Microleakage of Different Thickness of Restorative Materials Used in Endodontically Treated Teeth by Dye Penetration

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ABSTRACT
This study aimed to evaluate the coronal microleakage of different thickness of different restorative materials (glass ionomer cement (GIC, GC Gold Label 2), composite restoration (SDR, Dentsply Sirona) and (Filtek Z350 XT, 3M ESPE)) used as final restoration in endodontically treated teeth. 72 sound maxillary incisors were used. Following instrumentation, all canals were obturated with gutta-percha (Dentsply Maillefer) and Roth sealer (Roth International Ltd). After 2mm of gutta-percha removal below cemento-enamel junction, the crown was cut until length of 6mm from the gutta-percha to the incisal edge was achieved. The teeth were divided into four experimental groups (n=18) and access restorations were placed in different thickness combinations. Group 1 (G1): 2mm SDR + 4mm Filtek; Group 2 (G2): 4mm SDR + 2mm Filtek; Group 3 (G3): 2mm GIC + 2mm SDR + 2mm Filtek; Group 4 (G4): 6mm SDR. All samples were thermocycled (500 thermal cycles between 5⁰ and 55⁰C and dwell time of 30s), coated with nail varnish leaving 1mm margin around the filling material, immersed in 2% Rhodamine B solution and sectioned longitudinally. The dye penetration was observed under a stereomicroscope (Olympus SZX7) with 1.25x magnification. The data were analysed using Kolmogorov-Smirnov test, ANOVA test and post-hoc Tukey’s HSD test. There was significant difference of microleakage among all groups. G1 showed least microleakage but with no significant difference between G1 and G3 (p=0.513) and G1 and G4 (p=0.477). G2 showed significant microleakage compared to G1, G3 and G4 (p<0.05). In conclusion, sandwich technique between SDR and Filtek reduces microleakage in which the combination of 2mm SDR with 4mm Filtek in G1 had the least microleakage but with additional 2mm of GIC in G3 further reduces the microleakage.

Keywords: Coronal microleakage; Endodontic; Composite Resin; Smart Dentine Replacement; Glass Ionomer Cement; Dye Penetration

INTRODUCTION
It is crucial to have a maximum sealing ability of access restoration in endodontically treated tooth in order to prevent coronal microleakage. Microleakage is defined as the passage of fluids, debris, bacteria and salivary constituents into microscopic space in between the cavity preparation and the dental restoration. Thus, microleakage can cause recontamination of the root canal system mostly by bacteria due to incomplete sealing between the tooth interface and the restoration which later can lead to failure of endodontic treatments (1-2). An effective coronal seal is just as important as the apical seal for the ultimate success of endodontic treatment. It may also contribute to significant healing of periapical inflammation as compared to well obturated root canals (1).

Deepali and Hedge stated that composite resin is the most common choice of material for restoring access cavities due to its aesthetic value because it can provide a good match of colour and it simplify the procedure of restoration (1). However, composite resin has polymerization shrinkage which induces mechanical stress to tooth structures and
the thermal expansion is greater than the expansion of tooth which leads to formation of marginal gap (3).

Apart from that, GIC has little polymerization shrinkage compared to composite resin. It depends primarily on a chemical bond to the tooth structure in which they form an ionic bond to the hydroxyapatite at dentine surface where they obtain mechanical retention as well (1). It is shown that GIC has less microleakage when it is placed at the cervical margin. Therefore, it is better to place additional layer of GIC as intra-orifice barrier (4).

In addition to the newly developed bulk-fill resins, Dentsply Sirona Smart Dentine Replacement (SDR) is a self-levelling, flowable composite that requires no further manipulation and has excellent cavity adaptation. Moreover, SDR provide minimal polymerization stress that allowed it to be placed in 4mm increments, followed by a mandatory 2mm cover layer of conventional composite resin (3,5). A study has been conducted to compare SDR with two traditional flowable methacrylate-based composite and the result showed that SDR has lowest level of shrinkage stress, the longest pre-gel time and the lowest shrinkage rate (5).

In this study, sandwich techniques using different thickness of different types of materials were tested. This is because different restoration materials have different sealing properties which may influence the microleakage. Furthermore, sandwich technique has been suggested to reduce microleakage in restoration materials (6). The aim of this study is to evaluate the coronal microleakage of sandwich techniques involving combination of composite restoration (Filtek Z350 XT, 3M ESPE) with different thickness of bulk-fill flowable composite restoration materials, SDR (Dentsply Sirona) and glass ionomer cement (GIC, GC Gold Label 2) as access cavity restoration in endodontically treated teeth.

The null hypothesis for this study was there is no significant difference in the coronal microleakage between combinations of different thicknesses of different materials used as final restoration in endodontically treated teeth.

MATERIALS AND METHOD

1) Sample preparations

72 maxillary central incisors were used for this in vitro study. Teeth included in this study must be sound and free from caries or restorations. The canal must be patent and straight or slightly curved. Teeth with gross caries involving the roots and cracks on the root or crown surfaces were excluded. The samples were debrided by using ultrasonic scaler (Piezolux, KaVo, Germany) and stored in distilled water at room temperature. Periapical radiograph of each tooth was taken in order to identify and evaluate the canal.

Access cavity was done using diamond round bur, tapered and non-end cutting TC bur (NTI-Kahla GmbH, Germany) under air water cooling high-speed handpiece. Working length was determined by inserting #15 K-file up to the apical foramen and subtracting 0.5mm from the total file length. The canal was prepared using step-down technique until #40 K-file for MAF and tapered using step-back technique. The canal was irrigated using 2.5% sodium hypochlorite and saline alternatively in between each file. After completion of canal preparation, the canal was dried using paper points (Roeko, Coltene, Germany) and was obturated with gutta-percha (Gutta Percha points, Dentsply Maillefer, Switzerland) using cold lateral compaction technique.

For intra-orifice space preparation, 2mm of the coronal part of gutta-percha was removed (at the level of cemento-enamel junction) using a heated plugger (Friendo, DXM, Korea) in all teeth. After the gutta-percha was removed, the crown of the sample tooth was cut horizontally using a separate disc (Shofu, Japan) with a straight handpiece until the depth of 6mm from the gutta-percha to the incisal edge was achieved.

2) Restorative procedures

All the 72 samples were randomly divided into 4 experimental groups with 18 teeth each; Group I, II, III and IV (control group). Materials, manufacturer and chemical composition used in this study were listed in Table 1.

Table 1. Materials, manufacturers and chemical compositions used in this study

<table>
<thead>
<tr>
<th>Material name</th>
<th>Manufacturer</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Bond Universal Adhesive (SBU)</td>
<td>3M ESPE, Germany</td>
<td>MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Inititators, Silane</td>
</tr>
<tr>
<td>Smart Dentine Replacement (SDR)</td>
<td>Dentsply Sirona, Germany</td>
<td>SDR patented urethane di-methacrylate resin, Di-methacrylate resin, Di-functional diluent, Barium and Strontium alumino-fluoro-silicate glasses, Photoinitiating System, Colourant</td>
</tr>
<tr>
<td>Filtek Z350 XT (Filtek)</td>
<td>3M ESPE, Germany</td>
<td>Bis-GMA, UDMA, TEGDMA, bis-EMA(6), silica filler, zirconia filler</td>
</tr>
<tr>
<td>Dentine Conditioner</td>
<td>GC Corporation, Japan</td>
<td>Distilled water 90%, Polyacrylic acid 10%</td>
</tr>
</tbody>
</table>
3) Access restoration

**Group 1 (2SDR+4Filtek):** Access cavity was self-etched with SBU. The adhesive was applied into the cavity for 20s, air blown for 5s and light cured for 10s. 2mm of the access cavity was restored with SDR and light cured for 20 seconds. Another 4mm of the access cavity was restored with Filtek Z350 XT by increment of 2mm and another 2mm, then light cured for 40s in each increment (Figure 1.1).

**Group 2 (4SDR+2Filtek):** Access cavity was self-etched with SBU. The adhesive was applied into the cavity for 20s, air blown for 5s and light cured for 10s. 4mm of the access cavity was restored with SDR and light cured for 20 seconds. Another 2mm of the access cavity was restored with Filtek Z350 XT and light cured for 40s (Figure 1.2).

**Group 3 (2GIC+2SDR+2Filtek):** Dentine conditioner was placed at 2mm of the access cavity for 20s and rinsed with water and air blown without desiccating the dentine. After that, the 2mm of the access cavity was restored with GIC. Another 4mm of the access cavity was self-etched with SBU. The adhesive was applied into the cavity for 20s, air blown for 5s and light cured for 10s. 2mm of the access cavity was restored with SDR and light cured for 20s. Then, another 2mm was restored with Filtek Z350 XT and light cured for 40s (Figure 1.3).

**Group 4 (control group) (6SDR):** Access cavity was self-etched with SBU. The adhesive was applied into the cavity for 20s, air blown for 5s and light cured for 10s. Access cavity was restored with SDR by increment of 4mm and 2mm, then light cured for 20 seconds in each increment (Figure 1.4).

In order to control and standardise the placement of different thickness of the materials in the access cavity, a William’s probe was used to measure the remaining height of the access cavity after placement of the first material. The excess or deficiency was removed and added respectively until the desired remaining height was achieved.

After finishing, the restorations were polished and samples were subjected to thermocycling for 500 thermal cycles between 5°C and 55°C and dwell time of 30s.

4) Microleakage analysis

All of the 72 samples were dried and apices of roots were immersed in molten sticky wax to ensure apical seal. 2 coats of nail varnish were applied on the smooth surface of the teeth leaving 1mm margin around the filling to prevent dye penetration. After that, the samples were immersed in 2% Rhodamine solution with pH 7 for 48 hours at 37°C temperature. The samples were removed from dye solution, rinsed under running tap water for 30 minutes and nail
varnish and wax were removed. The samples were sectioned longitudinally in buccolingual direction using a low speed precision cutter (Micracut 125, METKON, Turkey).

After drying, the microscopic evaluations of the longitudinally sectioned samples were performed. The dye penetration was measured under a stereomicroscope (SZX7, Olympus, USA) with 1.25x magnification using Digital Image Twain software calibrated to 1-mm scale. Pictures of the images seen through the stereomicroscope were captured and measured in millimetres (Figure 2). The deepest penetration of the dye into the restoration was recorded, be it into the filling material or along the lateral walls.

![Score 4](image1)
![Score 4](image2)
![Score 4](image3)

Figure 2. Sample of microleakage in the sectioned teeth.

The data obtained was analysed using IBM SPSS Statistics 24 software. The data was subjected to Kolmogorov-Smirnov test to indicate a normal or non-normal distribution. One-way ANOVA and post hoc Tukey’s HSD test to check for any significant differences between the four groups. Level of statistically significance was set at $p<0.05$.

### RESULTS

The dye leakage score differed distinctly among all groups (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of sample</th>
<th>Dye leakage score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>0 0 14 4 0</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>0 1 1 11 5</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0 8 6 4 0</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>0 2 9 7 0</td>
</tr>
</tbody>
</table>

The mean leakage values (mm) and standard deviations (SD) of the degree of dye penetration of each experimental group is shown in Table 3. The mean values showed that the lowest microleakage was seen in Group 3 which used a sandwich technique of Filtek Z350 XT and SDR with additional layer of GIC and the highest microleakage was seen in Group 2 which used a sandwich technique of Filtek Z350 XT and SDR with measurement of 2mm and 4mm respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean microleakage (mm)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (2SDR+4Filtek)</td>
<td>3.07</td>
<td>1.06</td>
</tr>
<tr>
<td>G2 (4SDR+2Filtek)</td>
<td>5.55</td>
<td>1.62</td>
</tr>
<tr>
<td>G3 (2GIC+2SDR+2Filtek)</td>
<td>2.48</td>
<td>1.05</td>
</tr>
<tr>
<td>G4 (6SDR)</td>
<td>3.68</td>
<td>1.11</td>
</tr>
</tbody>
</table>

The data obtained for the microleakage in each group was analysed by Kolmogorov-Smirnov test to indicate a normal or non-normal distribution. Kolmogorov-Smirnov test revealed a normal data distribution between each group. Therefore, data was subjected to statistical analysis using One-way ANOVA test for microleakage comparison between the groups. The computed value is $p<0.05$ which indicates significant differences between the four groups.

Furthermore, the data was also subjected to post hoc Tukey’s HSD test to determine the intergroup
comparison as show in Table 4. The test revealed that there was significant difference between Group 1 and 2, Group 2 and 3, Group 2 and 4, and Group 3 and 4 (p<0.05). In relation to the thickness of SDR and Filtek Z350 XT used in Group 1 and 2, it showed that 2mm SDR is better than 4mm SDR. This result may also be influenced by the thickness of the SDR used. However, there were no significant differences between Group 1 and 3 (p=0.513) and Group 1 and 4 (p=0.477) but there are more reduced microleakage shown with additional of GIC used in Group 3. 6mm SDR restoration in Group 4 showed to be as good as combination of 2mm SDR and 4mm Filtek in Group 1 in reducing the microleakage.

Table 4. Intergroup comparison using post hoc Tukey’s HSD test

<table>
<thead>
<tr>
<th>Groups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>1 and 3</td>
<td>p=0.513</td>
</tr>
<tr>
<td>1 and 4</td>
<td>p=0.477</td>
</tr>
<tr>
<td>2 and 3</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>2 and 4</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>3 and 4</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In endodontically treated teeth, the success of endodontic therapy relies on the sealing ability and the properties of restoration and root filling materials. These materials should provide good apical and coronal seals in order to prevent the ingress of microorganisms and tissue fluids into root canal system via apical and coronal leakage which can lead to root canal treatment failure. This is because studies have shown that sealing the root canal alone using gutta-percha and root canal sealer is not sufficient to provide minimal resistance to microleakage (2). Therefore, it is vital to have a good coronal seal to improve the prognosis of endodontically treated teeth (7).

There are several methods that can be used to evaluate microleakage in restoration such as fluid filtration methodology, dye extraction method and dye penetration method (8). In this in vitro study, the microleakage of different thickness restoration materials used was evaluated by dye penetration method in order to compare their performances (9). It is preferable to conduct dye penetration method because of its low cost, simple, fast technique and ease of manipulation (10).

As in this study, Rhodamine B was used as the dye for microleakage evaluation. Rhodamine B is said to have more chemical stability, smaller dye molecules compared to methylene blue. Thus, making the penetration of Rhodamine B is greater than methylene blue. Other than that, Rhodamine B solution was standardized to pH 7 due to the fact that in acidic or alkaline condition may create spaces between tooth structure and restoration materials due to alterations of dentine structure and may increase the dye penetration. Rhodamine B concentration was set at 2% according to its preferable mass and volume relation (11).

In order to simulate the temperature changes in the oral environment research, thermocycling was done as it is universally used method in dental research (10). The samples were subjected to cyclic exposures of hot (55°C) and cold (5°C) temperatures. The rationale of doing thermocycling is because to mimic the aging process of the restorative material in vivo. The composite resin and adhesive systems are sensitive to temperature changes which induces bond fatigues and compromise the sealing ability of restoration (1).

In this study, self-etched technique was used for the adhesive placement using Single Bond Universal Adhesive (3M ESPE), according to the manufacturer’s instructions. The research by Moosavi et al. (2013) showed that the average microleakage in total-etch adhesive and self-etch adhesive based on dye penetration methods indicated that the microleakage of both adhesives was not significantly different (12). Similarly, a study done by Brackett et al. which also used dye penetration method to test the microleakage in dentine margins shows no significant difference between self-etch adhesive and total-etch adhesive (13).

The results of this study showed that the least microleakage was seen in Group 3 which is the combination materials of GIC, SDR and Filtek Z350 XT. The use of a cavity liner has been suggested can reduce the stress associated with polymerisation shrinkage (5). As in this study, GIC and SDR have been used as a cavity liner. GIC is used commonly as intra-orifice barrier for endodontically treated tooth. It has been recommended as an effective intracanal barrier to prevent coronal microleakage. It has demonstrated good sealing and antibacterial properties (14). It showed that with additional layer of GIC further reduced the microleakage. This observation is in accordance to study conducted by Parekh et al. where it was found that microleakage was less beneath a seal of GIC plus composite resin as opposed to composite resin alone (15).

Besides, flowable composites also have been recommended as cavity liners due to their low viscosity, low modulus elasticity and increased wettability (5). The low viscosity enables SDR to adapt well to the cavity margins and their low
modulus elasticity will compensate for contraction shrinkage stress when used as a cavity liner by acting as an elastic buffer and increase the flexibility of the bonded assembly and might also act as an absorber (16). SDR also has been proven to cause 60-70% less shrinkage stress compared to conventional methacrylate-based resins (5).

In this study, a cavity liner used has shown to decrease a microleakage in a restoration. The thickness of the SDR used as cavity liner also contributed to the microleakage in a restoration. In comparison between Group 1 and Group 2, Group 1 showed a reduction in microleakage when a thinner SDR used. This may be explained by the process of polymerization. When a thin layer of SDR is placed, it has better polymerization of the bottom surface of the increment and better distribution of stress to the surrounding (17).

Other than that, SDR has lower filler content that will shrink more when used in greater thickness. A thick layer of flowable composite SDR on the other hand, will cause a reduced perfect margin between the tooth surface and the flowable composite SDR which then reduces the marginal integrity (18). Based on a configuration factor (C-factor), the lower the C-factor, the more flow can occur and a lesser stress at the interface results. Thus, a thinner layer of flowable composite SDR used will reduce the C-factor as well as the polymerization shrinkage (19). However, in clinical situation, it is difficult to verify the thickness of flowable composite SDR placed due to its increased wettability.

Based on the result in this study between Group 1 and Group 4, the 6mm SDR showed higher microleakage compared to the 2mm SDR with 4mm Filtek Z350 XT. It can be suggested that the higher microleakage in Group 4 is due to SDR is not covered by the composite (Filtek Z350 XT) because it is mandatory for the SDR to be covered with Filtek Z350 XT (3,5). The explanation behind this is that the SDR has a higher wear rate than the Filtek Z350 XT, thus it has been proposed by the manufacturer that SDR should be used in only contact free areas. Other than that, the Filtek Z350 XT used in Group 1 was restored incrementally which aids in better distribution of polymerization shrinkage between the layers and reduces the configuration factor (20). Moreover, fully SDR restoration is not applicable in clinical situation due to its aesthetic-wise as final restoration as it only provides one universal shade. Plus, the cost of flowable composite (SDR) is more expensive than composite (Filtek Z350 XT).

Group 3 showed the least microleakage compared to the other 3 experimental groups in which with the additional layer of GIC as cavity liner underneath the SDR, it further reduced the microleakage. It can be explained by the bonding of GIC which is chemically bonded to the tooth structure which causing less water penetration into these bonds compared to bond that are formed between composite and tooth structure (21). However, there is no significant difference between Group 3 and Group 1 which is restored with 2 mm SDR and 4 mm Filtek Z350 XT. Therefore, clinically it is acceptable to exclude the additional GIC in order to ease the restoration procedure in clinical setting.

Some of the limitations in this study were the vision limitation as the size of the access cavity was small and difficulty to standardise to the size of the access cavity. Next, the placement of material was difficult because of the high wettability of SDR and GIC. Plus, both of it were placed at the most inner layer of the cavity. There was also limited number of samples as the ideal sample size calculated was 80 teeth. However, in this study only 72 teeth were used which lowered the sample power but still within the acceptable range.

CONCLUSION
Based on the findings in this in vitro study, it can be concluded that sandwich technique of composite (Filtek Z350 XT, 3M ESPE) and SDR (Dentsply Sirona) could reduce microleakage where 2mm of SDR with 4mm of Filtek Z350 XT had the least microleakage. However, with an additional GIC as the cavity liner (GIC, GC Gold Label 2) further reduced the microleakage.

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