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RESEARCH ARTICLE

COMPARISON OF MICROLEAKAGE IN CLASS 2 CAVITIES RESTORED WITH 2 METHACRYLATE BASED COMPOSITES USING BULK AND INCREMENTAL TECHNIQUES – AN INVITRO STUDY

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Key words:-

microleakage, confocal microscope, incremental technique, bulk technique, methacrylate, thermocycling, dye penetration.

Abstract

Introduction: The study aims to compare the microleakage in class 2 cavities restored with 2 methacrylate-based composites using bulk & incremental techniques.

Materials and method: Twenty six human premolars freshly extracted were used to prepare class 2 cavities of width and height of 3 mm on both mesial and distal sides with 1mm sound tooth structure in between them following which the cavities were etched bonded and restored with FILTEK Z350XT by incremental technique in mesial side and bulk technique in distal half group 1 and group 2 respectively, similarly in group 3 CERAM X DUO was restored in incremental technique on mesial side and in group 4 CERAM X DUO was restored using bulk technique on distal side and cured for 20 secs following which the samples were thermocycled and immersed in rhodamine dye and evaluated under confocal microscope for microleakage.

Results: The mean dye penetration values in descending order: group 3 (CERAM X DUO Incremental technique) $\geq$ group 1 (FILTEK Z350XT Incremental technique) $\geq$ group 4 (CERAM X DUO Bulk technique) $\geq$ group 2 (FILTEK Z350XT Bulk technique)

Conclusion: Based on the limitations of this study, it may be concluded that in Class II restorations, microleakage is observed regardless of the techniques used. Incremental techniques showed comparatively lesser microleakage than bulk placement technique and among the groups CERAM X DUO composite shows lesser microleakage at both cervical and occlusal walls than FILTEK Z350XT.

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Introduction:-

The basic purpose of the restorative materials is to substitute the biological, functional, and esthetic properties of healthy tooth structure.[1] Composites were introduced in the 1960's and since then have undergone a lot of research and development. Refinement in these materials led to the development of micro hybrid composite, with mean particle sizes in the 0.6- to 0.7-micrometer range.[2] Although successful techniques for posterior resin placement have been developed with these universal micro hybrid materials, they present many challenges when clinicians attempt to place morphologically correct and functional class II restorations, many hybrid composites are difficult to manipulate because of inherent stickiness and slumping.[3]

One of the most important requirements for the success of restoration is to prevent the microleakage, which is achieved with proper adherence of restorative material to the cavity walls. The inadequacy of the restorative materials to attain complete marginal seal leads to occurrence of micro fissures, in which the seepage of ions, fluids, and bacteria occurs, which causes secondary decay, sensitivity, and pulpal infections.^[4] Thus, the continuing quest for better restorative material, the newer materials were introduced with better biomechanical properties such as better marginal seal, good esthetics, easy polishability, biocompatibility, and compressive strength in evolution.^[5]

According to Kidd, microleakage is defined as the clinically undetectable passage of bacteria and bacterial products, fluids, molecules, or ions from the oral cavity along the various spaces present in the cavity restoration interface.^[6]

Two major properties of dental light cure composites that have to be improved are their polymerization shrinkage and the shrinkage-induced stress. The volumetric shrinkage of currently used composites ranges from 2 to 6%.^[7] This results in marginal gap formation leading to microleakage. If the contraction forces exceed the bonding strength at the interface, the resulting interfacial space can lead to staining, marginal leakage, postoperative sensitivity, and recurrent caries.^[8]

Several strategies have been proposed to reduce the microleakage; for instance, the use of ceramic inserts^[9] in the composites, the factor responsible for microleakage is the inherent shrinkage in the organic matrix. Methacrylate-based composite resins have linear reactive groups and polymerize by addition reaction which results in high polymerization shrinkage. Use of organically modified ceramic nanoparticles and nanofillers combined with conventional glass fillers in Ceram X has improved the natural aesthetics, reduced the monomer release, but polymerization shrinkage is still high, i.e., about 2.3% according to the manufacturers.^[10]

To reduce shrinkage stress effects, different restorative techniques have also been suggested. Among these are different types of sandwich restorations, different incremental placement techniques of the resin composite, and different light-curing regimens.^[11]

The technique involved in placing posterior composite presents many challenges^[13] the restoration of the large Class II mesial-occlusal-distal restorations with Resin Bonded Composites (RBC) materials is time consuming, in terms of placement, light-curing each increment, and the operator time required for separate etching, priming, and bonding techniques.^[12]

Therefore, this study is aimed at evaluating and comparing the microleakage of restorations using 2 methacrylate based composites i.e FILTEK Z350XT – methacrylate based nano composite (3M ESPE) and CERAM X DUO – nano ceramic-based composites (DENTSPLY) in class 2 cavities restored with incremental and bulk techniques.

Methodology:-

Specimen Preparation:-

A total of 26 human premolars which were removed for orthodontic purpose were selected for the study. Teeth with any visible caries, restoration, hypoplastic lesion, stains and cracks were excluded from the study.

DIVISION OF GROUPS

- Group 1: Incremental placement of FILTEK Z350XT on mesial class II cavity
- Group 2: Bulk placement of FILTEK Z350XT on distal class II cavity
- Group 3: Incremental placement of CERAM X DUO on mesial class II cavity
- Group 4: Bulk placement of CERAM X DUO on distal class II cavity

Teeth were randomly divided into 4 groups, after which class II cavities were prepared on both mesial and distal surfaces with 1mm sound tooth structure in between the 2 cavities, using straight diamond point with air/water cooled turbine.

The cavity was prepared according to below given dimension:-

- Occlusogingival height of 3mm
- Buccolingual width of 3mm

The cervical margin were located 1 mm coronal to the CEJ so that all the margins were located within enamel. buccal and lingual walls of the preparation were approximately parallel and connected to the gingival walls with rounded line angles. All the margins were kept as close as possible to a 90° cavosurface angle.

In group 1 (n=13) was restored with FILTEK Z350XT in incremental technique on mesial cavity and n=13 with bulk technique on distal side using the same composite

In group 3 (n=13) was restored with CERAM X DUO by incremental technique on mesial side and in group 4 (n=13) teeth were restored with bulk technique on distal side using same composite as group 3.

The specimens were stored at 37° C for 24 hrs and were submitted to 1000 thermal cycles at 5°C & 55°C for 1 min at each temperature.

To prevent dye penetration, two layers of nail varnish were used on all tooth surfaces, except on a distance of 1mm around the tooth composite resin interface, then the tooth was immersed in 0.6% aqueous rhodamine dye for 48 hrs and were divided into two halves mesiodistally using diamond disc at low speed.

Evaluation of Microleakage:-

The microleakage was measured using confocal microscopy at ×10 magnification (Confocal Fluorescence Imaging Microscope, Leica TCS-SP5, and DM 6000-CFS) in the fluorescent mode. Approximately, five photographs of each specimen were taken to obtain the full perimeter of the restoration. With a digital scale (image j digital scale), the highest width of interface between restoration and tooth surface was calculated

The microleakage scoring was also calculated using the method, as per Alavi and Kianimanesh.^[18] The scores are as follows:-

Score 1: No dye penetration.

Score 2: Dye penetration within one-half of occlusal or gingival wall.

Score 3: Dye penetration extending to the end of occlusal or gingival walls.

Score 4: Dye penetration through the gingival or occlusal wall to one-third of axial wall.

Score 5: Dye penetration through the gingival or occlusal wall to two-thirds of axial wall.

Score 6: Dye penetration throughout the axial wall.

Statistical Analysis:-

Collected data were analyzed using both descriptive and inferential statistical methods. Descriptive methods such as mean, standard deviation, confidence interval was calculated to summarize the data in these 4 groups.

Inferential method such as one-way analysis of variant was performed (ANOVA) to find significance difference between the groups followed by TUKEY HSD analysis or pair wise comparison.

Level of significance was 5% and analysis was performed in SPSS software VER 23.

MICROLEAKAGE

The statistical parameters such as mean and SD of microleakage of samples were obtained for each group as shown in the table 2 , the mean of group 3 (CERAM X DUO) Incremental was the lowest $31.24 \pm 1.38 \mu\text{m}$ followed by group 1 (FILTEK Z350XTIncremental) with a mean of $35.03 \pm 0.74 \mu\text{m}$ followed by group 2 (CERAM X DUOBulk) with the mean of $36.55 \pm 0.58 \mu\text{m}$ the group 2 (FILTEK Z350XTBulk) was the highest $38.78 \pm 0.65 \mu\text{m}$.

Analysis of variants was highly significant with the $P=0.000 \leq 0.001$.

Further Post hoc or Pairwise analysis by TUKEY HSD test was carried out as shown in (Table 3).

Table 1: microleakage score evaluated

Groups	Microleakage score
CERAM X DUO incremental	6
CERAM X DUO bulk	6
FILTEK Z350XT incremental	6
FILTEK Z350XT bulk	6

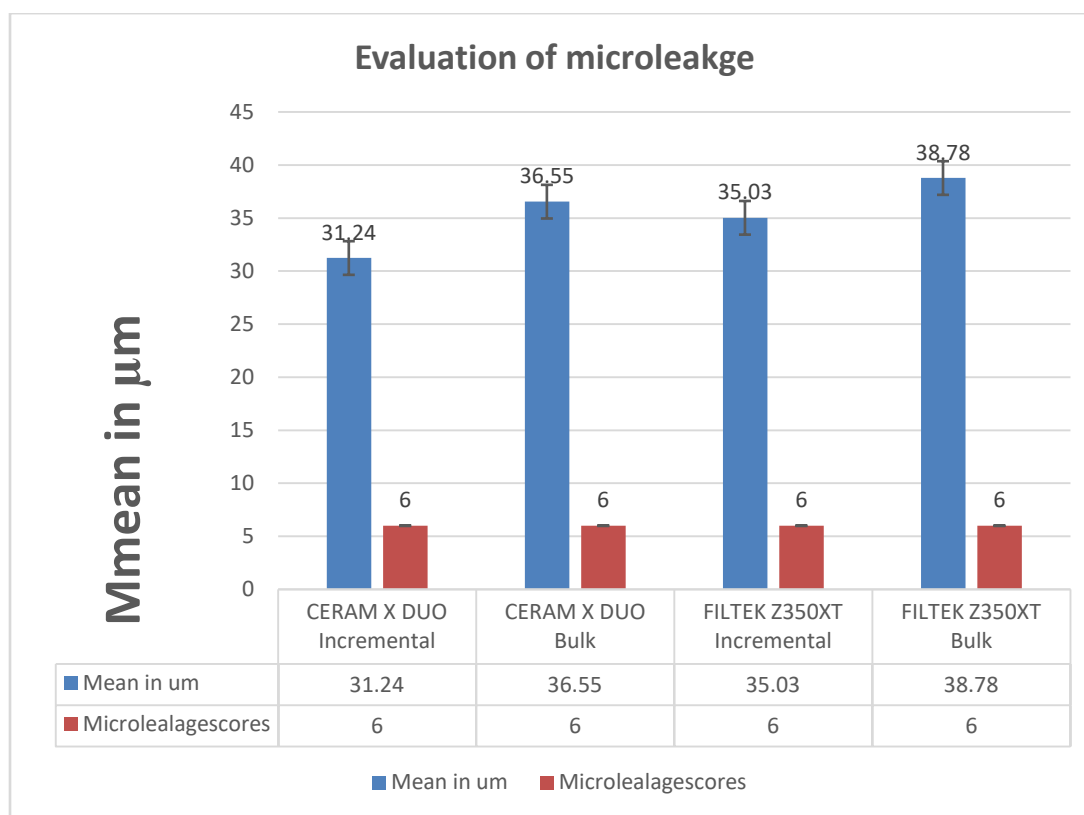
Results:-Table 2: Comparison of microleakage in 4 groups in μm

Groups	N ^a	Mean (SD)	F	P value
CERAM X DUO incremental	13	31.24 (1.38)	160.38	0.000
CERAM X DUO bulk	13	36.55 (0.58)		
FILTEK Z350XT incremental	13	35.03 (0.74)		
FILTEK Z350XT bulk	13	38.78 (0.65)		
Total	52	35.40 (2.90)		

Table 3: comparison in between the 4 groups

(I) groups	(J) groups	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
CERAM X DUO Incremental	CERAM X DUO Bulk	.000	-6.24899	-4.36470
	FILTEK Z350XT Incremental	.000	-4.73383	-2.84955
	FILTEK Z350XT Bulk	.000	-8.47745	-6.59317
CERAM X DUO Bulk	FILTEK Z350XT Incremental	.001	.57301	2.45730
	FILTEK Z350XT Bulk	.000	-3.17060	-1.28632

FILTEK Z350XTIncremental	FILTEK Z350XTBulk	.000	-4.68576	-2.80147
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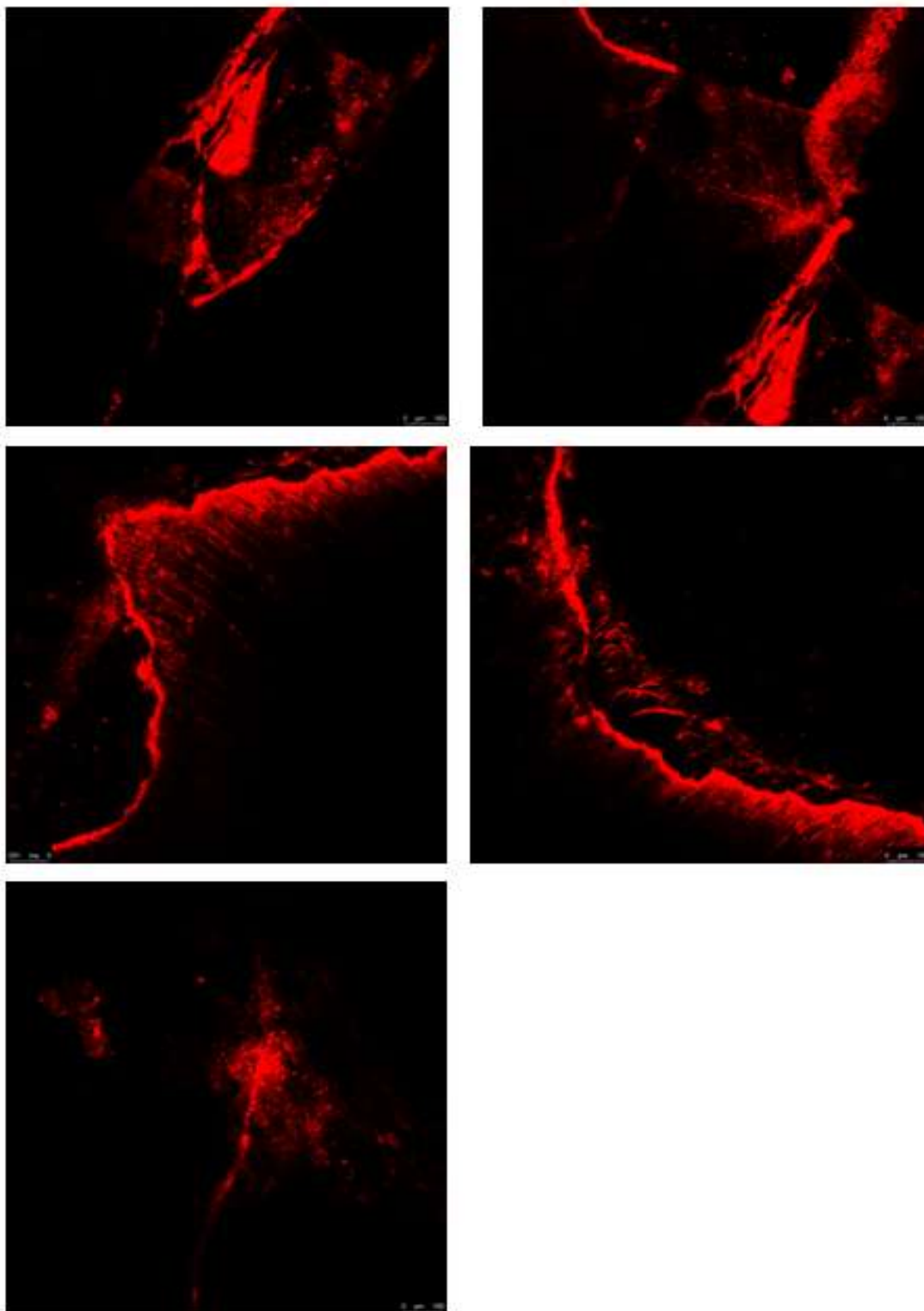
Graph 1: comparison between different groups and their scores

Discussion:-

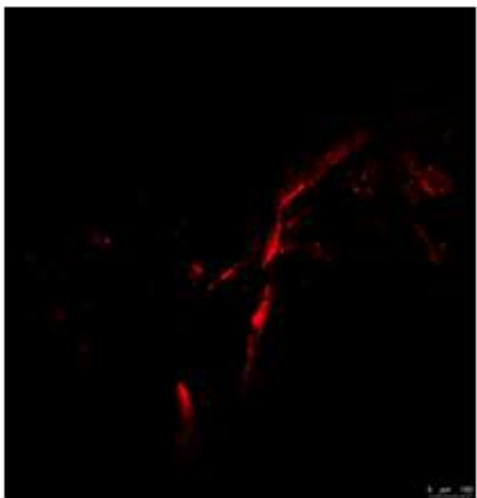
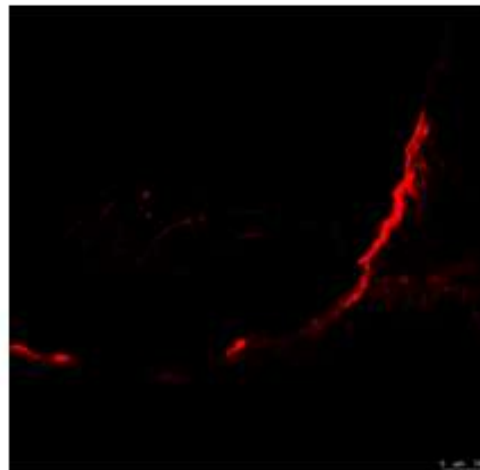
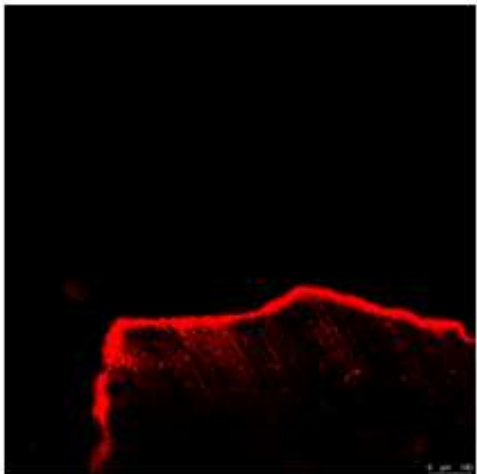
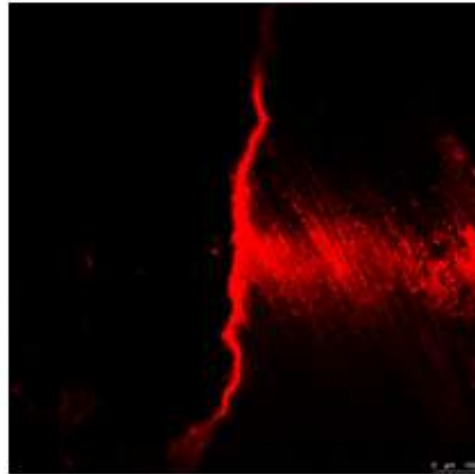
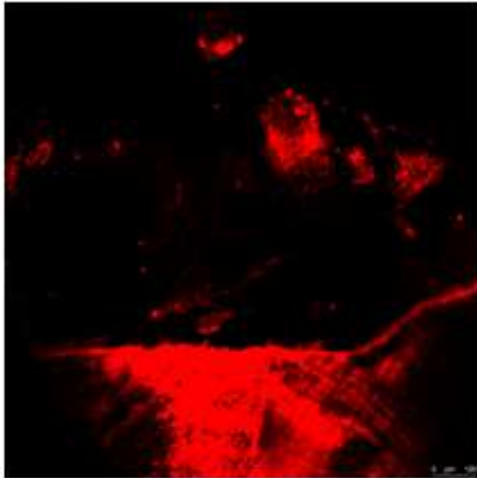
Composite resin has been increasingly utilized replacing amalgams as the posterior restorative material of choice for many dental patients. Both in educational and private practice dental settings, [19,20,14] However, the insertion of composite resin can be a very complex, challenging procedure for the dentist as a result of many material and clinical considerations. [20,21] One of the purposes of a dental restoration is to create a flawless seal at the material/tooth interface (margin), which should be resistant to contamination from oral fluids. [14] Leakage or microleakage occurs in conjunction with all dental restorations and has been defined as the “clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it.” [22] This potentially destructive process is associated with several limiting factors, Material component like filler particle size/form aggregates and resin loading. Physical characteristics like volumetric and postpolymerization shrinkage, modulus of elasticity, thermal expansion, methods of polymerization, cavity preparation design parameters and configuration factor, or “C-factor.” Clinical variables like material manipulation, insertion techniques, isolation constraints (usage of dental rubber dam), adequate knowledge of adhesive and composite resin science and

applicable technique. [13,23-29] Composite resin, used as a posterior restorative, is significantly influenced by polymerization shrinkage with amounts ranging from 1.5% to 5%. Shrinkage causes debonding of the material from tooth structure, precipitating clinical and radiographic sequelae including marginal staining and micro-gap formation (approximately 10 to 20 μm). This process, in turn permits bacterial ingress with recurrent caries, sensitivity, possible pulp inflammation and finally restoration removal and reinsertion as final outcomes. [23,42,30]

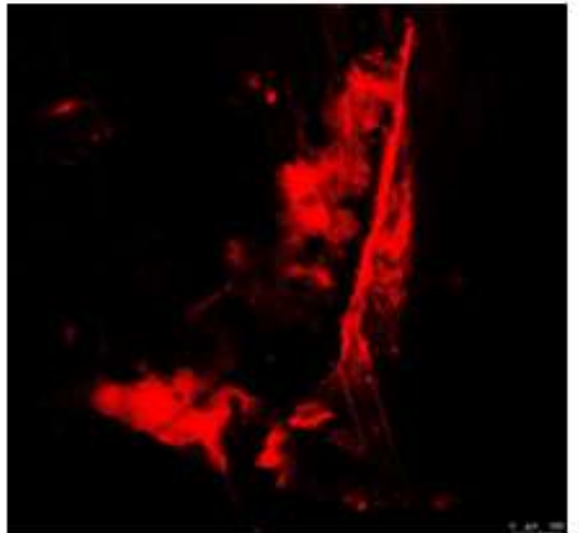
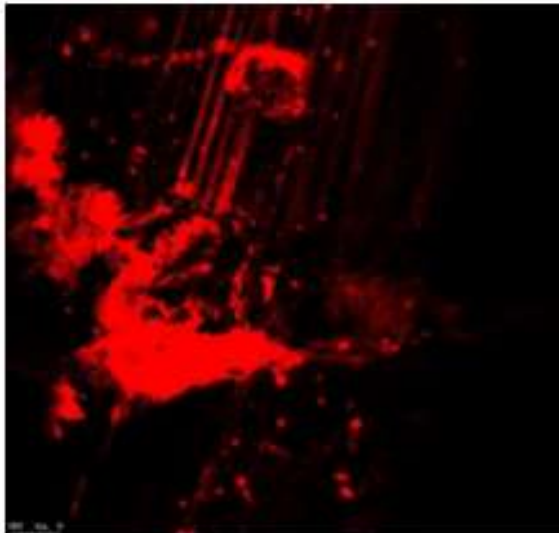
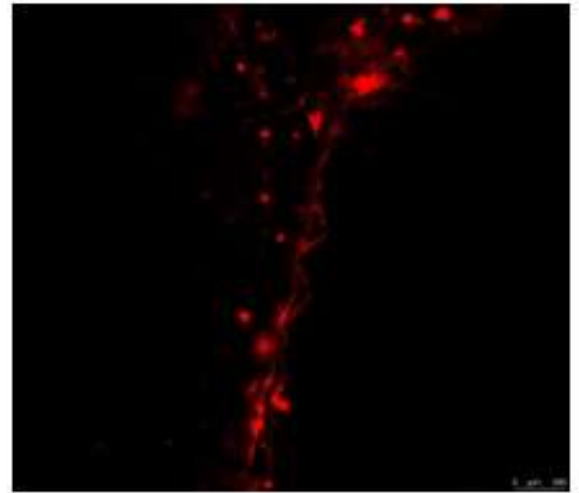
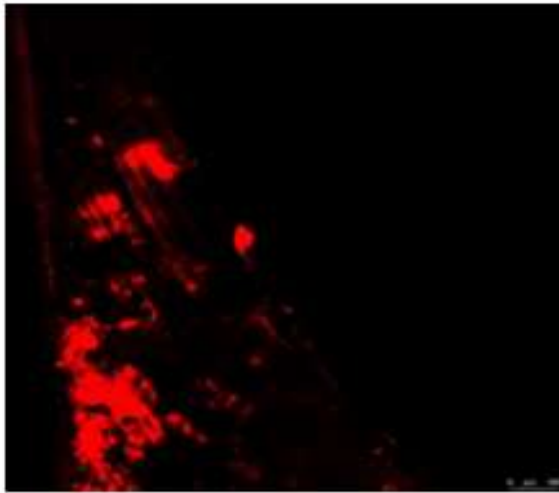
CONFOCAL IMAGES OF FILTEK Z350XT (INCREMENTAL)



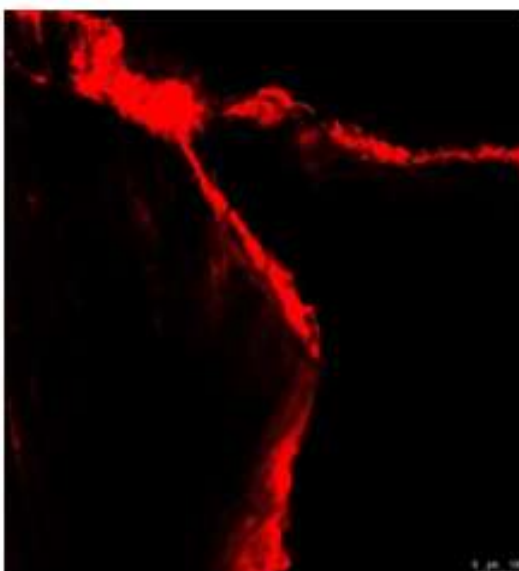
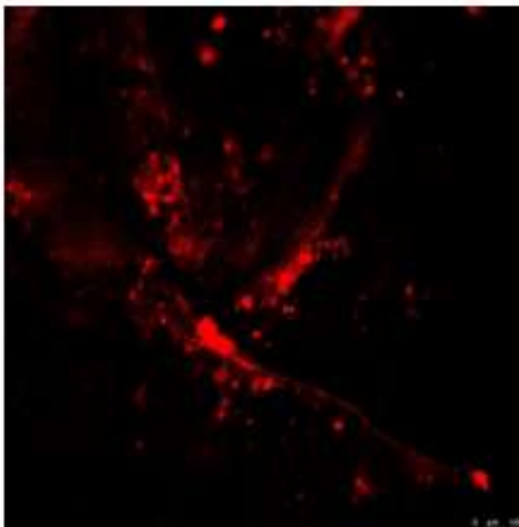
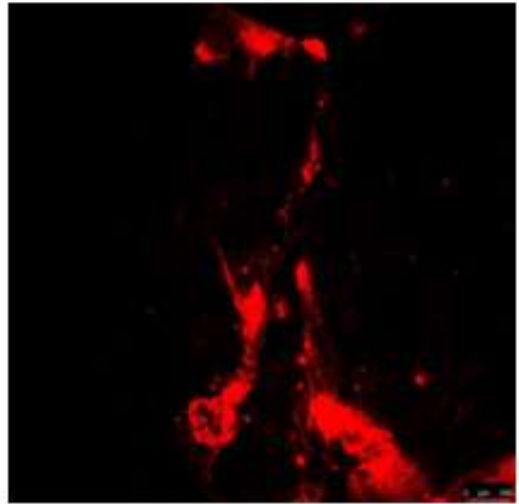
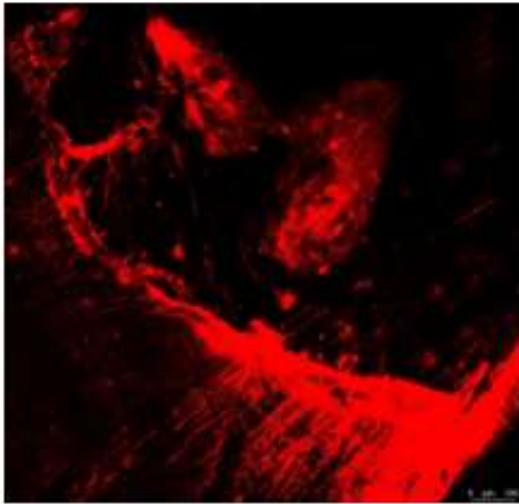
CONFOCAL IMAGES OF FILTEK Z350XT (BULK)



CONFOCAL IMAGES OF CERAM X DUO (BULK)



CONFOCAL IMAGES OF CERAM X DUO (INCREMENTAL)



In the present study, the cavity preparation was standardized to size 3x3x3 mm (occlusolingival x buccolingual x mesiodistal) respectively; and all the cavities were limited to enamel because adhesive restoration located below the cemento-enamel junction (CEJ) is questionable. Since the bond with dentin is weaker, the polymerization shrinkage of the resin-based composite may lead to separation of the resin from the preparation wall and the formation of gaps. [17,10] The same adhesive technique and polymerization technique was used for both resins, so that only the resin variable will influence the results. [10] In this study, prior to application of bonding, enamel margins were etched by 37% phosphoric acid for 15 seconds because previous studies showed that etching the enamel was efficient in bonding process and increased the marginal adaptation. [15] Adhesive used here was 3M SINGLE BOND UNIVERSAL, these use methacryloxydecyl phosphate (MDP). The MDP allows for better adhesion performance to enamel and also provides a high degree of conversion and more hydrophobic properties upon polymerization. Prior to curing and during application, Single Bond Universal adhesive is hydrophilic for optimum wetting of the tooth structure. After drying and curing, Single Bond Universal adhesive exhibits a high degree of conversion and is hydrophobic for a long-lasting bond. [31] In the present study, we eliminated the variability dependent on the tooth by performing the mesial filling with the increments of 1.5mm and the distal restoration with the bulk placement of increments 2.5 mm, using different composites. [10] Materials selected for the study are CERAM X DUO (DENTSPLY SIRONA) and FILTEK Z350 XT (3M) because according to manufacturer's claim, CERAM X® DUO universal nano-ceramic composite replicates the translucency and chroma of natural teeth. New and novel SphereTEC™ (stands for the process of manufacturing micro scaled, well-defined spherical super structures, comprising of submicron glass.) filler technology is used for enhanced handling properties, easy high-end esthetics, excellent cavity adaptation, sculptability and Improved slump resistance. [32] FILTEK Z350XT 3M ESPE builds its nanocomposite using a patented process that creates unique clusters of nanometre-sized particles.

The nanoclusters shear at a rate similar to the wear of the surrounding resin matrix during abrasion. This allows the restoration to maintain a smoother surface for long-term polish retention with minimal gap formation. [33] These composites were cured using VALO (ULTRADENT) polymerization unit. Microleakage can be aggravated by changes in temperature that occur in the mouth, due to the different coefficients of thermal expansion of dental tissues and composite resins. [17] Campos et al [34]. and Agarwal et al [35]., reported a satisfactory marginal adaptation values before thermomechanical loading, but these values were not maintained after subjecting the samples to thermocycling. In the current study thermocycling unit was used so that it accelerated the aging process of the teeth restorations. This mechanism increases the permeability of the hybrid layer and the porosity of the restorative material. This way, the stress that a restoration presents over the years can predict in vitro the longevity of a restoration in vivo. In the present study, the restored teeth were subjected to 1000 cycles of thermocycling, immersed alternately in cold water baths at a temperature of 5°C (±5°C) and hot water at a temperature of 55°C (±5°C) with a dwell time of 1 min in each bath for 1000 cycles. [17] Later the apex of the samples in the study was sealed with sticky wax and painted with 2 coats of nail varnish all over except 1mm distance throughout the restoration margin so that dye penetration occurs only at the tooth and resin interface. A variety of microleakage testing techniques are available. The simplest and most commonly used method is the dye penetration method. [16] Hence in this study dye penetration test was done, here 0.6% aqueous solution of rhodamine dye was used, the samples were immersed in the dye for 48 hours so that enough dye penetration takes place. As it is a fluorescent dye amber colored bottle was used to store the samples.

After retrieving the samples from the bottle, it was washed and cleaned and then sectioned mesiodistally so as to check the dye penetration within the tooth resin interface. The dye penetration was evaluated using scoring method as well as by calculating the interface between resin and prepared tooth surface using CLSM. Throughout history, there have been several microscopy techniques available to evaluate the restoration/dentin interface, including stereomicroscopy, scanning electron microscopy, transmission electron microscopy, and confocal laser scanning microscopy (CLSM). CLSM offers several advantages like the use of non-decalcified or hard tissue samples that do not require a specific section technique (sputter coating). It also provides detailed information about the presence and distribution of sealers at relative low magnification through the use of fluorescent Rhodamine-marked sealers and allows the exclusion of artifacts from the sample. [36] It does not require special specimen processing, and observations can be made under near normal conditions. [37] In the studies of dye penetration, the dentin staining was observed to be more different than the actual gaps between cavity walls and restoration materials.

This resulted in the use of a dye with a particle diameter equal to the bacterial size or smaller by researchers [38] CLSM requires a fluorescent dye for observing the specimens. The dye used in the present study was 0.6% aqueous Rhodamine B dye. Rhodamine B base is a colourless dye with undetectable fluorescence feature. In acidic condition, rhodamine B base gets transformed into a coloured rhodamine dye that acts as a stable and efficient laser dye. It is

often used as a tracer dye in water to determine the rate and direction of flow and transport.[39] In the present study it was used to check for the depth of microleakage occurred between the restoration and tooth. lesser the depth of penetration better the ability of the composites used. The CLSM used in the present study is the LEICA Microsystems DMI8, Germany. the microscope is easily adaptable for everything from simple fluorescent imaging to sophisticated super resolution applications.[40] The system is composed of a regular florescence microscope and the confocal part, including scan head, laser optics, computer.[41] 10x magnification was used to scan all the specimens. The zoom used was 0.75. There are different types of LASERS: Argon, Argon-Krypton, Helium, Neon, etc. [58] In the present study, Argon laser was used with an intensity of 545-609 nm. The scanning speed was 200Hz. All the images were analysed using the LEICA Application Suite X (LAS X) software. [58] This study was conducted using the CLSM in KMC, Manipal, Karnataka. With all the limitations in the study the results seen in the table 1 showed score 6 for all the samples i.e.: Dye penetration throughout the axial wall, this suggests that regardless of the techniques and materials used there is always microleakage seen in composites, It may be due to the type of restorative material which is partly determined by amount of filler particles. More filler particles increase the strength and modulus of elasticity and reduce polymerization shrinkage.

Shrinkage can be reduced by decreasing the filler size. Polymerization shrinkage creates significant stress intooth structure leading to bond failure in spite of a good adhesive system[42] Whereas in table 2, group 3 (CERAM X DUO Bulk placement) showed least microleakage at the mean of $31.24 \pm 1.38 \mu\text{m}$ followed by group 1 (FILTEK Z350XT incremental) with a mean of $35.03 \pm 0.74 \mu\text{m}$ followed by group 2 (CERAM X DUO Bulk) with the mean of $36.55 \pm 0.58 \mu\text{m}$ the group 2 (FILTEK Z350XTBulk) was the highest $38.78 \pm 0.65 \mu\text{m}$. After analyzing the results, it clearly states that incremental technique is always better than bulk placement, the results of some previous studies confirmed that regardless of the restorativesystem, employing the incremental technique resulted in significantly less microleakage than the bulk technique. [43, 44] Unlike other studies in which there was no significant difference. Heintze et al. [45] evaluated the quality of gingival margins of class II composite restorations, which placed in bulk or three increments, and showed that there was no significant difference between two techniques. Furness et al. [46] restored the cavities of class I with bulk fill (SDR, Quixx, Sonic Fill, TetricEvoC-eram) and a conventional composite, Filtek Supreme, and indicated that there was no significant difference between the marginal integrity of two composite placement methods. And by the overall results CERAM X DUO (DENTSPLY SIRONA) showed better results than FILTEK Z350XT (3M) this may be due to the presence of nanocomposites which were formulated by top-down approach, to make the filler particles of one nano meter in diameter. Due to this, nanocomposites show reduced polymerization shrinkage and low microleakage, thereby making them superior to other composite resins[16]

Study limitations and scope for further study:-

The present study was done under in vitro conditions and used natural extracted teeth for restoration, and thermocycling was used as part of test protocol. In vitro studies are very important for an early assessment of the dental material. However, only a clinical study takes into account, all the potential variables that vary from patient to patient. Some of the variables include masticatory forces, types of food, oral temperature, and humidity variations and presence of salivary enzymes and bacterial by-products. Many new restorative materials are evolving rapidly, each with better properties and promising results for better performance. Therefore, further studies are required to establish the factual clinical worth of these materials to validate their in vitro established results.

Conclusion:-

Based on the limitations of this study, it may be concluded that in Class II restorations, microleakage is observed regardless of the techniques used and incremental placement of composites showed comparatively lesser microleakage than bulk placement technique and among the groups CERAM X DUO composite shows lesser microleakage at both cervical and occlusal walls than FILTEK Z350XT.

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