Effect of Mechanical Load Cycling on Class V Glass-Ionomer and Composite Restorations; A Microleakage and Scanning Electron Microscopic Evaluation

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Abstract

Background and Aim: Microleakage is an important problem with direct restorations and familiarity with its contributing factors is of utmost importance. The aim of this study was scanning electron microscopic evaluation of marginal integrity in three types of class V tooth-colored restorations and the effect of load cycling on their microleakage.

Materials and Methods: In this in vitro study, class V cavity preparations were made on the buccal and lingual surfaces of 30 bovine incisors (60 cavities). The specimens were divided into three groups (n=10 each or 20 cavities) and restored as follows: group 1: with Filtek Z350(nanocomposite), group 2: Fuji IX/G Coat Plus(CGIC) , and group 3: Fuji II LC(RMGI).

All specimens were finished and polished immediately and were thermocycled (~2000,5-50 °C). In each group, half of the teeth were load cycled .Epoxy resin replicas of 12 specimens were evaluated under FE-SEM and interfacial gaps were measured. Finally the teeth were immersed in 0.5% basic fuchsin dye for 24 hours at room temperature, sectioned and observed under stereomicroscope. Data were analyzed with Kruskal-Wallis and Mann-Whitney U tests and a comparison between incisal and cervical microleakage was made with Wilcoxon test.

Results: It was shown that the mechanical load cycling caused a statistically significant increase in cervical microleakage of Fuji IX and Fuji II LC and in incisal microleakage of Fuji II LC. Microleakage in Z350 with load-cycling and Fuji IX with and without load-cycling was significantly higher in cervical compared with incisal area. Both incisal and cervical microleakage were significantly different among these materials under load-cycling.( Fuji II LC>Fuji IX>Z350).

Conclusion: It was concluded that the marginal sealing ability of Fuji IX under load-cycling was better than that of Fuji II LC. Z350 showed better marginal integrity while being load-cycled than both Fuji II LC and Fuji IX.

Key Words: Microleakage, Glass ionomer cement, composite resin
Introduction
Cervical lesions occur due to incorrect brushing, caries or excessive occlusal forces [1]. Microleakage is considered as the most important problem of cervical restorations [2]. The main reason for restoration failure is microleakage through the tooth-restoration interface [2, 3]. This can cause bacterial penetration through tooth-restoration interface thereby leading to secondary caries and pulpal irritation. Factors within the oral cavity such as occlusal forces, thermal changes, difference in physical properties of teeth and restorative materials such as polymerization shrinkage, coefficient of thermal expansion and modulus of elasticity are associated with microleakage [4]. Several studies have been conducted to eliminate the tooth-restoration gap, this goal has not yet been achieved. There is still a considerable challenge in marginal seal of even advanced bonded restoration systems with margins located beneath the CEJ [5-11]. Numerous types of materials have been introduced to establish a bond to tooth structure including glass ionomers. Which have the ability to bond to enamel and dentin [12, 13], release fluoride, and prevent caries. They are compatible with pulpal and periodontal tissues, have a coefficient of thermal expansion similar to that of dentin and a polymerization shrinkage less than that of composite [13,14]. The effect of restoration material on microleakage of class V cavities is a matter of debate, with some studies reporting better [15], some worse [16], and some others a comparable seal of glass ionomer in comparison with that of composite [17]. However, evaluation of changes in microleakage of these two materials while being subjected to occlusal forces is of paramount importance. Controversial issues exist concerning the effects of mechanical cycling on microleakage [18-20]. Few investigations have been performed on the effect of mechanical cycling on marginal adaptability of glass ionomers [21]. On the other hand, a novel restorative glass ionomer, Equia, has been introduced which is a combination of Fuji IX glass ionomer and a coating material (G Coat Plus). It has been claimed that better physical properties, fast contouring and decreased microleakage can be achieved following application of a nano-filled varnish. The objective of this study was to evaluate the effects of mechanical cycling on microleakage of three different tooth-colored restorative materials (i.e., Z350 composite, Fuji IX, and Fuji II LC) in class V cavities and evaluation of their marginal adaptability by scanning electron microscopy (SEM).

Materials and Methods
A total of 30 bovine central incisor teeth were used in this study. Teeth were inspected not to have any surface defects. Two class V cavities, one on buccal and the other on lingual surface of the teeth were prepared. Then, teeth were immersed in 0.5% chloramine T solution for disinfection and stored in normal saline at 4 degrees centigrade prior to the experiment. Standardized cavities had 3mm mesiodistal and 3mm occluso-gingival dimensions. The intradental depth was 1mm and the gingival margin was 1mm beneath the CEJ. Cavities were prepared by round diamond burs (DIAMIR,Italy,FGS 001018) with a high-speed handpiece and water spray. Burs were discarded after 5 preparations. Tooth pulps were also mechanically removed. Samples were randomly divided according to the type of restoration material and use of mechanical cycling.

Group 1: following desiccation, the enamel and dentin parts of the cavity were etched by 37% phosphoric acid (Kimia Co, Iran) for 30 and 15 seconds, respectively, rinsed for 15 seconds and dried with a cotton pellet. The procedure was done in a way that the cavity was not completely dried. Then, two layers of Single Bond adhesive (Adper Dental Products, ESPE, 3M, St. Paul, MN, USA) was applied and cured for 10 seconds, using a quartz-tungsten-halogen device (Optilux 501, Kerr, USA). Afterwards, each cavity was filled with three layers of Z350 composite (3M ESPE, Dental Products, USA) and
cured in each stage with the same curing device with an intensity of 520 mW/m² for 40 seconds. Group 2: In this group cavities were conditioned with a dentin conditioner (GC, Japan), rinsed and dried using an air syringe preventing over-desiccation of dentin and finally filled with self-curing injectable Fuji IX cement (GC Fuji IX GP EXTRA). One layer of coating (G Coat plus, GC Corp, Japan) was applied after complete hardening of the cement (2.5 minutes). Group 3: In this group the cavities were conditioned (Dentin Conditioner, GC, Japan) for 20 seconds, rinsed and dried with air spray and eventually filled with a glass ionomer cement (Fuji II, LC, Japan) and cured as stated previously. The glass ionomer cement was prepared in a way that 2 parts of powder were mixed with one drop of liquid on a glass slab to produce 3 minutes of working time. After curing, the surface was coated by Fuji Varnish (GC, Tokyo, Japan).

All teeth were polished using a super-fine grit diamond bur (FGSF 273012) under water spray. Coating were applied after polishing in groups 2 and 3. The teeth were thermocycled for 2000 cycles using 5-50 degrees centigrade. Subsequently, half of the samples underwent mechanical cycling (Germany, SD Mekanotronic). This procedure was performed as follows: initially a cylindrical tube was covered with a layer of wax, then the teeth were mounted up to a 1mm distance below the gingival margin of the restoration in a self-curing acrylic resin (Acropars 200, Malic Medical Industries Co, Tehran, Iran) at the middle and parallel to walls of the tube. Samples were then tested under 25000 axial and eccentric cycles using a force of 100 Newtons, a displacement of 1 millimeter and a frequency of 1 Hz. Afterwards, each tube was rotated in 180 degrees and another set of 25000 cycles was performed with the above mentioned specifications. Therefore, the total mechanical cycling time was about 14 hours for 50 cycle sets.

**Preparation of Replicas for Field Emission Scanning Electron Microscopy**

Before sectioning the teeth and in order to evaluate microleakage, an impression (Precise, Coltene, Switzerland) was made from two teeth in each group and positive epoxy resin (Epo-thin, Buehler Ltd., Lake Bluff, IL) replicas were prepared. Each replica was mounted on a metal stub and was gold sputtered. Samples were then visualized under a field-emission scanning electron microscope (FE-SEM) (Hitachi s4160, Japan) with 10,000x magnification to evaluate marginal adaptability. Before this evaluation, occlusogingival and mesiodistal dimensions of each restoration was measured using FE-SEM software and the results were compared with those of cavity dimensions, to rule out the presence of any overextended margin influencing on marginal gaps. Restoration margins were evaluated and gaps, if present, were recorded. Total length of interfacial gaps were pointed to as a percentage of total length restoration margins. The margins of the cavity was divided into 8 sections (see fig.1) each comprising 12.5% of the total length of restoration margins. Each section was visualized separately and percentage of gap formation was recorded. The total length of the interfacial gaps was considered as the sum of the total percentage of recorded marginal gaps for each section. (See fig.2)

**Microleakage evaluation**

After preparation of SEM replicas, the surfaces of all teeth were covered with three layers of nail varnish.

Figure 1: Cavity margins were divided into 8 sections and each section was visualized for evaluation of the percentage of gap formation.
Samples were immersed into 0.5% basic fuc-sin solution in 27 degrees centigrade for 24 hours.

**Figure 2:** A replica is shown on the right (20x) and interfacial gap of the same sample is shown on the left (10,000x)

Samples were rinsed with a physiologic solution and air dried. The teeth were mounted in self-curing acrylic resin molds. Two longitudinal sections (Presi, Mecatome, T 201 A, France) were made through the restored area so that a one-millimeter-thick cut was placed at the middle portion of the restoration, preparing two halves for microleakage evaluation [4]. Microleakage was visualized as dye penetration under a stereomicroscope (Nikon, SMZ 800, Tokyo, Japan) with 10 and 40x magnification settings according to the following scores:

Score 0. No dye penetration
Score 1. Dye penetration less than half of the incisal or gingival wall
Score 2. Dye penetration more than half of the incisal or gingival wall but not reaching the axial wall
Score 3. Dye penetration involving the axial wall

Data were analyzed by Wilcoxon, Mann-Whitney U and Kruskal-Wallis statistical tests and p<0.05 was considered a statistically significant difference.

**Results**

Results of incisal and cervical microleakage are shown in table 1. Microleakage was significantly more in cervical than in incisal margins of composite restorations while being under cycling and Fuji IX restorations with and without mechanical cycling. (See table 2) However, there was no statistically significant difference in incisal and cervical microleakage of Fuji II LC restorations with or without mechanical cycling and Z350 composite restorations without mechanical cycling.

Mechanical cycling significantly increased cervical microleakage of glass ionomer- restored samples. Besides, incisal microleakage of Fuji II LC was significantly increased following mechanical cycling. (See table 3) On the other hand this process did not have any statistically significant effect on incisal and cervical microleakage of Z350 composite and incisal microleakage of Fuji IX. (See table 3)

Kruskal-Wallis test indicated that there was a significant difference in cervical microleakage of the restorative materials while being mechanically cycled. (p=0.002) Also, there was a significant difference among all three restorative materials in incisal microleakage after mechanical cycling. (p=0.001) It has to be mentioned that the difference between incisal and cervical microleakage of restorative materials within the three groups was not statistically significant when no mechanical cycling was applied. (p>0.05)

**Results of field-emission scanning electron microscopic evaluation**

The results of field-emission scanning electron microscopic (FE-SEM) evaluation of the two random samples from each groups showed that in Z350 composite group after mechanical cycling 1.534% and 0.01% interfacial gap was observed, but in the same group without mechanical cycling both random samples showed 0% interfacial gap formation. Within the group restored with Fuji IX after mechanical cycling, the first sample showed 1.647% and the second 8.167% interfacial gap formation, but in those without mechanical cycling one sample showed 0% and the other 0.71% gap formation.
Table 1: Frequency of microleakage in each margin according to the use of restorative material and mechanical cycling

<table>
<thead>
<tr>
<th>Microleakage</th>
<th>Incisal microleakage</th>
<th>Cervical microleakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>scores</td>
<td>0 1 2 3 0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>Z350 Mechanical Cycling</td>
<td>16 4 0 0 11 7 2 0</td>
<td></td>
</tr>
<tr>
<td>Z350 No Mechanical Cycling</td>
<td>15 2 2 1 14 2 3 1</td>
<td></td>
</tr>
<tr>
<td>Fuji IX Mechanical Cycling</td>
<td>9 7 4 0 5 1 10 4</td>
<td></td>
</tr>
<tr>
<td>Fuji IX No Mechanical Cycling</td>
<td>14 0 5 1 9 5 5 1</td>
<td></td>
</tr>
<tr>
<td>Fuji II LC Mechanical Cycling</td>
<td>6 4 4 6 5 4 3 8</td>
<td></td>
</tr>
<tr>
<td>Fuji II LC No Mechanical Cycling</td>
<td>11 5 4 0 9 7 4 0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The results of Wilcoxon test in comparison of microleakage scores according to the use of the restorative material and mechanical cycling (a, b, and c are considered statistically significant)

<table>
<thead>
<tr>
<th>Restorative material</th>
<th>Loading</th>
<th>margin</th>
<th>number</th>
<th>Z value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z350</td>
<td>Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-2/333</td>
<td>0/02 a</td>
</tr>
<tr>
<td>No Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-0/557</td>
<td>0/557</td>
<td></td>
</tr>
<tr>
<td>Fuji IX</td>
<td>Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-2/924</td>
<td>0/003 b</td>
</tr>
<tr>
<td>No Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-2/236</td>
<td>0/025 c</td>
<td></td>
</tr>
<tr>
<td>Fuji II LC</td>
<td>Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-0/95</td>
<td>0/342</td>
</tr>
<tr>
<td>No Mechanical Cycling</td>
<td>Incisal</td>
<td>20</td>
<td>-0/44</td>
<td>0/66</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The results of Mann-Whitney U test about microleakage according to the application of mechanical cycling or lack thereof in any of the restorative materials in cervical or incisal margins (* shows significance of difference)

<table>
<thead>
<tr>
<th>Margin</th>
<th>Restorative material</th>
<th>Loading</th>
<th>number</th>
<th>Z value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incisal</td>
<td>Z350</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-0/594</td>
<td>0/678</td>
</tr>
<tr>
<td>No Mechanical Cycling</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-0/82</td>
<td>0/478</td>
<td></td>
</tr>
<tr>
<td>Fuji IX</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-2/252</td>
<td>*0/033</td>
<td></td>
</tr>
<tr>
<td>No Mechanical Cycling</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-0/533</td>
<td>0/659</td>
<td></td>
</tr>
<tr>
<td>Fiji II LC</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-2/196</td>
<td>*0/038</td>
<td></td>
</tr>
<tr>
<td>Z350</td>
<td>No Mechanical Cycling</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-2/435</td>
<td>*0/018</td>
</tr>
<tr>
<td>cervical</td>
<td>Fuji IX</td>
<td>Mechanical Cycling</td>
<td>20</td>
<td>-2/435</td>
<td>*0/018</td>
</tr>
</tbody>
</table>
In groups restored with Fuji II LC, the samples which had undergone mechanical cycling showed 1.712% and 6.861% interfacial gap formation, but in those without mechanical cycling one sample had 0.55% and the other 0.02% interfacial gaps.

Discussion
In this study bovine teeth were used because they have shown great similarities in structure with human teeth in literature [22-23]. In addition Kubo et al stated that there was no difference in mechanical cycling between human and bovine teeth. They also used repeated bucco-lingual loading and proposed that such loading can produce higher tensile strain in interfacial area compared with the time when axial loading I applied. They attributed this fact to the flexural load applied to the tooth [18] It is noteworthy that in the current study, both axial and bucco-lingual loadings have been used but due to the flattening of incisal surfaces in all samples in order to equalize the condition, flexure caused by bucco-lingual loading was far less than the time when no reduction is performed. This could have simulated an edge-to-edge occlusal relationship. On the other hand, during axial loading, teeth were subjected to flexural loading as well because they were mounted on a rigid platform [24].

According to Heymann et al factors related to the flexure of the teeth can be significantly influential in class V composite retention failures [25]. Additionally, one of the etiologic factors in non-carious cervical lesions is occlusal or incisal forces that can cause loads on dentin enamel junction in cervical areas [26]. Elastic or plastic deformations of restored teeth cause bond failures in restoration margins due to compressive stresses resulted from occlusal forces. [27]

In the current study, the effect of mechanical cycling on incisal and cervical microleakage of Single Bond/Z350 composite was not considered significant. This finding is in accordance with the results of previous studies [24,28,29] but statistically significant results were reported in microleakage of samples after mechanical cycling using 5000 cycles and 125 and 250 newton forces by Davidson and Abdalla [21]. Rigsby also stated that composites showed increased microleakage in cervical areas only when teeth were subjected to a combination of mechanical and thermal cycling [30]. It has also been shown that, 71% of class V restorations in third molars with opposing teeth indicate more leakage than those without opposing teeth [27].

Such differences in laboratory studies can be attributed to the difference in materials examined, the amounts of and methods, by which forces are applied, mechanical preparation of the cavities and/or the evaluation procedure.

In this study, microleakage of Fuji IX occurred only in cervical area following mechanical cycling. According to Yap et al, when condensable silver-reinforced glass ionomer (Shofu Hi Dense) was placed in class III cavities with intradentinal cervical margins and subjected to 100 cycles using a maximal force of 170 ±8 N, no significant difference was observed between experimental and control groups within enamel-restoration interface, but marginal microleakage at dentin-restoration interface was significantly higher in samples which had been subjected to cycling compared with those which had not (i.e., control). This can be attributed to the higher inorganic content of enamel which can provide a better seal with glass ionomer even when mechanical cycling is performed [31]. On the other hand, Ichim and co-workers who conducted a three-dimensional finite element analysis of cervical cavities filled with glass ionomer, in the cervical margin showed that more tensile stress was concentrated within the cervical margin- the area with increased susceptibility of restoration failure [22].

In Fuji II LC, both cervical and incisal microleakage was influenced by mechanical cycling. Nonetheless, in an investigation by Davidson and Abdalla, no significant effect of mechanical
cycling on Fuji II LC was observed when the samples underwent a 125-newton axial force for 5000 cycles, but following an increase in the force up to 250 newtons a significant effect on microleakage of this material was observed following mechanical cycling [21]. Of course, in the current study, the applied force was 100 N and the mechanical cycles were ten times that of Davidson et al’s study. Since it is stated by Xie and colleagues that Fuji II LC undergoes plastic deformation following application of compressive forces [33], such increase in microleakage following cycling can be due to plastic deformation of Fuji II LC glass ionomer and disruption of micromechanical bonds.

It was shown in this study that increased microleakage of composite was seen after being subjected to mechanical cycling. This was in agreement with Rigsby et al [30]. It has been declared by several investigators that adhesive strength in inferior in cemental substrate with respect to enamel in compositive restorations [34, 35]. Increased microleakage in cemental margins is expectable [36, 37]. Although Z350 is a low-shrinkage composite and oblique incremental technique was used in this study to minimize the effects of stresses resulting from C-factor, low polymerization shrinkage was not able to overcome the adhesive strength and microleakage ensued especially following mechanical cycling.

In the current study, cervical area microleakage of Fuji IX following mechanical cycling was significantly more than that of incisal area. This was congruent with Ichim et al who stated that parafunctional forces applied on teeth with class V cavities restored with gall ionomer can initially cause strain softening of the material in cervical area thereby leading to marginal weakening and finally cervical fracture of the restoration [28]. Likewise, significance of cervical and incisal microleakage of Fuji IX without application of mechanical cycling can be attributable to the higher inorganic content of enamel in incisal margin with respect to dentin and cementum in cervical region.

It can be declared by this study that mechanical cycling increases microleakage of Fuji II LC in both cervical and incisal and that of Fuji IX only in cervical areas. SEM evaluation also corroborated these results by showing increased percentage of marginal gap formation.

Lack of a significant difference between incisal and cervical microleakage in Fuji II LC with and without mechanical cycling, might have been related to destruction of both margins thereby equally increasing the microleakage.

When mechanical cycling was performed, incisal as well as cervical microleakage was significantly different among restorative materials. The minimal and maximal amount of microleakage in both incisal and cervical margins was recorded for composite and Fuji II LC, respectively. No statistically significant difference was observed among any of the restorative materials when no mechanical cycling was performed.

These results are congruent with Castro and co-workers who stated that Fuji IX had less microleakage compared with older standard glass ionomer cements but had similar microleakage in comparison with resin-modified glass ionomer cement and composite [17].

In the present study, there was no significant difference in microleakage of Fuji IX and Fuji II LC without application of mechanical cycling. This was in accordance with the results of Rodrigues who stated that microleakage of Fuji II LC was similar to that of other self-curing glass ionomer cements [39]. There are numerous controversial issues in the literature concerning comparison of self- and light-curing glass ionomer cements in terms of their microleakage, with some reporting self- and some others light-curing materials to have more microleakage [40]. This diversity can be related to different methods of investigation, different materials used as well as improvements in manufacturing process of glass ionomer cements.
Although there has not been any investigation to compare microleakage of composite and glass ionomer under similar cycling conditions, the reason for increased incisal and cervical microleakage of glass ionomer in comparison with composite under cycling condition is probably attributable to the strain softening of glass ionomer under bucco-lingual (such as parafunctional) forces that cause marginal weakening [28]. In the present study Z350 nanohybrid composite was used. This composite contains nanoclusters. Infiltration of silane into nanoclusters can modify the response to the applied loadings and increase failure tolerance, thereby improving clinical performance and providing an inherent strengthening mechanism in this type of composite in comparison with microhybrid and microfilled composite resins [42]. This can be a reason for superior results obtained in composite in our study compared with other similar works.

**Conclusion**

Based upon the obtained results, it appears that Fuji IX provides better marginal adaptability under mechanical cycling compared with Fuji II LC. Z350 showed the highest marginal adaptation under mechanical cycling. Enamel margins indicated a better seal in compared with dentinocemental counterparts in Fuji IX and Z350 composite after mechanical cycling. Also, mechanical cycling was found to increase cervical and incisal microleakage of Fuji II LC and cervical microleakage of Fuji IX.

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