Dental Research Journal

Original Article

Microbial microleakage assessment of class V cavities restored with different materials and techniques: A laboratory study

Hossein Nematollahi[1], Ali Bagherian[1], Kiarash Ghazvini[2], Habibollah Esmaily[3], Mina Azadegan Mehr[4]

[1]Dental Research Center, School of Dentistry, Mashhad University of Medical Sciences, [2]Antimicrobial Resistance Research Center, Medical School, Mashhad University of Medical Sciences, [3]Health Sciences Research Center, Medical School, Mashhad University of Medical Sciences, Mashhad, [4]Department of Pediatric Dentistry, School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

ABSTRACT

Background: The aim of this study was to compare microbial microleakage of class V cavities restored with different materials and techniques using a microbial leakage assessment method.

Materials and Methods: One hundred extracted, caries-free, human maxillary premolars were randomly divided into five groups. Group 1: Resin-modified glass ionomer (RMGI), Group 2: Closed sandwich with flowable composite + nanohybrid composite, Group 3: Nanohybrid composite, Group 4: Closed sandwich with RMGI + nanohybrid composite, and Group 5: Flowable composite + nanohybrid composite that were co-cured together ("snow-plow" technique). A microbial penetration method utilizing *Streptococcus mutans* as an indicator was tested for leakage assessment. Data were analyzed and the significance level was α =0.05.

Results: The log-rank test indicated a statistically significant difference in leakage rates among the five groups (P = 0.008). Mantel–Cox log-rank test indicated statistically significant differences in microleakage rates between Groups 1 and 3 (P = 0.029), between Groups 2 and 5 (P = 0.005), and between Groups 3 and 5 (P = 0.002).

Conclusion: With respect to the limitations of an *in vitro* study, our findings suggest that adding a thin layer of flowable composite or RMGI under nanohybrid composite in class V cavities did not decrease the bacterial leakage rate, whereas use of the "snow-plow" technique caused an increase in the microleakage rate.

Key Words: Dental leakage, Dental restoration, Filtek Z250 composite resin, fuji glass-inomer lining cement

Received: January 2017
Accepted: July 2017

Address for correspondence:
Dr. Ali Bagherian, Department of Pediatric Dentistry, School of Dentistry, Park Square, Mashhad, Iran.
E-mail: drbagherian@yahoo.com

INTRODUCTION

Cervical lesions due to early childhood caries, adult caries, or erosion present a special challenge to any pediatric and restorative dentist, because in such cavities, the restorative material is usually required to adhere to dentin or cementum in the cervical margin of a class V cavity.[1,2] Resin-based composites are widely used for restoring cervical lesions, as they are esthetically pleasing and bond to the tooth structure.[3] Restoring a cervical lesion with resin composites has always been a challenge, particularly where no enamel is present for efficient bonding at the gingival margin to counteract polymerization shrinkage, temperature variables, and masticatory forces. Thus, microgaps

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Nematollahi H, Bagherian A, Ghazvini K, Esmaily H, Mehr MA. Microbial microleakage assessment of class V cavities restored with different materials and techniques: A laboratory study. Dent Res J 2017;14:344-50.

created at the gingival margins cause marginal microleakage, which is defined as the transport of bacteria, fluids, molecules, or ions through the tooth tissue-restoration interface, leading to recurrent caries, pulpal irritation, postoperative sensitivity, etc.[3-6]

A thin layer of flowable composite or glass-ionomer under the main part of restoration (sandwich technique) as an elastic buffer or a shock absorber and to reduce the C-factor of a class V cavity has been used in several studies, but with different conflicting results in their ability to reduce microleakage.[3,7-14]

Applying the "snow plow" technique, the use of flowable composite under the main composite and co-curing them together, is another clinical approach that has been advocated for polymerization shrinkage control; however, the results of the studies are conflicting.[6,15-17]

Glass ionomer cements (GICs) are alternative materials to composites for cervical lesions because of their chemical adhesion to tooth structure, fluoride release, lower shrinkage values, and nearly acceptable esthetics. Resin-modified glass ionomer (RMGI) cements were introduced to overcome the problems of moisture sensitivity and low early mechanical strengths associated with the conventional GIC in restoration treatments.[1,18-20]

Many microleakage measurement methods have been tested and performed over the years. Although the color dye penetration method has been used as the gold standard in most leakage studies, this method has two major disadvantages. The first is that it provides only a limited number of sections for leakage assessment, possibly leading to leakage underestimation. The second is that organic dyes, such as methylene blue and Rhodamine-B, have a smaller molecular size than average-sized bacteria, possibly leading to leakage overestimation.[21-24]

The microbial penetration method introduced by Bagherian *et al*.[25] for coronal restoration leakage assessment is used for this study, which uses bacteria (as the main etiologic cause of dental caries) as indicators of leakage and thus, may mimic oral conditions more accurately.

The purpose of this study was to compare microleakage of class V cavities restored with different materials (resin composites and RMGI) and techniques (conventional sandwich technique and snow plow technique) using a microbial leakage assessment method to find the method with less microleakage for treating the cervical lesion of teeth.

MATERIALS AND METHODS

This laboratory study was conducted using 100 freshly extracted human maxillary premolar teeth and its protocol was approved by the Ethics Committee of the Mashhad University of Medical Sciences. Inclusion criteria included sound, caries-free, and restoration-free maxillary premolars extracted for orthodontic purposes, whereas teeth with the presence of carious lesions, fractures, cracks, and hypoplasia were excluded. Teeth were disinfected in 5.25% sodium hypochlorite (Golrang, Tehran, Iran) for 15 min, rinsed under tap water for 1 min, and stored in distilled water at room temperature for 12 h prior to be used.

The teeth were cut with a long diamond bur (Tees Kavan Co, Ltd., Tehran, Iran) 5 mm apical to the cementoenamel junction, after which a K-file and 5.25% sodium hypochlorite were used to remove the soft tissue from the coronal pulp chamber. In each tooth, a box-shaped class V cavity with the dimensions of 3.0 mm (mesiodistal), 3.0 mm (occluso-gingival), and 1.5 mm depth at the occlusal margin and 0.75 mm depth at the cervical margin was prepared in the buccal surface. The cervical margin was located 0.5 mm apical to the cementoenamel junction (on dentin/cementum). For cavity preparation, diamond burs with 1-mm diameter (Tees Kavan Co., Ltd) in a high-speed handpiece and copious amount of water as coolant were used. Each bur was used for five preparations and then replaced by a new one. The depth of cavities was standardized to the nearest millimeter using a periodontal probe. A thin fissure bur (0.6 mm in diameter) was used to establish a channel from the midgingivoaxial line angle surface to the pulp cavity, followed by application of a thin layer of blue inlay wax (Azar Teb, Tabriz, Iran) to seal the entrance of the channel to prevent restorative material penetration. Prior to restorative treatment, a random sequence generation was performed at www.randomizer.org to obtain a series of randomized real numbers corresponding to teeth 1-100 and then they allocated into five groups (20 per group).

In Group 1 (RMGI), all the cavity surfaces were conditioned for 10 s using 20% polyacrylic acid conditioner (GC America Inc., Alsip, IL., USA),

rinsed and dried gently without desiccation, and restored with Fuji II RMGI (GC America Inc.).

In Groups 2 (closed sandwich with flowable composite) and 3 (nanohybrid composite), 35% phosphoric acid (3M ESPE, St. Paul, Minn., USA) was used for cavity etching. The etching, rinsing, and drying periods were 20 s each. Adper Single Bond (3M ESPE) as an adhesive system was applied in accordance with the manufacturer's instructions. In Group 2, a thin layer of Filtek Z350 (3M ESPE) was placed in axial and gingival walls of the cavity and cured for 20 s, and then nanohybrid Filtek Z250 XT (3M ESPE) composite was applied in a bulk increment and cured for 20 s to restore the cavities. In Group 3, the cavities were completely restored in a bulk increment with Filtek Z250 XT (3M ESPE) and cured for 20 s.

In Group 4 (closed sandwich with RMGI), the axial and gingival walls of the cavities were conditioned for 10 s using the same polyacrylic acid conditioner, rinsed and dried gently without desiccation, and a thin layer of Fuji II RMGI was placed on these walls and cured for 20 s. Then, Filtek Z250 XT composite was applied in a bulk increment and cured for 20 s after the same etching, rinsing, drying, and priming as in Group 2.

In Group 5 ("snow-plow" technique), the same etching, rinsing, drying, and priming as those in Groups 2 and 3 were applied. A thin layer of Filtek Z350 XT (3M ESPE) was placed in axial and gingival walls of the cavity without curing and then nanohybrid Filtek Z250 XT composite was applied in a bulk increment to fill the cavity and they were co-cured (light cured simultaneously) for 20 s.

An LED KY-L036 light-curing unit (Foshan Cicada Technology Development Co. Ltd., Guangdong, China) with 1700 mW/cm^2 output and a wavelength of 430–485 nm was used for all curing procedures. The tip of the light was held as closely as possible to the applied materials without actually touching them. All the preparation and restoration procedures were performed by the same operator.

The inlay wax was washed out through the pulp chamber using boiling water. To seal the probable existing microscopic defects around the crown of the tooth sample, the restored area and 1 mm beyond it were covered with a piece of pink wax and the rest of the crown was etched and sealed with a thin layer of Masterdent sealant (Dentonics, Inc., Monroe, NC, USA). To increase the root length, a sectioned capillary tube was next inserted into the pulp canal cavity through the root and stabilized with inlay wax.

The root portion of each specimen, including the capillary tube, was embedded in one end of a plastic microtube (Sigma-Aldrich, Darmstadt, Germany) until it reached 2 mm under the gingival border of class V restoration and stabilized with the wax. Then, for preventing unwanted bacterial leakage, the epoxy resin glue (Donyaychasb, Mashhad, Iran) was poured with a syringe into the microtube around the capillary tube and allowed to set for 24 h. These assemblies were coded according to the tooth and group numbers, packed, and sterilized for 12 h with ethylene oxide using Anprolene N74i (Andersen Products Inc., Haw River, NC, USA). On the other side, plastic centrifuge falcon tubes, as container of brain–heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany), were sterilized in a vertical-type 75 steam sterilizer (Zaim Teb, Tehran, Iran) at 121°C for 20 min. Then, under laboratory conditions, with sterile gloves and near a flame, sterile BHI was poured into a falcon tube and the crown occlusal portion of the specimen was inserted through a foramen into the head of falcon tube containing sterile BHI broth until the broth was at least 2.0 mm above the gingival border of the class V cavity. The assembly used to assess microbial leakage in this investigation is depicted in Figure 1.

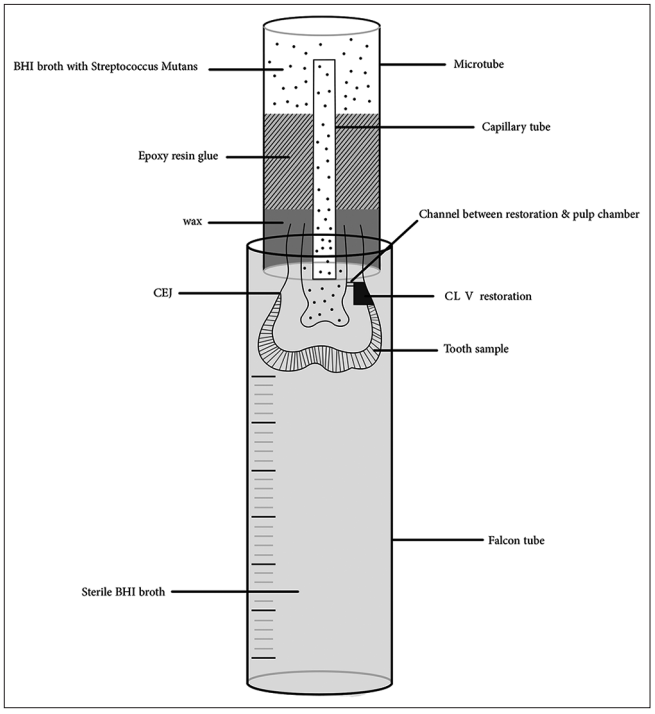

Figure 1: The assembly used for microbial leakage assessment of class V restorations.

Specimens were placed in five laboratory tube racks, one for each group, and were then incubated at 37°C for 40 days, and 0.5 ml of BHI broth containing approximately 1.5×10^8 *Streptococcus mutans* (PTCC 1683)/ml was introduced into the other end of the microtubes with a sterile syringe to reach through the capillary tube under the restoration. Fresh microorganisms were added to the tube at daily intervals. Bacteria migrated through the capillary tube and the channel established between the pulp chamber and midaxiogingival surface, subsequently reaching the tooth-restoration interface. Daily examination was performed by a dentist blinded to the different groups for bacterial leakage, as evidenced by turbidity in BHI broth in the centrifuge tube surrounding the tooth-restoration interface.

To verify that the cause of turbidity was *S. mutans*, all turbid samples were streaked on blood agar culture plates, where streptococci were identified and confirmed by colony morphology and Gram staining under microscopy.

To ensure that the bacteria were viable and capable of inducing turbidity in the BHI broth, a positive control assembly was used in conjunction with the other specimens, but without any restoration material on the class V cavity so that bacteria could easily pass through the channel into the broth. To ensure that bacteria could not pass through the glue or any other part of the assembly (with the exception of the tooth-restoration interface), the same tooth without any class V cavity represented the negative control specimen.

The number of days required for turbidity to appear was recorded as an indication of microleakage. Data from observations of the centrifuge tube broth were recorded daily for 40 days and analyzed using SPSS 20.0 software (SPSS Inc., Chicago, Ill, USA). Leakage rates were estimated using the nonparametric Kaplan–Meier product-limit method, and mean leakage time in days was estimated for all groups. Bacterial leakage rates were compared by log-rank test across the five experimental groups. Pairwise comparisons over strata were subsequently performed using the Mantel–Cox log-rank test with a Bonferroni correction. Results with $P < 0.05$ were considered statistically significant.

RESULTS

The positive control exhibited microleakage on the first day of the investigation, whereas the negative control showed none throughout the investigation period (40 days). All the specimens of the five groups indicated microleakage by 40 days. Table 1 summarizes the mean day of leakage occurrence in the five experimental groups.

Figure 2 depicts the Kaplan–Meier plot of microleakage occurrence rates across the five study groups over the 40 days of the investigation. As can be seen in the figure, the highest microleakage rate occurred in Groups 5 and 1 and the lowest in Groups 2 and 3. The overall log-rank test suggested a statistically significant difference between the five groups ($P = 0.008$). The Mantel–Cox log-rank test showed statistically significant differences in microleakage rates between Groups 1 and 3 ($P = 0.029$), between Groups 2 and 5 ($P = 0.005$), and between Groups 3 and 5 ($P = 0.002$), but no significant difference between Groups 2 and 3 ($P = 0.336$) or between Groups 3 and 4 ($P = 0.342$). In addition, microleakage rates were lower in Groups 2 and 4 than in Group 1; however, the difference was not statistically significant ($P > 0.05$).

DISCUSSION

Since the beginning of dentistry, many researches had been done to overcome the problem of microleakage in dental restoration, especially in class V cavities,

Table 1: Frequency distribution and the mean days of microleakage occurrence in the five experimental groups over a 40-day period

Groups	Sample size	Minimum days of microleakage occurrence	Maximum days of microleakage occurrence	Mean days of microleakage occurrence±SD	95% CI
Group 1: RMGI	20	1	20	8.15±6.87	5.09-11.21
Group 2: Closed sandwich with flowable composite	20	2	22	12.35±6.37	9.50-15.20
Group 3: Nanohybrid composite	20	1	40	14.30±11.23	9.28-19.32
Group 4: Closed sandwich with RMGI	20	1	40	10.60±11.35	5.52-15.68
Group 5: “Snow plow”	20	1	20	5.70±5.24	3.36-8.04

RMGI: Resin-modified glass ionomer; SD: Standard deviation; CI: Confidence interval

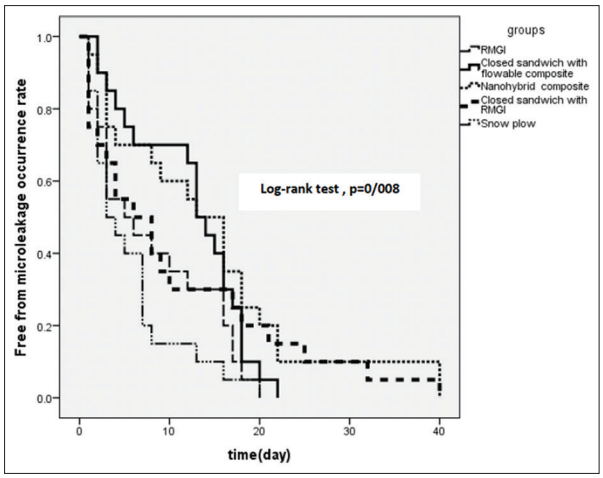

Figure 2: Microleakage occurrence rates across the five study groups over the 40 days of the investigation. Group 1: Resin-modified glass ionomer; Group 2: Closed sandwich with flowable composite + nanohybrid composite; Group 3: Nanohybrid composite; Group 4: Closed sandwich with resin-modified glass ionomer + nanohybrid composite and Group 5: Flowable composite + nanohybrid composite that co-cured together ("snow-plow" technique).

but until today, there have been no dental restorative materials or techniques that can truly eliminate microleakage.[4]

Considering the limitations of an *in vitro* study (e.g., maintaining teeth in a storage medium for different lengths of time and our lack of tooth thermo- and load-cycling equipment), the results of this study indicated that the nanohybrid composite and sandwich groups had the least microbial microleakage and adding a thin layer of flowable composite or RMGI under the nanohybrid composite did not improve the leakage rate.

In theory, it seems that using a liner of flowable composite or RMGI with more wetability that causes better adaptation of restoration to cavity walls, and also with reduction of the C-factor, will lead to fewer cavity wall stresses, and subsequently less microleakage, as several studies have shown.[3,6,9,14,17,26] In contrast, some studies, including the present one, have indicated that the use of flowable material as an intermediate layer does not reduce microleakage in composite restorations.[7,10,27,28] They have explained that flowable composites have higher polymerization shrinkage due to their lower filler content and this may disrupt the bond to the cavity walls. In this study, it also can likely be attributed to the lower degree of polymerization shrinkage of the nanohybrid composite used (Filtec Z-250 XT). The manufacturer has claimed that because of the replacement of some molecules of tetraethyleneglycol dimethacrylate with polyethyleneglycol dimethacrylate in the resins and the use of different filler particle sizes; this composite has less polymerization shrinkage.[29] Thus, it is possible that, for this reason, it has shown less microleakage, even without a liner under it.

In the present study, the "snow-plow" technique group showed an increased microbial microleakage rate. Some other studies have shown the same results.[16,17,26,27] They showed that use of flowable composite light cured simultaneously with the main composite did not improve marginal sealing, in contrast with other studies, which hypothesized that co-curing the flowable liner and the overlying composite together would help to improve the penetration of uncured liner and leads to improved sealing at the margin due to hydraulic pressure of the overlying viscous composite.[6,15] This may be explained by displacement of flowable composite into the main bulk of the composite, which leads to a heterogeneous increase of resin contents of the main bulk of the composite restoration and subsequently, an increase of polymerization shrinkage and microleakage. This may also be attributed to polymerization shrinkage of the overlying composite, which causes contraction forces and dislodges the bond of uncured flowable composite liner from the cavity walls.

Our findings indicated that, except for the "snow-plow group," the resin composite groups had less microleakage than the RMGI cement group, in accordance with studies by Parolia and Prabhakar *et al*.[22,30] In contrast, a meta-analysis of the clinical trials on tooth-colored materials in restoration of a class V cervical lesion indicated that, according to the retention aspect, GIC showed the best results in the mouth.[31] The superior performance of GICs in the mouth compared with laboratory conditions can be explained by their closer modulus of elasticity and linear thermal coefficient to tooth structure in comparison with the composite.[3] The presence of more leakage in the glass ionomer group in our laboratory study may be explained by a lack of load- and thermo-cycling and a lack of load cycling in the Parolia and Prabhakar studies, respectively, which could have had more pronounced adverse effects on the composite groups than on the glass ionomer group because of their larger differences in modulus of elasticity and linear thermal coefficient to tooth structure.

Many microleakage measurement methods have been tested and performed over the years, such as dye penetration, bacterial leakage, electrochemical methods, fluid filtration, and micro-computed tomography. Although the color dye penetration method has been used as the gold standard and most widely used because of ease of use, and convenience, this method has major disadvantages.[21,22,25,32,33]

In the present study, a coronal microbial penetration method was used for class V restoration leakage assessment. The dye penetration method is a destructive method, and the tooth requires sectioning for leakage assessment, while the microbial penetration method is nondestructive and reassessment of leakage is possible. Data on the microbial penetration method are quantitative (number of days elapsing before turbidity can be observed). This method uses cariogenic bacteria instead of color dye, which may mimic oral conditions more accurately. This method also has several disadvantages, such as a relatively long duration and the need for daily checking of the samples and confirming of penetrated bacteria by colony morphology and Gram staining under microscopy. Furthermore, this method can only evaluate bacterial passage through the tooth-restoration interface, and not that of bacterial metabolites, toxins, fluids, or ions that are defined within the term "microleakage." In addition, the properties of BHI broth do not completely mimic the oral cavity condition regarding defensive chemical components of saliva.[25]

In this study, all the restorations showed bacterial leakage by 40 days. This result can be attributed to the daily exposure of the restoration to high concentrations of fresh bacteria that finally passed through the tooth-restoration interface specifically from the shallower and weaker adhesion of the gingival margins of the class V cavities. In a study by Bagherian *et al*.[25] on microbial leakage assessment of fissure sealant restoration, some samples did not show leakage until the end of the follow-up period (60 days). There are two possible explanations for this. First, in fissure sealant treatment, the composite resin sealant material made a strong adhesion with the enamel. The second is that they refreshed bacteria every 5 days that may affect the available vital and motile bacteria that should pass tooth-sealant interfaces.

Bagherian *et al*. used a steam sterilizer for sterilization of the tooth assemblies in their sealant studies of the tooth-sealant interface on enamel. However in the present study, because of concerns about the adverse effect of heat on the dentin of a class V cavity, as in many other leakage studies, ethylene oxide gas sterilization was used. The carcinogenic and mutagenic nature of this gas and the need for 10 to 16 h sterilization periods are other disadvantages of this method.[25]

The authors of this study tried, in a pilot study, to use BHI with *S. mutans* from the buccal surface of the restoration instead of the pulp chamber side (as it is more similar to mouth conditions). However, the short length of the remaining root could not induce the proper attachment to prevent leakage. Therefore, we used this type of assembly, and because the bacteria should cross the entire tooth-restoration interface, it seems that there is no difference between sides.

CONCLUSION

Considering the limitations of an *in vitro* study, our findings suggest that adding a thin layer of flowable composite or RMGI under nanohybrid composite in class V cavities did not decrease the bacterial leakage rate, whereas use of the "snow-plow" technique caused an increase in the microleakage rate.

Financial support and sponsorship

This study was supported by a grant from Mashhad University Research Council.

Conflict of interests

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial, in this article.

REFERENCES

1. Gupta SK, Gupta J, Saraswathi V, Ballal V, Acharya SR. Comparative evaluation of microleakage in Class V cavities using various glass ionomer cements: An *in vitro* study. J Interdiscip Dent 2012;2:164.
2. Manhart J, Garcia-Godoy F, Hickel R. Direct posterior restorations: Clinical results and new developments. Dent Clin North Am 2002;46:303-39.
3. Lokhande NA, Padmai AS, Rathore VP, Shingane S, Jayashankar DN, Sharma U. Effectiveness of flowable resin composite in reducing microleakage - An *in vitro* study. J Int Oral Health 2014;6:111-4.
4. Ian AT, Muttlib NA, Bakar WZ, Alam MK. Comparison between microleakage of composite and porcelain in Class V restoration: An *in vitro* study. Int Med J 2013;20:359-62.
5. Kidd EA. Microleakage: A review. J Dent 1976;4:199-206.

6. Sadeghi M, Lynch CD. The effect of flowable materials on the microleakage of Class II composite restorations that extend apical to the cemento-enamel junction. Oper Dent 2009;34:306-11.
7. Arslan S, Demirbuga S, Ustun Y, Dincer AN, Canakci BC, Zorba YO. The effect of a new-generation flowable composite resin on microleakage in Class V composite restorations as an intermediate layer. J Conserv Dent 2013;16:189-93.
8. Beznos C. Microleakage at the cervical margin of composite Class II cavities with different restorative techniques. Oper Dent 2001;26:60-9.
9. Leevailoj C, Cochran MA, Matis BA, Moore BK, Platt JA. Microleakage of posterior packable resin composites with and without flowable liners. Oper Dent 2001;26:302-7.
10. Neme AM, Maxson BB, Pink FE, Aksu MN. Microleakage of Class II packable resin composites lined with flowables: An *in vitro* study. Oper Dent 2002;27:600-5.
11. Soubhagya M, Goud KM, Deepak BS, Thakur S, Nandini TN, Arun J. Comparative *in vitro* evaluation of internal adaptation of resin-modified glass ionomer, flowable composite and bonding agent applied as a liner under composite restoration: A scanning electron microscope study. J Int Oral Health 2015;7:27-31.
12. Tredwin CJ, Stokes A, Moles DR. Influence of flowable liner and margin location on microleakage of conventional and packable Class II resin composites. Oper Dent 2005;30:32-8.
13. Ziskind D, Adell I, Teperovich E, Peretz B. The effect of an intermediate layer of flowable composite resin on microleakage in packable composite restorations. Int J Paediatr Dent 2005;15:349-54.
14. Majety KK, Pujar M. *In vitro* evaluation of microleakage of Class II packable composite resin restorations using flowable composite and resin modified glass ionomers as intermediate layers. J Conserv Dent 2011;14:414-7.
15. Reddy SN, Jayashankar DN, Nainan M, Shivanna V. The effect of flowable composite lining thickness with various curing techniques on microleakage in Class II composite restorations: An *in vitro* study. J Contemp Dent Pract 2013;14:56-60.
16. Sensi LG, Marson FC, Monteiro S Jr., Baratieri LN, Caldeira de Andrada MA. Flowable composites as "filled adhesives:" A microleakage study. J Contemp Dent Pract 2004;5:32-41.
17. Yazici AR, Baseren M, Dayangaç B. The effect of flowable resin composite on microleakage in Class V cavities. Oper Dent 2003;28:42-6.
18. Abd El Halim S, Zaki D. Comparative evaluation of microleakage among three different glass ionomer types. Oper Dent 2011;36:36-42.
19. Wilson AD, Kent BE. A new translucent cement for dentistry. The glass ionomer cement. Br Dent J 1972;132:133-5.
20. Yap AU, Lim CC, Neo JC. Marginal sealing ability of three cervical restorative systems. Quintessence Int 1995;26:817-20.
21. Chen X, Cuijpers VM, Fan MW, Frencken JE. Validation of micro-CT against the section method regarding the assessment of marginal leakage of sealants. Aust Dent J 2012;57:196-9.
22. Parolia A, Adhauliya N, de Moraes Porto IC, Mala K. A comparative evaluation of microleakage around Class V cavities restored with different tooth colored restorative materials. Oral Health Dent Manag 2014;13:120-6.
23. Wimonchit S, Timpawat S, Vongsavan N. A comparison of techniques for assessment of coronal dye leakage. J Endod 2002;28:1-4.
24. Memarpour M, Derafshi R, Razavi M. Comparison of microleakage from stainless steel crowns margins used with different restorative materials: An *in vitro* study. Dent Res J (Isfahan) 2016;13:7-12.
25. Bagherian A, Ahmadkhani M, Sheikhfathollahi M, Bahramabadinejad R. Microbial microleakage assessment of a new hydrophilic fissure sealant: A laboratory study. Pediatr Dent 2013;35:194-8.
26. Bore Gowda V, Sreenivasa Murthy BV, Hegde S, Venkataramanaswamy SD, Pai VS, Krishna R. Evaluation of gingival microleakage in Class II composite restorations with different lining techniques: An *in vitro* study. Scientifica (Cairo) 2015;2015:896507.
27. Boruziniat A, Gharaee S, Sarraf Shirazi A, Majidinia S, Vatanpour M. Evaluation of the efficacy of flowable composite as lining material on microleakage of composite resin restorations: A systematic review and meta-analysis. Quintessence Int 2016;47:93-101.
28. Moazzami SM, Sarabi N, Hajizadeh H, Majidinia S, Li Y, Meharry MR, *et al.* Efficacy of four lining materials in sandwich technique to reduce microleakage in Class II composite resin restorations. Oper Dent 2014;39:256-63.
29. Filtek Z250XT Nano Hybrid Universal Restorative. Available from: http://www.tinyurl.com/jdvo3tc. [Last accessed on 2017 Jan 20].
30. Prabhakar AR, Madan M, Raju OS. The marginal seal of a flowable composite, an injectable resin modified glass ionomer and a compomer in primary molars – An *in vitro* study. J Indian Soc Pedod Prev Dent 2003;21:45-8.
31. Santos MJ, Ari N, Steele S, Costella J, Banting D. Retention of tooth-colored restorations in non-carious cervical lesions – A systematic review. Clin Oral Investig 2014;18:1369-81.
32. Alani AH, Toh CG. Detection of microleakage around dental restorations: A review. Oper Dent 1997;22:173-85.
33. Taylor MJ, Lynch E. Microleakage. J Dent 1992;20:3-10.