



Evaluation of the Adaptability and Nanoleakage among Lining Materials at the Cervical Dentin in Open Sandwich Technique

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Abstract

Resin composite is the most popular dental material. The polymerization shrinkage is a vital disadvantage resulting in the gap formation. Bonding agent is used along with resin composite to decrease the gap. However nanoleakage is found and related to the hydrolytic degradation of bonding agent. An open sandwich technique has been suggested to address this problem.

Aim: to evaluate the degree of nanoleakage and adaptability of different lining materials in open sandwich technique.

Method: the slot cavities were prepared on the occluso-distal surface of teeth with the gingival margin 1 mm below the CEJ. The teeth were divided into 4 groups (n=5) following types of lining materials: Group I a flowable resin composite, Group II a bulk-fill flowable resin composite, Group III no lining material and Group IV a resin modified glass ionomer cement. The lining material was applied with 1 mm thickness and then all samples were restored with nanofilled resin composite, thermocycled and processed with silver nitrate solution. The deposition of silver nitrate and gap formation were observed under SEM.

Result: the silver nitrate deposited entire thickness of hybrid layer, in the dentinal tubules and the resin tags in group I, II and III. In group IV, it deposited within the modified hybrid layer and in the dentinal tubules. The silver nitrate deposition was highest in group III (70.44%) while the group II (55.29%) was the lowest. The gap formation was found in almost all outer 1/3 of samples. The width of gap was different among materials and the location in the cavity.

Conclusion: type of lining materials did not effect on the nanoleakage of resin composite. The bulk-fill flowable resin composite could improve the adaptability of the restoration to cervical dentin margin.

Keywords: adaptability, nanoleakage, bulk-fill resin composite, open sandwich technique, cervical dentin

Introduction

Resin composites are the most popular restorative material at present due to the satisfied mechanical properties and the outstanding tooth-colored similarity. The tooth-structure preservation is another advantage when preparing the cavity for the resin composite. It is undeniable to agree that the role of the resin composite is likely to take over that of an amalgam. However the polymerization shrinkage is a vital imperfection of the light cured resin composite that is associated with the shrinkage stress and resulting in the occurrence of the gap formation, the post-operative sensitivity, secondary caries and the bond failure (Bausch, de Lange, Davidson, Peters, & de Gee, 1982; Van Meerbeek et al., 2003). Many studies reported the marginal leakage at the cervical area when the margin of the cavities located on the root dentin particularly in the proximal cavities (Dietschi, De Siebenthal, Neveu-Rosenstand, & Holz, 1995; Mahrous, Eltiti, Ahmed, & Alagha, 2015). The moisture contamination and



the incomplete light curing at the bottom of the proximal cavities influence on this defect. The stiffness of the resin composites may not establish the proper adaptation to the internal surfaces, cavosurface and cervical margin of the proximal cavities (Tredwin, Stokes, & Moles, 2005). Applying the adhesive system into dentin is challenged due to the difference components in the natural structure of dentin (Mahrous et al., 2015). The different techniques have been suggested to improve the quality of resin composite restorations such as the technique of light curing, placing of materials into cavities or applying other materials along with resin composite namely the open sandwich technique. For the latter technique, the materials with low modulus of elasticity such as the flowable resin composite or resin modified glass ionomer cement (RMGIC) are advocated as a the gingival liner or intermediate layer (Aggarwal, Singla, Yadav, & Yadav, 2014; Civelek, Ersoy, L'Hotelier, Soyman, & Say, 2003; Dietrich, Losche, Losche, & Roulet, 1999; Karaman & Ozgunaltay, 2014; Leevailoj, Cochran, Matis, Moore, & Platt, 2001; Sadeghi & Lynch, 2009; Yoshikawa, Sano, Burrow, Tagami, & Pashley, 1999).

RMGICs have a chemical adhesion to the dentin and an anti-cariogenic effect. When applying RMGIC as the gingival lining materials along with the resin composite restoration, the total volume of the resin composite decreases and resulting in the reduction of the shrinkage stress within the resin composite materials (Tanumiharja, Burrow, Cimmino, & Tyas, 2001). Some studies found the substantial improvement of the marginal adaptation at the gingival margins (Aggarwal et al., 2014; Dietrich et al., 1999). However some studies showed the remaining gap formation at the RMGIC-dentin interface (Soubhagya et al., 2015).

An elastic cavity wall concept has been presented by using the flowable resin composite as a lining material. Due to the low modulus of elasticity, this gingival liners function as a stress absorbing layer and reduce shrinkage stress at resin-dentin interface (Karaman & Ozgunaltay, 2014). Various studies reported that the placement of flowable resin composite as the gingival liner minimizes the leakage at the gingival floor (Civelek et al., 2003; Leevailoj et al., 2001; Sadeghi & Lynch, 2009) and reduces the gap formation at the internal margins (Yoshikawa et al., 1999). However some studies did not find any advantages from flowable resin composite because of the low filler content and high polymerization shrinkage (Miguez et al., 2004; Pecie, Onisor, Krejci, & Bortolotto, 2013).

Bulk fill resin composites have been recently developed to facilitate the clinical process. This material has the modification of fillers by either reducing the filler content or increasing the filler-particle size in order to reduce the light scatter at the filler-matrix interface and increase the degree of light transmission (Bucuta & Ilie, 2014; Ilie & Hickel, 2011; Kim, Kim, Choi, & Lee, 2015; Scotti et al., 2014). They are subsequently able to fill into the cavity with the thickness 4 - 5 mm. Some studies reported that the bulk fill resin composites had less polymerization shrinkage stress and better marginal adaptation (Agarwal, Hiremath, Agarwal, & Garg, 2015; Kim et al., 2015). This material has been also suggested to use as an alternative gingival lining material.

A nanoleakage investigation is a common method to investigate the quality of an adhesive system. It had been showed as nanometer-sized spaces within a hybrid layer even there was a gap-free margin. A silver nitrate is the most popular reagent to be used to detect these nano-spaces by observing the deposition of the silver nitrate under a high magnification Scanning Electron Microscope (SEM) (Sano, Shono, Takatsu, & Hosoda, 1994; Sano et al., 1995). This leakage is the result of an incomplete polymerization and infiltration of adhesive resin including the contamination at the bonding area. The nanoleakage is the considerable pathway for the penetration of bacterial products, oral fluid and dentinal fluid related to a hydrolytic degradation of adhesive resin and the bond failure (Li, Burrow, & Tyas, 2000; Sano et al., 1995).



Although using the liner materials in the class II resin composite open sandwich techniques improve the marginal adaptability, there are some studies reported nanoleakage at the cervical dentin margins (de Mattos Pimenta Vidal, Pavan, Briso, & Bedran-Russo, 2013). Both the marginal adaptability and the nanoleakage influence on the quality of the resin composite restorations. Nevertheless, there have been few studies to evaluate both adaptability and degree of nanoleakage of different liner materials in the class II resin composite open sandwich technique at the cervical dentin margin. Since the limitation of the information, the aims of this study were 1) to evaluate a degree of nanoleakage at a cervical dentin margin of three liner materials in class II resin composite open sandwich technique and 2) to evaluate adaptability to a cervical dentin of three liner materials in class II resin composite open sandwich technique. The null hypotheses tested were that 1) there was no significant differences in the degree of nanoleakage at the cervical dentin margin among three liner materials in the class II resin composite open sandwich technique and 2) there was no significant differences in the interfacial gap width at the cervical dentin margin among three liner materials in the class II resin composite open sandwich technique.

Materials and methods

2.1 Sample preparation

The maxillary premolar teeth of patients aged above 20 years old recently extracted for orthodontic reasons were collected from the dental clinic in Amphoe Mueang, Phitsanulok and the Faculty of Dentistry, Naresuan University. This study was approved by the Ethics committee of Naresuan University (IRB No. 578/59). The inclusion criteria were teeth with normal morphological feature, no cavity and no restorations. The exclusion criteria were teeth with abnormal morphologic feature, cavitated lesion, restorations and crack line or craze line. The extracted teeth were collected in 10% formalin solution no longer than one month. Calculus and soft tissue were removed from the extracted teeth. All teeth were then submerged in fresh 10% formalin solution for 2 weeks and stored in 0.1% Thymol solution at room temperature.

All teeth were mounted with sticky wax in silicone blocks. After that occluso-distal slot cavities were prepared with 4 mm in bucco-lingual width parallel to opposing walls. The gingival wall was finished at 1 mm cervically to the CEJ to keep gingival margin on dentin. The width of gingival wall was 1.5 mm in mesio-distal width by using fissure diamond bur (#835 FG 016 Jota, Switzerland) with a high speed handpiece. Each bur was replaced with a new one after five cavity preparation. After that all prepared teeth were horizontally sectioned at occlusal surface by using low speed diamond saw device with water coolant in order to receive the tooth samples with 4 mm occluso-cervical height. Then a tofflemire matrix holder and metal band were placed. The teeth were assigned into 4 groups (N=20).

Group I: Conventional flowable resin composite (FC+Co), n=5

Group II: Bulk fill flowable resin composite (BF+CO), n=5

Group III: No liner material, conventional nanofilled resin composite (Co), n=5

Group IV: Resin-modified glass ionomer cement (GI+Co), n=5

All cavities in group I, II and III were etched with 37% phosphoric acid (Scotchbond™ Etching liquid, 3M ESPE) for 15 s then rinsed with water jet for 20 s and gently air dried. The bonding agent (Adper™ Single Bond2, 3M ESPE) was applied according to the manufacturer's instruction. For the group IV, the GC conditioner liquid was applied to gingival floor for 10 s then rinsed with water jet for 20 s and gently dried. The thickness of lining materials in all groups (except group III) was 1 mm. The depth was checked with periodontal probe and light cured for 20s.



After placement the lining materials, the cavities (group I, II and III) were restored by incremental technique with 2 mm increments of nanofilled resin composite (Flitek™Z350 XT Universal restorative, 3M ESPE) and light cured for 20s on each layer. For the group IV, the cavities were treated with 37% phosphoric acid (Scotchbond™Etching liquid, 3M ESPE) and the bonding agent (Adper™Single Bond2, 3M ESPE) as mentioned above before incremental restored with nanofilled resin composite (Flitek™Z350 XT Universal restorative, 3M ESPE).

After the tofflemire matrix holder and metal band were removed, all samples were light cured for 20 s at buccal and palatal aspects by using LED light curing unit (Mini LED ACTEON, France) with light intensity 2,000 mW/cm². Then all samples were stored in distilled water at 37°C for 24 h and subjected to thermal cycling for 2000 cycles with temperature range of 5°C to 55°C with dwell time of 15s and 7s transferred time (Roggendorf, Kramer, Appelt, Naumann, & Frankenberger, 2011; Wattanawongpitak, Yoshikawa, Burrow, & Tagami, 2007).

2.2 Nanoleakage evaluation (Duarte, Phark, Varjao, & Sadan, 2009)

The samples in group I, II and group IV were coated with two layers of nail varnish excepted 1 mm around the liner material and 1 mm around the cervical dentin margin in group III. All samples were immersed in a 50% ammoniacal silver nitrate solution (pH=9.5) for 24 h in the dark. Then, they were thoroughly rinsed with distilled water and immersed in a photo-developing solution for 8 h under fluorescent light to reduce diamine silver ions to metallic silver grains (Peliz, Duarte, & Dinelli, 2005).

The samples were fixed in 2.5% glutaraldehyde in 0.1 M PBS buffer at pH 7.4 for 12 h at 4°C. After fixation, the specimens were rinsed by distilled water for 1 min. The samples were sectioned longitudinally, in a mesio-distal direction through the center of the restorations. The specimens received the second fixation in 2.5% glutaraldehyde in 0.1 M PBS buffer at pH 7.4, 4°C for 12 h followed by rinsing with distilled water for 1 min. The specimens were dehydrated in ascending concentrations of ethanol as follows: 25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min and 100% for 60 min. The specimens were polished to high gloss with water silicon carbide papers of decreasing abrasiveness (600, 800 and 1,200 grits) followed by the soft cloth with increasingly fine diamond suspensions of 2 µm and 1 µm. After polished, the specimens were sonicated in 100% ethanol for 10 min to remove residual debris, thoroughly dried, and demineralized in 0.5% silica-free phosphoric acid for 1 min. The specimens were dried by immersed in hexamethyldisilazane for 10 min, placed on filter paper and air dried at room temperature (Tay, Pashley, & Yoshiyama, 2002). The specimens were mounted on aluminum stubs, sputter-coated with gold and observed under SEM using backscattered electron mode (magnification x1000).

The SEM micrographs were measured the length of silver nitrate deposition along the gingival floor by using Image J software program. The extension of leakage was calculated as the percentage of silver nitrate deposition on the gingival floor. In addition, The SEM micrographs were measured gap width in three points of each sample (inner point, middle point and outer point of silver nitrate deposition).The data was analyzed by descriptive statistics using SPSS software. Normal distribution was verified with the Shapiro-Wilk test and homogeneity by Levene's test. The mean percentage of silver nitrate deposition was performed by using Kruskal-Wallis Test. The mean gap width at gingival floor was performed by using Kruskal-Wallis Test followed by Mann Whitney U test. ($p < 0.05$).



Results

The SEM micrographs presented the thickness of hybrid layer, pattern of silver nitrate deposition and gap formation (Fig. 1). In group I, II and III, the silver nitrate deposited entire thickness of hybrid layer, penetrated into the dentinal tubules and deposited on the resin tags. The inner and middle areas presented the thicker hybrid layers compared with that of the outer area. In group IV, all specimens presented modified hybrid layer which is clearly thinner than the hybrid layers of group I-III. These modified hybrid layer had the silver nitrate deposition and it also penetrated into the dentinal tubules.

The percentages of silver nitrate deposition were shown in Table 1. Group III (Co, control) had the highest percentage of silver nitrate deposition (70.44%), followed by group I (64.78%), IV (62.49%) and II (55.29%). However there was no statistically significant difference among the restorative materials ($p < 0.05$).

The gap formation was found in almost all outer-area samples and in some inner- and outer-area samples. The gap formation presented between the hybrid layer or modified hybrid layer and materials (Fig. 1). The width of gap was different among materials and the positions in a cavity.

The gap width of different materials and areas of gingival floor was presented in Table 2. In group I-III, the largest gaps were found in the outer area (5.23, 1.35 and 3.88 μm respectively) while the inner area of group IV showed the largest ones (4.45 μm). The gap width of the outer area of group I was significantly larger than other areas ($p=0.043$). The group III also had the largest size of gap in the middle area, significant difference when compared with group II and group IV ($p=0.019$). In the group IV had significantly larger size of gap in the inner area ($p=0.045$) when compared among materials, however there was no gap formation in the middle area in all samples.

Table 1 : The mean and standard deviation (SD) of percentage of silver nitrate deposition at cervical dentin

Type of liner material	N	% of silver nitrate deposition
		Mean (SD), %
Group I (FC+Co)	5	64.78 (14.14) ^a
Group II (BF+Co)	5	55.29 (13.47) ^a
Group III (Co)	5	70.44 (16.37) ^a
Group IV (GI+Co)	4	62.49 (4.14) ^a

Lower case characters represent statistically significant differences ($p < 0.05$)



Table 2 : The mean and standard deviation (SD) of gap width at cervical dentin margin

Type of liner material	N	Gap width between hybrid layer & liner		
		mean (SD), μm		
		Outer	Middle	Inner
Group I (FC+Co)	5	5.23 (2.94) ^{A,a}	0.41 (0.92) ^{B,a,b}	0.00 (0.00) ^{B,a}
Group II (BF+Co)	5	1.35 (1.30) ^{A,a}	0.00 (0.00) ^{A,a}	0.18 (0.26) ^{A,a}
Group III (Co)	5	3.88 (1.56) ^{A,a}	1.18 (0.74) ^{A,b}	2.38 (2.19) ^{A,a}
Group IV (GI+Co)	4	2.70 (3.12) ^{A,a}	0.00 (0.00) ^{A,a}	4.45 (2.36) ^{A,b}

Lower case characters represent statistically significant differences ($p < 0.05$) within columns

Upper case characters represent statistically significant differences ($p < 0.05$) within rows

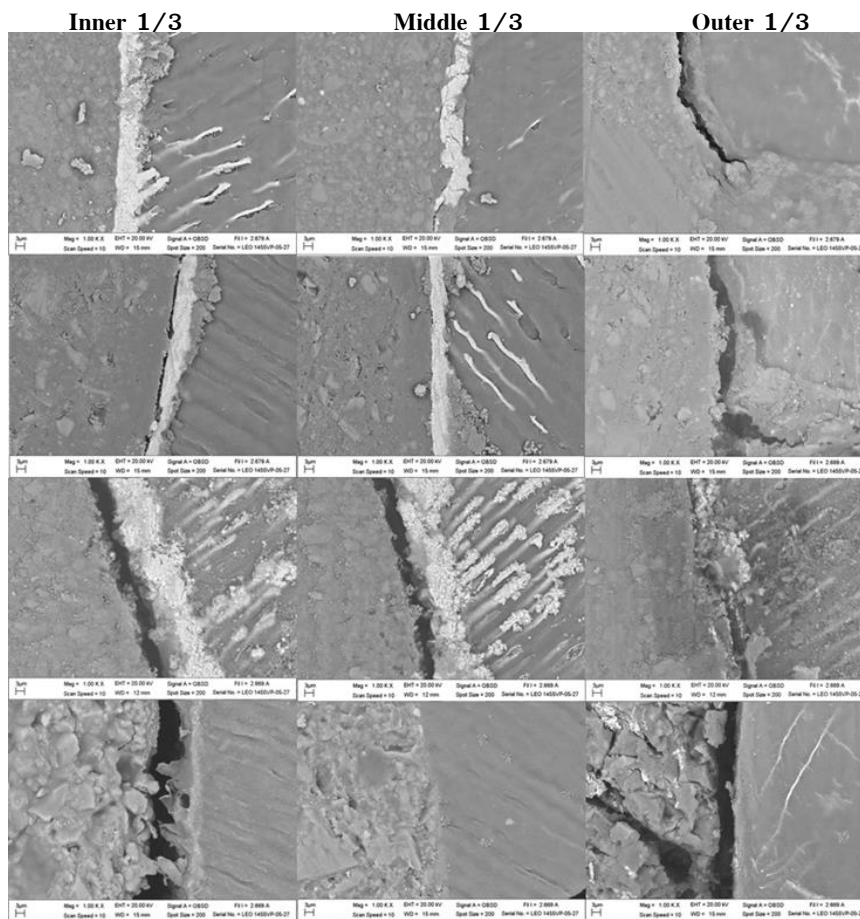


Fig 1 : The Backscattered SEM micrographs (1000x) present the thickness of hybrid layer, pattern of silver nitrate deposition and gap formation. Figure a-c demonstrate a pattern of silver nitrate deposition and gap formation of group I (FC+Co). At the inner area (a), silver nitrate deposits entire thickness of hybrid layer, penetrates into dentinal tubule and deposits on the resin tags (arrow). At the middle area (b), silver nitrate discontinuously deposits within a hybrid layer including resin tags and the dentinal tubules. Some area has a thin



layer of silver nitrate deposited at the top of hybrid layer. At the outer area (c), thin layer of silver nitrate deposits at the base of hybrid layer and/or at the top of hybrid layer. There is a gap formation between hybrid layer and bonding layer. Figure d-f demonstrate a pattern of silver nitrate deposition and gap formation of group II (BF+Co). There was a similar pattern as group I. At outer area (f), thin layer of silver nitrate deposits at the base of hybrid layer (arrow). There is also a gap formation between hybrid layer and bonding layer (f). Figure g-i demonstrate a pattern of silver nitrate deposition and gap formation of group III (Co). There was a similar pattern as group I and II. Some samples showed the spotted type of silver nitrate deposition in a hybrid layer (data not shown). There is also a gap formation along the gingival floor between the hybrid layer and bonding layer. Figure j-l demonstrate a pattern of silver nitrate deposition and gap formation in group IV (GI+Co). The SEM micrographs present modified hybrid layer. Silver nitrate deposits at the base of modified hybrid layer (arrow) and penetrates into dentinal tubule. In addition silver nitrate deposits within resin modified glass ionomer cement mass. There is a gap formation above modified hybrid layer at inner and outer area (j,l). (D=Dentin, FC=conventional flowable resin composite, BF=bulk fill flowable resin composite, Co=conventional nanofilled resin composite, GI=resin modified glass ionomer cement)

Discussion

Silver nitrate deposition represents the incomplete bonding of either an adhesive system or restorative material. These defects occur as the nanometer-sized spaces around the collagen fibrils within the hybrid layer. These spaces result of incomplete infiltration of adhesive resin into a demineralized dentin (Eick, Gwinnett, Pashley, & Robinson, 1997; Li et al., 2000; Pashley, Ciucchi, Sano, & Horner, 1993; Sano et al., 1994; Sano et al., 1995).

The percentages of silver nitrate deposition among three liner materials in class II resin composite open sandwich technique were no statistical difference. The pattern of silver nitrate deposition in group I, II and III had the similar pattern because these three groups were applied the same adhesive system.

This study used the total etched adhesive system (Single Bond2) in group I, II and III. This adhesive system applied 37% phosphoric acid for 15 s resulting in the exposure of collagen fibers. The combined primer-adhesive resin agent was then applied on moist dentin for the formation of hybrid layer.

The majority of silver nitrate deposited at the dentin side of hybrid layer which was probably denoted the shrunk collagen fibers which were accumulated on the dentin surface after applying etchant (Pashley et al., 1993). This thin layer (0.2-0.3 μm) might interfere the adhesive resin infiltration, consequently the silver ion precipitation (Sano et al., 1995). In addition, the components of adhesive reagent probably affected the silver nitrate deposition. Adhesives with high percentage of hydrophilic monomers demonstrated a higher degree of permeability after polymerization, consequently more silver nitrate deposition (Tay, Pashley, Suh, Carvalho, & Itthagarun, 2002). The Single Bond2 contains HEMA, this is hydrophilic monomer improved their infiltration into moist substrate. However, HEMA decreases the water vapor pressure resulting in retaining water at the interface. Finally, the hybrid layer acts as a hydrogel which promotes silver nitrate deposition (Tay, King, Chan, & Pashley, 2002).

These results presented the thick hybrid layer and much silver nitrate deposition at the inner area. Using the air blowing for solvent evaporation probably causes the accumulation of adhesive resin at the inner line angle. The thick layers of adhesive resin might prevent the proper evaporation of solvent, resulting in poor polymerization (Zheng, Pereira, Nakajima, Sano, & Tagami, 2001). The residual monomer probably caused the infiltration of silver nitrate within the resin (Ferracane, 1994; Lee, Greener, & Menis, 1995) consequently the deposition of silver nitrate within a hybrid layer or entire thickness of hybrid layer. Additionally, using of the moist bonding



technique may leave the excess water along the line angle, interfering the evaporation of solvent evaporation and leading to the incomplete resin polymerization.

The group IV (GI+Co) showed the deposition of silver nitrate within the modified hybrid layer and within the resin modified glass ionomer cement mass. The hydrophilic functional monomers contained in resin modified glass ionomer cement can absorb water resulting in hydrolytic degradation and increasing silver nitrate deposition (Cattani-Lorente, Godin, & Meyer, 1994). In addition, the porosity of material probably causes silver nitrate deposition within the material mass (Alster, Feilzer, De Gee, Mol, & Davidson, 1992; Miyazaki, Fukuishi, & Onose, 1999).

Almost all specimens demonstrated a gap formation at outer area of cervical dentin margin. The marginal gap width of group I (5.23 μm) was larger than group III (3.88 μm). The marginal gap width of group II (1.35 μm) and IV (2.70 μm) were smaller than that of group III. However they were insignificant differences. These results might imply that the bulk fill flowable resin composite and the resin modified glass ionomer cement using as a liner in the class II resin composite open sandwich technique can improve the marginal adaptability. In the other hand, using of a conventional flowable resin composite as a liner in the class II resin composite open sandwich technique cannot improve the marginal adaptability.

The conventional flowable resin composite has a low modulus of elasticity. A placement of flowable resin composite as a liner material can dissipate stress and reduce shrinkage stress at tooth-restoration interface (Basavanna, Garg, & Kapur, 2012; Karaman & Ozgunaltay, 2014; Olmez, Oztas, & Bodur, 2004). In addition, the flowable resin composite has low surface tension therefore this material can penetrates into the irregularity surface resulting in better adaptability (Civelek et al., 2003; Estafan, Estafan, & Leinfelder, 2000; Leevailoj et al., 2001; Sadeghi & Lynch, 2009). In the other hand, the conventional flowable resin composite contains 20-25% less filler than conventional materials and larger amount of diluent monomers resulting in high polymerization shrinkage (Pecie et al., 2013; Xavier, Monteiro, & Montes, 2010). The diluent monomer especially TEGDMA, which contains in Filtek™ Z350 XT flowable, has small molecule with more active sites leading to negative effect on polymerization shrinkage (Asmussen & Peutzfeldt, 1998).

The present results were similar to the previous studies which report that the flowable bulk fill resin composites showed better dentin marginal adaptation and less gap formation compared with conventional flowable resin composite (Agarwal et al., 2015; Roggendorf et al., 2011; Scotti et al., 2014). The bulk fill resin composites demonstrated low polymerization shrinkage stress and high degree of light transmission because of the reduction of light scattering at filler-matrix interface by either reducing the filler contents or increasing the filler particle size (Bucuta & Ilie, 2014; Kim et al., 2015). The present study used SureFil SDR Flow® as a liner in the class II resin composite open sandwich technique. This material added a modified urethane dimethacrylate in organic part together with photoactive groups leading to reduce shrinkage stress (Ilie, Bucuta, & Draenert, 2013). The occurrence of gap formation might be related with the polymerization shrinkage of materials. The SureFil SDR Flow® has less polymerization shrinkage probably resulted in small gap formation compared with others. Nevertheless, the bulk fill flowable resin composite has significantly lower mechanical properties than high viscosity bulk fill nanohybrid and conventional flowable resin composite (Bucuta & Ilie, 2014; Ilie et al., 2013). Therefore, the manufacturers recommend using this material as the intermediate layer.

Additionally, the implication of this study was the resin modified glass ionomer cement was an alternative lining material for class II resin composite open sandwich technique which may improve the marginal adaptability.



These results were corresponded with the previous studies (Aggarwal et al., 2014; Dietrich et al., 1999; Goldman, 1983; Sampaio et al., 2011).

The limitation of this study is the small sample size which resulting in abnormality in data distribution and insignificant difference of data. However this is a preliminary study, it might need further studies with increase sample size.

Conclusion

A conventional flowable resin composite and bulk fill flowable resin composite could not improve the nanoleakage when used with the same bonding system as conventional resin composite. The bulk fill flowable resin composite (SureFil SDR Flow®) created narrower gaps which might be related with the smaller polymerization shrinkage.

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