Microleakage evaluation of a hydroxyapatite base in comparison with a resin modified glass ionomer cement in vitro

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Abstract
Background and Aim: Many attempts have been done to improve the quality, properties and composition of dental materials to decrease the microleakage and its consequences. The objective of this invitro study was to compare microleakage of light cure resin modified glass ionomer cement and a hydroxyapatite containing base.

Materials and Methods: In this experimental study 40 intact human premolars were divided into 4 groups. On the buccal surface of each tooth, around cavity was prepared with 3 mm in diameter and at least 1 mm deep into dentin. Teeth were restored using L.C resin modified glass ionomer lining cement, L.C resin modified glass ionomer restorative cement, Lime-Lite with bonding agent, and Lime – Lite without bonding agent. After thermocycling the specimens were immersed in AgNO3 solution for 2 hours. After sectioning the specimens, dye leakage was evaluated by a stereomicroscope. Microleakage was graded according to ISO 11 405 in occlusal and gingival cavity margins. Obtained data were statistically analysed by Kruskal-Wallis, Wilcoxon and Mann-Whitney tests.

Results: Most of the samples in group 3 (lime – lite with bonding agent) demonstrated zero microleakage grades in gingival and occlusal cavity margins and in group 4 (Lime – Lite – without bonding agent) the majority of samples showed grade 3 microleakage in both margins. These findings in both margins were significantly higher than those of other groups (p< 0.05), but there were not significant differences among glass ionomer groups in marginal microleakage (p> 0.05).

Conclusion: Hydroxyapatite containing base showed more microleakage compared with glass ionomer cement, but application of a bonding agent prior to its placement was effective in reducing its microleakage.

Key Words: Microleakage - Glass Ionomer - Hydroxyapatite cement

Introduction
Microleakage at tooth-restoration interface is a common problem in clinical dentistry that results in recurrent caries and restoration failure [1]. Microleakage causes passage of microorganisms, saliva, molecules and ions through tooth-restoration interface that results in hypersensitivity, discoloration, recurrent caries, pulpal damage, and rapid
destruction and degradation of some restorative materials [1-3]. Sometimes, there is a need to place a layer of cement as liner and/or base before application of the restorative material in the cavity, to protect the pulp against thermal, electrical or mechanical insults. This can also be effective to oppose polymerization shrinkage of composite resins [1]. Minimizing the number of interfaces between tooth and restorative material gives rise to increased restoration longevity. In this regard, On the other hand, integrity of the restoration reduces the likelihood of microleakage through the dental materials used. One of the most frequent liners and bases in use today are light-activated resin-modified glass ionomer cements with superior simplicity of use, higher bond strength and fluoride release in comparison with self-cured glass ionomer cements [3]. Light-activated resin modified glass ionomer cements are used as insulators in amalgam restorations. They are also used to oppose polymerization shrinkage of composite resins and to remove undercuts in indirect restorations. They are available in powder-liquid form in which the powder-to-liquid ratio should be carefully determined during preparation of the material. They should be mixed within the suggested time to give rise to the favorable consistency. Preparation of this cement in its recommended consistency requires experience and care. On the other hand, glass ionomer cements are moisture-sensitive - a feature that renders them difficult to manipulate [4]. Therefore, materials capable of being used as a bases or liners with properties similar to those of glass ionomer cements, but with easier application and less technique sensitivity can serve as appropriate alternatives. Several changes, including changes in chemical composition, have been made in order to improve the properties of bases and liners. Incorporation of hydroxyapatite has been taken into consideration to improve mechanical properties of different dental materials. Lime-lite is a novel resin-based hydroxyapatite-containing material that can be used as a liner or base according to the manufacturer’s claim. It releases hydroxyl, fluoride and calcium ions. It is radiopaque and is delivered through a syringe. The manufacturer has stated that the use of an adhesive resin prior to this material is optional, but it is not also clear whether the use of an adhesive before its application is influential in reducing microleakage [5]. Limited information is available about the properties of this material. Some authors evaluated its compressive strength with and without application of an adhesive, and some investigated its shear bond strength to composite resin [6,7]. No information is available about microleakage of this material. On the other hand, several investigations were available regarding microleakage of different dental materials including glass ionomer cements and composite resins [1,8-13].

According to the information gap regarding microleakage of this novel hydroxyapatite-containing base, this study was aimed to compare microleakage of this material with a commonly used resin-modified glass ionomer cement.

Materials and Methods
This experimental in vitro study involved 40 human intact premolar teeth which were extracted for orthodontic purposes. The teeth were visualized under 10x magnification to rule out any cracks, fractures, hypocalcifications, or carious lesions. This study was designed according to ISO TR 11405 (2003) specifications [14]. Characteristics of the materials used in this study are shown in table 1. Teeth were stored under distilled water after extraction. Remaining periodontal tissues were then removed by a scaler. Afterwards, all teeth were cleansed using a rubber cup and non-fluoridated pumice. Teeth were disinfected by being immersed in 0.2% thymol solution for 48 hours at room temperature [1]. A round cavity 3±0.2 mm in diameter was prepared at least 1 mm deep into dentin at the midbuccal area of each tooth using a cylindrical diamond bur (D&Z, Germany). Each bur was discarded after 5 cavity preparations. Then, the samples were randomly divided into four groups (n=10 each) and restored as follows:
Group 1: Cavities were filled by base/liner type light-activated resin-modified glass ionomer
cement (Fuji lining LC, GC corporation, USA). The powder and liquid were mixed according to the manufacturer’s recommendation.

After drying the cavity with a cotton pellet, the material was placed within the cavity. Then, each layer of the material was photo-cured by a quartz-tungsten-halogen light-curing unit (Coltolux, Switzerland) with an intensity of 400 mw/cm² for 30 seconds.

Group 2: cavities were restored by restorative type of light-activated resin-modified glass ionomer cement (Fuji II LC, GC corporation, USA). Powder and liquid were mixed according to the manufacturer’s recommendation and the cement was applied within the cavities as in group 1. Then, the material was light cured as previously mentioned.

Group 3: The cavities were filled using a hydroxyapatite-containing base (Lime-lite, Pulpdent, USA) without use of any bonding agent. After rinsing and drying the cavities, a 0.5-mm thick layer of the material was applied within the cavity using the specified syringe and cured for 20 to 30 seconds. A second layer was applied upon the first layer of the material to completely fill the cavity. Then, the excessive material was removed from the cavity margins and cured for 30 seconds.

Group 4: The cavities were filled with a hydroxyapatite-containing base (Lime-lite, Pulpdent, USA) accompanied with application of a bonding agent suggested by the manufacturer (Dentastic, Pulpdent, USA). In each sample, after cavity preparation and isolation, enamel and dentin surfaces were etched by the use of a 38% phosphoric acid in the bonding kit for 15 seconds. Then, the cavity was rinsed and dried for another 30 seconds. Excessive moisture was removed by a cotton pellet. Two drops of Dentastic bonding agent were applied in two separate stages in the cavity using a microbrush. Excessive bonding agent was removed by microbrush and air-blast. Photo-curing was carried out for 10 seconds. Then, Lime-lite was placed in the cavity as in group 3 and cured for 20 seconds.

All prepared samples were polished using coarse to fine composite polishing discs (KerrHawe, USA). Samples were immersed in distilled water for 24 hours at room temperature. Subsequently, the samples were thermocycled (Malekteb, Iran) for 5000 cycles under 5-55 degrees centigrade with a dwell time of 30 seconds. Apical end of the root in each sample was sealed with sticky wax and all surfaces were covered with nail varnish except 1 mm around the restoration margin. Samples were placed in 50%(w) silver nitrate solution for 2 hours and then immersed in a radiographic fixing solution under fluorescent light in order to facilitate reduction of silver ions. Samples were then longitudinally sectioned using a 0.5 mm sectioning de-

| Table 1: Characteristics of the composites used in the study |
|---|---|---|
| **Components** | **Manufacturer** | **Material** |
| Powder: aluminofluorosilicate glass | GC, USA | base/liner type light-activated resin-modified glass ionomer cement |
| Liquid: polyalkenoic acid, HEMA | | |
| Powder: aluminofluorosilicate glass | GC, USA | restorative light-activated resin-modified glass ionomer cement |
| Liquid: polyacrylic acid, HEMA | | |
| 2-2-4 trimethylhexamethylene dicarbonate, TEGDMA, water | | |
| Hydroxyapatite, urethane dimacrylate resin, fluoride salt, barium sulfate, photoinitiator | Pulpdent, USA | Lime-lite |
| Hydrophilic resin-containing bonding agent, PMGDM, acetone solution | Pulpdent , USA | Dentastic |
vice (Mecatom, Persi Co., France) Dye penetration was evaluated using a stereomicroscope (Nikon, Japan) under 100x magnification, by a blinded and calibrated examiner. Microleakage was scored according to ISO 11405 (2003) specifications as follows: \[8,14\]
Score 0: No penetration of the dye material
Score 1: Dye penetration up to the enamel wall of the cavity.
Score 2: Dye penetration through the dentinal wall of the cavity without involvement of the pulpal or axial wall.
Score 3: Dye penetration up to the pulpal or axial wall of the cavity.

Comparison of microleakage was statistically carried out using Mann-Whitney, Kruskal–Wallis, and Wilcoxon Signed tests.

**Results**

Within this experimental study evaluation of microleakage of two types of light-activated resin-modified glass ionomer cements (liner and restorative types) was carried out in comparison with a hydroxyapatite-containing base (with and without application of a bonding agent) according to ISO 11405 (2003) specifications. The major findings are illustrated in graphs 1 and 2.

Graph 1 shows the number of samples showing different amounts of leakage in occlusal margins of the study groups. As illustrated, group 4 (Lime lite with Dentastic bonding agent) has the highest number of zero-score microleakage. Group 3 (Lime-lite without bonding) showed the highest number of score 3 microleakage. Also, in this group none of the samples showed score 0 and 1. There was no score 0 in glass ionomer groups, but only one sample showed score 3. Kruskal-Wallis test showed that there was a significant difference between microleakage of group 3 in comparison with other groups (p<0.05). There was no significant difference between glass ionomer groups (groups 1 and 2) and group 4 in microleakage of occlusal margins (p>0.05).

Microleakage findings in gingival margins of the study groups are depicted in graph 2. It was shown that the maximum number of samples showing score 0 microleakage in gingival margin was in group 4, but this group showed a higher number of score 3 microleakage in comparison with glass ionomer groups. Groups 1, 2, and 3 indicated no score 0 microleakage in gingival as in occlusal margins. On the other hand, all samples in group 3 had score 3 microleakage in gingival margins. Kruskal-Wallis test showed that the difference between gingival microleakage of group 3 was significant in comparison with other groups (p<0.05).
Wilcoxon Signed Ranks test showed that there was no significant difference in microleakage of occlusal and gingival margins in any of the groups (p>0.05). Mann-Whitney test showed that use of Dentastic adhesive prior to application of Lime-lite can significantly cause a decrease in occlusal and gingival microleakage of Lime-lite (p<0.05).

Discussion
There has long been a trend toward elimination of microleakage through increase bonding capabilities of the dental materials. Microleakage of a material is dependent upon several factors including the chemical, mechanical and thermal properties of a material, as well as the forces applied to the tooth and the characteristics of the tooth [2,3].

Glass ionomer cement is well known for its physicochemical bond to the enamel and dentin, fluoride release and anti-caries properties and is used as a liner, base or a restorative material. (2 The physicochemical bond of the glass ionomer cement to the tooth structure is extremely resistant as an intermediate layer of glass-ionomer-tooth is formed. On the other hand, closeness of the linear coefficient of thermal expansion in tooth structure and the glass ionomer cement results in a decreased likelihood of percolation and microleakage in tooth-restoration interface, thereby enhancing the restoration longevity.

Resin-modified glass ionomer cements represent superior resistance against acid solubility and moisture sensitivity. They have increased capability to release fluoride, resulting in enhanced anti-caries ability [4,15]. The major disadvantage of this kind of glass ionomer cement is difficulty in manipulation of this material. Recently, a light-cured liner/base material is presented in a syringe with easier application with respect to conventional glass ionomer cements. This material contains hydroxyapatite in a urethane di-metacrylate base and has the capability to release fluoride, calcium and hydroxyl ions. Therefore, it has anti-caries properties [5-7]. The results of the current study indicated that all materials show different levels of microleakage. Glass ionomer cements used in this study did not show any significant difference between each other in their microleakage. Perhaps the use of conditioner prior to the application of this material can have a positive role in reducing microleakage. Application of Lime-lite without use of bonding agent showed a significantly higher microleakage in both occlusal and gingival margins in comparison with other groups. Although application of the dental adhesive Dentastic before use of Lime-lite is not definitely advocated by the manufacturer, [5] it could cause a significant decrease in occlusal and gingival microleakage in this study. Dentastic is a hydrophilic adhesive containing PMGDM. This composition is capable of establishing a stronger bond compared with other available materials incorporated in chemical composition of adhesives. As seen in composite resins, it appears that adhesion of Lime-lite can also be more predictable if an adhesive used before its application. Definitely, more extensive studies are required for this idea to be approved, such as shear bond strength tests and SEM evaluations. In addition, the need for prior application of an adhesive should be positively supported by the manufacturer. Information about Lime-lite and its properties is scarce [6,7]. On the other hand, the non-significant difference between Lime-lite and resin-modified glass ionomer cement used as base/liner indicates that this material can be used in certain clinical circumstances. It appears that in deep cavities with thin remaining dentin thicknesses, use of a calcium hydroxide liner can prevent cytotoxic effects of the resinous base, thereby minimizing pulpal damage and cold sensitivity [16]. Indeed, evaluation of cytotoxicity of this material is still highly required.

It should be kept in mind that each material be used in its proper situation. It is likely that incomplete polymerization ensues while using Lime-lite in deep cavities- a fact that requires more investigations especially using FTIR technique to be elucidated.

Microleakage of glass ionomer cements has been extensively studied in comparison with composite resins in the literature. Mali et al showed that con-
ventional glass ionomer cement had the highest and glass ionomer resin cement the lowest amount of microleakage, with no statistically significant difference between glass ionomer cement and composite resin [1]. Ajami and colleagues also showed that resin-modified cement had a lower microleakage in comparison with compomer in gingival and axial walls [17].

In the current investigation, both liner-type and restorative-type glass ionomer cements indicated similar microleakage. In this investigation, cavity dimensions, bur type selection, storage condition of the teeth before and after restoration and microleakage scores were all performed according to the ISO11405 specifications [14]. This can strengthen the possibility to generalize the results of this study.

The selected dye is important in evaluation of microleakage. Fuchsin, methylene blue and silver nitrate have been utilized in various investigations [8-10,13,17-20]. Fifty percent (w%) silver nitrate was used for evaluation of microleakage which was in accordance with Mali et al. Use of silver nitrate for detection of microleakage is considerable due to the small size of silver ion (0.059 nm). Common bacterial species of the oral cavity are usually 0.1 to 0.5 micrometers long. Therefore, the silver ion is more penetrable. The restorative material should have a high bonding ability to prevent ion penetration [1]. Evaluation of microleakage was carried out according to ISO11405 standards as in Gerdolle DA, Ashvin R, Magni E and Wahab F et al’s studies [8,9,14,18,19]. Other microleakage scoring systems are also available in the literature but the one proposed by ISO is more valid and more capable of being generalized. Evaluation of microleakage in both occlusal and gingival margins highlights the importance of dental tissue and effectiveness of the material of interest in adhesion to these areas. Occlusal margins show less microleakage and better adhesion with restorative materials due to the presence of enamel, but in gingival margins, decreased enamel thickness and presence of dentin leads to microleakage [2,3]. In the current study, the cavities were located in the midbuccal areas of the teeth according to ISO standards, therefore no significant difference was observed in microleakage of occlusal and gingival margins. On the other hand, similar adhesion behaviors of the materials indicate proper bonding of the materials used. Definitely, scanning electron microscopic evaluations provide a more detailed evaluation of the interface between Lime-lite and tooth structure in comparison with microleakage studies.

**Conclusion**

According to the conditions of this study, it can be concluded that all tested materials showed various degrees of microleakage. Lime-lite without bonding had the highest microleakage value. Lime-lite with bonding and light-activated resin modified glass ionomer showed similar values of microleakage. Use of Dentastic bonding agent before application of Lime-lite significantly decreased microleakage when compared with the time when this material was used without bonding agent.

**References**

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