Chapter 5 Sealing Ability of Occlusal Resin Composite Restoration Using Four Restorative Procedures

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ABSTRACT

The purpose was to investigate fluid flow after restoration using four restorative procedures. Micro-gap, internal dye leakage and micro-permeability of bonded interfaces were also investigated. Each tooth was mounted, connected to a fluid flow measuring device and then an occlusal cavity prepared. Fluid flow after cavity preparation was recorded as baseline, and the cavity was restored using one of four restorative procedures: bonded with total-etch (Single Bond 2) or self-etch (Clearfil SE Bond) adhesives without lining; lined with resin-modified GIC (Fuji Lining LC) or conventional GIC (Fuji IX) and then bonded with the total-etch adhesive. Fluid flow after restoration and up to 6 months was recorded. Micro-gap was examined from resin replicas using scanning electron microscopy. Internal dye leakage with 2% methylene blue was observed using a light microscope. In the micro-permeability test, fluorescent-dye penetration was investigated using laser confocal microscopy. None of the restorative procedures provided a perfectly sealed restoration. Glass ionomer lining did not reduce fluid flow after restoration, and micro-gaps were frequently detected. Using a self-etching adhesive failed to provide a better seal than the total-etching adhesive even initial gap formation was rarely observed for the former. Methylene blue and fluorescent dye penetrations were detected in most restorations.
INTRODUCTION

Resin composite is widely used to restore anterior and posterior teeth because conservative cavity preparation can be achieved, it can be bonded to tooth structure when used with an adhesive and is tooth-coloured. However, polymerization shrinkage stresses are a major problem for these restorations; if the bond is unable to withstand the forces from polymerization shrinkage, micro-gaps are likely to be formed, and the seal of the restoration will deteriorate (1). Nevertheless, the adhesive layer does not provide a complete seal even when a micro-gap is not observed. This layer is still permeable to molecules such as water, silver nitrate or fluorescent dye (2-4). Several studies have investigated the sealing ability of resin composite restorations by measuring fluid flow after restoration (5-7). It is believed that fluid moves to fill micro-gaps and/or through the permeable adhesive layer. Therefore, a restoration which provides a superior seal is required.

Currently, adhesive systems for resin composite restorations are divided into two broad types: total-etching and self-etching. In the former, two-step single-bottle systems are commonly used. In the latter, two-step self-etching systems have recently become popular as an alternative adhesive (8). The acidic primer in two-step self-etching adhesive systems partially removes the smear layer, but the smear plugs still remain in the dentinal tubules (9). Under simulated pulpal pressure, dentin permeability after priming does not significantly increase (9). On the other hand, phosphoric acid etching in a total-etching adhesive system entirely removes the smear layer and causes a dramatic increase in dentin permeability which affects bond quality
Therefore, using a self-etching adhesive system may achieve a better dentinal seal and less fluid movement after restoration than using a total-etching adhesive system.

In occluso-proximal restorations using a two-step single-bottle total-etching adhesive, RATIH et al. (10) proposed that placing a glass ionomer cement (GIC) liner can completely stop fluid movement soon after restoration. However, compared with restorations without a lining, a higher incidence of gap formation has been reported when GIC liner is used (11-13). Furthermore, long-term degradation of GIC, which might affect bond quality and other physical properties, has also been of concern (14, 15). Hence, the benefit of placing a GIC liner to achieve sealing restoration is still questionable.

The purpose of this study was to investigate fluid flow up to 6 months after occlusal resin composite restorations were placed using four restorative procedures. In addition, the bonded interfaces were investigated using field-emission scanning electron microscopy, internal dye leakage and a micro-permeability test. The null hypothesis was that there is no significant difference in fluid fluxes after restoration over time, percentages of gap formation and percentages of dye leakage among the four restorative procedures.
CHAPTER 5: SEALING ABILITY RESIN COMPOSITE

MATERIAL AND METHODS

One-hundred and twenty intact human third molars extracted from patients 18 to 30 years-old were used. The teeth were cleaned, disinfected in a 2% thymol solution and then stored at 4 °C in phosphate buffered saline solution (PBS). The study was approved by the Ethics in Human Research Committee of the University of Melbourne, Australia, and patient consent was obtained to retain their teeth.

Fluid flow measurement

Thirty-two teeth were prepared as follows: each tooth was sectioned 3 mm below the cemento-enamel junction (CEJ), using a slow-speed diamond blade with water coolant (Struers, Ballerup, Denmark). Pulpal tissue was carefully removed using tweezers and a barbed broach. The teeth were immersed in PBS and cleaned in an ultrasonic cleanser (Model 2009, L&R, Kearny, New Jersey, USA) for 5 min.

Figure 5-1 shows a diagrammatic representation of the test apparatus for measuring fluid flow. Each tooth was mounted on a polymethylmethacrylate plate, containing an 18-gauge needle in the centre, using cyanoacrylate glue (Bostik, Thomastown, Victoria, Australia) and subsequently covered with epoxy resin (Araldite, Selleys, Padstow, NSW, Australia) above the level of the CEJ. The needle was connected via a silicone tube to a glass capillary tube, 30 cm long and internal diameter 0.84 mm, filled with PBS and mounted horizontally in an automated fluid flow measurement device (Flodec, De Marco Engineering, Geneva, Switzerland). The other end of the glass tube was connected to a PBS reservoir used to simulate an intrapulpal pressure of 0 or 1.3 kPa (16). Fluid flow measurement was conducted at
room temperature 24±1°C. Prepared teeth were covered during the experiment with a humidified container to minimize outward fluid flow from evaporation.

The system was tested to detect any leakage under a pressure of 1.3 kPa for 10 min. After that, a box-shaped occlusal cavity, 4±0.2 mm x 4±0.2 mm square and 3±0.2 mm deep (measured at the mesial central groove), was prepared using a high-speed fissure diamond bur size 010 standard grit (Heico, Steinach, Switzerland) under air-water coolant. After preparation, an impression of the cavity using an addition silicone light-body impression material (Take1, Kerr, Orange, CA, USA) was obtained. The dentin surface area of the pulpal floor and cavity walls was measured approximately from digital photographs of the impression material using the UTHSCSA ImageTool software version 3 (The University of Texas Health Science Center, San Antonio, USA), and a standardized 0.4 mm² square grid was used for calibration.

Initially, fluid flow through the prepared cavities was recorded for 10 min under 1.3 kPa as the baseline flow. After that, the cavity-prepared teeth were equally divided in a random order into four groups. Without lining, groups 1 and 2 were bonded with a two-step total-etching adhesive system, Single Bond 2 (SB2), or a two-step self-etching primer adhesive system, Clearfil SE Bond (SE), respectively. In groups 3 and 4, a light-cured resin-modified GIC, Fuji Lining LC (FLC), or, a conventional high powder-liquid ratio GIC, Fuji IX (FIX), was placed as a lining and subsequently bonded with SB2. Table 5-1 shows the components, batch numbers and manufacturers of the materials used. The restorative procedures are listed in Table 5-2 and described below in detail. Bonding and restorative procedures were performed under a pressure of 0 kPa.
In group 1, the entire cavity was etched with 37% phosphoric acid (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA) for 15 s and rinsed thoroughly with 5 mL of PBS. Fluid movement through the acid-etched dentin was recorded at the simulated pressures of 0 and 1.3 kPa for 10 min each. The cavity was gently air blown to remove excess PBS, and a moist dentin surface was obtained. SB2 adhesive was applied according to the manufacturer’s instructions and light cured for 10 s (C8 Blue Phase, Ivoclar Vivadent, Schaan, Lichtenstein). The light intensity was checked regularly using the internal light indicator. In group 2, the cavity was treated with Clearfil SE Primer for 20 s, gently air blown, applied with Clearfil SE Bond and then light cured for 10 s.

In groups 3 and 4 for lining placement, the cavity was treated with Dentin Conditioner for 20 s before placing FIX, while the cavity was left untreated prior to FLC application. FLC or FIX was mixed according to the manufacturer’s instructions and then applied to a thickness of 0.5-1.0 mm covering the entire dentin surface. FIX was allowed to chemically set for 6 min, whereas FLC was light cured for 20s. Fluid movement during GIC setting, i.e. from application until light curing for FLC and 6-min chemical setting for FIX, was recorded.

Filtek Supreme XT, a nano-filled, hybrid resin composite, was placed in the bonded cavity in two horizontal increments to ensure effective light curing and reduce void incorporation. The first layer was approximately 0.5-1 mm thick. The second increment entirely filled the cavity and was contoured to produce anatomical form. Each increment was light cured for 20 s.

After restoration, the simulated intrapulpal pressure was immediately raised to 1.3 kPa. Fluid movement after restoration was recorded during 0-15 min and 45-60
min after restoration, and then at 24h, 1 week, 1 month and 6 months, for 10 min each. The restored teeth were immersed in PBS and kept in a chamber at 90% humidity and 37 °C between measurements.

Fluid flow volume in nL was calculated using the formula below:

Fluid flow volume (nL) = \( \pi r^2 \times D \times 10^{-3} \)

Where \( r \) = radius of the glass tube (0.42 mm); \( D \) = linear displacement (mm) of the bubble. Fluid flux in nL.s\(^{-1}\).cm\(^{-2}\) was obtained from volume in nL divided by measuring time in second and estimated dentin surface area in cm\(^2\).

**Micro-gap formation**

Sixty-four intact third molars were prepared and equally divided into four groups, restored as previously described for the fluid flow experiment and examined at 24 h, 1 week, 1 month and 6 months after restoration. The sixteen restored teeth (four of each restorative technique) were sectioned bucco-lingually at the center of the restoration using the diamond blade with water coolant (Struers). Each sectioned surface was polished with wet 600-grit Si-C paper for 10 strokes and 1000-grit paper for 5 strokes, cleaned in an ultrasonic cleaner for 5 min and then the smear layer was removed with neutral EDTA 5% (Dentavision, Castle Hill, NSW, Australia) for 15 s. The surface was blot dried and an impression taken with the addition silicone light-body impression material (Take 1). The impression was removed, checked for defects, and then epoxy resin (Epoxy resin, Polymer Daystar, Mortdale, NSW, Australia) was poured into the impression to obtain a replica. After 24 h, the replica was mounted, gold sputter-coated and examined using a Field–Emission Scanning Electron Microscope (FE-SEM), (Philips XL30 FE-SEM, Eindhoven, the Netherlands) at 70X.
and 1,000 X magnifications. Overlapping images were obtained at 70X and combined to create a montage of the entire cavity. Images at higher magnification were used to examine the regions where gaps had been identified. Gap formation was calculated as a percentage of the total length of the bonded interface, measured using ImageTool software. Examination for micro-gap formation between resin composite and GIC liner was also performed.

**Internal dye leakage**

Sixteen intact third molars were mounted, prepared and restored in exactly the same manner as the fluid flow experiment; four restorations were placed for each restorative procedure. The restored teeth were tested for internal dye leakage immediately after restoration. Under a pressure of 1.3 kPa, each restored tooth was connected to a reservoir containing 2% methylene blue dye (2g per 100 mL of PBS) for 24 h. The tooth was covered with a container to humidify and minimize any drying effects. Afterwards, the tooth was removed, rinsed and sectioned buccolingually. The sectioned tooth was examined and photographed under a light microscope (Leica S8AP0, Leica Microsystems, Heerbrugg, Switzerland). Bonded interfaces exhibiting dye leakage were reported as a percentage of the total length of the bonded cavity walls, as measured using the ImageTool software.

**Micro-permeability test**

Eight intact third molars were prepared and restored in exactly the same manner as fluid flow measurement, two for each restorative procedure. After restoration, each restored tooth was connected to a tubing system filled with Rhodamine B fluorescent dye (Chem-supply, Gillman, South Australia, Australia) at a
concentration of 1% (1 g per 100 mL of PBS) under a pressure of 1.3 kPa. After 24 h, the specimen was removed, rinsed thoroughly to remove excess dye in the pulp chamber, and then serially sectioned bucco-lingually at the center of the restoration, using a diamond blade with water coolant, to obtain a 0.5mm-thick specimen. The specimen was sequentially polished with Si-C paper grit up to 2500-grit, then with 3 and 1 μm diamond pastes. All specimens were kept in the dark, under humidification. After fixation on a glass slide with glycerin, each specimen was examined using a confocal laser scanning microscope (Olympus FV 1000, Olympus Optical Co. Ltd., Tokyo, Japan) at 40X magnification to observe dye penetration at the bonded interface.

**Statistical analysis**

Fluid fluxes of different restorative procedures and time intervals, percentages of gap formation and percentages of dye leakage were analyzed using General Linear Model ANOVA (Minitab14, Minitab Inc., State College, PA, USA) and multiple comparisons by Tukey’s test at the 0.05 level of significance.
RESULTS

Fluid flow measurement

Under the pressure of 1.3 kPa, fluid flux after acid etching increased significantly ($P < 0.001$) from baseline, 0.53 (±0.18) to 4.22 (±1.39) nL.s$^{-1}$.cm$^{-2}$. However, no significant difference in the fluid flux was found at the pressure of 0 kPa ($P > 0.05$); a modest increase from 0.04 (±0.03) to 0.06 (±0.03) nL.s$^{-1}$.cm$^{-2}$ was reported.

Fluid flux during setting of either FLC or FIX significantly increased from baseline ($P < 0.001$); the changes were from 0.03 (±0.05) to 3.21 (±0.93) nL.s$^{-1}$.cm$^{-2}$ and from 0.13 (±0.15) to 3.23 (±0.71) nL.s$^{-1}$.cm$^{-2}$, respectively. However, there was no significant difference in the fluid flux between these materials ($P > 0.05$).

Fluid flux after restoration in each group was reported as a negative or positive percentage change from baseline flow rate. To obtain a normal distribution, all data were transformed using log 10 values. Moreover, outlier data (SB2 tooth no.1, SE tooth no.4, FLC tooth no. 7 and FIX tooth no.3) were removed. According to the individual data of each group, a trend of change over time was not clear except the FLC group in which a consistent trend of changes was observed.

Means of fluid fluxes after restoration, expressed as % change from baseline, are presented in Figure 5-2. Seemingly, significant differences between the fluid fluxes would have been detected among groups, however only a few differences were reported because of wide variation in the data and subsequent high standard deviations. Within each restorative procedure, there was no significant difference in
fluid flux between the time periods ($P > 0.05$) except the group lined with FLC which showed the fluid flux at 24 h was significantly lower than at 15 min ($P < 0.001$). Comparisons among the restorative procedures showed that fluid fluxes at 24 h and 1 month in the FLC group were significantly lower than those in the FIX group ($P < 0.001$ and $P = 0.0056$ respectively), but the fluid flux was not significantly different from those of the SB2 and SE groups ($P > 0.05$). No significant difference in fluid flux between restorative procedures was found at the other periods ($P > 0.05$).

**Micro-gap formation**

Micro-gap formation as a percentage of total length of cavity walls is presented in Table 5-3. Gap formation significantly increased after 6 months storage in the SB2 group ($P = 0.0322$). Gap formation in the SE group was minimal until 1 month storage and then increased significantly after 6 months storage ($P < 0.05$). Gap formation was initially high in the FLC group and then significantly increased at 6 months storage ($P = 0.0093$). In contrast, the percentage of gap formation did not significantly change in the FIX group over 6 month’s storage ($P > 0.05$).

Comparisons among restorative procedures, significant differences in gap formation were observed. Until 1-month storage, SE showed significantly less gap formation than FLC and FIX ($P < 0.05$). However, there was a significant difference in gap formation between SE and SB2 at 1 week ($P = 0.007$). Gap formation at 6 months for SE was not significantly different from FIX ($P > 0.05$), but these were significantly less than SB2 or FLC ($P < 0.05$).

Micro-gap formations between resin composite and GIC liners are presented in Table 5-4. Minimal gap formations were initially observed at 24 h in the two
groups. After 1 week storage, the gap formation significantly increased and then changed little thereafter. Furthermore, gap formation up to 6 months between resin composite and GIC liner were not significantly different between the two groups.

Representative bonded interfaces of the SEM images are shown in Figure 5-3. Micro-gap formation was occasionally found at the bonded interface of the SB2 group (Figure 5-3A). In the SE group, less gap formation was observed; most bonded interfaces showed good adaptation (Figure 5-3B). In the group lined with FLC, gaps were commonly observed as a cohesive failure within FLC, and there usually was smear-layer incorporated GIC remaining on the dentin (Figure 5-3C). In the FIX group, a larger micro-gap was detected. Moreover, porosities and irregularities were observed in the GIC adjacent to the de-bonded interface (Figure 5-3D).

**Internal dye leakage**

Dye penetration varied among restorative procedures and also individual teeth. Methylene blue dye was located mainly on the pulpal floor while minimal leakage was detected on the cavity walls. Table 5-5 shows semi-quantitative dye leakage results as a percentage of the total length of cavity walls. No statistically significant difference was found among the four groups ($P > 0.05$).

**Micro-permeability test**

Figure 5-4 represents Rhodamine B dye penetration at the bonded interfaces of the four restorative techniques. Fluorescent and grey images were simultaneously digitally photographed observing penetration of the dye through dentinal tubules to the bonded interfaces of the restorations. Cavities bonded with SB2 (without GIC lining), showed stained interfaces with the fluorescent dye, which related to micro-gap
detected in the reflected light image (Figure 5-4A). Even though a micro-gap was not clearly noticeable, Rhodamine B penetrated through the interface bonded with SE (Figure 5-4B). In the restoration lined with FLC, the fluorescent dye was detected at the interface, as well as in the bulk of the GIC (Figure 5-4C). When lined with FIX, the interface was stained with Rhodamine B, which related to the defective areas presented in the reflected light (Figures 5-4D).
DISCUSSION

As a control group, the two-step total-etching adhesive, SB2, was selected due to its wide use, and the two-step self-etching adhesive system, SE, was chosen to compare the result obtained from the two commonly used types of resin-based adhesive systems. Moreover, there were groups using GIC liners, either conventional or resin-modified, to simulate the clinical situation when a dentist applies a lining material before restoration. Each material used was considered representative of its class or type.

None of the restorative procedures in this study provided a perfectly sealed restoration. Continuous fluid flow after restoration and micro-gaps were observed. This was also confirmed by the result of the micro-permeability test where the fluorescent dye penetrated through the bonded interfaces. Moreover, internal leakage by methylene blue dye was detected in the majority of restorations.

Lining with GIC did not stop fluid flow after restoration, as one other study has reported (10). In our study, an occlusal cavity possesses the highest C-factor and greater polymerization shrinkage stress corresponding with an occluso-proximal cavity (17). Hence, a higher shrinkage stress and greater deterioration of the bonded interface could be expected. In addition, the teeth used in the other study were relatively impermeable since no significant increase of fluid flow after phosphoric acid etching was reported. Neither using conventional (FIX) nor resin-modified GIC (FLC) reduced fluid flow after restoration in our study. Similarly, SIDHU, et al. reported that there was continuous fluid flow after restoration with a conventional GIC (FIX) (18).
The imperfect sealing of the GIC linings might be explained by their hydrophilic properties, micro-gaps and/or porosities. GIC is a hydrophilic, water-based material and sets by an acid-base reaction (8). During setting, both GICs absorb a considerable amount of water, which may affect their sealing ability and other physical properties. Silica hydrogel forming around the glass particles is likely to be a fluid reservoir. The monomer, such as HEMA, added to resin-modified GIC is likely to increase water sorption of the cement compared with a conventional GIC (19). Images obtained from laser confocal microscopy confirmed that the fluorescent dye clearly stained into the bulk of FLC.

Micro-gaps were frequently detected in the restorations lined with GIC. Dentinal fluid might flow through incompletely sealed dentinal tubules to the interfacial gap (11). Presumably, initial bond strengths to dentin of GICs were not strong enough to withstand the polymerization shrinkage stress of resin composite. In a pilot study, we found that good adaptation between the GIC liner and dentin was observed when resin composite was not placed over the lining where no polymerization shrinkage stress is present. In addition, porosities in the GIC, which are dependent on the mixing and viscosity of the material (20), are also critical. From the SEM images, the porosities observed on the conventional GIC surface adjacent to the interface were highly likely to act as a reservoir for fluid to move into. The bulk of the GIC contained less porosity than at the bonding interface. The defects may lead to greater water sorption shortly after setting.

Comparing the two GICs, micro-gaps usually formed at the interface between the conventional GIC (FIX) and dentin whereas the gap was often seen as a cohesive failure within the resin-modified GIC (FLC). Microscopically, FLC remained on the
dentin as a thin protective layer. In addition, the smear layer, which is a barrier providing a short-term seal (21), was partially removed by conditioning before FIX was placed while it was not removed before placing FLC. Furthermore, FIX at the cavity interface was observed to be quite porous. Hence, these explain the differences in fluid fluxes after restoration between the FLC and FIX groups.

Although the incidence of micro-gap formation in the total-etch adhesive (SB2) group was higher than the self-etch adhesive (SE) group, an insignificant difference in fluid flow after restoration was noted, which corresponds to the results of a study comparing the self-etch adhesive, SE with four single-bottle total-etching adhesives (22). No difference in sealing capacity between both adhesives was confirmed by a similar incidence of internal dye leakage and pattern of micro-permeability. Even though there was a substantial increase in dentin permeability after phosphoric acid etching while only a modest increase was observed after priming with SE primer (9), it seems that SB2 is able to seal the highly-permeable etched dentin as well as SE on the less-permeable primed surface.

Both adhesives SB2 and SE contain HEMA, which is a hydrophilic monomer that absorbs water even after polymerization (23). Due to the water sorption, the dyes were able to be absorbed even though there was no micro-gap formation. Moreover, HEMA-containing adhesive layers are vulnerable to hydrolysis (24), and this might explain the steady increases in fluid flows for the SB2 and SE groups after long term storage. Furthermore, self-etching adhesive, SE also contains another functional monomer, MDP-10, that has a hydrophobic, long carbonyl chain and may resist hydrolysis more effectively (24).
During the first hour after restoration, the high initial fluid flow rate in the group lined with FIX was possibly a result of its water sensitivity and imbalance. A lower initial flow rate in the FLC group may be explained by the maturation of the resin-modified GIC is affected by the additional setting reaction from the resin components, so the water sensitivity and imbalance may decrease. For the resin-based adhesives, the initial reduction in fluid flow rates at the 24 h measurement may be a consequence of less water absorption. The increase in fluid flow at 1 week and thereafter is probably due to hydrolytic degradation, which can affect bond quality and subsequent sealing ability. A decrease in dentin bond strength after long-term storage has been reported (25, 26).

Increase in micro-gap formation of the restorations lined and/or bonded with the resin-based materials (SB2, SE and FLC) after 6-months storage may be explained by water absorption into the resin causing expansion and subsequent stresses affecting the bond. In contrast, micro-gap formation of the restorations lined with FIX, which is a conventional GIC, was insignificantly changed over time. The higher incidence of gaps existing between the GIC and resin composite in the restorations lined with FIX than FLC is possibly due to the bond strength to resin composite of the conventional GIC is lower than that of the resin-modified GIC (12).

A high occurrence of micro-gaps in restorations lined with GIC could be of clinical concern. This phenomenon has been reported in previous studies (12, 13). Micro-gaps were usually noticed at the pulpal floor in the bonded restoration with a resin-based adhesive while gaps were also frequently detected on the surrounding vertical walls between the GIC lining and dentin. This implies that distance from the cavosurface margin to such gaps is shorter when a GIC lining is present. If leakage is
present, restorative failure may be expected to occur more rapidly in GIC-lined resin composite restorations.

In conclusion, the null hypothesis, which proposed no significant difference in fluid fluxes after restoration over time and percentages of gap formation among the restorative procedures, were rejected. However, the null hypothesis which proposed there was no significant difference in percentages of dye leakage was accepted. Even though the fluid fluxes over time and dye leakages were not significantly different, SE provided less short-term gap formations than SB2, which might due to a more stable bond to dentin of SE. Interestingly, lining application with GIC did not provide superior seal on occlusal resin composite restoration, and gap formation was more frequently detected than the restoration bonded with resin-based adhesive only. Therefore, application of GIC lining prior resin composite restoration may need to be reconsidered. Its advantages of pulpal protection and reduction in postoperative sensitivity may also be doubtful. Further laboratory and clinical investigations are required to increase the evidence.
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REFERENCES


Figure 5-1 Diagrammatic representation of the test apparatus for measuring fluid flow.
Figure 5-2 Fluid fluxes after restoration, expressed as % change from baseline, of the four restorative procedures measured up to 6 months. Overall, fluid flow tended to decrease for the first 24 hours and then gradually increase again over six months. Within each experimental group, there was a significant difference only in the FLC group between fluid fluxes at 15 min and 24 h \((P < 0.001)\). Comparisons among the restorative procedures showed significant differences only between FLC and FIX at 24 h and 1 month \((P < 0.001\) and \(P = 0.0056\), respectively). The X axis is not linear, and the points are joined within each experimental group only for clarity.
Figure 5-3 Representative SEM images from resin replicas of sectioned teeth (DT-dentin, XT- Filtek Supreme XT, FLC- Fuji Lining LC and FIX- Fuji IX). A- There were gaps found at bonded interface of the SB2 group while some areas showed good adaptation. B- In the SE group, good adaptation along the bonded interface was commonly observed; less gap formation was observed. C- Gap was commonly formed like cohesive failure within FLC, and there was remaining GIC on the dentin (asterisk). D- In the FIX group, the bonded interface frequently showed large gap formation and signs of degradation, such as porosities and irregularities.
Figure 5-4 (A-D) Representative bonded interfaces labelled with Rhodamine B fluorescence dye and obtained from confocal laser scanning microscope at 40X magnification (an indicating bar is 50 micron). Simultaneously, fluorescence and reflected light images were digitally photographed. A- In the specimen bonded with SB2, Rhodamine B dye moved through dentinal tubules and accumulated along the interfacial gap (observed in the reflected image). B- Bonded interface of SE showed dye penetrations through the bonded layer even micro-gaps were not clearly detected from the reflected image. C- In the FLC group, fluorescence-dye staining was not only observed at the interface, but also distributed into the GIC. D- Lined with Fuji IX, bonded interface was stained with Rhodamine B, which related to defective areas presented in the reflected light image.
Table 5-1 Materials, components, batch numbers and manufacturers.

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<th>Materials</th>
<th>Components</th>
<th>Batch no.</th>
<th>Manufacturers</th>
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<tr>
<td>Dentin</td>
<td>10% polyacrylic acid</td>
<td>0507291</td>
<td>GC Corp., Tokyo, Japan</td>
</tr>
<tr>
<td>Conditioner (DC)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fuji Lining LC Paste Pak (FLC)</td>
<td>Paste A- Alumino silicate glass 70-80%, HEMA 10-15%, Urethane dimethacrylate 5-10%;</td>
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<td></td>
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<td></td>
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<tr>
<td>Fuji IX GP capsule (FIX)</td>
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<td>GC Corp., Tokyo, Japan</td>
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<td>3M ESPE, St. Paul, MN, USA</td>
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<td>Bonding- Bisphenol-A diglycidyl ether dimethacrylate, HEMA, dimethacrylate, colloidal nanofiller 10%, solvent, water</td>
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<td></td>
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<td>Clearfil SE Bond (SE)</td>
<td>SE Primer (SEP)- 10-MDP, HEMA, hydrophilic dimethacrylate, dl-camphoquinone, N,N-diethanol-p-toluidine, water</td>
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<td></td>
<td>SE Bond (SEB)- 10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphoquinone, N,N-diethanol-p-toluidine, silanated colloidal silica</td>
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<td>Filtek Supreme XT (XT)- A2B</td>
<td>BIS-GMA, UDMA, TEGDMA, Bis-EMA, inorganic fillers 59.5% (by volume)</td>
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<td>3M ESPE, St. Paul, MN, USA</td>
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10-MDP- 10-methacryloyloxydecyl dihydrogen phosphate; HEMA- 2-hydroxyethyl methacrylate; BIS-GMA- Bisphenol-A diglycidyl methacrylate; UDMA- urethane dimethacrylate; TEGDMA- triethylene glycol dimethacrylate; Bis-EMA- Bisphenol-A polyethylene glycol dimethacrylate
Table 5-2 Restorative procedures in fluid flow measurement and the related experiments.

<table>
<thead>
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<th>Group</th>
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<th>Bonding</th>
<th>Restoration</th>
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<td>-</td>
<td>Single Bond 2</td>
<td>Filtek Supreme XT</td>
</tr>
<tr>
<td>2- SE</td>
<td>-</td>
<td>Clearfil SE Bond</td>
<td>Filtek Supreme XT</td>
</tr>
<tr>
<td>3- FLC</td>
<td>Fuji Lining LC</td>
<td>Single Bond 2</td>
<td>Filtek Supreme XT</td>
</tr>
<tr>
<td>4- FIX</td>
<td>Fuji IX GP</td>
<td>Single Bond 2</td>
<td>Filtek Supreme XT</td>
</tr>
</tbody>
</table>
Table 5-3 Micro-gap formations at bonded interfaces observed from SEM montage images in percentages of total length of cavity walls (means and standard deviations in parenthesis) at 24 h, 1 wk, 1 month and 6 months after restorations using the four restorative procedures. Gap formations at 6 months were significantly different from baselines observed at 24 h in the first three groups \((P < 0.05)\). Gap formation in the FIX group was insignificantly changed \((P > 0.05)\).

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h</th>
<th>1 wk</th>
<th>1 month</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-SB2</td>
<td>13.90 (11.20) (^{a,c,d})</td>
<td>22.35 (11.39) (^{a,b,d})</td>
<td>20.81 (16.12) (^{a,b,d})</td>
<td>32.55 (8.55) (^{b,e})</td>
</tr>
<tr>
<td>2-SE</td>
<td>0.68 (1.30) (^{c,f})</td>
<td>1.32 (1.11) (^{c,f})</td>
<td>2.89 (3.81) (^{a,f})</td>
<td>23.25 (15.75) (^{d,g})</td>
</tr>
<tr>
<td>3-FLC</td>
<td>36.59 (14.04) (^{b,g,h})</td>
<td>41.49 (8.86) (^{c,g,h,i})</td>
<td>29.01 (12.82) (^{b,d,h})</td>
<td>57.19 (10.42) (^{c,i})</td>
</tr>
<tr>
<td>4-FIX</td>
<td>31.46 (7.77) (^{b,d,h})</td>
<td>25.11 (10.16) (^{b,d,h})</td>
<td>27.13 (8.70) (^{b,d,h})</td>
<td>25.96 (5.18) (^{h,d,h})</td>
</tr>
</tbody>
</table>

The same letter shows no significant difference.
Table 5-4 Gap formations between GIC liner (FLC or FIX) and resin composite observed from SEM montage images in percentage of total length of the interfaces (mean and standard deviation in parenthesis) at 24 h, 1 wk, 1 month and 6 months after restorations. Gap formations of FLC and FIX significantly increased at 1 wk \((P = 0.0384\) and \(P = 0.0314\), respectively\) and then were slightly changed thereafter \((P > 0.05)\).

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h (mean, SD)</th>
<th>1 wk (mean, SD)</th>
<th>1 month (mean, SD)</th>
<th>6 months (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-FLC</td>
<td>0.83 (2.35)\textsuperscript{a,d}</td>
<td>10.61 (1.96)\textsuperscript{b,c,e}</td>
<td>5.27 (4.91)\textsuperscript{a,b,d}</td>
<td>7.08 (7.98)\textsuperscript{a,b,c,d}</td>
</tr>
<tr>
<td>4-FIX</td>
<td>3.82 (4.71)\textsuperscript{d,e}</td>
<td>14.18 (2.81)\textsuperscript{b,c}</td>
<td>14.14 (10.63)\textsuperscript{b,c}</td>
<td>15.89 (5.17)\textsuperscript{c}</td>
</tr>
</tbody>
</table>

The same letter shows no significant difference.
Table 5-5 Internal dye leakage results in percentages of total length of cavity walls (means and standard deviations in parenthesis) of restorations using the four restorative procedures. No significant difference in dye leakage was found among the four restorative procedures ($P = 0.109$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dye leakage (% of total length of cavity walls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-SB2</td>
<td>34.14 (6.98) $^a$</td>
</tr>
<tr>
<td>2-SE</td>
<td>33.67 (5.09) $^a$</td>
</tr>
<tr>
<td>3-FLC</td>
<td>22.09 (17.24) $^a$</td>
</tr>
<tr>
<td>4-FIX</td>
<td>29.24 (8.87) $^a$</td>
</tr>
</tbody>
</table>

The same letter shows no significant difference.
Author/s:
Banomyong, D.

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