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Research Article

COMPARATIVE EVALUATION OF MICROLEAKAGE BETWEEN DIFFERENT RESTORATIVE MATERIALS USED IN CERVICAL LESIONS – A CONFOCAL LASER SCANNING MICROSCOPIC STUDY

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A B S T R A C T

Marginal integrity of restorative materials plays an important role in success of restoration and also improves the longevity of restorations by decreasing the chances of microleakage.
Aim - To compare and evaluate the microleakage in class V cavities which were restored with different restorative materials (packable microhybrid composite resin and flowable nanohybrid composite resin) under confocal laser scanning microscope.
Methodology - This in-vitro study was performed on twenty human maxillary premolars which were extracted for orthodontic reasons. A standard wedge shaped cavity was prepared on the buccal surfaces of teeth with the gingival margin placed near Cemento Enamel Junction (CEJ). Teeth were divided into two groups of 10 each and restored with packable microhybrid and flowable nanohybrid composite resin and were subjected to thermocycling. Teeth were then immersed in 0.5% Rhodamine B dye for 48 hours. They were sectioned longitudinally from the middle of cavity into mesial and distal parts. The sections were observed under Confocal Laser Scanning Microscope (CLSM) to evaluate microleakage.
Statistical Analysis - One Way Analysis of variance (ANOVA) was done to compare the mean values across the two groups for numerical data (using the F distribution).
Results – microhybrid composite resin showed less microleakage which was statistically insignificant when compared to nanohybrid composite resin (p=1.0000).
Conclusion - Within the limitations of the current study it can be concluded that although all the tested groups showed microleakage in class V cavities, microhybrid packable composite resin showed comparatively less microleakage than nanohybrid flowable composite resin.

INTRODUCTION

Cervical lesions are lesions, occurring at the cervical aspect of the buccal or lingual surfaces of teeth.[1] Conventionally, based on the etiology, a cervical lesion is broadly classified as Carious and Non-Carious lesions.[2]

These lesions present a special challenge to any clinician as, the restorative material is usually required to adhere to dentin or cementum in the cervical margin. Also, because of the high flexural forces acting at the cervical region of the tooth, which leads to greater debonding forces on the tooth restoration interface. The goal of restorative dentistry is to restore the tooth to its form, function and esthetics by biocompatible materials that do not compromise the pulp integrity and also maintain the marginal adaptation.

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With the advent of ASPA in 1969, Glass Ionomer Cement (GIC) has been the material of choice for restoring cervical lesions, but with the ever growing advancements in restorative dentistry, composite resin is now used in conjunction with GIC to restore these lesions.

In case of the resin based composites, an interplay of polymerisation shrinkage and stress, elastic modulus, viscous flow capacity and conversion degree of the material as well as the adhesive ability, cavity configuration and tensile stresses exerted on the restoration is considered responsible for marginal integrity.[3] This marginal integrity is the interface between the restoration and dental hard tissue and is an area of clinical concern as insufficient sealing can lead to hypersensitivity, marginal discolouration, secondary caries and pulpitis due to microleakge. Microleakage, as defined by Kidd, is the clinically undetectable passage of bacteria, bacterial products, fluids, molecules or ions from the oral environment along the various gaps present in the cavity restoration interface.[4]

Restoring a cervical lesion with resin composites has always been a challenge, particularly at the cervical margin where no enamel is present for efficient bonding.[5] The higher organic component, variation in tubular structure, fluid pressure and the lower surface energy of dentin makes bonding to dentin more difficult than to enamel.[6,7] Similarly the high organic component of cementum makes bonding more difficult than in dentin or enamel. Poor adhesion between dentin/cementum and restorative material predisposes gap formation leading to microleakage.[8] Also there is difficulty seen while maintaining isolation for cervical lesions.

Many attempts have been made to reduce the microleakage of composite resins which includes restoring the cavity in increments or in different layering techniques or by using materials of different viscosity and flow or by using different intensities of the curing light.

In late 1996, flowable composite resin, was introduced. Due to its low modulus of elasticity it can undergo plastic deformation to flex and absorb polymerization shrinkage stress.[9] On the other hand, since it has less filler content, the coefficient of thermal expansion of flowable composites is close to that of the tooth structure[10] and thus was used for restoring class V cavities.

One of the most important advances in recent years is the application of nanotechnology to resin composites. Nanotechnology is known as the production of materials and structures in the range of about 0.1–100 nm by various physical and chemical methods. The size of the filler particles lies around 8–30 µm in hybrid composites, and 0.7–3.6 µm in microhybrid composites, new fillers with size ranging from around 5–100 nm have been developed which are incorporated in nanohybrid composite resin.[11]

The present study was aimed to compare and evaluate the microleakage in class V cavities which were restored with different restorative materials (packable microhybrid composite resin and flowable nanohybrid composite resin) under confocal laser scanning microscope.

The null hypothesis was that there will be no difference in microleakage in class V cavities restored with the two tested materials.

MATERIALS AND METHODS

Twenty human maxillary first premolar teeth extracted for orthodontic or periodontal reasons were collected from the Department of Oral and Maxillofacial Surgery (Fig 1). Immediately after extraction the soft tissue attached to the tooth surface was carefully removed with wet cotton. They were checked for any caries, abrasion, attrition, fluorosis, or other enamel defects, which, if present, were discarded. OSHA and CDC recommendations and guidelines were followed. After collection, the samples were transferred to 100ml of 5.25% sodium hypochlorite solution (Prime Dental Products Pvt Ltd, Thane, India) stored in amber coloured bottle. The solution was discarded after 30 minutes and the teeth were transferred into separate jars containing artificial saliva (Wet Mouth, ICPA Health Products Ltd, Ankleshwar, India. Batch No: C30101) to simulate the oral environment with added 0.1% thymol as antifungal agent. The samples were removed with cotton pliers and rinsed in tap water. The samples were dried by placing them over paper towels and blotted for a few minutes before using them for study.

Fig 1 Twenty maxillary premolars chosen for the study.

Wedge shaped cavities were prepared using diamond points using a high speed handpiece utilizing water spray coolant at the gingival third of the buccal surfaces of the teeth with air water spray. Cavities were prepared with standardized dimensions of 3 mm occluso-cervically, 3 mm mesio-distally, and 2 mm depth of the axial wall, 1mm beyond the cementoenamel junction. The depth of the preparation was assessed using a William's periodontal probe. The gel etchant of 37.5% phosphoric acid (Kavo Kerr Corporation, Orange, CA, USA) (Fig II) was applied to the prepared tooth surfaces for 15 seconds after which it was washed off with water from the three way syringe and dried with absorbent paper without dessicating the dentin.

Fig 2 Materials used for the study.

From top to bottom:
Bonding Agent - Te-Econom Bond (5th generation, Ivoclar Vivadent, Amherst, NY, USA)
Etchant - 37.5% phosphoric acid (Kavo Kerr Corporation, Orange, CA, USA)
Packable composite resin – Polofil Supra (VOCO GmbH, Germany) (microhybrid composite resin)
Flowable composite resin - Tetric N Flow (Ivoclar Vivadent, Amherst, NY, USA) (Nanohybrid flowable composite resin)

Te-Econom Bond (5th generation, Ivoclar Vivadent, Amherst, NY, USA) (Fig 2) was applied on the dentin using the applicator brush according to the manufacturer's instructions. The material was gently brushed for 10s. The bonding agent was reapplied and the same process was repeated following which it was light cured for 20 seconds using a LED curing unit (Coltolux LED, Coltene, 1400watt/square cm) (Fig 3).

Fig 3a Procedure for sample preparation
Clockwise -Standardized Class V tooth preparation
Application of etchant Application of Bonding Agent

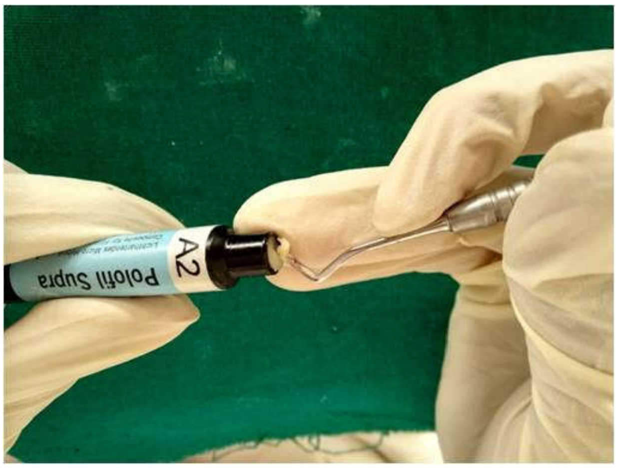

Fig 3b Restoring with Packable microhybrid composite resin

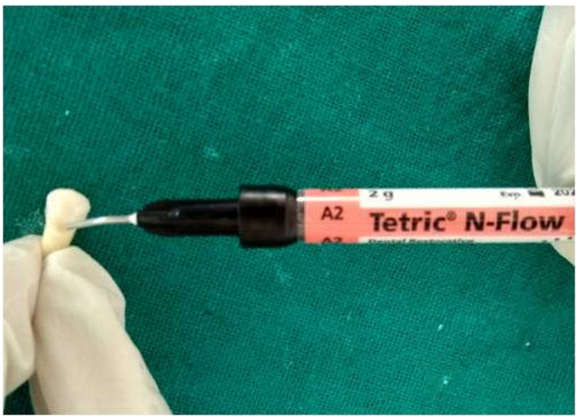

Fig 3c Restoring with Flowable Nanohybrid composite resin

The samples were then divided into two groups based on the restorative material used:

Group I: (n = 10) samples were restored with Polofil Supra (VOCO GmbH, Germany) (composite resin) in 1 mm increment layer. Each increment was light cured for 40 seconds by the same LED curing unit.(Fig 4)

Fig 4 Thermocycling unit

Group II : (n = 10) samples were restored with Tetric N Flow (Ivoclar Vivadent, Amherst, NY, USA) (flowable composite resin) in similar 1 mm increments and each increment was light cured for 40 seconds using the same LED curing unit. (Fig 5)

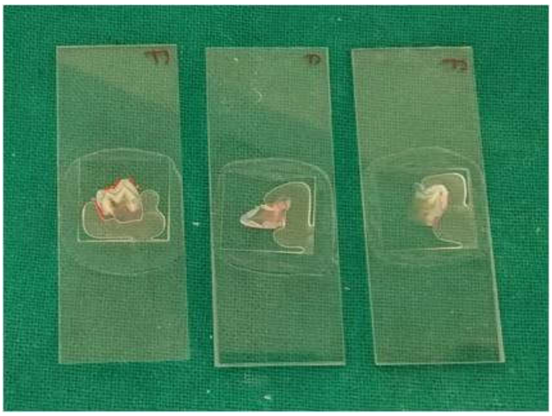

Fig 5 Slides preparation for viewing under confocal laser scanning microscope

Table I enlists the various products used in this study.

Table I Materials used in this study

Material	Product	Composition	Manufacturer
Etchant	Gel Etchant	37.5% phosphoric acid	Kavo Kerr Corporation, Orange, CA, USA Lot No. 6115991
Bonding Agent	Te-Econom Bond	HEMA, di- and monomethacrylates, inorganic fillers, initiators and stabilizers in an alcohol solution	Ivoclar Vivadent, Amherst, NY, USA Lot No. V51485
Packable Composite Resin	PolofilSupra– Microhybrid composite resin.	BIS GMA, UDMA, TEGDMA, sintraglass multifiller	Voco - Germany Lot No. 1711578

	Shade A2	76.5% by weight 0.05 um – microfillers 0.5-2 um - macrofillers	
Flowable Composite Resin	Tetric N Flow – Nanohybrid composite resin. Shade A2	27.8% - Urethane dimethacrylate, Bis-GMA 7.3% - Triethyleneglycol dimethacrylate 63.8% - Barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide 1.1% - Additives, stabilizers, catalysts, pigments	Ivoclar Vivadent, Amherst, NY, USA. Lot No. W42746

The restorations were then finished after 24 hours to contour with 12 fluted finishing bur (Kavo Kerr Corporation, Orange, CA, USA) with air–water spray in a high-speed handpiece. Later, medium, fine, and superfine Sof-Lex discs (3M ESPE, St. Paul, MN, USA) were used in sequence with air–water spray in a slow-speed handpiece. All the teeth were stored in distilled water for 24 hours, at 37˚C, and subjected to 1,000 thermal cycles between water baths of 5˚C and 55˚C, with a dwell time of 30 seconds (Fig 6).

Fig 6 Confocal Laser Scanning microscope unit

All the specimens were covered with two coats of nail varnish leaving 1 mm of the tooth-restoration margin and the root apices were sealed with modelling wax. All specimens were immersed in 0.1% Rhodamine B dye (Sisco Research Laboratories Pvt Ltd, Maharashtra, India) for 48 hours. The radicular portion was removed and the coronal portion was embedded in resin blocks. These blocks were sectioned bucco lingually into two mesial and distal parts (Fig 7).

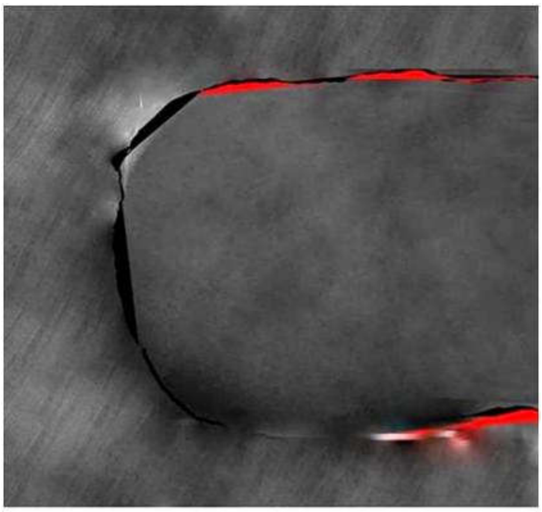

Fig 7 Packable microhybrid composite resin under CLSM

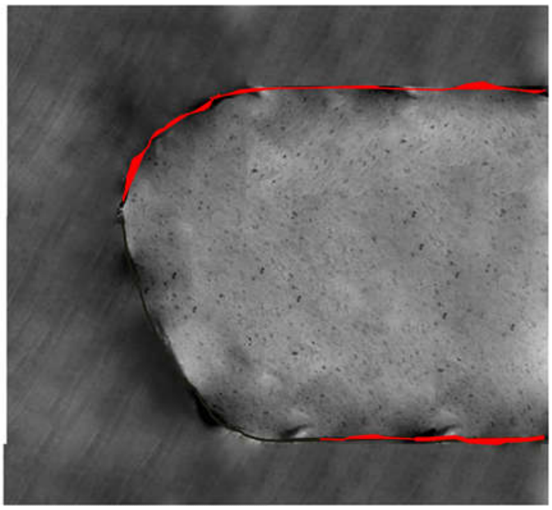

Fig 8 Flowable nanohybrid composite resin under CLSM

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 (Fig 8). Approximately, six photographs of each specimen were taken to obtain the full perimeter of the restoration. For each restoration, the sectioned half with greater leakage was selected for scoring.[12]

The depth of dye penetration was analyzed according to a 0-3 scale scoring system as suggested by Silveira de Araújo C:[13]
Score 0 = No evidence of dye penetration
Score 1 = Dye penetration along the occlusal/gingival wall to less than half of the cavity depth
Score 2 = Dye penetration along the occlusal/gingival wall to more than half of the cavity depth, but not extending on to the axial wall
Score 3 = Dye penetration along the occlusal/gingival wall to the full cavity depth and extending on to the axial wall.

RESULTS AND STATISTICAL ANALYSIS

The microleakage score was calculated for each specimen depending on the amount of dye penetration seen as shown in figure IX and X for the two groups respectively. The score achieved for both the groups have been tabulated in Table no. II.

Table II Raw Data-scoring for each specimen

GROUP	SCORE 0	SCORE 1	SCORE 2	SCORE 3
Group 1 (packable microhybrid composite resin)	0	0	6	4
Group 2 (flowable nanofilled composite resin)	0	0	2	8

Descriptive statistic analysis was done using Statistical Package for the Social Sciences (SPSS) 19.0. One Way Analysis of variance (ANOVA) was done to compare the mean values across the two groups for numerical data (using the F distribution).

The p-value corresponding to the F-statistic of one-way ANOVA (Table III) is higher than 0.05, suggesting that the results are significantly indifferent.

Table III The one way ANOVA table

Source	Sum of squares (ss)	Degrees of freedom (vv)	Mean square (ms)	F statistic	P-value
Treatment	0.0000	1	0.0000	0.0000	1.0000
Error	20.0000	2	10.0000		
Total	20.0000	3			

DISCUSSION

Resin composites are widely used for restoring cervical lesions. They are esthetic, mercury free and bond to tooth structure with the use of bonding systems.[9]

Unfortunately, the coefficient of linear thermal expansion of resin composites is three or four times that of tooth structure. In addition to the differences in thermal expansion coefficients, the shrinkage of composites during curing induces stresses at the tooth/restorative interface and generally results in gap formation. Therefore, polymerization shrinkage and the thermal expansion coefficient of these restorative materials have been suggested as major causes of microleakage.[14-16]

Flowable composites were introduced in late 1996.[10] They have a filler size similar to hybrid composites but lower filler content (60%-70% by weight and 60%-75% by volume). The less filler loading reduces elastic modulus and helps in enhanced flow. The lower elastic modulus provides it with greater ability to flex with the tooth than stiffer restorative materials.[17] Also, this material seems to wet the cavity walls more completely than conventional packable resin-based restorative materials.[18]

Ferdianakis (1998) compared the microleakage performance of flowable resin composite with that of hybrid resin composite and found significantly less leakage in cavities restored with flowables in class I cavities on the permanent molars.[19]

However, Mazer & Russell (1998) have reported that flowable composites and hybrid composites performed equally well in terms of microleakage.[20] Also, Estafan et al[21] and Chimello et al[22] found no difference in the occlusal or cervical microleakage of cavities restored with flowable or hybrid resin composites. Braga et al[23] showed that flowable composites produced polymerization contraction stress similar to hybrid composite and in a study by Niket A Lokhande *et al* similar leakage values were shown by flowable and hybrid composites, which showed that they performed equally well in terms of microleakage in class V cavities.[24]

In the present study, microhybrid composites exhibited less microleakage at the cervical margins as compared with the flowable nanocomposite resin though it was statitstically insignificant. These results can be attributed to higher filler loading with smaller particle size of nanocomposites in comparison with comparatively larger-sized filler particles and lesser filler loading in the microhybrid composite.[25] Another factor is the monomer size, larger the molecule (microhybrid composite), lesser is the polymerization shrinkage and associated stress resulting in lesser microgap formation.[26] Also, smaller-sized particles in nanocomposites cause scattering of light and decrease its absorption, thereby reducing the overall polymerization and increasing the microleakage in the material.[27] Also, nanocomposites contain a high amount of tri-ethylene glycol dimethacrylate (TEGDMA) in comparison with the minor amounts present in the microhybrid composite. The low-molecular weight TEGDMA and resultant high number of double bonds per unit weight create a high degree of crosslinking, creating a rigid resin with a relatively high shrinkage. Also, the majority of TEGDMA monomers elute within few hours, which may again contribute to microgap formations and, ultimately, increased microleakage.[28,29]

In a study carried out by Mahapatra *et al* (2006) the micro-leakage scores for micro-hybrid composites were more (0.9±0.7) than for nano-composites (0.4±0.5).[30] In a study conducted by Abdul Majeed *et al* (2005) the mean rank of microleakage seen in cavities restored with micro-hybrid composites was more (2.36±0.74) than restorations done with nano-composites in dentine and cementum (1.68±0.82).[30] However, Awliya & El-Sahn *et al* (2008) assessed micro-leakage in class V lesions using flowable nano-composite and micro-hybrid composites. They reported that the mean micro-leakage score around the tooth restoration interface with the micro-hybrid composite was 2.10±7.2 and for a nano-filled composite, it was 25.8±7.5. Indicating that micro hybrid composite resin caused less microleakage than nanofilled composite resin.[31]

Several techniques have been developed to assess the tooth restoration interface, in vitro and in vivo. One of the oldest and most frequently used method for the study of microleakage around restorations was the use of organic dyes. Eosin , methylene blue, methyl violet, hematoxylin and mercuric chloride, Prontosil soluble red, aniline dye, basic fuchsin, chromotrope 2R , crystal violet dye, and fluorescent dyes are a few of the many dyes that have been used by countless investigators. Fluorescent dyes are found to be particularly useful as tracers for the demonstration of leakage around dental restorations, because they are detectable in dilute concentrations, are sensitive to ultraviolet light, are easy to photograph, permit more reproducible results, are inexpensive, contrast sharply with the natural fluorescence of teeth, permit direct observation of the total marginal interface during evaluation and scoring of marginal leakage, are non toxic and permit clinical as well as laboratory investigations. In the present study, 0.1% rhodamine B dye (fluoroscent dye) was used to evaluate the microleakage.[32]

Confocal Laser Scanning Microscope offers several advantages over stereomicroscope and the Scanning Electron Microscope, like the ability to control depth of field, elimination or reduction of the background information away from the focal plane (which leads to image degradation), the capability to collect serial optical sections even from thick sections and having a simpler sample preparation procedure and therefore was used in this study.[33]

CONCLUSION

Within the limitations of the current study it can be concluded that although all the tested groups showed microleakage in class V cavities, microhybrid packable composite resin showed comparatively less microleakage than nanohybrid flowable composite resin. Higher microleakage in nanohybrid flowable composite resin can be attributed to higher filler load and decreased particle size.

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