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Research Article

Comparative Evaluation of Microleakage of G-Aenial, Bis-GMA Nanohybrid and Bis-GMA Microhybrid Flowable Composites in Class I Cavities – An in Vitro Study.

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Abstract: The most vulnerable area while restoring a tooth surface lies at the interphase between the restoration and the tooth margins. This area is the weakest junction in a tooth and serves as a potential pathway for microorganisms to invade into the tooth. This leads to failures in existing restorations paving way for the initiation of secondary caries progression. An advanced restorative material bridges this gap and seals off the weak interphasic junctions. Flowable composites having adequate strength and lesser polymerisation shrinkage were chosen. The aim of this study was to find a material having lesser microleakage deemed suitable for restorative purposes. Three types of flowable composites were chosen, which had the objective of being tested as a flowable material for evaluation of microleakage amongst the three tested groups. Dye penetration test was carried out to evaluate the amount of microleakage occurring under flowable resins which were then evaluated under a light reflecting digital compound microscope. A total of 45 extracted human premolars were selected for the study; scoring of specimens was done with the help of an Ordinal scale to detect microleakage. The results obtained from the study was that G-aenial Universal Flo, performed superior than Tetric N flow which was then followed by Kulzer Charisma Flo which showed highest dye penetration. The amount of dye penetration reflects directly the microleakage occuring at the interphases of restoration. The novelty of this study lies in the fact that this is one of its kind of study evaluating the amount of microleakage occurring between three different flowable composites. It was concluded that G-aenial Universal Flo, showed the least amount of microleakage when compared against two similar flowable composites to provide an adequate marginal seal.

Keywords: G-aenial universal flo, tetric n flow, kulzer charisma flow, flowable composite, microleakage, nanohybrid, microhybrid.

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1. INTRODUCTION

Flowable composites have recently been a significant boon in the field of restorative dentistry and endodontics. The continuous research on the formulations of this versatile material has led to an increase in its physical and mechanical properties which have directed its use in a wide variety of applications. The use of flowable composites in inaccessible areas has been of great advantage since it offers high viscosity, which tends to reduce polymerisation shrinkage [1]. With the advent and introduction of new materials, there has been a rapid shift from using composite materials in various restorative procedures. The various reasons reported for substituting the usage of these materials with the newer flowable ones have provided the clinician wide range of options for selecting a material. The era of nanotechnology has introduced nanohybrids[2] which display superior mechanical properties like enhanced wear resistance, increased strength, polishability and retention for a more extended period. Superadded to this is its low viscosity, which provides excellent handling characteristics, the ability to flow in deepened pits and fissures and easy syringe delivery, which aids in faster placement of the material into small to moderate class 1 and class 2 cavities and inaccessible areas. [3] A quirky feature demonstrated by the flowables is its unique ability to diminish stresses incorporated at the junctions of the restorative material and the cavity design. This benefits the material as it has the potential to eliminate the thermal and occlusal stresses, which favours making the material prolong its durability in the oral cavity. The essential property behind achieving these clinical benefits lies in the material's ability to lower the modulus of elasticity of flowables compared to traditional ones.[4] Recently introduced highly filled G-aenial Universal Flo Nanocomposites are flowable, light-cured radiopaque restorative material. The superior properties offered by this material include its high strength, high wear resistance and high gloss retention when compared to the currently leading flowable and conventional composites.[5] On the other hand, another Microhybrid flowable composite, Kulzer Charisma Flow, has been tested, which also has low viscosity and good thixotropy. This enables it to be an ideal material of choice for many applications like restoration of cavities, pit and fissure sealing, Class V fillings and as an intermediate liner material in deep cavities.[6] On similar grounds, a nanohybrid flowable composite Tetric N-flow was selected. It is known for its outstanding stability and is thus ideal for Class V restorations.[7] It has good adhesion properties but is also easy to remove and clean. The added benefit of having excellent wetting behaviour allows for convenient application in all areas. It also comprises a high level of radiopacity for a proper diagnosis. [2] Despite the ongoing research on its mechanical and physical properties, minimal emphasis was made on the marginal microleakage of the flowable resins. The evaluation of this parameter will thus help in having a broader understanding regarding the selection and the type of material to be used in specific dental restorations, which will help in the long-term sustainability of the restoration in the oral cavity.

2. MATERIALS AND METHODS

2.1. Material

This study stands out as being a one of its kind to evaluate and analyse the microleakage amongst novel flowable composite resins. Its uniqueness lies in proving experimentally the distinctive nature of the study when a set of three newly tested flowable composite resins have been studied for detection of microleakage. No studies have been previously evaluated based on these parameters employed to evaluate microleakage amongst the aforementioned flowable composites. A total of three flowable composites: G-aenial nanohybrid flowable composite, Tetric N-Flow Bis-GMA containing Nanohybrid flowable composite, Kulzer Charisma Flow Bis-GMA containing Microhybrid flowable were selected for the study. Methylene blue dye (Merck Millipore, Germany) was selected for microleakage evaluation. Dye penetration was observed under a binocular reflective digital compound microscope (BIOLUX-CX, KYOWA, Japan). An experimental, in vitro study was conducted on January 18, 2023. The tests for microleakage for the desired specimens were carried out at N.E.E.R.I. (National Environmental Engineering Research Institute), and statistical analysis was further evaluated at Sharad Pawar Dental College & Hospital, Sawangi (Meghe), Maharashtra 442001. An institutional ethical committee approval was obtained (IEC Ref No. D.M.I.M.S. (DU)/IEC/2022/879).

2.2. Inclusion criteria

Intact, non-carious, unrestored maxillary and mandibular premolars extracted for therapeutic reasons were included in the study.

2.3. Exclusion criteria

Maxillary and mandibular premolars that are carious, restored, or that weren't intact were excluded from the study.

2.4. Methodology

Forty-five non-carious human mandibular premolars were extracted and cleaned with tap water. Polishing was done with pumice, and teeth were stored in containers containing normal saline at room temperature until they were used for the study.

2.5. Cavity Preparation

Class I cavities were prepared using straight fissure bur (SF-12, Mani, Japan) with a high-speed handpiece on the occlusal surfaces of 45 premolars, under profuse water cooling, to produce a flat surface perpendicular to the long axis of the tooth. The measurements of the final cavity preparation were approximately 3.0 mm buccolingually, 3.0 mm mesiodistally, and 2 mm in depth. Dimensions of each cavity were measured using William's graduated periodontal probe to maintain uniformity among the size of the cavities. The teeth were randomly divided into three experimental groups of 15 teeth each.

2.6. Restoration Procedure

The preparations were etched using 37% phosphoric acid (Scotch bond Etchant, 3M ESPE) for 20 seconds, followed by rinsing with water for 15 seconds and then blot dried, ensuring that the dentin remaining is moist and shiny (Figure 2). Two simultaneous generous coatings of a mixture of ethanol & water comprising an adhesive system (ADPER single bond 2, 3M ESPE) were applied onto the entire preparation (Figure 2). The cavity was gently air-dried after 10 seconds of application of the ADPER bonding agent and then light-cured for 20 seconds. Finally, the three groups were categorized as follows:

Group I - G-aenial nanohybrid flowable composite (n=15)
Group II - Tetric N-Flow Bis-GMA containing Nanohybrid flowable composite (n=15)
Group III- Kulzer Charisma Flow Bis-GMA containing Microhybrid flowable composite (n=15)

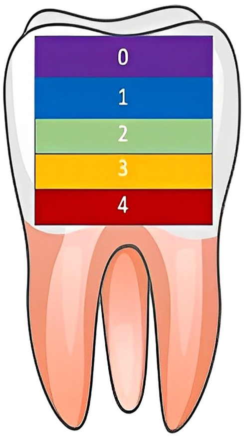
Following the manufacturer's instructions, the specimens of each group were restored with the corresponding allocated composites. After that, the Immersion of restored specimens was stored in normal saline at 37 degrees Celsius for 12 hours. The restorations were finished and polished using aluminium oxide disks (Sof-Lex Pop On, 3M ESPE). A coating with two layers of nail varnish was done, leaving approximately a gap of 1.0-millimetre width around the restoration.

2.7. *Staining of the samples*

Thermocycling of specimens was carried out for 1000 revolutions/cycles at 50 degree Celsius and 55 degree Celsius with a dwell time of 30 seconds. The specimens were then immediately submerged in 2% Methylene blue dye for 24 hrs.

2.8. *Microleakage analysis*

Specimens were sectioned in the buccolingual direction with the help of sectioning discs. Restorations were analyzed at 10x magnification and with the help of a binocular reflective digital compound microscope. They were scored according to an ordinal ranking system (Figure 1) for the amount of dye penetration along the occlusal surface of the cavity preparations.



Score	Tooth restoration interface
0	No evidence of dye penetration at the tooth restoration interface
1	Dye penetration along the cavity wall, upto 1/3 th of the cavity depth
2	Penetration > 1/3 but <2/3 of the cavity depth
3	Penetration >2/3 of the cavity depth, but not along the dentinal tubules.
4	Penetration to cavity depth and along the dentinal tubules.

Fig 1- A figure of the tooth depicting its scoring criteria for evaluating the depth of dye penetration at the tooth restoration interface which has been carried out by dividing the tooth into 4 different zones from coronal to the apical area. The colour codings depict the various scorings done onto the surface of the restoration.

3. STATISTICAL ANALYSIS

The data were processed and analyzed using SPSS software version 19-SPSS Inc. Chicago, IL, USA. Comparison using Kruskal Wallis ANOVA showed statistically significant results (Kruskal Wallis=10.916, p=0.004) (Table 1). Comparison using Mann Whitney U test showed a statistically significant difference between Groups 1 & 2 (Mann Whitney u=59.500, p=0.026) and 3 (Mann Whitney u=39.500, p=0.002). Still, there was no statistically significant difference between Groups 2 & 3 (Mann Whitney u=85.000, p=0.242) (Table 2).

Table 1: Table depicting the usage of Kruskal Wallis Test utilized for data interpretation from samples where standard deviation and means amongst the three groups was calculated and tabulated. 15 specimens was the sample size per group.

Group	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Kruskal Wallis	P value
				Lower Bound	Upper Bound		
Group I	15	.6000	.73679	.1920	1.0080	10.916	**0.004**
Group II	15	1.8667	1.55226	1.0071	2.7263		
Group III	15	2.5333	1.59762	1.6486	3.4181		
Total	45	1.6667	1.55212	1.2004	2.1330		

Table 2: Table showing utilization of Mann Whitney Test for finding statistical differences between the three groups of samples. It can be inferred from this table that statistically significant differences were obtained between Group 1 and 2, whereas no statistical difference was present between Group 2 and 3.

(I) G roup	(J) group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	2.00	-1.26667*	.49463	**0.026**	-2.5001	-.0332
	3.00	-1.93333*	.49463	.002	-3.1668	-.6999

Group I	2.00	-1.26667*	.49463	**0.026**	-2.5001	-.0332
	3.00	-1.93333*	.49463	.002	-3.1668	-.6999
Group II	1.00	1.26667*	.49463	**0.026**	.0332	2.5001
	3.00	-0.66667	.49463	0.242	-1.9001	.5668
Group III	1.00	1.93333*	.49463	**.001**	.6999	3.1668
	2.00	0.66667	.49463	0.242	-.5668	1.9001

4. RESULTS

Figure 2 displays microleakage of three different groups seen under 10x binocular reflective digital compound microscope (BIOLUX-CX, KYOWA, Japan) and an inter-group comparison of various samples showing the depth of dye penetration was done using Kruskal Wallis ANOVA & Mann Whitney U test. (Table 3 & Figure 2). Amongst 15 samples in each group, eight in Group 1 & 4 samples in Group 2 showed no evidence of dye penetration at the tooth restoration interface (Score 0). Whereas six samples in Group 3 showed penetration of dye at a depth of the cavity and along the dentinal tubules (Score 4). (Figure3)

Table 3- Comparison amongst three groups based on the depth of dye penetration using an ordinal ranking system. Out of a total of 45 total specimens (n=15), the highest score no of samples was 8 (Group 1), which showed the least microleakage (Score 0). The lowest score (Score 4) was seen in (Group 3), having the highest no. of samples among all tested specimens.

Group	Score 0	Score 1	Score 2	Score 3	Score 4	Total
Group 1	8	3	2	1	1	15
Group 2	4	3	2	3	3	15
Group 3	3	1	2	3	6	15

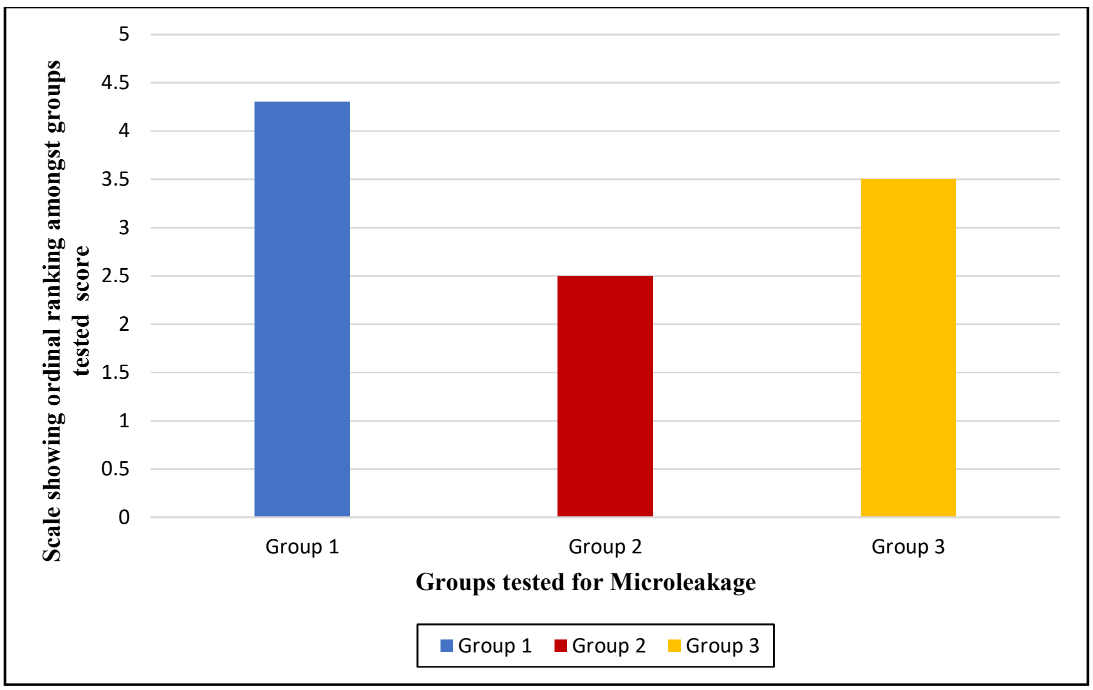
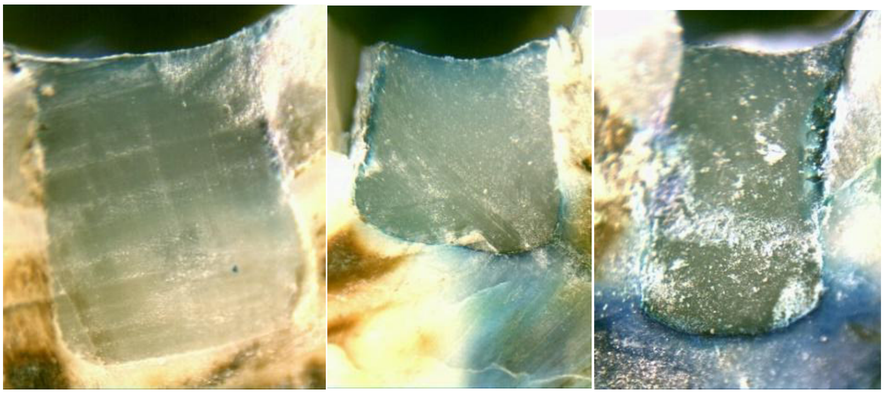


Fig 2: Bar graph depicting the mean dye penetration score amongst the three groups tested. The coordinates indicate the highest no. of samples included in Group 1 and lowest in Group 3.



(a) (b) (c)

Fig 3: Microleakage of (a) g-aenial universal flo, (b) tetric n-flow, (c) kulzer charisma flow as seen under the binocular reflective digital compound microscope (x10).

5. DISCUSSION

Composites are applied to various surfaces of a tooth during the restoration of a tooth. An ideal material for restoration should show good marginal integrity and the ability to resist fracture after its placement into the oral cavity. Even after the introduction of newer and better modifications of composites, it is expected that polymerisation shrinkage would reduce with it. A good marginal seal acts as a barrier and protects the restoration from seepage of various fluids and saliva is known to all. This is achieved by adequately contouring the restorations to the margins of the tooth. The ability to bond well to the cavity walls ensures that the material will provide an excellent marginal seal and prevent microleakage between the tooth and the restorative material, which could lead to secondary caries and restoration failure.[8] Microleakage is the clinically undetectable passage of bacteria, fluids, molecules, or ions in micro gaps (10−6 μm) between a cavity wall and the restorative material applied to it. As far as the resistance to microleakage is considered, this can be checked clinically by subjecting the samples to a dye penetration test wherein the depth of penetration of the dye can be examined. A dye penetration test is used as an auxiliary by physicians and researchers to anticipate the effectiveness and durability of restorative material in terms of allowing the passage of fluids. This is further used to determine the success or failure of the restoration.[9] In the present study, Methylene Blue dye was used due to the fact that the diameter of dye molecules is 0.80 nm, which is less than the diameter of dentinal tubules (1–4 μm). (10) Added to this is that it is cost-effective, readily available and easy to perform. Also, due to the low molecular weight of the dye, known to be smaller than bacteria, it is helpful in detecting leakage in places where even bacteria cannot penetrate. Accordingly, this test has been considered a reliable method to understand the sealing ability of these materials when placed into the prepared cavities.[11] However, a proper method of understanding the cause of microleakage and its exact role in determining the clinical durability of three newly introduced composite restorations still needs to be explored. Therefore, the present was devised to evaluate performance. The results of the study indicate that the performance of the nanohybrid flowable composite (G roup I) is superior in comparison to the nanohybrid flowable composite with Bis-GMA (Group II) and Microhybrid flowable composite (Group III) tested. The mean penetration score of Group I specimens was minimum (0.600 ± 0.73679), and Group III specimens showed the maximum depth of penetration (2.5333± 1.59762), which was done using Kruskal Wallis ANOVA (Table 2). This indicates that Group I showed superior resistance to microleakage compared to Group III. According to ANOVA, a statistically significant difference was observed between Groups I and III, whereas no statistically significant difference was present between Group II and Group III ($P < 0.004$). According to Post Hoc analysis using Man Whitney Test [Table 3], the mean penetration score of Group II specimens (0.667) and Group III (0.667) was not statistically significant, but in comparison with that of Group I (1.933), a statistically significant difference was noted. Group II performed inferior to Group I but showed decreased dye penetration compared to Group III. The highest amount of dye penetration was seen in Group III amongst the three tested materials used in the study. Labella & others (1999)[14] have demonstrated that the elastic moduli for adhesive resins and flowable composites and microfills were relatively low compared to the hybrid materials. This explains that Group II, comprised of Nanohybrid flowable containing Bis-GMA having a lesser elastic modulus, was stiffer, thereby increasing the strain capacity of the composite to show less polymerisation shrinkage. Thus a lower amount of microleakage occurred in Group II than in Group III. The property of microleakage is directly related to the amount of filler content present in the composite material. This filler loading capacity enhances the strength of the flowable composite. On the basis of this knowledge, the study justifies the fact that G-aenial Universal Flo (Group I), having a filler loading of 50% (vol%), showed the least amount of microleakage in comparison to the other groups.[12] On similar grounds, Tetric N-Flow (Group II) showed more microleakage than the G-aenial Universal Flow (Group I) but lesser than the Charisma Flow (Group III). This is related to the filler loading of 43% (vol%) present in Tetric N-flow (Group II). About 41% (vol%) in Charisma Flow (Group III), [13] thus additionally proves the fact that the more the amount of filler (vol%), the lesser will be the microleakage experienced at the material margins.[15] Size and type of particle also influence the microleakage of composites.[13] The type of filler seen in G-aenial Universal flow (Group I) contains Silica and Strontium Glass, which were significantly larger. TetricN-Flow (Group II) contains Barium glass, ytterbium fluoride, and silica, which have a smaller particle size. On the other hand, Charisma Flow (Group III) contains the smallest filler size comprising Barium-alumino-fluorosilicate glass, ytterbium trifluoride and silica. Therefore, the larger the size of the filler particles, the lesser the amount of microleakage. On the contrary, this has a deleterious effect on the polishability of the composites.[15] This study, therefore, was conducted to compare the mechanical properties and evaluate the microleakage amongst three flowable composite resin materials. Further studies are required to explore the future perspectives of marginal microleakage in class 2 cavities. Also, a long-term follow-up in vivo study is required to check for the integrity of the flowable in various cavity designs.

5.1. Limitation

The study's limitations were that the results were carried out in in-vitro settings, which may vary in In-vivo conditions. This is of great importance in determining the interaction of the tested flowable materials with other bodily fluids, particularly saliva in the mouth. Pre-operative microscopic analysis of the specimens was not carried out to determine whether the restoration had been adapted well to the tooth. Thus the correlation between oral fluids and restorative materials needs to be done.

5.2. Future scope

The future scope of this study lies in the fact that the limitations of this study should be thoroughly evaluated for microleakage testing in combination with other flowable composite resins. A long-term follow-up pooled with advanced microscopic evaluation with help shed light on the future treatment modalities and areas of interest of these materials.

6. CONCLUSION

Considering the limitations of the study, G-aenial universal flow can be used as an efficient restorative material. However, utilising the best material does not assure the clinician of a complete marginal seal; microleakage remains an inevitable phenomenon. The evaluation of mechanical properties of newer flowable composites thus should be tested to further establish them as a benchmark in selecting it as a material of choice.

7. ACKNOWLEDGEMENTS

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8. ETHICAL APPROVAL STATEMENT

The study design was an In-Vitro study design approved by the Institutional Ethical Committee, Datta Meghe Institute of Higher Education & Research (DMIHER), (IEC Ref No. D.M.I.M.S. (DU)/IEC/2022/879). Since this was an In-Vitro study, no informed written consent was needed. The study was conducted according to the Institutional Ethical Committee guidelines.

9. AUTHORS CONTRIBUTION STATEMENT

Conceptualization of this topic was suggested by Dr Aditya Patel & Shraddha Patel. Dr Pradnya Nikhade & Dr Manoj Chandak helped me gather resources throughout the study. Dr Ladusingh Rajpurohit provided the statistical evaluation for the study. Dr Swayangprabha Sarangi for giving me constant support throughout the study.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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