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Effect of Different Application Techniques on Marginal Adaptation of Class II Cavities Filled with Three Different Bulkfill Composite Filling Material: An *In Vitro* Study using Confocal Microscopy

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ABSTRACT

An experimental study was conducted to assess marginal adaptation of various bulkfill composite materials, at cervical and occlusal margins of class II cavity preparation, applied by two different application techniques. Seventy-two (72) extracted premolar teeth were selected, prepared and filled with three different bulkfill composite materials, and inserted using incremental and bulk techniques. Specimens were thermocycled and bisected mesio-distally. All specimens were observed using a confocal fluorescence imaging microscope at 10× magnification. The data was analyzed using the Mann-Whitney U test. $P < 0.05$ was used to indicate statistically significant differences. The study results revealed that, no significant differences in marginal adaptation were detected among the two application techniques (incremental and bulkfill) at cervical margins p=1.000 and the occlusal margins p=0.639 with the lowest gap width formation achieved by X-trafil® bulkfill composite among the three different bulkfill composite filling materials, while Filtek™ Flowable composite material had the highest gap width. This study signifies that good marginal seal depends on the proper cavity preparation, good manipulation of filling materials and skill of the operator whatever is the application technique.

Keywords: Adaptation, application techniques, bulkfill composite materials, cervical margin, confocal microscope, occlusal margin

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INTRODUCTION

The long-standing overall performance of the restoration depends on the tight fitting of the restorative material to internal cavity surfaces and margins (Roulet, 1994). Despite outstanding advances in the field of composite restorative materials technology and its applications in restorative dentistry, there are many factors that may influence the stability of composite restoration, such as low strength, technique sensitivity, high wear rate, and polymerization shrinkage (Pitel, 2013). Polymerization shrinkage generates a reduction in volume of the material 1.7% to 5.7% (Alvarez-Gayosso et al., 2004), as a result of changing the material density during the polymeric network formation process. The shrinkage stress may lead to generating gap (10–15 μm) (Ferracane, 2005). This gap permits the escape of fluids as well as bacteria between the oral environment and the dentin pulp complex (Amaral et al., 2004), which are considered to be harmful.

Many studies have looked at methods to reduce the rate of polymerization shrinkage and improve marginal adaptation. These include placement of liners (Chuang et al., 2004), replacement of the dentin with a glass-ionomer cement in the sandwich technique (Dijken & Pallesen, 2012) and incremental application techniques. Most of these studies have been done with conventional resin based composites (RBCs), even though conventional RBCs have typically been placed in layers not exceeding a thickness of 2 mm, due to these techniques being time-consuming and complicated when used to fill large and voluminous cavities in posterior teeth. Additionally, many dentists prefer the use of an alternative to this highly sensitive multiple layering technique, the one-step insertion of a 4 mm bulkfill composite resins.

Bulkfill composite resins have been developed, in an effort to overcome polymerization shrinkage problems. Lower filler loading, lower viscosity, high flowability and a self-leveling property of the material that adapts well to the cavity wall are the main advantages of the bulkfill material (Chuang et al., 2001). Placement of composite restorative material at thicknesses greater than 4 mm has become possible, by altering the initiator in bulkfill composite resins, resulting in considerably shorter chair times during the filling procedure (Tiba et al., 2013).

Due to inherent polymerization shrinkage and contraction stress, deboning and increased risk of gap formation at the tooth-composite interface could be compromised to posterior teeth particularly in class II restorations. Gingival Cavo-surface margins of Class II restorations can be an area of failure (Moffa,1989). Possible causes include insufficient polymerization of the RBCs at the gingival wall, the high C-Factor characteristic of the box shape, limited access of proximal boxes making the placement of the material more challenging and the adhesive bonding to the cervical tooth structure. These contribute to the increase in polymerization shrinkage stresses during the setting reaction of the material (Sabatini et al., 2010).

It is believed that effective marginal seal has been obtained by incremental application technique over the bulkfill method by decreasing the stresses generated within the tooth-restoration system (Dijken & Pallesen, 2011; Dietschi et al., 2002). On the other hand, class I and class II cavities can be restored with bulkfill materials in a mono-increment technique, they are predictable to be capable to produce proper marginal integrity. Therefore, research conclusions need to be confirmed by means of experimental work that mimics clinical environments. Consequently, this is an experimental study conducted with the aim of assessing the effect of incremental and bulkfill application techniques on marginal adaptation of class II cavities filled with three different types of bulkfill composite resins. Confocal microscopy had been used for accurate and closer examination of the restoration margins. The null hypothesis is there is no difference in the marginal adaptation achieved by the examined placement techniques.

MATERIALS AND METHODS

Specimen Preparation

Seventy-two premolar teeth were stored for no more than three months after extraction for an orthodontic purpose. Exclusion criteria were teeth with developmental defects, caries or microcracks. Ethical approval was from the Research Ethics Committee of the University of Sciences and Technology prior to study execution (MECA NO: EAC\UST141). The teeth were cleaned by hand scaling and immersed in a 0.1% chloramine T solution for one week, followed by immersion in normal saline at room temperature during the experimental time.

Cavity Preparation

A dental manikin with upper and lower jaws was used, the teeth were set with crowns in the long axis parallel to each other and in proximal contact (Sabah & Baban, 2013), and a metal matrix band used to adopt teeth. A standardized class II cavity preparation was prepared to all teeth using coarse diamond fissure points (F80710M, ökoDENT, Thuringia, Germany) with a high-speed hand piece (W&H, Bữrmoos, Austria) under profuse water cooling and finished with finishing diamond points (#2203, Dentex, Taipei, Taiwan). Roundation and beveling were applied to the enamel margins and the inner-angles of the cavities. All cavities received 1 mm distance preparation below the cemento-enamel junction (CEJ). For every four cavities, one new bur was used to maintain the cutting efficiency (Borges et al., 2012). A width of 4 mm bucco-lingually and a length of 4 mm occluso-gingivally with a depth of 2 mm axially were prepared in the cavities as shown in Figure 1. A periodontal probe was used to confirm dimensions.

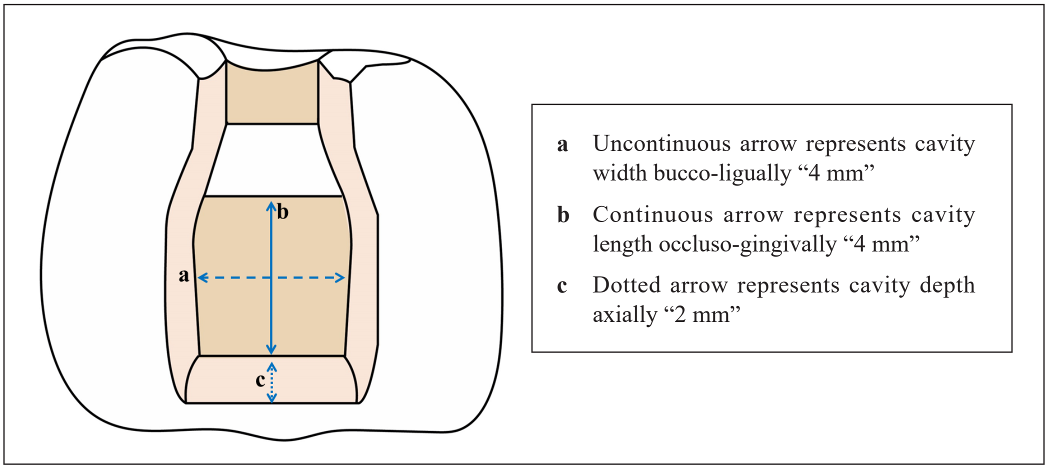


Figure 1. Schematic diagram of cavity preparation dimensions

Specimens Grouping

The specimens were assigned to one of two main study groups with 36 tooth in each group corresponding to two different application techniques (incremental and bulkfill). The specimens were further randomly sub-divided into three subgroups for each technique, with 12 teeth in each subgroup based on the different bulkfill restorative materials used in this study as shown in Figure 2. The characteristics of the materials used in this study are shown in Table 1.

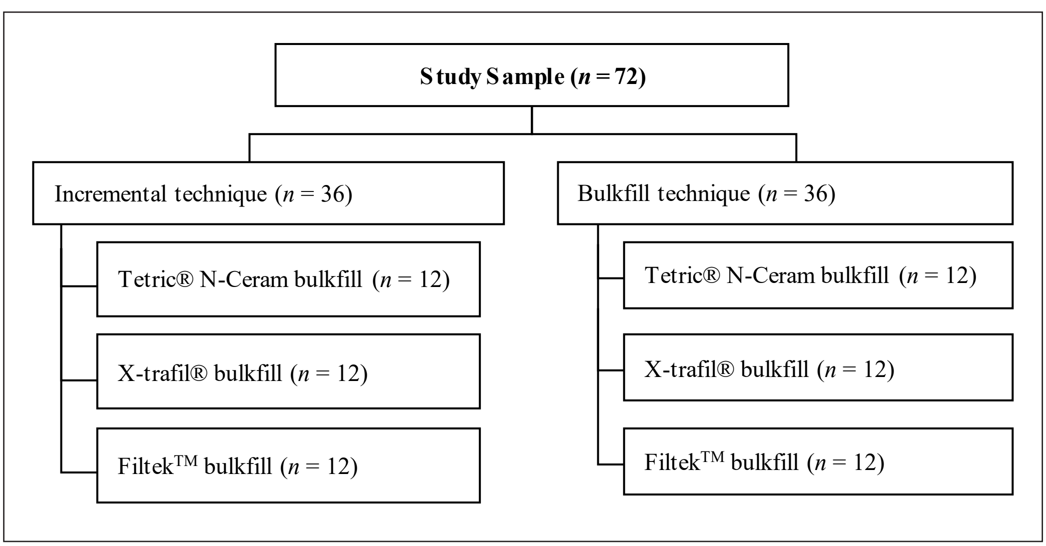


Figure 2. Flow chart demonstrating specimens grouping

Table 1
Characteristics of the materials used in this study

Material	Manufacturer	Composition	City\Country
Tetric® N-Ceram bulkfill	Ivoclar Vivadent	BIS-GMA, Urethane Di methacrylate, Barium glass filler, Ivocerin, shrinkage stress reliever, Light sensitivity filter filler, Pigments	Liechtenstein, Germany
Filtek™ bulkfill Flowable Restorative	3M ESPE	BIS-GMA, Urethane Di methacrylate, procrylat resin fillers are combination of Zirconia /Silica (.01 to 3.5 micro meter) &Ytterbium tri fluoride (0.1 to 5)	St. Paul, MN, USA
X-trafil®	Voco	BIS-GMA, Urethane di methacrylate, TEGDMA	Cuxhaven, Germany

Restorative Procedure

A solution of 37% phosphoric acid gel (Ivoclar Vivadent, Liechtenstein, Germany) was applied to all cavities as an etching process for 30 seconds on the enamel and 15 seconds on the dentin, followed by 5 seconds washing with a water jet and drying with a gentle stream of air which left the surface moistened. Corresponding self-etch bond adhesive systems were applied: Adhe SE One F (Ivoclar Vivadent, Liechtenstein, Germany), Scotch Bond Universal (3M Espe, St. Paul, MN, USA), and Futura bond DC (Voco, Cuxhaven, Germany). A LED light curing unit (LY-C240 Foshan City, China) of 1200 mw\cm² light intensity which was monitored and checked periodically with a radiometer (Dymax, Torrington, USA) was used. A 10 seconds was a distance which was determined by a periodontal probe, where the specimens were light-cured from the occlusal and cervical margins with the light tip contacting the margins. Two techniques were employed in this study:

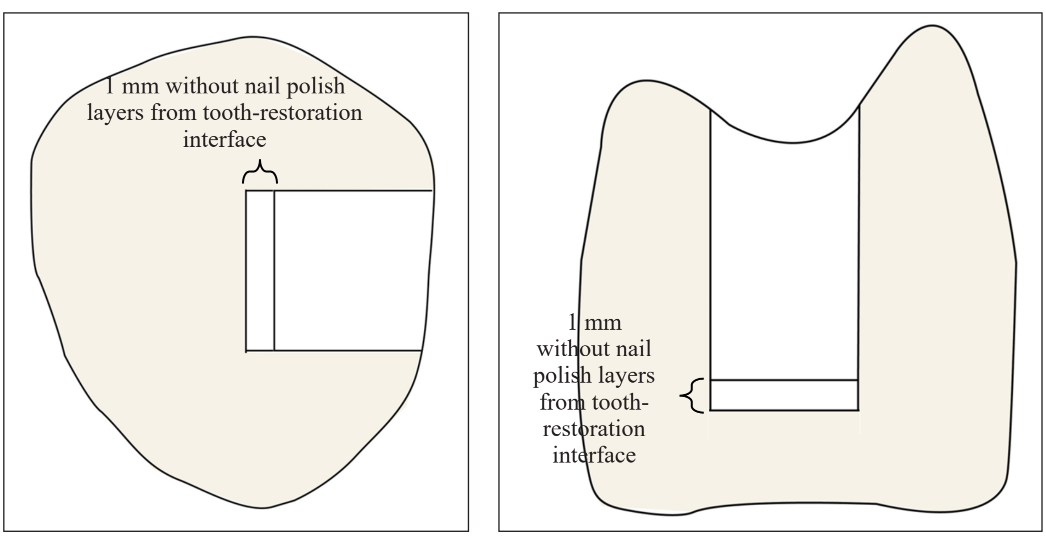
(a) A 4 mm single step placement of the bulkfill composites.
(b) A 2 mm thickness of bulkfill composites were used for the incremental technique. Both techniques were cured for 40 seconds, and all restorations were performed by one operator.

Thermocycling Procedure

In simulation to the temperature changes in the oral environment and development of micro-space between the tooth margins and the restorative material, two water baths, 55°C and 5°C, with a 30 seconds dwell time, were selected to performe 1000 cycles according to the International Organization for Standardization (ISO)TR 11405 (Loguercio et al., 2004).

Evaluation of Marginal Adaptation

Once the thermocycling process was completed, the specimens were dried, and two layers of nail polish were applied 1 mm above and below the interface between the tooth and restorative material, as shown in Figure 3. Afterwards, a slow speed of 300 rpm with a diamond disk (D-12203 Berlin, Germany) under constant cooling (Opdam et al., 2010), was used to bisect the specimens mesio-distally through the midpoint of restorative material parallel to the occlusal surface. Rhodamine B Isothiocyanate (Aldrich Chemical Co., Milwaukee, WI, USA), the fluorescent material was applied to the specimen based on the manufacturer instruction. Then, a Confocal Fluorescence Imaging Microscope (Leica TCS-SP5, DM6000-CFS) at 10X magnification was used to examine the specimens to determine marginal gaps along the restoration-cavity wall interfaces in the cervical and occlusal margins (Zarrati & Mahboub, 2010), the specimens were sent to Malaysia and the work was done in conjunction with Dr Alshawsh who is an Associate Professor at department of pharmacology, Faculty of Medicine, Unversiti Malaya for examination under a Confocal Fluorescence Imaging Microscope. Three points on the occlusal and cervical margins of the tooth-restorative interface were selected to facilitate the determination of the marginal gap width (the distance between the tooth axial wall and the restorative material), and the full perimeter of the restoration was bought by way of taking approximately six photos of each specimen (Usha et al., 2011). Image analysis software (Scope Photo 3.0 USA) was used to record the marginal gap width from the three points in each region. The mean marginal gap in micrometers (μm) for the occlusal and cervical margin was calculated. Confocal microscopic examination was used to be accomplished via one operator with experience in quantitative analysis of margin and who used to be blind to the application procedures. The marginal fitting surface between composite restoration and dentin used to be presented as a percentage of the whole margin length in the enamel and dentin.



(a) (b)

Figure 3. A schematic diagram to show the exact place where two layers of nail polish were applied "colored area" at occlusal and cervical margins: (a) occlusal view; and (b) proximal view

Marginal qualities along the outer periphery of the restorations were classified according to the criteria “continuous margin”, “noncontinuous margin” and “not judgeable\artifact”. The percentage of “continuous margin” in relation to the individual noncontinuous margin was calculated as marginal integrity according to the formula:

$$Marginal\ integrity = \frac{Noncontinuous\ margin}{Continuous\ margin} \times 100$$

Statistical Analysis

All data were collected and statistically analyzed with the aid of SPSS version 25.0 (Chicago, USA). The assumption of normality was not met, because the data did not approximate a normal distribution curve. This was further confirmed using the Shapiro-Wilk test. All specimens of the two groups were evaluated and compared for marginal adaptation along with the occlusal and cervical wall using a Mann-Whitney U test. A p value of < 0.05 was used to indicate statistically significant differences.

RESULTS

The quantitative assessment of marginal adaptation exposed that, no gap formation was executed in several regions (Figure 4), even though noncontinuous margins categorized as “marginal fissures” were observed in all restorations (Figure 5). The mean of three points that shows the largest marginal gap width of the cervical region and the point that represented the largest marginal gap width at the occlusal region for the three different bulkfill materials were recorded and the data were introduced in Table 2.

Table 3 reveals marginal adaptation scores expressed as a percentage of gap formation at the occlusal and cervical margins of various bulkfill composite materials inserted with incremental and bulkfill techniques.

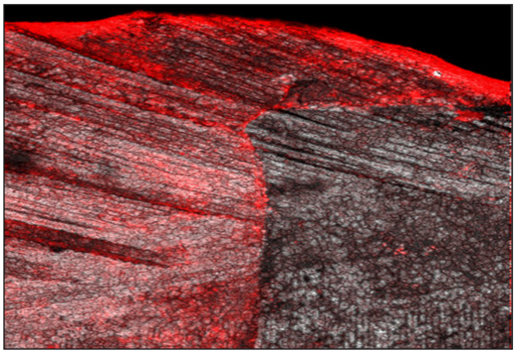


Figure 4. Confocal microscope image shows continuous marginal adaptation (no gap formation) in certain restoration

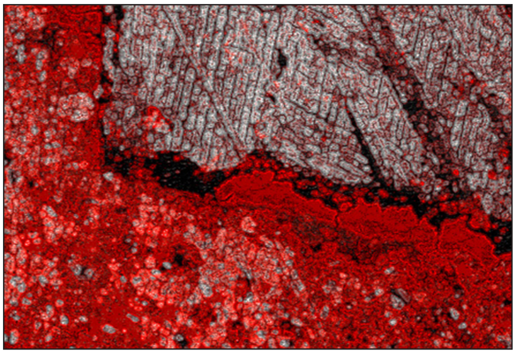


Figure 5. Confocal microscope image shows non-continuous marginal adaptation (gap formation) in all restorations

Table 2
Mean and standard deviation values for marginal gap width at occlusal and cervical regions

Application techniques	Margins	Descriptive statistics of bulkfill materials m±St		
		Filtek™ Flowable m±St	Tetric®N- Ceram m±St	X- trafil® m±St
Incremental	Occlusal	1.77±0.718	0.75±0.754	0.3±0.00
	Cervical	2±0.00	1.5±0.522	1±0.00
Bulkfill	Occlusal	1.5±0.522	1.67±0.492	0.5±0.00
	Cervical	2±0.00	1.5±0.522	1±0.00

Table 3
Marginal adaptation scores expressed as percentage of gap formation at occlusal and cervical margins of various bulkfill composite materials inserted with incremental and bulkfill techniques

Margins	Marginal adaptation	Incremental application						Bulkfill application					
		Filtek™ Flowable		Tetric® N-Ceram		X-trafill®		Filtek™ Flowable		Tetric® N-Ceram		X- trafill®	
		f	(%)	f	(%)	f	(%)	f	(%)	f	(%)	F	(%)
Occlusal	Continuous margin	0	0%	5	41.7%	9	75%	0	0%	2	16.7%	10	83.3%
	Non-continuous margin	4	33.3%	5	41.7%	3	25%	6	50%	6	50%	2	16.6%
	Not judgeable\ artifact	8	66.7%	2	16.7%	0	0%	6	50%	4	33.7%	0	0%
Cervical	Continuous margin	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
	Non-continuous margin	12	100%	6	50%	3	25%	12	100%	6	50%	3	25%
	Not judgeable\ artifact	12	100%	6	50%	0	0%	12	100%	6	50%	0	0%

No significant differences in marginal fitting among the three different bulkfill composite materials were detected between the two application techniques at the cervical margins $p > 1.000$ and occlusal $p > 0.639$ using the Mann-Whitney U test, Table 4.

A statistically significant difference was found in the gap width analysis scores between the occlusal and cervical margins with $p < 0.000$ for all groups of tested materials utilizing the Mann-Whitney U test, Table 5.

The assessment of marginal gaps width in the cervical and occlusal regions within each bulkfill restorative material using the Mann-Whitney U test exposed a statistically significant difference among the tested margins, Tables 3 and 6. X-trafill® bulkfill composite showed the lowest gap width formation, while Filtek™ Flowable composite material had the highest gap width.

Table 4
Gap formation scores comparison between incremental and bulkfill application techniques at occlusal and cervical margins

Margins	Mann-Whitney U	Z	Sig	Incremental application method	Bulk Application method
Occlusal margin	609.00	-0.470	0.639	35.42	37.58
Cervical margin	648.00	0.000	1.000	36.5	36.5

* No significant differences of gap formation between incremental and bulkfill application techniques at occlusal and cervical margins.

Table 5
Marginal adaptation comparison between occlusal and cervical margin

Marginal adaptation	Mann-Whitney U	Z	Sig	Occlusal margin	Cervical margin
	1458.00	-4.860	0.000*	56.75	88.25

*significant differences of marginal adaptation between occlusal and cervical margins $p < 0.000$

Table 6
Marginal gaps width among the three bulkfill composite materials filled with incremental and bulkfill techniques at each margin

Margins	Bulkfill materials	Incremental application		Bulkfill materials	Bulkfill application	
		Mean differences	P-value		Mean differences	P-value
Occlusal	A-B	1.47	0.000*	D-E	-0.17	0.000*
	A-C	1.02	0.011*	D-F	1	0.003*
	B-C	-0.45	0.000*	E-F	-1.17	0.000*
Cervical	A-B	1	0.000*	D-E	1	0.000*
	A-C	0.5	0.001*	D-F	0.5	0.000*
	B-C	-0.5	0.001*	E-F	-0.5	0.000*

A: Teeth restored with Filtek™ Flowable using the incremental technique.
B: Teeth restored with X-trafil® bulkfill composite using the incremental technique.
C: Teeth restored with Tetric®N-Ceram bulkfill using the incremental technique.
D: Teeth restored with Filtek™ Flowable using the bulkfill technique.
E: Teeth restored with X-trafil® bulkfill composite using the bulkfill technique.
F: Teeth restored with Tetric®N-Ceram bulkfill using the bulkfill technique.
* significant differences of marginal gaps width among the different three bulkfill composite materials.

DISCUSSION

The quest for a good adaptation between the restoration and cavity walls remains a goal of many researchers (Ferrari & Garcia-Godoy, 2002). Therefore, the present *in vitro* study evaluated marginal adaptation of various bulkfill composite restorative materials applied by incremental and bulkfill methods at the occlusal and cervical margins in class II restoration.

Class II cavities were prepared on the teeth of the current study because the occlusal and cervical margins are often distinguished and designated for composite restorations. They involve both the enamel and the dentin, and therefore, the nature of adaptation of composite resin can be compared at both regions. To ensure standardization to all restorative procedures, the same degree of cure and polymerization reaction between the studied groups was achieved by using a single LED light curing unit. The self-etch technique was used for all restorations, and the adhesives used in this study combine the functions of both primer and adhesive components which reduced the procedure time. A thermocycling procedure was applied to all specimens at a specific temperature range according to (ISO) TR11405 (Loguercio et al., 2004), with the goal of thermally accentuating the junction at the tooth-filling interface.

Quantitative analysis of the amount and width of gaps generated at the margins and marginal irregularities provided by marginal adaptation measurement had been chosen for this study rather than the qualitative isolated analysis provided by microleakage. To minimize the errors during scoring and calculation of marginal data, the number of criteria used to detect differences between tested groups was collapsed and narrowed down to the criteria of no-gap (continuous margin) against gap (noncontinuous margin), to make interpretation and statistical analysis of results easier.

The present study measured adaptation using confocal microscopy at 10X magnification. Confocal laser scanning microscopy (CLSM) is a technique used for picturing subsurface tissue characteristics. An advantage of this technique is the use of lens focus which can focus a few microns under the observed surface, thus avoiding the spread of stain due to specimen sectioning and avoid polishing artifacts (Lopes et al., 2009). The six images taken for analysis were nonoverlapping to avoid replication of the same gap score of a previous image.

The hypothesis stating that there are significant differences in marginal adaptation between the two techniques was not supported by this study results. The use of a bulkfill application technique showed gaps with an amplitude similar to that of the incremental technique. There were no statistically significant differences between the two application techniques. The study results are in agreement with other studies done by Roggendorf et al. (2011), Campos et al. (2014) and Furness et al. (2014) comparing the different placement techniques (incremental and bulkfill) with different RBC systems (conventional versus bulkfill composites). However, the findings in the present study contrasted to a study done by Mullejans et al. (2003), comparing the application techniques (incremental against bulk) using single conventional composite resin, which exhibited that incremental application reduced gap formation. These results may be different from the current study because only the conventional composite resin was used and the types of composite resin systems used were different.

The study results exhibited satisfactory marginal adaptation to all investigated bulkfill materials at the occlusal margin, unlike the cervical margin. The values of continuous margins in the occlusal region were higher than the cervical region (Roggendorf et al., 2011). This could be attributed to the beveling of the enamel margins in order to increase the surface area of the enamel to bond to the composite. The findings of the current study have been corroborated by a study conducted by Oskoee and colleagues, who reported that occlusal marginal adaptation was superior to that observed in the cervical enamel margins (Oskoee et al., 2012). This could be explained by that, the adhesion of the restorative materials to the proximal aspect at the Cavo-surface margin is greater to dentin rather than enamel, due to low enamel thickness at that area (Bogra et al., 2012). In addition, the distance of the light source from the material is lower at the occlusal surfaces compared to that at the proximal box base, thereby reducing the percentage of degree of conversion (Coutinho et al., 2013).

In this study, X-trafill® bulkfill composite material, exhibited a great significant reduction in the width of marginal gaps alongside the restoration-enamel interface at the gingival and occlusal regions after thermocycling procedure in relation to the Filtek™bulkfill restorative material. This ought to be accredited to an inadequate adaptation to the enamel walls, as a result of the high viscosity of the Filtek™bulkfill restorative material, because of the increase in the amount of filler particles (Radhika et al., 2010; Majeed, 2012). On the other hand, the better adaptation exhibited by X-trafill® restorative material was owing to the low viscosity of the material that facilitate plastic flow during the early phases of polymerization (Scotti et al., 2014). The present study results contradict the study conducted by Patel et al. (2018), who evaluated marginal fitting integrity of three bulkfill composite materials in Class II cavities, and found that Filtek™ bulkfill composite material showed better marginal adaptation than Tetric®N-Ceram and X-trafill® bulkfill composites. This could be due to their study design as stereomicroscopy was used for evaluation.

The limitations of the present study are that this study tested the cervical marginal adaptation underneath the level of the CEJ. However, in a practical work, it is not-indicated to apply composite restorations underneath the CEJ, as normally open sandwich technique is recommended in clinical application, where suggested modifications had been done in this study. As well as, the current study evaluated adaptation using only a self-etching system. Therefore, the results cannot be extrapolated to other systems. Future research should be carried out *in vivo* to confirm the current study results.

CONCLUSIONS

It can be concluded from the results that the marginal adaptation scores were not affected by the various tested application techniques. On the other hand, as anticipated, with the both application techniques, the marginal adaptation in the occlusal surface was higher than that in the cervical surface, and among the restorative materials, X-trafill® restorative material exhibited the highest score of adaptation, irrespective of the filling materials investigated in this study.

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