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Comparison of Microleakage with Three Different Intermediary Base Materials Used in Open Sandwich Technique for Class II Composite Restorations- An In-Vitro Confocal Analysis.

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ABSTRACT

Background: The aim of the study was to determine out of the three intermediary base materials; GC IX, RMGIC, Biodentine which one will give least microleakage in Class II restorations using open sandwich technique. **Methods**: Standardized sixty Class II proximoocclusal cavity preparations were prepared in permanent maxillary and mandibular premolars. Teeth were restored using three base materials (n=15): GC Fuji IX, GC Fuji II LC; Resin Modified Glass ionomer cement and Biodentine followed by nanohybrid composite resin is done using open sandwich technique. The restorations were subjected to thermocycling procedure (500 cycles; 5°C-55°C) in hot and cold. Sealing of apices with sticky wax, coating with nail varnish and soaked in freshly prepared 0.25% Rhodamine B fluorescent dye for 24 h at 37°C. Teeth were sectioned mesiodistally longitudinally and seen under confocal microscope for dye penetration. Statistical Analysis: Data was evaluated by using One-way ANOVA, Post Hoc multiple comparison test and t Test. **Results**: Biodentine and RMGIC produced the minimum microleakage when compared to GCIX in Class II restorations on permanent premolars using open sandwich technique. **Conclusion**: Both Biodentine and GC Fuji II LC; RMGIC were more effective in sealing the gingival margins of Class II open sandwich restorations as compared to GC Fuji IX and Nanohybrid composite resin.

Keywords: Nanohybrid composite, Class II cavities, GC fuji IX, Resin modified glass ionomer cement, Biodentine, Open sandwich technique.

INTRODUCTION

Clinical dentistry has been facing the challenges of replacing lost tooth structure for years. To resolve this major problem in restorative dentistry, many materials have been developed.[1] Dental composite restorative resins are popular worldwide because of their excellent esthetic value and needing minimal tooth preparation due to micromechanical bonding to tooth structure. Their specific use in posterior teeth was introduced in early 1980s.[2] Poor marginal adaptation and considerable microleakage have been shown in cavities with the cervical margin located at or below the cervico-enamel junction.[3,4] Microleakage may be clinically undetectable, but is a major factor influencing the longevity of restorations by affecting the property of materials, resulting in recurrent caries, post-operative sensitivity, pulpal pathosis and discoloration and that is one of the major reasons for failure of Class II restorations restored with composites.[2,5-7]

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Other considerable problem of the direct resin is the shrinkage that occurs during polymerization (1-5% volume). Volumetric polymerization shrinkage that occurs upon curing and produces stress between bonded restorations and tooth walls which result in powerful forces that can separate restoration from tooth resulting in gap formation especially at cervical margins. This is due to lack of enamel at cementoenamel junction and incomplete adhesion between dentine and composite restoration. Clinical effects of the shrinkage stress and lack of sealing allows the occurrence of marginal microleakage.[3,4]

In an effort to reduce the side effects caused by polymerization shrinkage stress and to improve interfacial adaptation of direct composite restorations, Mclean and Wilson (1997) suggested sandwich or laminate technique8 in which an intermediary layer between composite restoration and the tooth is placed that acts as a stress breaker resulting in reduction of gap formation produced by polymerization shrinkage.[9] This layer acts as a buffer and thus decreases microleakage. Various materials like GIC, flowable composites, self or dual-cured composite resin material and RMGIC have been used in sandwich technique as stress absorbing intermediary layer and act as a buffer.[3]

Glass ionomer cements (GIC) are better capable of reducing the contraction stresses and are therefore recommended as base material. They failed soon due to moisture sensitivity and progressive loss of the glass ionomer cement. Light cured Resin modified glass ionomer cement (RMGIC, Kerr Kaviton LC), introduced during the early nineties showed improved mechanical and physical properties compared to the conventional cements and higher resistance to early moisture contact and desiccation. RMGIC is preferred because of better marginal adaptation due to its chemical adhesion and low polymerization shrinkage and thus less microleakage.[10,11]

GC Fuji IX is an advanced radioopaque high strength packable posterior self-curing glass ionomer can be used in open sandwich technique because of strong chemical bonding, good compressive and bond strength. It also provides micromechanical interlocking via the formation of hybrid layer and resin tags between highly viscous glass ionomer cement and conditioned dentin surface.[12]

Contemporary dentine substitutes like Pro Root Mineral Trioxide Aggregate (MTA) can also be used as a dentine substitute under restorations. However, mechanical properties and long setting time of Pro Root MTA (2.75 hrs) restricts its clinical use as a dentine substitute in open sandwich restorations. Recently, various new commercial calcium silicate materials have been introduced including Biodentine, Bio Aggregate, Doxadent (Doxa; Uppsala, Sweden), MTA Angelus and MTA Plus. A new Ca_3SiO_5 based cement, Biodentine (Septodont; St-Maur, France), is a biocompatible, bioactive material which may stimulate dentin regeneration by inducing odontoblast differentiation from pulp progenitor cells. According to the manufacturer, its setting characteristics and mechanical behaviour make it suitable as a dentine substitute for direct posterior restorations.[1,12,13]

The mechanical properties and abrasion resistance of resin-based composites have improved considerably over the years. Recently, a new brand of composite resins nano filled composites or Nano Hybrid Universal restorative composites (FiltekTM Z250 XT, 3M ESPE) with single bond universal adhesive, nanofiller technology and formulated with nanomer and nanocluster filler particles has been developed. This combination reduces the interstitial spacing of the filler particles and gives the restoration better wear resistance and a surface that is easier to polish for good esthetic results.[2,14,15]

Over the past few years there have been efforts to investigate microleakage three dimentionally by using confocal microscopy, fluid filtration and micro CT to detect bond failure at enamel-resin interface. Confocal laser scanning microscope is a non-destructive 3-D imaging technique which offers several other advantages over conventional optical microscopy. This type of microscopy enables high-resolution images to be made of samples with minimum requirements for specimen preparation. For all these reasons, this technology was utilized for microleakage testing of the current study. Different leakage tracer dyes are used to assess microleakage. Rhodamine B5 is better than other dyes because of greater microleakage marker capacity, deeper dye penetration depth, small particle size and hard tissue non-reactivity.[16,17]

The clinical success of newer materials which shrinks minimally and bonds best to tooth structure with least microleakage especially at the cervical margins still continues. Thus the purpose of this in-vitro study was to microleakage evaluation with three different base materials using RMGIC, GC Fuji IX and Biodentine with Nanohybrid composites used in open sandwich technique for Class II composite restorations using dye penetration under Confocal microscopy.

MATERIALS AND METHODS

Sample collection:

Non carious freshly extracted 60 intact human maxillary premolars of approximately same dimensions were collected from the Department of Oral and Maxillofacial Surgery of SDDHDC, Panchkula & nearby clinics and all the selected teeth were cleaned, washed, polished with pumice and stored in distilled water.

Figure 1: Sample collection, tooth preparation and restoration

Grouping and restorative procedure of samples:

Tooth Preparation:

Standardized Class II cavities were made involving the proximal and occlusal surfaces using No. 245 and 169 L tungsten carbide burs in a high speed air rotor with water spray. The overall dimensions and depth of cavities were standardized as follows: Pulpal Depth: 2.5 mm, Bucco-Lingual Width: 2mm, Axial Wall Height: 3mm till CEJ, Gingival Seal: 0.8 mm into dentin at CEJ. [Figure 1]

GROUP 1- Bonding agent (ScotchbondTM Universal Adhesive 3M ESPE St Paul, MN, USA) was applied on 15 Class II cavities with a microbrush for 35 sec, thinned by mild air pressure for 5 sec, and then polymerized for 20 sec. In each sample tofflemire metal matrix band and wedge was applied and restored with Filtek™ Z250 XT Nanohybrid composite (3M ESPE, ST Paul, USA) in increments gingivo-occlusally (each increment being 2 mm). Each layer or increment was polymerized for 40 sec from the occlusal surface with a LED light curing unit (Celalux 2 High-Power LED curing-light, Voco GmbH, Cuxhaven, Germany) at a light intensity of 1000 mW/cm2 according to manufacturers' instructions. Bonding agent (ScotchbondTM Universal Adhesive 3M ESPE St Paul, MN, USA) was applied on 15 Class II cavities with a microbrush according to the manufacturer's instructions, especially on enamel edges. It was applied for 35 sec, thinned by mild air pressure for 5 sec, and then polymerized for 20 sec. [Figure 2]

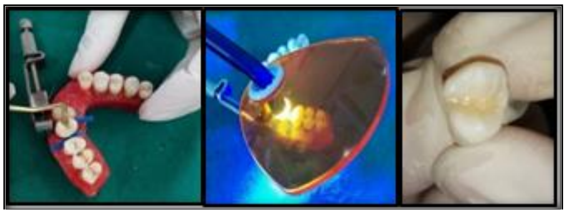

Figure 2: Different steps in restorative procedure

GROUP II- 15 Class II cavities were restored with an intermediary layer of self-curing glass ionomer cement (Fuji IX, GC, Tokyo, Japan) in the gingival half of the proximal box (Open Sandwich Technique). The rest of the cavity was then restored with Filtek™ Z250 XT Nanohybrid composite resin (3M ESPE, ST Paul, USA) in an incremental manner as described for Group 1.

GROUP III- 15 Class II cavities were restored with an intermediary layer of RMGIC (Fuji II LC, GC America Inc., Alsip, IL, USA) in a horizontal increments in the gingival half of the proximal box (Open Sandwich Technique). It was polymerized for 20 seconds using a LED curing light. The rest of the cavity was then restored with Filtek™ Z250 XT Nanohybrid composite resin (3M ESPE, ST Paul, USA) in an incremental manner as described for Group I.

GROUP IV- Biodentine (Septodont, Saint-Maur-des-Fosses Cedex, France) was mixed according to manufacturer's recommendations. An intermediary layer of Biodentine was placed in 15 Class II cavities in the gingival half of the proximal box (Open Sandwich Technique). After 12 minutes the prepared cavity was restored with Filtek™ Z250 XT Nanohybrid composite resin (3M ESPE, ST Paul, USA) in an incremental manner as described for Group I.

Finishing and polishing of samples: The finishing and polishing of all restorations were achieved with the sequential use of finishing and polishing burs, Diamond or Aluminum oxide discs, rubber cups, wheels and pastes. Proximal margins were finished with Sof-Lex discs (3M ESPE, St. Paul, MN, USA).

Thermocycling: The teeth were stored in isotonic saline solution at 37°C water bath for 24 h. The samples were subjected to thermocycling in customized thermocycling device i.e. 500 thermal cycles at 5°C, 37°C and 55°C in water with a dwell time of 30 sec and transfer time of 15 seconds. [Figure 3]

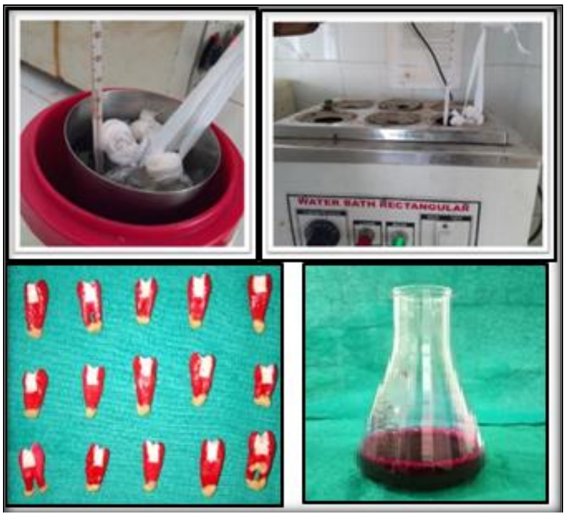

Figure 3: Cold and hot water bath for thermocycling

Fig 4: Coating the teeth with varnish, sealing of the apices with Sticky wax and samples soaked in 0.25% Rhodamine B dye

Apices of all the teeth were sealed with sticky wax and two coats of Nail varnish were applied on the entire sample surface except for the restoration and 1mm area beyond the margins of restoration to limit dye penetration to cavity margins. Further the samples were soaked in freshly prepared 0.25% Rhodamine B Fluorescent dye for 24 hours. The teeth were removed from the staining solutions and washed under running water for 5 minutes and dried at ambient temperature for 24 hours to allow the dyes to fix. All the samples were then sectioned in Mesio-Distal dimension longitudinally from the centre of the restoration with diamond coated disc mounted in straight hand piece under constant water flow. [Figure 4,5]

Figure 5: Z- Stack sections of samples seen under Confocal Microscope

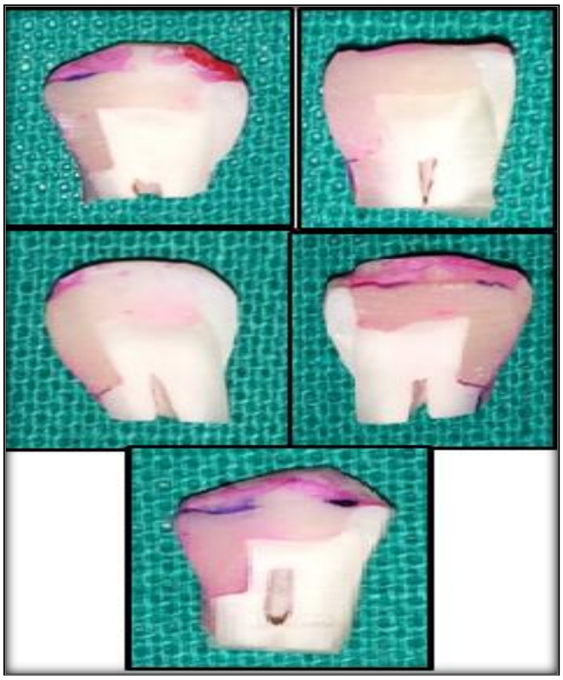

Figure 6: Confocal 3-D Images: Scoring Criteria from 0-4 in which Red colour depicts the amount of leakage demarcated by Yellow line

Microleakage Evaluation: Depth of microleakage was noted & tabulated. Dye penetration was assessed according to the scoring criteria given by Leevaloj C, Cochran MA et al.

Scoring Criteria for Assessing Dye Leakage at the Gingival and at the Junction of Restorations

Score 0: perfect adaptation/no dye penetration at tooth restoration interface

Score 1: dye penetration upto 1/3rd of gingival wall

Score 2: dye penetration upto 2/3rd of gingival wall

Score 3: dye penetration all along the gingival wall

Score 4: dye penetration along the axial wall towards the pulp [Figure 6]

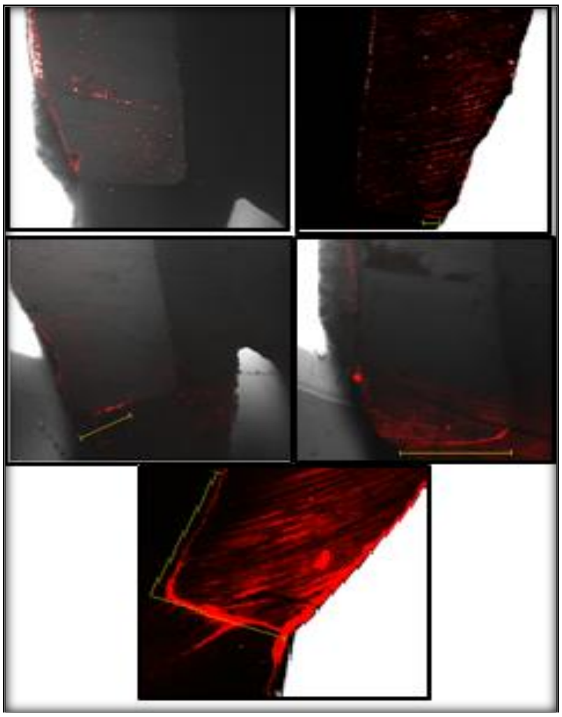

Figure 7: ?

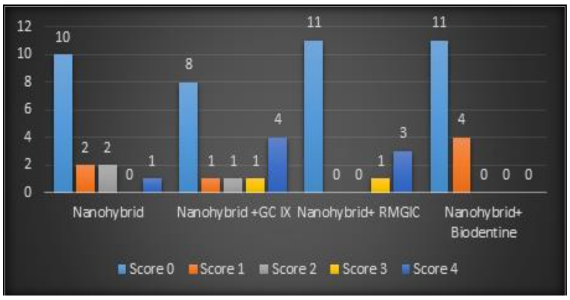

Table 1: Microleakage in different restoration groups (Gingival Level)

The highest of microleakage scores were considered to characterise a given restoration. Depth of penetration was evaluated using NIS - Elements D (Advance Research Microscope Imaging Software) BR Ver4.13.05 32 bit edition. The tabulated results of microleakage were then put to statistical analysis using One-way ANOVA, Post Hoc multiple comparison test and t Test.

RESULTS

The present study was conducted to compare microleakage with three different base materials used in open sandwich technique for Class II composite restorations under confocal microscopy.

Analysis of Variance (ANOVA) carried out among the different restorative materials at the gingival level. The analysis revealed significant differences between Nanohybrid + Biodentine and Nanohybrid + RMGIC. Further, after applying a post-hoc test [Fisher's LSD (Least Significant Difference)], it was revealed that the material causing the least microleakage are ranked according to superiortity is NH+Biodentine > NH+RMGIC > Nanohybrid > NH+GCIX.

Analysis of Variance (ANOVA) carried out among the different restorative materials at the junctional level revealed significant differences between Nanohybrid + RMGIC. After applying a post-hoc test [Fisher's LSD (Least Significant Difference)], it was revealed that the material causing the least microleakage are ranked according to superiortity is Nanohybrid +RMGIC > Nanohybrid + GC IX > Nanohybrid + Biodentine.

Table 2: Analysis of Variance (ANOVA) among the different restorative materials at the gingival level

ANOVA (Gingival Level)		Sum of Squares	df	Mean Square	F	Sig.	Post-Hoc Fishers Least Significant Test
Nanohybrid	Between Groups	12.333	3	4.111	1.354	.370	Nanohybrid +Biodentine > Nanohybrid +RMGIC > Nanohybrid > Nanohybrid + GC IX
	Within Groups	33.400	11	3.036			
	Total	45.733	14				
Nanohybrid +RMGIC	Between Groups	8.400	3	2.800	.917	.045*	
	Within Groups	33.600	11	3.055			
	Total	42.000	14				
Nanohybrid + GC IX	Between Groups	4.833	3	.278	1.455	.641	
	Within Groups	54.100	11	.191			
	Total	4.947	14				
Nanohybrid +Biodentine	Between Groups	.833	3	.278	1.455	.000*	
	Within Groups	1.997	11	.191			
	Total	2.567	14				

Table 3: T-test for comparison between groups (gingival level)

Paired Samples t- Test (Gingival Level)		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Standard Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NHGS – NHRMGICGS	-.800	2.336	.603	-2.094	.494	-1.326	14	.026*
Pair 2	NHGS - NHGCIXGS	-.333	1.718	.444	-1.285	.618	-.751	14	.465
Pair 3	NHGS - NHBGS	.400	1.056	.273	-.185	.985	1.468	14	.014*
Pair 4	NHGCIXGS - NHRMGICGS	.467	2.669	.689	-1.011	1.945	.677	14	.509
Pair 5	NHGCIXGS - NHBGS	1.200	2.007	.518	.088	2.312	2.316	14	.365
Pair 6	NHRMGICGS - NHBGS	.733	1.668	.431	-.190	1.657	1.703	14	.111

Application of paired samples t-test used at the Gingival level, a significant difference was seen between Nanohybrid and Nanohybrid + RMGIC restorative material (p=0.026) and between Nanonhybrid and Nanohybrid + Biodentine (p=0.14).

DISCUSSION

Dental composite restorative resins have been available for nearly 50 years. Their specific use in posterior teeth was introduced in early 1980s and has become increasingly popular in restorative dentistry. Recently, improvements in adhesive systems and properties of resin composites with increasing esthetic demands by patients have increased the use of composites instead of amalgam on the posterior segment. Adequate adhesion and endurance (longevity) of dentin bonding have always been the topic of interest for dentistry. This is mainly because imperfect bonding leaves a microscopic gap that allows the infiltration of bacteria, fluids, molecules and ions between the restoration and the tooth structure, commonly referred to as microleakage.[14]

Margins that are placed apical to the cementoenamel junction, on dentin or cementum are more problematic because of a very thin layer of enamel is present in the gingival margin for bonding. Lokhande et al in 2014 stated that polymerization shrinkage of conventional composite causes stresses of approximately 18 MPA. These stresses challenge the integrity of resin-dentine bond and tend to pull the resin from the dentine, leading to formation of marginal gaps.[18] To overcome the problems associated with shrinkage stresses, modified techniques like three-sited curing technique, directed shrinkage polymerization technique and sandwich restorations (open or close) have been recommended. Use of an incremental liner of GIC, RMGIC or calcium silicate cement has been suggested to overcome the problems associated with polymerization shrinkage. Karaman et al (2013), Ozgunaltay (2011) and Kasraei et al (2011) advocated the placement of liners in Class II cavities.

So in the present study we evaluated the in vitro microleakage of open sandwich restorations based on calcium silicate-cement Biodentine TM, Resin-modified glass ionomer GC fuji II LC and GC fuji IX with that of Nanohybrid composite under Confocal laser scanning electron microscopy (CLSM). P values ≤ 0.05 found after statistical analysis are considered to be statistically significant in all the tests.

The results of our study showed that microleakage was minimum with (Group IV) Nanohybrid with Biodentine and maximum with Group II (Nanohybrid with GC fuji IX) when gingival margin is at CEJ in Class II restorations as compared to other groups. The glass ionomer maintains its bulk volume through internal microcracks. With water sorption, the cracks close to repair cohesive strength, and the dimensional stability of glass ionomer cement is maintained, resulting in excellent adaptation with tooth structure. In in vitro condition, absence of water and lower cohesive strength can alter the properties of glass ionomer cement, which may have resulted in leakage in the present study.

The analysis revealed significant differences between Biodentine and GC fuji IX. It was revealed that the material causing the least microleakage are ranked according to superiortity is Nanohybrid+Biodentine > Nanohybrid +RMGIC > Nanohybrid > Nanohybrid +GCIX. The results of Group IV (p=0.00) and Group III (p=0.045) were statistically significant as compared to Group I and Group II.

When microleakage was evaluated among the different restorative materials at the junctional level, analysis revealed significant differences between Group III- Nanohybrid with RMGIC than Group II (Nanohybrid with GC fuji IX) and Group IV (Nanohybrid with Biodentine). It was revealed the material causing the least microleakage and ranked according to superiortity is Nanohybrid +RMGIC > Nanohybrid + GC IX > Nanohybrid + Biodentine. The significance (p) values of Group III (p=0.005) were statistically significant as compared to Group II and Group IV.

In this study when two materials were compared to check microleakage at the gingival level, a significant difference in microleakage score between Nanohybrid and Nanohybrid + RMGIC (p=0.026) restorative material and between Nanonhybrid and Nanohybrid + Biodentine (p=0.014) was found. The results indicate better prevention of marginal leakage among RMGIC and Biodentine when used with Nanohybrid Composite. In our current study, when two materials were compared at the junction of restorations there was significant difference seen between Nanohybrid with GC Fuji II LC; RMGIC base material as compared to Nanohybrid with GC IX (p=0.049). The results indicate better prevention of marginal leakage among Nanohybrid with RMGIC as compared to Biodentine and GC IX. Also, no significant difference was seen between Biodentine & GC fuji IX and between RMGIC & Biodentine. RMGIC; GC Fuji II LC owing to its adhesive properties like GIC and good wear resistance like composite may be the reason for the better performance. Moreover Fuji II LC; RMGIC has rigid framework and less capability of elastic deformation at the initial stage of polymerization and chemical bonding with dentine which is not satisfactory with composite restorations.

It can be concluded from the results of the present study that materials used for Class II cavities restoration should not be affected by moisture contamination. Group IV (Nanohybrid with Biodentine) showed significantly minimum microleakage and Group II (Nanohybrid with GC Fuji IX) showed significantly maximum microleakage when cervical margins were at CEJ. Group III (Nanohybrid with RMGIC) showed significantly minimum microleakage and Group II (Nanohybrid with Biodentine) showed significantly maximum microleakage when margins were at the junction of restorations.

An important point to be noted is solubility of biodentine due to pronounced ion release. However the deposition of substances such as hydroxyapatite on the material surface when it comes in contact with tissue fluids will compensate for that release.[19,20] Still its application as a restorative material is challenging. As our study was in vitro study and in order to achieve a valid comparison, the experimental protocol must reproduce optimal clinical conditions. Thus, for clinical relevance, further in vivo studies with a larger sample size are required.

CONCLUSION

Within the limitations of the present study, it can be concluded that:

1. Both Biodentine and GC Fuji II LC; RMGIC were more effective in sealing the gingival margins of Class II open sandwich restorations as compared to GC Fuji IX and Nanohybrid composite resin.
2. Recent calcium silicate cement i.e. Biodentine turns to be the best for sealing Class II gingival margins than rest of the groups and showed minimum microleakage.

3. GC Fuji II LC; RMGIC proved to be the best in sealing margins at the junction of restorations as compared to Biodentine and GC Fuji IX.
4. No material was able to totally eliminate microleakage in Class II restorations with gingival margin ended in dentine.

   A definite conclusion as to which material should be preferred among GC Fuji IX, GC Fuji II LC (RMGIC) and Biodentine, can be withdrawn after correlating the findings of the present study with clinical research. Therefore more studies are advocated.

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