=0.0001) was statistically significant, but no such correlation was detected in cervical microleakage between RelyX-Unicem and Maxcem after 24 hours (P=0.65).

DISCUSSION

At present, use of resin adhesive cements has greatly increased due to their improved physical properties and favorable marginal seal.

Table 2. Comparison of the Occlusal and Cervical Microleakage in the Three Cements After 24 Hours and Two Months

	Number	Cement	Mean Scale	Chi-Square		P-Value
Occlusal microleakage after 24 hours	10 10 10	RelyX-Arc RelyX-Unicem Maxcem	11.45 17.25 17.80	4.501	2	0.11
Occlusal microleakage after 2 months	10 10 10	RelyX-Arc RelyX-Unicem Maxcem	15.50 14.0 17.0	1.208	2	0.55
Cervical microleakage after 24 hours	10 10 10	RelyX-Arc RelyX-Unicem Maxcem	5.95 22.15 18.4	21.708	2	0.0001 (significant)
Cervical microleakage after 2 months	10 10 10	RelyX-Arc RelyX-Unicem Maxcem	17.0 12.25 17.25	2.398	2	0.31

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In RelyX-Unicem, additional acidic reactions occur between the phosphoric acid methacrylates and inorganic fillers with 72 wt% [28].

Luting properties of Maxcem are due to the combination of several adhesive monomers like glycerol dimethacrylate dihydrogen phosphate that is also found in other adhesive products such as Optibond/Optibond FL, Optibond Solo Plus, and Solo Plus Self-Etch [28]. Hydrophilic monomers have also been added to create adequate moisture when adhering to the dental substrate. Phosphoric acid's glycerol dimethacrylate esters are also capable of etching enamel and dentin [29]. There is not enough data neither regarding the pH of Maxcem cement and primary hydrolysis process of its acidic monomers nor about the chemical details of redox activator used in Maxcem cement [30]. Details about the acidic reactions between the self-cure activator and acidic resin monomers are obscure [31]. Both Rely X-Unicem and Maxcem benefit from the combination of dimethacrylate acidic monomers and phosphoric acid groups. It looks like the demineralizing capacity of multi-purpose monomers in Maxcem cement is low and cannot etch the newly formed smear layer that quickly buffers the monomers. Thus, only a surface reaction occurs between the cement and dental substrate [32].

In our study, in all samples, microleakage was similar on the occlusal and cervical surfaces and only in Rely X-Unicem and Maxcem, after 24 hours, microleakage was greater on the cervical surface compared with the occlusal surface, which is in contrast with the findings of Behr et al, in 2004 [14] who evaluated the marginal adaptation (through evaluation of microleakage) of universal self-adhesive cement RelyX-Unicem in comparison with previously used cements. They concluded that with no conditioning, this cement had marginal adaptation similar to conventional luting agents in the dentin.

In 2012, Inukai et al. evaluated the microleakage of MOD indirect composite restorations bonded with self-adhesive and self-etching resin cements (Panavia F 2.0, SA Cement, and RelyX-Unicem) with or without acid etching of the proximal enamel margins and found that acid etching had no effect on microleakage and the two tested self-adhesive cements showed similar bond strengths to the self-etching resin cement [33].

Piwowarczyk et al. in 2005 [34] evaluated microleakage and marginal gaps of restorations bonded with a self-adhesive universal resin cement compared with well-tried systems and showed that RelyX-Unicem had the lowest degree of microleakage when compared with glass ionomer, Panavia F 2,0 and Rely X-Arc. The difference between these findings and ours can be attributed to the fact that specimens were subjected to different time periods of incubation and full crowns were evaluated instead of composite inlays.

In addition, our obtained results may be due to the fact that self-adhesive systems can only remove a part of the smear layer and as a result the formed hybrid layer would have a lower quality. The microscopical consequence of such a weak bond is an increased microleakage and a decreased bond strength. Another possible explanation for increased microleakage in self-adhesive systems is insufficient penetration of resin between the enamel rods for formation of resin tags due to the presence of acid-resistant mineral deposits that are also resistant to the pH (acidity) of the adhesive system and especially high viscosity of these cements. Another reason for decreased bonding of these cements to the tooth structure is that their chemical reaction is dependent to the presence of water on the substrate surface. Water prevents full penetration of resin into the dentin collagen and the space that is supposed to be filled with resin, is filled with water that results in a decreased bonding seal [28, 35, 36].

Despite the manufacturer's claim regarding the flawless marginal adaptation of these cements, their complex chemical reaction can be

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responsible for cervical microleakage after 24 hours. De Munck et al. (2004) compared RelyX-Unicem with a conventional resin cement (Panavia F 2,0) and demonstrated that RelyX-Unicem only superficially reacts with dentin and enamel and it requires a little pressure for better adaptation of the cement with cavity walls. The best bond was achieved where enamel surface was etched before cementation (12). In the mentioned study, despite the very low pH of the mixture (less than 2 in the first minute), almost no demineralization was seen on the dentin surface (12) that per se can be responsible for decreased bond strength and microleakage in this area. In our study, the microleakage of RelyX-Unicem was significantly greater than that of RelyX-Arc (control) that can be due to the high viscosity of the cement and short duration of penetration and reaction time since it has to be cured directly immediately after application.

Self-adhesive cements have high viscosity and small penetration into the tooth structure. In previous generations of resin cements like Panavia F 2,0, a self-etching primer containing acidic monomers renders complete penetration of the monomer into the demineralized dentin [37]. For cements like RelyX-Arc, the contact area between the resin and dentin or enamel is fully conditioned using bonding agents and use of a layer of hydrophobic bonding below the resin cement helps in decreasing microleakage and completion of the seal [37].

Considering the technique sensitive nature of self-adhesive cements, when moisture control or pressure application during curing is not feasible, coating the tooth surface with bonding agents (Resin Coating Technique) may improve the properties of these materials [38].

In our study, the difference between cervical and occlusal microleakage in RelyX-Unicem (P=0.0001) and Maxcem (P=0.001) after 24 hours was statistically significant. This finding is in contrast with that of Ibarra et al. in 2006 [13]. They evaluated microleakage of porcelain veneer restorations bonded to enamel and dentin with a new self-adhesive resin-based dental cement and reported decreased micromechanical retention due to the high viscosity and improper pH of the cement. However, in a study by Moezzyzadeh and Moayedi (2007) on microleakage of various bonding systems in enamel and dentin margins of class V composite restorations, it was revealed that in all groups, microleakage in the dentin margin was greater than in the enamel margin [39].

In a study conducted by Gerdolle et al. (2005), microleakage in composite inlays cemented with four bonding agents was evaluated in vitro and it was shown that microleakage in enamel margins was significantly lower than microleakage in cementum margins for the understudy bonding systems. In addition, thermocycling was reported as the main cause of increased microleakage [40]. In our study, microleakage of RelyX-Unicem and Maxcem was greater in dentin margins (cervical) than in enamel (occlusal) margins. It can be concluded that use of these cements on the enamel can decrease microleakage.

Fabianelli et al. in 2005 evaluated wall-to-wall adaptation of a self-adhesive resin cement used for luting gold and ceramic inlays in-vitro and compared it with Fuji Cem and Variolink and attributed its optimal seal to its hydrophilic properties. The hydrophilic nature of this cement results in water sorption after curing that per se causes swelling or in other words enlargement of the material [27] and in long term seals the primary gaps. This finding indicates improvement in the properties of self-adhesive cements over time. In our study, such a result was not observed in the control group (RelyX-Arc). The reason seems to be the chemical formulation of these cements. Although it is expected that by completion of the polymerization process these hydrophilic self-adhesive resin cements become hydrophobic, in our study, the improved seal and decreased microleakage in these cements were somehow indicative of continuation of chemical reactions beyond the first 24 hours.

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It seems that in addition to the formation of complex compounds with calcium ions, other types of physical interventions including hydrogen bonds or bi-polar reactions play a role in the adhesion of self-adhesives [33].

Frankenberger et al. in 2008 evaluated luting of ceramic inlays in-vitro and they compared the marginal quality of self-etch and etch and rinse adhesives versus self-etch cements [41]. They reported that the percentage of gap-free margins was significantly higher in the etch-and-rinse systems.

Goracci et al. in 2006 assessed the microtensile bond strength and interfacial properties of self-etching and self-adhesive resin cements used to lute composite onlays under different seating forces and reported that the adaptation of these cements can be improved by applying a force greater than finger pressure throughout the initial self-curing period; which is in contrast to what was done in our study [42].

Since our study had an in-vitro design, generalization of results to clinical setting should be carried out with caution. Although in-vitro studies have rarely demonstrated a complete seal, most cements usually show an acceptable function.

Also, it should be remembered that leakage of fluid is not necessarily equal to leakage of bacteria and their proliferation. Chemical formulation of the cement also plays a significant role in microleakage through releasing metal ions and fluoride. Final assessment of the properties and function of restorative materials especially cements should only be done through long term clinical studies.

CONCLUSION

-None of the understudy cements resulted in microleakage-free restorations.

-The degree of cervical microleakage in RelyX-Unicem and Maxcem was greater than the occlusal microleakage after 24 hours.

-The greatest level of cervical microleakage was observed in RelyX-Unicem after 24 hours and the lowest occlusal microleakage was observed in the control cement (RelyX-Arc) after 24 hours.

-In RelyX-Unicem and Maxcem self-adhesive cements, a greater microleakage was detected after 24 hours compared with 2 months. This difference for cervical microleakage was statistically significant.

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