International Journal Dental and Medical Sciences Research
Volume 3, Issue 2, Mar-Apr. 2021 pp 1140-1148 www.ijdmsrjournal.com ISSN: 2582-6018

Comparative Evaluation Of Microleakage In Class II Restoration Using Different Bulk-Fill Resin-Based Composites Using Confocal Fluorescence Microscopy And Fesem:An In-Vitro Study

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Submitted: 01-05-2021 Revised: 10-05-2021 Accepted: 12-05-2021

ABSTRACT: The aim of this study was to compare and evaluate micro leakage in class II preparations restored with bulk-fill resin-based composites using confocal fluorescence microscopy and FESEM.Class II cavity was prepared in 40 caries free premolar teeth on the mesial and occlusal surface in each of the premolars, with the gingival margin of the cavity being 1 mm below cementoenamel junction. The cavities were restored with SDR Surefil, Tetric N Ceram bulk fill composite, Filtek bulk fill posterior and Solare sculpt. The teeth were immersed in rhodamine B dye for 48 hours. Sectioned samples were examined under an open frame laser scanning confocal microscope and Dentin surface evaluation was done using Field Emission Scanning electron microscopy (FESEM). SDR plus, showed highest micro leakage among the bulk fill composites followed by Filtekbulkfillflowable which showed comparable microleakage with control group and Tetric N-Ceram Bulk Fill showed least micro leakage values compared to all the other groups including the control group.Based on the results, Tetric N Ceram showed the least micro leakage compared to other conventional Bulkfill composites.

KEYWORDS: Bulk fill composite, microleakage, scanning electron microscopy, field emission scanning electron microscopy, flowable composite

I. INTRODUCTION

The longevity of dental restorations is dependent on the interface between the restorative material and tooth structures.[1]The oral cavity, with its associated temperature changes, chewing loads and chemical attacks by acids and enzymes, creates a rather severe challenge for tooth composite bonds resulting in degradation of bonding at the tooth-restoration interface and the formation of gaps can result in the passage of bacteria, fluids, or ions between the cavity wall and the resin composite, a process known as microleakage.[2]Marginal microleakage is commonly observed with various restorative materials especially resin based restorations. Marginal microleakage can lead to discolouration as well as secondary caries and if not treated in time, it can cause pulpal pathology. Controlling microleakage has always been an important goal of operative dentistry.

Marginal microleakage in composites occurs because of polymerization shrinkage. During the setting process, polymerization shrinkage of a resin composite can create forces that may disrupt the bond to cavity walls.[3]This competition between contracting forces built up in the polymerizing resin and the bonds of adhesive resins to the wall of the restoration is one of the main causes of marginal failure and subsequent microleakage.[3][4]

Alternatives are being studied to try to minimize or eliminate the indices of microleakage in restorations of composite resins. A modern example has seen the increasing popularity amongst dental practitioners of so-called ‘‘bulk-fill’’ materials, which are claimed to enable the restoration build-up in thick layers, up to 4 mm. There currently exists a growing trend in the use of bulk-fill materials amongst practitioners due to a more simplified procedure.[5] Bulk-fill resin-

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Volume 3, Issue 2, Mar-Apr. 2021 pp 1140-1148 www.ijdmsrjournal.com ISSN: 2582-6018

bonded composites were introduced in an attempt to reduce the interfacial gap formation of incremental technique thereby improving physical and mechanical properties of composite restorations particularly in the posterior areas.[6]These bulk-fill resin bonded composites enable to cure to a depth of 4mm to 5mm and thereby reducing the chair side time of layering process as seen with conventional composites. Manufacturers mentioned that the main advancement of bulk-fill composite materials are related to modifications in the filler content and/or organic matrix with the help of advanced technology.[7]The aim of the present study was to compare microleakage bulk-fill resin composite in class II cavities using laser scanning confocal microscope.

II. MATERIALS AND METHODS

60 recently extracted maxillary premolar teeth were collected for the study. After excluding teeth with caries, anatomical deformities, crack, decay, fracture, abrasion, previous restorations, or structural deformities, 40 intact teeth were selected for the study. Immediately after extraction, the teeth were cleaned by scraping to remove debris and stored in saline before use. The teeth were mounted in plaster models to carry out the restorative procedure.

Class II cavity preparations were performed involving the mesial and occlusal surfaces using a carbide bur (number 245) under water spray. For every five preparations a new bur was used. The buccolingual extension of the cavities was 3 mm; axial depth was 6mm and the gingival seat was located in dentin/cementum (1 mm below the cementoenamel junction/CEJ).The dimensions of the prepared cavities were checked with a verniercaliper. A ±0.3 mm tolerance in the measurements was considered acceptable for including the specimen in the trial. No bevels were added to any margin of the preparations.The teeth were divided randomly into four groups Group I – control; Groups II, III and IV as depicted in Table 1.

Scotch bond universal (3M ESPE, St Paul, MN, EUA) system adhesive self-etch primer was applied to the enamel and dentin surfaces. After waiting for 20 seconds, the excess solvent was evaporated with a gentle blast of air for 10 seconds, and the primer was light cured for 10 seconds using an LED photopolymerizer (IvoclarVivadentBluephase N M) at a 1 mm distance, according to manufacturer's instructions.

Following application of the adhesive system, all cavities were filled with the bulk fill resins of each group in single increments of 4mm: G2, Filtek Bulk Fill; G3, SDR plus ; G4, Tetric N-Ceram Bulk Fill, and the incremental technique of 2 mm, the control group (G1) using Solare sculpt (GC), also photoactivated using the LED device (IvoclarVivadentBluephase N M) for 20 or 40 seconds, according to manufacturer's instructions as shown in Table 2. Finally, the finishing and polishing of the restorations, using Sof-Lex (3M/ESPE) aluminum oxide sanding disks in fine and ultra-fine granulations, was performed. The apices were thensealed with acrylic resin and the teeth were stored for 24 hours in distilled water at 37°C. All procedures were performed by the same operator.

Table 1: The experimental study groups

MATERIALS	GROUPS
Solare sculpt (GC)	Group I (control)
Filtek Bulk Fill Posterior Flowable (3M/ESPE Seefeld Germany)	Group II
SDR plus (Dentsly, Knstanz, Germany)	Group III
Tetric N-Ceram Bulk Fill (Ivoclarevivadent, Schaan, Liechtenstein)	Group IV

Table 2: Chemical Compositions and Application Instructions for the Materials Tested

Materials	Composition	Method of application
Filtek Bulk Fill flow 3M/ESPE	Bis-GMA, Bis-EMA, UDMA and polyacrylic resin; Ytterbium trifluoride (0.1-5.0μm), zirconia/silica (0.01-3.5μm); 64% by weight and 42.5% by	Insertion in single 4mm increments, photoactivation for 20s.

	volume.	
SDR plusDentsply	Glass of barium boron fluoride aluminum silicate, glass of strontium aluminum fluoride silicate; Modified urethane dimethacrylateresin; ethoxylatedbisphenol A dimethacrylate (EBPADMA); triethylene glycol dimethacrylate (TEGDMA); camphorquinone (CQ) as photoinitiator; butyl hydroxy toluene; UV stabilizers; titanium dioxide; iron oxide pigments.	Insertion in single 4mm increments, photoactivation for 20s
Tetric N-Ceram Bulk Fill	Monomer matrix is composed of dimethacrylates(19–21% weight). The total content of inorganic fillers is 75–77% weight or 53-55% volume. The fillers consist of barium glass, prepolymer, ytterbium trifluoride and mixed oxide. Additives, catalysts, stabilizers and pigments are additional contents (< 1.0% weight). The particle size of the inorganic fillers is between 0.04 and 3 µm. The mean particle size is 0.6 µm.	Tetric N-Ceram Bulk Fill should be applied in increments of maximum of 4 mm with curing time of ≥ 500 mW/cm2 20 s and ≥ 1'000 mW/cm2 10 s
Solare sculpt	Bis-GMA, Bis-MEPP, UDMA, TEGDMA, Silica fine particle, barium glass, pre-polymerized filler, pigments and Photo initiator.	Insertion in 2mm increments for shade A1, A2, A3 and 1.5 mm increments for shade A3.5 Followed by curing time of 20s halogen/LED at $700 mW/cm^2$

Bis-GMA, bisphenol-A-glycidyl-dimethacrylate; EBPADMA, ethoxylatedbisphenol-A-dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.Bis-MEPP,2,20-Bis-4-methacryloylethoxyphenyl propane
Source: Data were extracted from the package inserts of each material, following the technical specifications provided by each manufacturer.

Confocal microscopic analysis

The unprepared coronal and root surfaces were painted with one coat of nail polish placed as close as possible to the restoration margins to prevent dye penetration anywhere other than the interfaces. Then the tooth was immersed in 0.5 % rhodamine B fluorescent dye for 48 hours. A longitudinal mesiodistal section of the tooth was accomplished by a microtome (Buehler, Isomet 4000) using a high speed diamond after being washed thoroughly with water. The sections were mounted and examined immediately (i.e., before any drying could take place) with open frame laser scanning confocal microscope.[8]

We first obtained the reflected light image followed by the fluorescence image of the tooth section. The specimen is scanned over 120 x 120 pixels covering an area equal to 156 µm x 156 µm with an acquisition time of about 18 seconds per image. Approximately 10 images were captured corresponding to different parts of specimen along a particular direction by moving the samples laterally in known amounts with a micrometre stage (with precision of 10 micrometre)MATLAB Programme was used to draw a line plot across the interface (between dentin and restoration) at 10 locations and the corresponding full width at half maximum (FWHM) was calculated. The average value of FWHM will be the measured width of the interface.

FESEM analysis

For evaluation of surface interface between composite and the dentin, five specimens from each group were randomly selected. All samples were attached to aluminium stubs and then sputtered with a 2000Å thick layer of gold under the sputtering unit (JEOL JFC- 1600, Auto fine coater). Dentin surface evaluation was done using FESEM (GeminiSEM 300) with a magnification of ×10–×2000.

III. RESULTS

Confocal microscopic

Fig 1.depicts the mean width of the tooth-restoration interface in the experimental groups, as measured by confocal microscopy. The pairwise comparison of the mean values of all groups as measured from confocal microscopic images (Table 3). LSD post hoc test at 0.05 significance level shows that the difference between Tetric N ceram and SDR is statistically significant (p = 0.014). However, there was no statistical difference when the other groups were compared.

FESEM

The mean width of the interface measured by FESEM of the four experimental groups is shown in Fig 2. The variances in the four mean values were compared using analysis of variance test at 0.05 significance level(Table 4). The pairwise comparisons shows highly significant difference in mean width between Solare Sculpt and Filtek bulk fill (p < 0.001), between Solare Sculpt and Tetric N-Ceram (p < 0.001) and significant difference between Solare Sculpt and SDR plus(p = 0.001). Difference in mean width between Filtekbulkfill and Tetric N-Ceram is found to be highly significant (p< 0.001) and between Filtekbulkfill and SDR plus is found to be significant (p = 0.019). Difference in mean width between Tetric N-Ceram and SDR plus is found to be highly significant (p< 0.001).

Fig. 1 illustrates the false colorFig.s combining the reflected Fig. (red channel) and the corresponding fluorescent Fig.s(green channel) of the tooth- restoration interface as captured using confocal microscope. The least microleakage was observed in Tetric N-Ceram (Fig. 1D) followed by FiltekBulkfill (Fig. 1C) and Solare sculpt (Fig. 1B). The highest microleakage was seen in SDR plus (Fig. 1A).

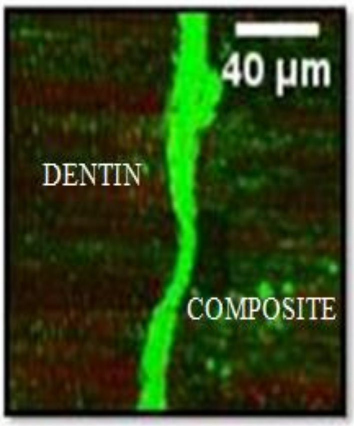

Fig.. A

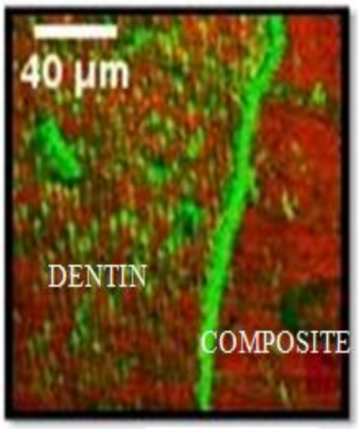

Fig. B

International Journal Dental and Medical Sciences Research
Volume 3, Issue 2, Mar-Apr. 2021 pp 1140-1148 www.ijdmsrjournal.com ISSN: 2582-6018

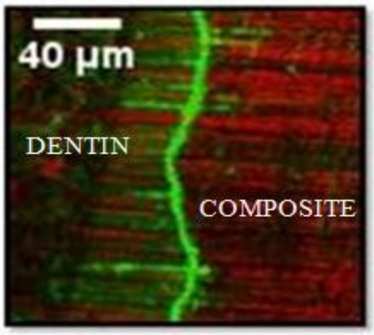

Fig. C

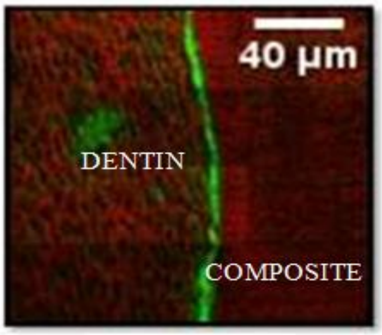

Fig. D

Table 3: The pairwise comparison of the mean values of all groups as measured by confocal microscopy

	EXPERIMENTAL GROUPS	Mean	Std. Dev	Min	Max	LSD test		
						Difference in means	p value	Inference
1	Solare sculpt	17.19	6.7	8.1	24.6	1.86	0.551	Not significant
	Filtekbulkfill flowable	15.33	4.8	7.5	23.4			
2	Solare sculpt	17.19	6.7	8.1	24.6	4.54	0.150	Not significant
	Tetric N-Ceram	12.65	4.1	8.4	20.7			
3	Solare sculpt	17.19	6.7	8.1	24.6	3.45	0.272	Not significant
	SDR plus	20.64	10.3	8.1	36.0			
4	Filtek bulkfill flowable	15.33	4.8	7.5	23.4	2.68	0.392	Not significant
	Tetric n ceram	12.65	4.1	8.4	20.7			
5	Filtekbulkfill flowable	15.33	4.8	7.5	23.4	5.31	0.094	Not significant
	SDR plus	20.64	10.3	8.1	36.0			
6	Tetric N-Ceram	12.65	4.1	8.4	20.7	7.99	0.014	Significant

Table 4: The pairwise comparison of the mean values of all groups as measured by FESEM

	EXPERIMENTAL GROUPS	Mean	Std. Dev	Min	Max	Difference in means	p value	Inference
1	Blue - SDR surefil	8.31	0.69	7.65	9.47	2.71	P< 0.001	Highly significant
	Red - filtekbulkfillflowable	5.59	0.92	4.19	6.79			
2	Blue - SDR surefil	8.31	0.69	7.65	9.47	7.19	P< 0.001	Highly significant
	Green - tetric n ceram	1.11	0.10	0.97	1.27			

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3	Blue - SDR surefil	8.31	0.69	7.65	9.47	1.63	0.001	Significant
	Orange - Solare sculpt	6.67	0.59	6.13	7.49			
4	Red - filtekbulkfillflowable	5.59	0.92	4.19	6.79	4.48	P< 0.001	Highly significant
	Green - tetric n ceram	1.11	0.10	0.97	1.27			
5	Red - filtekbulkfillflowable	5.59	0.92	4.19	6.79	1.07	0.019	Significant
	Orange - Solare sculpt	6.67	0.59	6.13	7.49			
6	Green - tetric n ceram	1.11	0.10	0.97	1.27	5.56	P < 0.001	Highly Significant
	Orange - Solare sculpt	6.67	0.59	6.13	7.49			

Fig. 2 illustratesquantitative analysis of tooth restoration interface under field emission scanning electron microscopy. The least microleakage was observed in Tetric N-Ceram (Fig. 2D) followed by FiltekBulkfill (Fig. 2B) and Solare sculpt (Fig. 2C). The highest microleakage was seen in SDR plus (Fig. 2A).

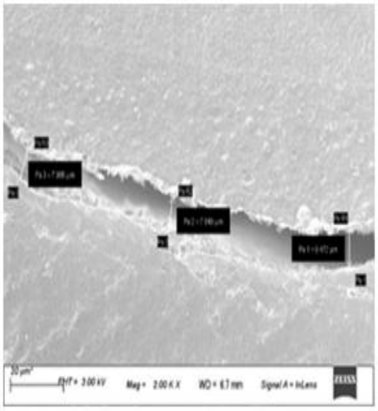

Fig. A

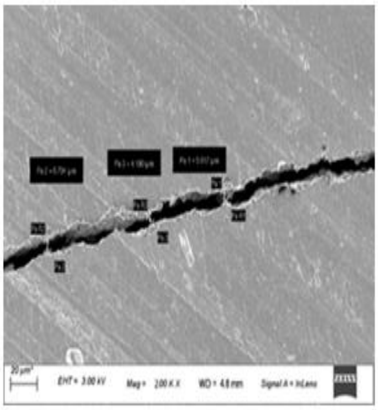

Fig. B

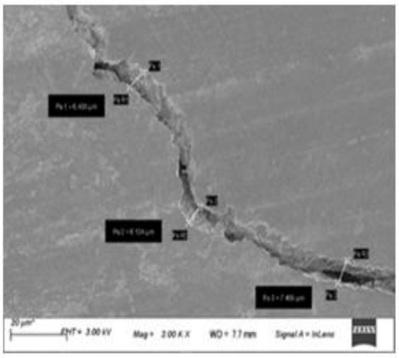

Fig. C

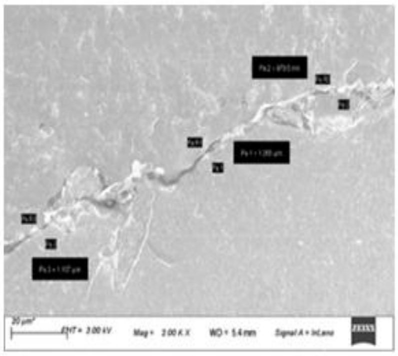

Fig. D

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Volume 3, Issue 2, Mar-Apr. 2021 pp 1140-1148 www.ijdmsrjournal.com ISSN: 2582-6018

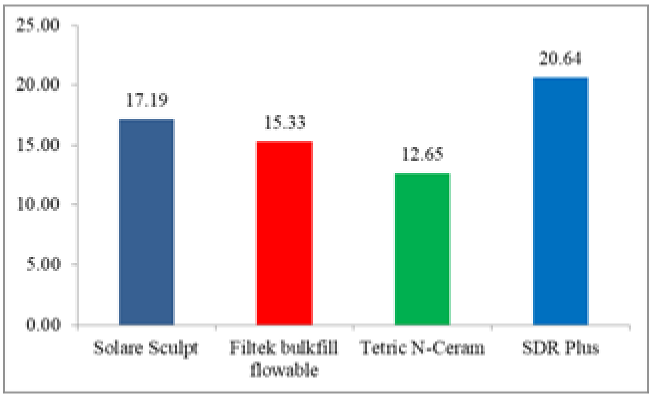

Fig 3: Bar Chart showing the mean width values of the four groups as measured from confocal microscopic images

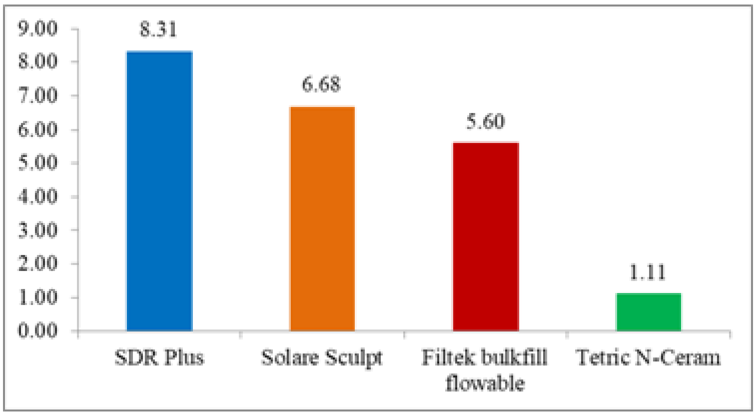

Fig 4: The mean width of the interface measured by FESEM of the four experimental groups.

IV. DISCUSSION

The durability of a restoration mainly depends on the preservation of the marginal integrity of a restoration. But this integrity is compromised by micro leakage due to marginal failure occurring as a result of polymerization shrinkage. [9]This process has proved to be the most common cause of failure of direct posterior composite restorations. Internal contracting forces that are generated due to polymerization shrinkage are capable of causing an adhesive failure or micro cracking of the restorative material as well as the enamel. [10]

For many years, clinicians and researchers have believed that shrinkage stresses can be reduced by placing resin composite in small increments.[11] But this concept was later reprehended by several researchers, recommending the use of larger increments and even bulk filling of the entire cavity with one increment. But there was one major concern about the bulk filling technique and it was that the deeper layer of the increments suffered from reduced polymerization due to light attenuation. Some of the bulk-fill materials recently introduced were claimed to have more potent initiator systems and most of them were characterized by low viscosity and high translucency.[11]Three such new bulk fill composites were used in this study.

In case of Class II carious lesions, most of them extend upto CEJ or beyond it and into deeper dentin along the proximal pulpal wall and therefore the restorations were prepared so that the cervical margins were placed 1 mm below the DEJ.[12]In order to enhance the process of micro leakage thermocycling has been proved to be the most influential factor in the previous studies. Laser scanning confocal microscopy is used as a technique for visualizing subsurface tissue characteristicswiththe advantage of imaging up to a few microns beneath the observed surface,[13]avoiding the spread of stain during specimen sectioning and polishing artefacts. Another technique applied for evaluation of the microleakage was Field Emission Scanning electron microscopy. It allowed a qualitative

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evaluation of the adhesive interfaces created between the dentin bio-structure and surface prepared from the composite resins by evaluating the width of the interface at the resin-dentin junction. As this width is directly proportional to the amount of resin polymerization, it is used as a parameter to access the polymerization shrinkage occurring at resin-dentin interface.[14]

In this study, the bulk-fill composite resins and their marginal sealing capabilities in case of class-II restorations were evaluated. This study showed that none of them were able to completely eliminate marginal micro leakage. Though there was significant statistical difference among the Bulk Fill resins used in this study. The evaluations and comparisons made using FESEM showed more statistically significant results compared to confocal fluorescence microscopy. The control group (G1) filled using incremental technique of 2 mm showed micro leakage indices similar to those of groups II, III and IV, filled using single increments of 4mm, all formed by bulk-fill resins marketed as having low contraction. The similarity of the results between the methacrylate-based resins may be explained by the similar compositions and by other factors like the use of adhesive systems that involve acid etching of the dentin.

Tetric N-Ceram Bulk Fill showed lowest micro leakage values among all the other groups including the control group (Group I) while SDR plus showed highest micro leakage values. The results were in agreement with the study by Juloski et al[15]but contradictory to the studies of Scotti et al.[16]and Roggendorf et al.[17]Filtek bulkfill flowable showed comparable microleakage with control group (Group I). The values were lower than SDR plus but higher than Tetric N-Ceram bulk fill.

In case of bulk technique, the most important issue is whether or not the deeper portions of the composite resin get cured. And there are few studies that evaluate the degree of conversion and polymerization kinetics of bulk-fill composites. Some of them evaluated that SDR plus, Tetric N-Ceram Bulk Fill and Filtek bulk fill flowable showed adequate curing at the deepest portion of 4-mm increments. Regarding the depth of polymerization of bulk-fill composites, the claims made by their manufacturers can be considered reliable.The least micro leakage seen in Tetric N-Ceram may be attributed to the pre polymer shrinkage stress reliever, the filler technology which includes polymer fillers and a new photosensitizer - Ivocerine – Dibenzoyl germanium derivative.

The resin system of Filtek™ Bulk Fill Flowable is a combination of four high molecular weight monomers BisGMA, BisEMA, Procrylat and UDMA which have been used in clinically successful composites. In case of SDR plus, increased polymerization shrinkage than the other groups seen may be due to the reduced filler volume and increased amount of low molecular weight monomer. The proposed mechanism of reducing shrinkage even though filled in bulk have been proven correct with Tetric N-Ceram bulk fill composites (Group IV)and proven incorrect for SDR (Group III). However the following study was performed in vitro, the in vivo results may vary.

V. CONCLUSION

Thus, within the limitations of the study, it can be concluded that micro leakage is observed regardless of the technique used. All the restored groups showed micro leakage, out of which Tetric N-Ceram showed the least micro leakage followed by Filtek bulk fill flowable composite and control group. The highest microleakage was observed in SDR composite proving to be the weakest material. Conventional composites showed more microleakage than TetricN-Ceram and Filtek Bulk Fill but the values are not statistically significant.

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