Section A-Research paper

EFFECT OF VARIOUS INSERTION TECHNIQUES ON THE MARGINAL ADAPTATION OF CLASS II CAVITIES WITH DIFFERENT COMPOSITE FILLING MATERIALS USING CONFOCAL LASER SCANNING MICROSCOPE-AN INVITRO STUDY.

[1]Dr.Sowmya Kallepalli[*], [2]Dr. Baljeet Singh Hora, [3]Dr. Wasayf Hammad A.Almarzuqi, [4]Dr. Leena Ibrahim M. Alakhtar, [5]Dr. A. Jagadeesh Kumar, [6]Dr Swathi Lakshmi Rongala

[1]PhD Scholar, Department of Conservative Dentistry and Endodontics, Rama Dental College, Hospital and Research Centre, Rama University, Kanpur,UP, India.
[2]Principal &HOD, Department of Conservative Dentistry and Endodontics, Rama Dental College, Hospital and Research Centre, Rama University, Kanpur, UP, India.
[3]General Dentist, Njad medical complex, Qassim Province, KSA
[4]Board resident at Qassim Reginal Dental Centre, Qassim Province, KSA
[5]Assistant Professor, St. Joseph Dental College and Hospital, Oral and Maxillofacial pathology, Eluru, AP, India.
[6]BDS, General Dentist, Visakhapatnam, AP, India
[*]Corresponding Author: Dr.Sowmya Kallepalli, Email: sowmyakutedoll@gmail.com

Abstract

Aim: To evaluate various insertion techniques on the marginal adaptation of class II cavities with different composite filling materials using CLSM. Materials and method: Standardized class II cavities (MO) were prepared in eighty sound extracted human upper premolars. The cervical margin of the proximal box was located at 1mm occlusal to the cementoenamel junction (CEJ). The prepared teeth were divided into two groups of 40 teeth each and then each group was subdivided into four subgroups of 10 teeth each. The samples were subjected to etching process using 3M ESPE Scotchbond Universal etchant followed by bonding with 3M ESPE Adper™ Single Bond Plus Adhesive and then Group I was restored with 3M ESPE Filtek™ Supreme Ultra Universal, a Conventional nanohybrid resin-based dental composite and Group II was restored with 3M ESPE Filtek™ Bulk Fill Posterior Restorative,a Bulk-fill Nanohybrid high viscosity composite using four different insertion techniques. GP_1- Horizontal insertion technique GP_2- Vertical insertion technique GP_3- Oblique insertion technique and GP_4- Bulk fill technique. All the restored teeth were stored in distilled water for 24 hours at room temperature, thermocycled and then soaked in Rhodamine B dye for 48 hours. Teeth were then sectioned for evaluation of marginal adaptation along the tooth-restorative interface in the occlusal and gingival regions using a CLSM. Data were collected and statistically analyzed using ANOVA followed by Post hoc Tukey HSD test. Results: Statistically significant difference was observed between the four insertion techniques when conventional nanohybrid resin-based dental composite was used with Oblique insertion technique (GPI_3) performing better than other subgroups with highest mean score (29.67+/-0.02; $P<0.001$) followed by horizontal(GPI_1-

29.44+/-0.04; P<0.001) then vertical (GPI_2-29.08+/-0.06; P<0.001) insertion techniques with poor marginal adaptation observed using bulk fill technique (GPI_4-27.57+/-2.60; P<0.001) but no statistically significant difference in the marginal adaptation observed when bulk fill nanohybrid high viscosity composite was used. However bulk fill technique ($GPII_4$) had the highest mean score (29.59+/-0.05; P=0.077). The horizontal ($GPII_1$-29.44+/-0.04; P=0.077), oblique (GPI_3-28.58+/-0.30; P=0.077) and vertical (GPI_2-28.53+/-0.10; P=0.077) insertion techniques had lower scores compared to bulk fill technique respectively. Conclusion: Marginal gaps could not be eliminated by any of the tested insertion techniques. Incremental techniques showed better marginal adaptation compared with the bulk fill technique when conventional nanohybrid resin-based dental composite was used and bulk fill technique showed better marginal adaptation compared with incremental placement techniques though statistically not significant when bulk fill nanaohybrid composite material was used.

Keywords: marginal adaptation, class II cavity, conventional nanohybid composite, bulk fill nanohybrid composite, insertion techniques.

Introduction

In dentistry, a damaged or decayed tooth is restored with a restorative material to bring back its original form, function and aesthetics. A wide variety of materials are available among which silver amalgam and composite resins are extensively used as direct restorations. The growing urge to have a beautiful smile led to the development of posterior composites and have taken an edge over silver amalgam restorative materials and gained popularity due to their ability to replace the tooth structure in both appearance and function[1].

Resin composites have undergone enormous progress since the introduction of bisphenol A glycidyl methacrylate (Bis-GMA) to dentistry and have been modified into different formulations by varying the functional groups which contributed to the vast progress of present day composites[2].

Despite the improvements of restorative material in recent times, the satisfactory marginal adaptation of restorations remains a challenge for clinicians.Marginal adaptation is defined degree of approximation of a restorative material to the tooth surface. The marginal failure of composite resin restorations is related mainly to the quality of bonding to the dental structures and to stress generated on the restoration. A close marginal adaptation and seal at the interface is important for successful dental restoration[3].So, many attempts have been made to improve the marginal adaptation and restoration placement techniques are universally recognized as one of the major factors in the modification of marginal seal. Various insertion techniques which reduce the level of stress due to resin composite polymerization shrinkage have been proposed over years, which include incremental layering techniques like oblique, horizontal, vertical, three site, successive cusp buildup along with centripetal buildup technique and bulk-fill technique[1].

The incremental layering technique has been accepted as the gold standard for the placement of resin composite restorations[4].During an incremental layering technique, the composite resin material is placed in layers of 2 mm or less. This approach has a number of advantages; such as, it results in better light penetration and better polymerization of the composite resin, reduction of the cavity configuration factor, cuspal deflection, polymerization shrinkage stresses; and ensure that the resin adheres better to cavity walls. However, in addition to these advantages, there are a number of drawbacks associated with the use of incremental approach. These include voids can be trapped between the increments, bonding failure could occur between the increments, it can be difficult to place composite after conservative cavity preparation, and the time taken to complete the procedure is lengthier due to the time required to place and polymerize each increment[5]. In this study horizontal, oblique and vertical incremental techniques have been compared.

To overcome the disadvantages of incremental layering techniques,a new category of resin based composites called ‘bulk-fill’ composites have been introduced which offer a single increment placement of 4-5mm thickness instead of the conventional 2mm increment[6]. The rationale of the bulk-fill composite resin material would be to reduce interfacial gap formation of incremental technique and clinical steps by filling the cavity in a “single” increment leading to a reduced porosity and a uniform consistency for the restoration, further reducing the clinical time taken and cost factor of the patient[5, 7]. In addition, studies reported that bulk fill composites produce less shrinkage and cuspal flexure in class II cavities[8].

In this study, Class II cavity preparation is considered to test the in vitro performance of marginal adaptation of the composite resins as one of the reasons is gingival cavo-surface margins of Class II restorations could be a factor for an early area of failure due to its limited access of proximal boxes making the placement of the material more challenging.The other reason is the critical isthmus portion can be a challenging area for any restorative materials[9].

In the present study, CLSM, a nondestructive technique for visualizing subsurface tissue characteristics, is considered as a reliable tool to assess the extent of marginal adaptation was used at low magnification (×10)[10].

As there is no much literature available on the effect of using various incremental placement techniques of bulk-fill composites on marginal adaptation, this in vitro study was conducted to evaluate the marginal adaptation quantitatively by measuring the gaps between different type of composite materials with the tooth structure using different insertion techniques using conventional composites and the hypothesis of the study is that different insertion techniques will have an effect on the marginal adaptation in class II composite restorations.

Materials and Methods

Materials

The materials used in this study were as shown in Table 1.

Table 1. Materials

S.NO.	MATERIALS	MANUFACTURER	COMPOSITION	DESCRIPTION
1.	Filtek™ Supreme Ultra Universal	3M ESPE	Bisphenol-A diglycidyl ether dimethacrylate, urethane dimethacrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), and bisphenol A polyethylene glycol dietherdimethacrylate (6) resins. The filler is a combination of silica filler and zirconia filler	Conventional (Methacrylate based) nanohybrid resin-based dental composite
2.	Filtek™ Bulk Fill Posterior Restorative	3M ESPE	Aaromatic dimethacrylate (AUDMA), addition-fragmentation monomer (AFM), urethan dimethacrylate(UDMA), dodeconated dimethacrylate (DDDMA), Silica (20 nm), zirconia (4-11 nm), zirconia/ silica clusters, ytterbium fluoride (100 nm agglomerate particles)	Bulk-fill (Methacrylate based) Nanohybrid high viscosity composite
3.	Adper™ Single Bond Plus Adhesive	3M ESPE	Bis-GMA, HEMA, dimethacrylates, silica nanofiller (5 nm), polyalquenoic acid copolymer, initiators, ethanol, water	Etch-and-rinse adhesive
4.	Scotchbond™ Universal Etchant - Etching Gel	3M ESPE	34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide (Scotchbond Universal Etchant)	Etchant

Specimen preparation

A total of 80 human maxillary premolars, extracted for periodontal/orthodontic reasons were selected and were cleaned with a hand and ultrasonic scaler (Wood Pecker Medical Instrument. Co. Ltd China) from any soft tissues or hard calculus deposits, then immersed in 10% formalin for 5 days for disinfection , then finally stored in normal saline solution at room temperature and were used for the study within six months[8]. The teeth were fixed with sticky wax to the base of plastic cylinder. The cylinder was filled with modelling wax so that only root was embedded within the modelling wax.

A standardized class II mesio-occlusal cavity preparation was prepared in all teeth using coarse diamond fissure points with a high-speed hand under profuse water cooling and finished with finishing diamond points. The overall dimensions of the cavities were standardized as follows: 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. The gingival margin of the proximal box was located 1mm occlusal to the cementoenenamel junction (CEJ). Periodontal probe was used to confirm dimensions. All the cavosurface margins were prepared without beveling and all internal line angles were rounded. 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.

Restorative procedure

The teeth were randomly assigned into the two experimental groups (n=40) each based on the type of composite resin selected. Each group was again, divided into following 4 subgroups (n=10) according to the type insertion technique used. Universal Tofflemire retainer (AISI 420 German stainless steel) with a metal matrix band of 0.05 mm (No 1001/30, Kerr Hawe SA, Bioggio, Switzerland) was applied to all cavities.

All the samples selected for Group I and II have been restored with Nanohybrid Conventional composite resins and Nanohybrid high viscosity Bulk Fill composite resins respectively.The teeth were subjected to etching process using 3M ESPE Scotchbond Universal etchant for 20 seconds followed by rinsing with distilled water for 15 to 20 seconds and further blot dried for 20 seconds. The Adper Single Bond plus Adhesive (3M ESPE, St Paul, MN, USA) bonding agent, was applied and light cured using LED light curing unit for 20 seconds. Then the each group was divided into following subgroups based on different composite insertion techniques used.

In subgroups I_1,I_2 and I_3, the Filtek™ Z350 XT Universal Restorative composite resin is applied in two horizontal, vertical and oblique increments with approximately 2 mm thickness respectively, and in Subgroup I_4, it is applied in single increment of 4 mm thickness.In Group II_1,II_2, II_3 and II_4 , the Filtek™ Bulk Fill Posterior Restorative composite resin is applied in two horizontal, vertical and oblique increments with approximately 2 mm thickness respectively, and in Subgroup IA4, it is applied in single increment of 4 mm thickness.

Each increment was gently condensed with clean teflon coated composite condenser in order to ensure complete adaptation to the underlying resin and tooth structure. The occlusal anatomy was carved as exactly as possible avoiding overhangs. The 2-mm increment are cured for 20s while the 4-mm increment is light-cured for 40 s with a LED light curing unit (LED.D, Woodpecker) with output irradiance of approximately 800 mW/cm2 held in contact with the cavosurface of the tooth[11]. After removal of the matrix band, the restoration was light-cured from their buccal and lingual aspects for an additional 20 seconds on mesial side to ensure complete polymerization, followed by finishing and polishing using finishing discs and polishing pastes. All the restored samples were stored in distilled water for 24 hours before testing to ensure a complete polymerization process.

Thermocycling

All samples are subjected to thermocycling where they were alternately immersed in 5°C to 60°C water bathes for 1000cycles with a dwell time of 30 seconds[12]. Thermocycling was done to mimic intra-oral temperature variations. Then, the specimens were dried, and two layers of nail polish was applied except on the resin composite restoration and 1 mm area around it, and the apex was sealed with sticky wax, to avoid any dye penetration from invisible cracks, areas devoid of enamel or cementum. The teeth were then immersed in Rhodamine-B dye for 48hrs hours[1].

Marginal adaptation analysis using CLSM

The samples were then taken out and washed with distilled water and sectioned mesio-distally through the center of restorations with a slow speed of 300 rpm with a diamond disk under constant cooling[1]. Then, the specimens were examined under Confocal Laser Scanning Microscope at 10X magnification to determine the marginal adaptation of the samples.

To evaluate marginal adaptation along the tooth-restorative interface in the occlusal and gingival regions, three points were selected to facilitate the determination of the marginal gap width (the distance between the tooth axial wall and the restorative material). The full perimeter of the restoration was calculated by taking approximately six photos of each specimen[19].Image analysis software 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[13].
Total marginal adaptation=Total perimeter of the restoration – Mean marginal gap for the occlusal and cervical margin[13].
The percentage of marginal adaptation was calculated using the following equation[13]:
Marginal adaptation (%) = (sum of marginal adaptation for occlusal and cervical margins / total perimeter of the restoration) × 100

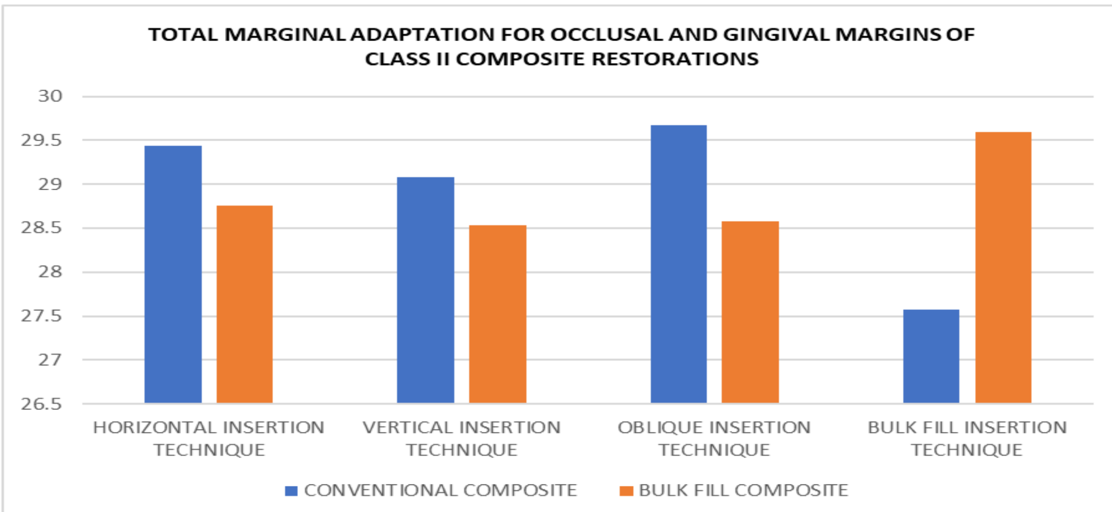
Statistical Analysis

The results of marginal adaptation were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. The mean scores of marginal adapation microleakage of the two subgroups were compared using Kruskal Wallis's ANOVA followed by Mann-Whitney U test and non-parametric Kruskal-wallis test to determine the significant difference at occlusal and gingival margins. $P < 0.05$ will be considered to be statistically significant.

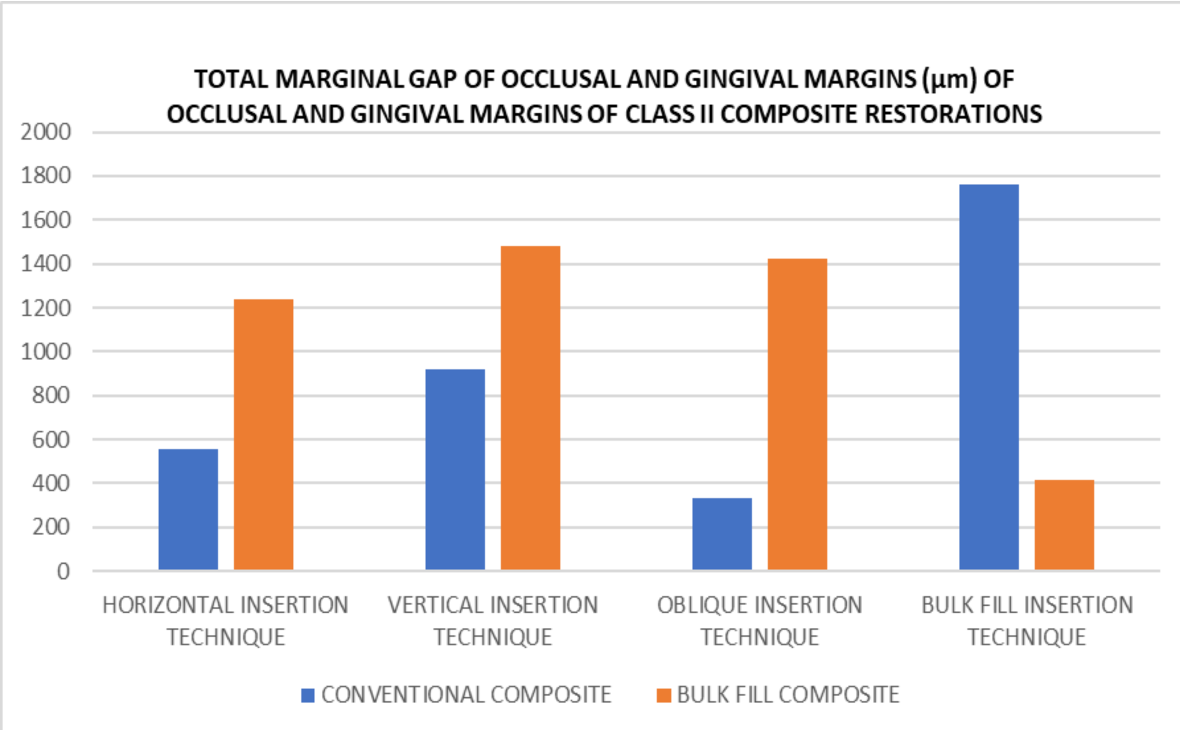
Results

None of the test groups showed 100% perfect margins regardless of the test material or location of the margin or placement technique. Statistical analysis was done among the subgroups for total marginal adaptation, mean marginal gap and percentage of marginal adaptation using one way analysis of variance (ANOVA) followed by post hoc Tukey HSD test shown as in table 2 & 3 for Group I , table 4 & 5 for Group II, Graph1,2 & 3 and table 6, Graph 4 & 5, for the marginal adaptation between occlusal margins and gingival margins.

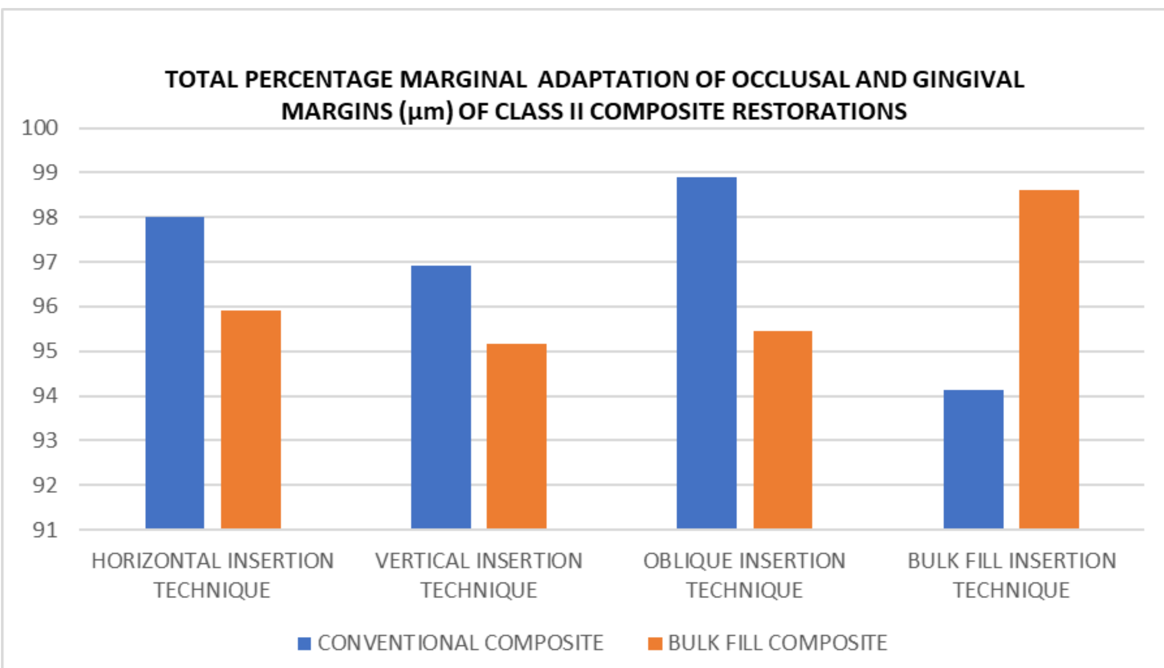
GRAPH 1



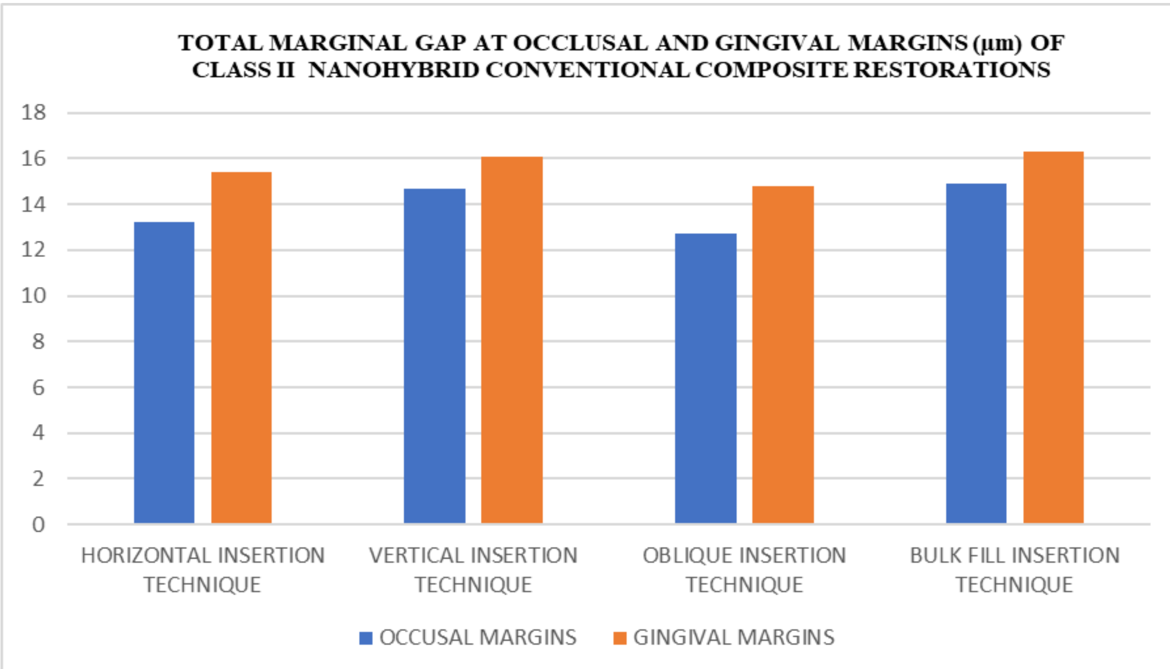
GRAPH 2:


TOTAL MARGINAL GAP OF OCCLUSAL AND GINGIVAL MARGINS (µm) OF OCCLUSAL AND GINGIVAL MARGINS OF CLASS II COMPOSITE RESTORATIONS
2000
1800
1600
1400
1200
1000
800
600
400
200
0
HORIZONTAL INSERTION TECHNIQUE
VERTICAL INSERTION TECHNIQUE
OBLIQUE INSERTION TECHNIQUE
BULK FILL INSERTION TECHNIQUE
CONVENTIONAL COMPOSITE
BULK FILL COMPOSITE

GRAPH 3


TOTAL PERCENTAGE MARGINAL ADAPTATION OF OCCLUSAL AND GINGIVAL MARGINS (µm) OF CLASS II COMPOSITE RESTORATIONS
100
99
98
97
96
95
94
93
92
91
HORIZONTAL INSERTION TECHNIQUE
VERTICAL INSERTION TECHNIQUE
OBLIQUE INSERTION TECHNIQUE
BULK FILL INSERTION TECHNIQUE
CONVENTIONAL COMPOSITE
BULK FILL COMPOSITE

GRAPH 4



GRAPH 5

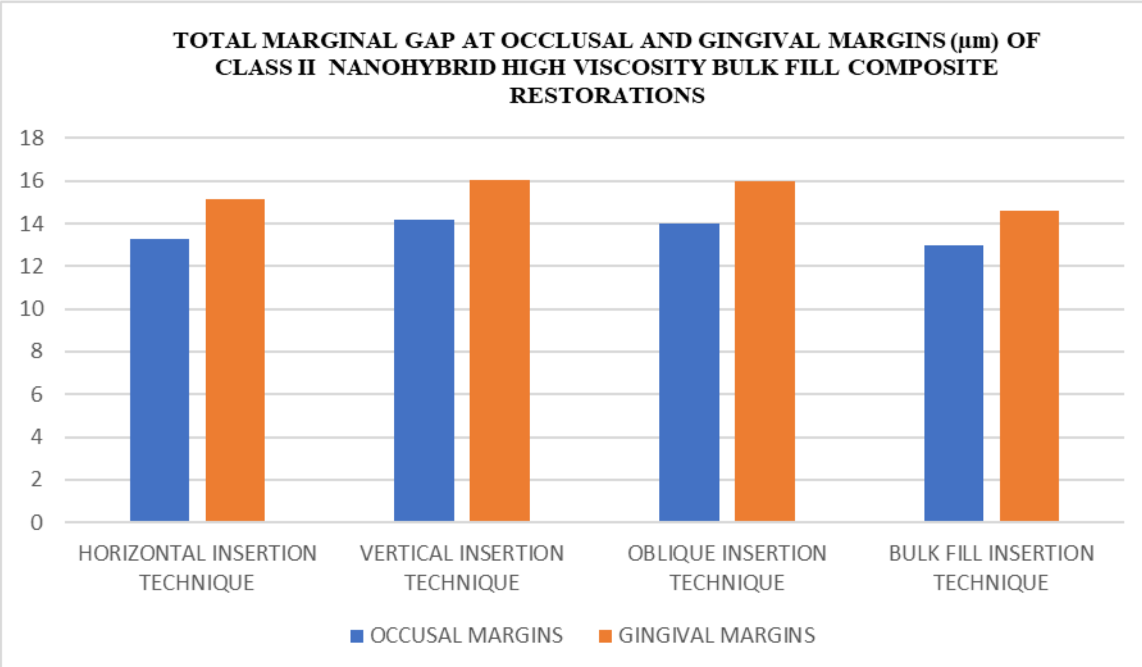


Table 2. Comparison of total marginal adaptation for occlusal and gingival margins, Marginal gap of occlusal and gingival margins (in µm) and Percentage of marginal adaptation of class II composite restorations between different layering technique of among Conventional nanohybrid resin based dental composite.

Parameter	Subgroup	N	Mean	SD	F	P Value
Marginal adaptation	Horizontal layering technique	10	29.44	0.04	7.925	**<.001***
	Vertical layering technique	10	29.08	0.06		
	Oblique layering technique	10	29.67	0.02		
	Bulk Insertion technique	10	27.57	2.60		
Marginal gap of occlusal and gingival margins (in µm)	Horizontal layering technique	10	554.49	43.89	2922.076	**<.001***
	Vertical layering technique	10	921.20	49.19		
	Oblique layering technique	10	333.13	21.23		
	Bulk Insertion technique	10	1764.14	57.74		

Percentage of marginal adaptation of class II composite restorations	Horizontal layering technique	10	98.01	0.14	892.517	**<.001***
	Vertical layering technique	10	96.92	0.18		
	Oblique layering technique	10	98.89	0.08		
	Bulk Insertion technique	10	94.12	0.48		

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation

Table 3. Multiple comparison of total marginal adaptation for occlusal and gingival margins, Marginal gap of occlusal and gingival margins (in µm) and Percentage of marginal adaptation of class II composite restorations between different layering technique among Conventional nanohybrid resin based dental composite.

Tukey HSD							
Dependent Variable	(I) Sub groups	(J) Sub groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Marginal adaptation	Horizontal layering technique	Vertical layering technique	0.36333	0.48	0.87	-0.90	1.62
		Oblique layering technique	-0.224	0.48	0.965	-1.48	1.03
		Bulk Insertion technique	1.876*	0.48	**0.001***	0.62	3.13
	Vertical layering technique	Oblique layering technique	-0.58733	0.48	0.607	-1.85	0.67
		Bulk Insertion technique	1.51267	0.48	**0.012***	0.25	2.77
	Oblique layering technique	Bulk Insertion technique	2.1*	0.48	**<.001***	0.84	3.36
Marginal gap of occlusal and gingival margins	Horizontal layering technique	Vertical layering technique	-366.71467*	16.46	**<.001***	-410.30	-323.13

(in µm)		Oblique layering technique	221.35533*	16.46	**<.001***	177.77	264.94
		Bulk Insertion technique	-1209.65000*	16.46	**<.001***	-1253.24	-1166.06
	Vertical layering technique	Oblique layering technique	588.07000*	16.46	**<.001***	544.48	631.66
		Bulk Insertion technique	-842.93533*	16.46	**<.001***	-886.52	-799.35
	Oblique layering technique	Bulk Insertion technique	-1431.00533*	16.46	**<.001***	-1474.59	-1387.42
Percentage of marginal adaptation of class II composite restorations	Horizontal layering technique	Vertical layering technique	1.08733*	0.10	**<.001***	0.83	1.35
		Oblique layering technique	-.87800*	0.10	**<.001***	-1.14	-0.62
		Bulk Insertion technique	3.88467*	0.10	**<.001***	3.63	4.14
	Vertical layering technique	Oblique layering technique	-1.96533*	0.10	**<.001***	-2.22	-1.71
		Bulk Insertion technique	2.79733*	0.10	**<.001***	2.54	3.06
	Oblique layering technique	Bulk Insertion technique	4.76267*	0.10	**<.001***	4.50	5.02

* The mean difference is significant at the 0.05 level.

Table 4. Comparison of total marginal adaptation for occlusal and gingival margins, Marginal gap of occlusal and gingival margins (in µm) and Percentage of marginal adaptation of class II composite restorations between different layering technique of Etch and Rinse adhesive strategy among Nanohybrid high viscosity Bulk Fill composite resin.

Parameter	Subgroup	N	Mean	SD	F	P Value
Marginal adaptation	Horizontal layering technique	10	28.76	0.05	133.467	0.077
	Vertical layering technique	10	28.53	0.10		

	Oblique layering technique	10	28.58	0.30		
	Bulk Insertion technique	10	29.59	0.05		
Marginal gap of occlusal and gingival margins (in µm)	Horizontal layering technique	10	1238.16	53.48	142.99	0.059
	Vertical layering technique	10	1481.23	52.79		
	Oblique layering technique	10	1420.34	304.88		
	Bulk Insertion technique	10	415.54	58.60		
Percentage of marginal adaptation of class II composite restorations	Horizontal layering technique	10	95.92	0.24	435.776	0.082
	Vertical layering technique	10	95.17	0.44		
	Oblique layering technique	10	95.45	0.25		
	Bulk Insertion technique	10	98.61	0.18		

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation

Table 5. Multiple comparison of total marginal adaptation for occlusal and gingival margins, Marginal gap of occlusal and gingival margins (in µm) and Percentage of marginal adaptation of class II composite restorations between different layering technique of Etch and Rinse adhesive strategy among Nanohybrid high viscosity Bulk Fill composite resin.

Tukey HSD							
Dependent Variable	(I) Sub groups	(J) Sub groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Marginal adaptation	Horizontal layering technique	Vertical layering technique	.22533	0.06	0.146	0.07	0.38
		Oblique layering technique	.18200	0.06	0.124	0.02	0.34
		Bulk Insertion technique	.17733	0.06	0.056	0.02	0.21
	Vertical layering	Oblique layering	.04333	0.0	0.88	0.20	0.12

	technique	technique		6	9		
		Bulk Insertion technique	1.05267	0.06	0.531	0.21	0.89
	Oblique layering technique	Bulk Insertion technique	1.00933	0.06	0.649	0.17	0.85
Marginal gap of occlusal and gingival margins (in µm)	Horizontal layering technique	Vertical layering technique	243.07467	58.32	0.562	0.50	0.65
		Oblique layering technique	182.17800	58.32	0.668	0.60	0.73
		Bulk Insertion technique	822.61600	58.32	0.847	0.69	0.94
	Vertical layering technique	Oblique layering technique	60.89667	58.32	0.724	0.53	0.82
		Bulk Insertion technique	1065.69067	58.32	0.911	0.64	0.98
	Oblique layering technique	Bulk Insertion technique	1004.79400	58.32	0.850	0.37	0.92
Percentage of marginal adaptation of class II composite restorations	Horizontal layering technique	Vertical layering technique	.75000	0.11	0.297	0.17	0.36
		Oblique layering technique	.47733	0.11	0.372	0.19	0.76
		Bulk Insertion technique	2.69067	0.11	0.116	0.09	0.41
	Vertical layering technique	Oblique layering technique	0.27267	0.11	0.064	0.05	0.23
		Bulk Insertion technique	3.44067	0.11	0.345	0.29	0.56
	Oblique layering technique	Bulk Insertion technique	3.16800	0.11	0.775	0.45	0.87

* The mean difference is significant at the 0.05 level.

Table 6. Comparison of marginal gaps at occlusal and gingival margins using

Kruskal-wallis test

Group	Subgroup	Site	N	Mean	P Value
Nanohybrid Universal composite resin	Horizontal layering technique	Occlusal	10	13.23	0.109
		Gingival	10	15.42	
	Vertical layering technique	Occlusal	10	14.7	0.59
		Gingival	10	16.1	
	Oblique layering technique	Occlusal	10	12.71	0.094
		Gingival	10	14.77	
	Bulk layering technique	Occlusal	10	14.9	0.691
		Gingival	10	16.3	
Nanohybrid high viscosity Bulk Fill composite resin	Horizontal layering technique	Occlusal	10	13.26	0.471
		Gingival	10	15.12	
	Vertical layering technique	Occlusal	10	14.19	0.264
		Gingival	10	16.02	
	Oblique layering technique	Occlusal	10	13.98	0.59
		Gingival	10	15.97	
	Bulk layering technique	Occlusal	10	12.96	0.264
		Gingival	10	14.62	

*****Statistical significance set at 0.05

In Group I, oone way analysis of variance (ANOVA) followed by *post hoc* Tukey HSD test displayed a statistically significant higher total marginal adaptation for occlusal and gingival margins with oblique layering technique with highest mean score (29.67+/-0.02; P<0.001) among all the techniques used in Group I followed by Horizontal (29.44+/-0.04; P<0.001) and Vertical (29.08+/-0.06; P<0.001) with least marginal adaptation with Bulk fill technique (27.57+/-2.60; P<0.001). Similarly, in Group II, one way analysis of variance (ANOVA) followed by *post hoc* Tukey HSD test displayed a higher total marginal adaptation for occlusal and gingival margins were observed with Bulk insertion technique with mean score (29.59+/-0.05; P=0.077) followed by Horizontal (28.76+/-0.05; P=0.077), Oblique (28.58+/-0.30; P=0.077) and vertical (28.53+/-0.10; P=0.077) insertion techniques though the scores are not statistically significant. Kruskal-wallis test displayed a no significant difference in marginal adaptation for occlusal margins when compared to gingival margins though the marginal gaps detected were higher at gingival margins in both the groups.

Discussion

Rapid development and improvement of restorative materials led to a paradigm shift in the way the teeth are restored today. The inclination towards esthetic procedures of teeth, conservation of tooth structure along with the advancements in the field of adhesive dentistry led to the use of direct composite restorative materials predominantly. Despite of the improved properties of composite resins, the marginal adaptation of the restoration remains a challenge even today. One of the primary factors for this drawback is polymerization shrinkage when the forces of polymerization stress exceed interfacial bond strength, gaps are created between composite restoration and cavity walls. Long term thermal and mechanical stresses on the restorations too can be other factors causing gap formation by altering thermal and physical properties of the composite material and cause dimensional changes leading to gaps at tooth-restoration interface[14]. So, a good adaptation between the restoration and cavity walls remains a goal of clinicians for the long term success of the restoration.

Therefore, the present in vitro study evaluated the marginal adaptation of two different composite materials applied by various incremental and bulk fill methods in a class II cavities using Confocal Laser Scanning Microscope at 10 x magnification. 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 strain due to specimen sectioning and avoid polishing artifacts[10,11,15]. The six images taken for analysis were none overlapping to avoid replication of the same gaps score of a previous image.

The hypothesis stating that there are significant difference in marginal adaptation among different insertion techniques using conventional nanohybrid composites has been supported by the results of this study. The results have shown significantly better marginal adaptation with conventional composites placed incrementally compared to bulk fill insertion technique. Conventional nanohybrid composite tested in bulk placement had significantly larger gap interfaces and less adaptation to the cavity walls. This outcome might be related to the reduced material volume and c-factor of each increment which reduced the generated contraction stresses and is in agreement with previously reported studies[16]. Moreover, the light may adequately reach he deepest composite layers and lead to more significant polymerization, thus minimizing possible marginal degradation that might occur if polymerization was incomplete.

However, the findings in the present study contrasted to few studies done by other researchers[17,18] comprising the different placement techniques (incremental and bulk fill) with different resin based composite systems(conventional versus bulk fill composites). The results could be varying due to different experimental conditions like different materials, cavity preparation and adhesive techniques used.

In this study, among the incremental techniques used, oblique technique showed better marginal adaptation followed by horizontal technique and vertical technique with no statistically significant difference between these two techniques. This could be attributed to lower configuration factor of the oblique technique. The oblique technique proposed by Lutz et al[19] in 1986 relies on placing small amounts of composite which increases the adhesive-free surfaces, allows a better flow of the resin and reduces the shrinkage at a low volume. This procedure minimizes the configuration factor of the preparation, assisting in the adaptation of the composite to the bonded surfaces. The oblique layering placement technique is accomplished by placing a series of wedge shaped composite increments. This technique reduces the c-factor and residual stresses at the tooth-restoration interface and then increases the marginal adaptation by reducing the bulk of material cured at one time. Similar results were found in studies by Tjan et al[20] supporting the findings in this study. The oblique layering technique reduces the c-factor and limits the development of contraction forces between opposing walls and hence decreases the polymerization shrinkage stresses[21]. The horizontal placement technique has been reported to increase the shrinkage stresses between the opposing cavity walls. Vertical layering technique reduces the gap formed at the gingival margin, hence reduces postoperative sensitivity and secondary caries[22].

Some studies found no influence of placement techniques of the composite resin on marginal adaptation[23] whereas other investigators found that oblique layering technique had he most gap-free margins when the proximal box ended on enamel[24]. Other investigators reported better results with the vertical layering technique compared to oblique layering technique[25].

The bulk placement technique shows more marginal gaps and these results could be attributed to the fact that when composite was placed inside the cavity in a single increment, the material contacted four walls at a time leaving only two free unbounded surfaces. In such a case, the c-factor is high and therefore possibility of gap formation and adhesive bond failure[26]. Another possible explanation could be related to ineffective or inadequate curing at the deeper layer of the composite restoration[26]. Our findings are in line with the previous studies that demonstrated that the placement of a large increment of bulk fill resin composite into a cavity increased the potential of creating high shrinkage stress and induced more strain[26].

Statistical analysis revealed that there were no statistically significant difference in marginal adaptation among the different placement techniques, although incremental placement techniques showed less gaps than bulk placement technique when bulk fill nanohybrid composite restorative material was used. These results coincided with previous studies and found no significant difference between bulk and incremental techniques when evaluating gaps of class II composite restorations[27]. The horizontal placement technique of the bulk fill composite showed better adaptation among all test groups. Our findings were in agreement with Frakenberg et al 2007, who found that the horizontal layering technique had the best marginal and bond qualities compared to the vertical and oblique layering techniques[28]. Studies reported that the shrinkage of a single horizontal thin layer of composite generates remarkably less tensile force than the contraction of a bulk of composite that fills the whole cavity[29].

Perhaps, the most important contribution of horizontal incremental technique would be an adequate polymerization for bulk fill composites and an adequate degree of conversion of the material in this thickness, as it was postulated by Campus et al 2014 in their study[20].

In this regard, the result could be related to the benefits claimed by the manufacturer that higher translucency and light transmission properties of bulk fill resin were enhanced, and modifies by adding prepolymer shrinkage stress relievers, polymerization modulators chemically embedded in the centre of polymerizable resin backbone, high-molecular weight base monomer to optimize flexibility and network structure and highly light-reactive photoinitiator system enable rapid polymerization and greater curing depth[20].

On the contrary to our result, several authors reported that using this technique for composite application leads to an increase in the c-factor, and there upon increases the shrinkage stresses between the opposing cavity walls which lead to gaps and microleakage[30, 31].

When marginal adaptation values were evaluated in terms of different cavity margins, the results showed that marginal gaps are more at the gingival margins compared to occlusal margins but this increase was not statistically significant. In this study, gingival margins were placed approximately 1mm coronal to the cementoenamel junction; in other words, at enamel. The more marginal gaps, which were not statistically significant at the gingival margins, could be attributed to the difficulty in bonding and placement of restoration and compromised visibility in the cervical region relative to the occlusal margins. These findings are in agreement with studies done by other researchers[32, 33].

The marginal adaptation of resin composite restoration might be affected by various factors including the cavity size, the angle at which enamel prisms and dentinal tubules are cut based on their location, the procedure in which dental hard tissues are conditioned, the layering protocol and the polymerization technique used[34]. Therefore, in the present study it appears that differences in the placement techniques were responsible for differences in gap formations[35].

Conclusion

Within the limitations of this in vitro study and based on the results, it can be concluded that: Marginal gaps could not be eliminated by any of the tested placement techniques using conventional and bulk fill composites despite the significant advances in composite materials and adhesive systems. Incremental placement techniques showed lesser marginal gaps compared to bulk placement technique in both the groups which oblique technique performing better than other techniques when used with conventional composites and horizontal technique performing better than other techniques when used with bulk fill composite materials. The marginal adaptation in the occlusal surface was higher than that in the gingival surface in all the groups, further in vivo studies are recommended to add more insight into this research and to evaluate other clinical parameters like post-operative sensitivity and discoloration which cannot be judged in vitro.

Bibliography

1. Radhika M, Sajjan GS, Kumaraswamy BN, Mittal N. Effect of different placement techniques on marginal microleakage of deep class II cavities restored with two composite resin formulations. J Conserv Dent 2010; 13(1):9-15.
2. Davidson, C. L. and A. J. Feilzer. Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. J Dent. 1997; 25(6): 435-440.
3. Lutz F, Krejci I, Barbakow F. Quality and durability of marginal adaptation in bonded composite restorations. Dent Mater 1991; 7(2):107-13.
4. Giachetti L, Scaminaci R, D., Bambi C, Grandini R. A review of polymerization shrinkage stress: Current techniques for posterior direct resin restorations. Journal Contemporary Dental Practice 2006; 7(4):79-88.
5. Jaganath BM, Krishnegowda SC, Rudranaik S, Madanan S, Kurup NB, Manjula CG. Influence of Composite Insertion Techniques (Bulk-fill and Incremental Nano- filled Composites) on Adaptability to the Pulpal Floor and Interfacial Gap Formation. Int J Prev Clin Dent Res 2017; 4(3):225-231.
6. Katarina Kelić, Sanja Matić, Danijela Marović, Eva Klarić and Zrinka Tarle. Microhardness of bulk-fill composites. Acta Clin Croat 2016; 55(4): 607-614
7. Kapoor N, Bahuguna N, Anand S. Influence of composite insertion technique on gap formation. J Conserv Dent 2016; 19(1):77-81.
8. Leprince JG, Palin WM, Vanacker J, et al. Physico-mechanical characteristics ofcommercially available bulk-fill composites. Journal of Dentistry 2014; 42(8):993–1000.
9. Hunt PR. A modified class II cavity preparation for glass ionomer restorative materials. Quintessence Int Dent Dig. 1984; 15: 1011-8.

10. Rashid, H. Application of confocal laser scanning microscopy in dentistry. J. Adv. Microsc. Res. 2014; 9:245–252.
11. Teng X., Li F., Lu C. Visualization of materials using the confocal laser scanning microscopy technique. Chem. Soc. Rev. 2020; 49:2408–2425.
12. Wahab FK, Shaini FJ, Morgano SM. The effect of thermocycling on microleakage of several commercially available composite Class V restorations in vitro. J Prosthet Dent. 2003; 90:168–74.
13. Aschheim, Kenneth W. Esthetic dentistry: A clinical approach to techniques and materials. 3rd ed., St. Louis, Elsevier Mosby, 2015.
14. Malarvizhi D, Karthick A, Gold P, Mary, N., Venkatesh A. Shrinkage in composites: An enigma. Journal of International Oral Health 2019; 11(5):244-8.
15. Aulfat Ahmed Albahari1, Mohammed Ahmed Dubais, Ahmed Abdullah Madcap, Waled Abdul Malek Alanesi. 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. Pertanika J. Sci. & Technol.2020; 28 (2): 703 – 716.
16. Alqudaihi FS, Cook NB, Diefenderfer KE, Bottino MC, Platt JA. Comparison of Internal Adaptation of Bulk-fill and Increment-fill Resin Composite Materials. Oper Dent. 2019; 44(1): E32-E44.
17. Campos, E. A., Ardu, S., Lefever, D., Jassé, F. F., Bortolotto, T., & Krejci, I. Marginal adaptation of class II cavities restored with bulk-fill composites. Journal of Dentistry 2014; 42(5), 575-581.
18. Furness, A., Tadros, M. Y., Looney, S. W., & Rueggeberg, F. A. Effect of bulk/incremental fill on internal gap formation of bulk-fill composites. Journal of Dentistry 2014; 42(4), 439-449.
19. Lutz E, Krejci I, Oldenburg TR. Elimination of polymerization stresses at the margins of posterior composite resin restorations: a new restorative technique. Quintessence Int. 1986; 17(12):777-84.
20. Tjan AH, Bergh BH, Lidner C. Effect of various incremental techniques on the marginal adaptation of class II composite resin restorations. J Prosthet Dent 1992; 67(1):62-6.
21. Sarfi S, Bali D, Grewal MS. Effect of different layering techniques on microleakage of nanofilled composite in class i restorations: An In Vitro study. Journal of international Clinical Dental Research Organization 2017; 9(1):8-11.
22. Roulet JF. The Problems Associated with Substituting Composite Resins for Amalgam: A Status Report on Posterior Composites. Journal of Dentistry 1988; 16(3):101-03.
23. Usha H, Kumari A, Mehta D, Kaiwar A, Jain N. Comparing microleakage and layering methods of silorane-based resin composite in class V cavities using confocal microscopy: An in vitro study. Journal Conservative Dentistry 2011; 14(2):164-8.
24. Eakle WS, Ito RK. Effect of insertion technique on microleakage in mesio-occluso-distal composite resin restorations. Quintessence International 1990; 21(5):369-74.

25. Bagis YH, Baltacioglu IH, Kahyaogullari S. Comparing microleakage and the layering methods of silorane-based resin composite in wide class II MOD cavities. Operative Dentistry 2009; 34(5):578-85.
26. Armaghan Naghili, Navid Yousefi and Elham Zajkani et al. Influence of Cavity Dimensions on Microleakage of Two Bulk-Fill Composite Resins. Pesqui. bras. Odontopediatria clín. Integr. 2019. Vol. 19.
27. Kader MA, Altheeb A, Al-Asmry AA, Luqman M. Microleakage evaluation of class II composite restoration with incremental and bulk fill technique Journal Dental Restoration and Review 2015;2(4):153-5.
28. Frankenberger R, Kramer N, Lohbauer U, Nikolaenko SA, Reich SM. Marginal integrity: Is the clinical performance of bonded restorations predictable in vitro. Journal of Adhesive Dentistry (Supplement 1) 2007:107-16.
29. Yumei N, Xue M, Mingwen F, Sijie Z. Effect of layering techniques on the micro-tensile bond strength to dentin in resin composite restorations. Dental Materials 2009; 25(1):129-34.
30. Han S-H, Sadr A, Junji T, Park S-H. Internal adaptation of resin composites at two configurations: Influence of polymerization shrinkage and stress. Dental Material 2016; 32(9):1085-94.
31. de O, Correia. Ayla. Macyelle., Maciel PVE, Bresciani E, et al. Influence of cavosurface angle on the stress concentration and gaps formation in class V resin composite restorations. Journal of the Mechanical Behavior of Biomedical Materials 2019; 97:272–77.
32. Manal I. Faraj, 1 Naeima M. Betamar, Nagat H. Bubteina. Influence of Different Placement Techniques of Bulk-fill Resin Composite on Microleakage of Class II Cavity Preparation. Libyan Journal of Dentistry. 2021;5(2):1-7
33. Coutinho, M., Trevizam, N. C., Takayassu, R. N., Leme, A. A., & Soares, G. P.Distance and protective barrier effects on the composite resin degree of conversion. Contemporary Clinical Dentistry 2013; 4(2): 152-155.
34. Oskoee SS, Bahari M, Navimipour EJ, et al. Factors affecting marginal integrity of class II bulk-fill composite resin restorations. Journal Dental Research 2017; 11(2):101-09.
35. Poggio C, Chiesa M, Scribante A, Mekler J, Colombo M. Microleakage in Class II composite restorations with margins below the CEJ: in vitro evaluation of different restorative techniques. Medicine Oral Pathology oral y cirugía bucal. Ed. Inglesa 2013; 18(5):e793-e98.