Microleakage Evaluation of Two Different Nano-Restorative Materials in Primary Molars: *In Vitro* Study

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Abstract: The **aim** of this *in vitro* study was to compare the microleakage values of a nano-resin modified glass ionomer, nano- composite alone and in contact with nano-resin modified glass ionomer base (sandwich technique) versus conventional- resin modified glass ionomer and conventional- composite in primary molars. **Methods:** Seventy five extracted primary molars were selected, and class V cavities were prepared on the buccal/lingual surfaces. Teeth were randomly distributed to 5 groups according to the type of restorative materials (n=15). Group 1: nano-resin modified glass ionomer (nano-RMGI) Group 2: nano- composite. Group 3: nano-resin modified glass ionomer (nano-RMGI) as a base followed by bonded nano- composite as a sandwich technique. Group 4: conventional- resin-modified glass ionomers (conv-RMGI). Group 5: conventional- composite. After thermocycling assessment of gap surface area was done. The teeth were immersed in basic fuchin dye then sectioned and evaluated under a stereomicroscope. Microleakage was assessed using linear dye penetration in (μm) and on a scale from zero to three. **Results:** There was statistically significant difference between the five groups gap surface area and gap surface fraction**.** Nano-RMGI /nano composite sandwich technique group exhibited the lowest value followed by nano composite group while conv-RMGI showed the highest value. Regarding linear dye penetration and microleakage scores, no significant differences were found between the tested materials. The degree of leakage in the gingival margins was significantly higher than that of occlusal margins for nano-RMGI, nano-RMGI / nano-composite and conv –RMGI groups. **Conclusion:** Complete marginal sealing could still not be reached with any of the tested restorative materials. Nano-RMGI / nano-composite sandwich technique showing the least microleakage followed by nano-composite when compared to the other four materials tested.

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Keywords: Microleakage, nano composite, nano glass ionomer, primary teeth

1. Introduction

For many decades, silver amalgam has been the standard restorative material in pediatric dentistry. However, the detrimental environmental effects of mercury, debates on possible health effects of amalgam, and the growing interest of patient and parents in enamel-colored restorations have resulted in a considerable reduction in the use of amalgam in dentistry.[1,2] The most frequently used alternatives to amalgam for restoring primary teeth have been glass-ionomer cements,[3] resin modified glass-ionomer cements (RMGICs)[4], compomers[5] and resin composites.[6]

Microleakage is a common problem in restorative dentistry. It is defined as the leakage of microorganisms and toxins between the restoration and walls of cavity preparation. It influences the restoration longevity leading to postoperative sensitivity, recurrent caries and negative pulpal seque.[7] Microleakage is considered mainly as the result of polymerization shrinkage. Techniques to reduce the effects of polymerization shrinkage in clinical practice include incremental placement of light-curing composite resins[8], sealing restoration margins with a 'glaze' of unfilled resin[9], beveling enamel margins[10], and use of staged light curing[11]. Other techniques involve use of the sandwich technique where a glass ionomer is placed as a base[12] or liner[13] with a bonded composite resin forming the outer, functional surface of the restoration. Tolidis et al[14] showed that use of an RMGI liner significantly reduced volumetric polymerization contraction for all the light-curing composite resin restorative materials tested.

The field of nanotechnology has expanded dramatically as nanostructured materials exhibit unique properties on the macroscale that offer high-potential technological benefits. Typically, the critical properties of nanomaterials are attributable to internal structures between 1 and 100 nanometers in dimension, defining the nano world. As size is decreased to nanoscale dimensions, physical properties, e.g. optical characteristics, get altered, especially when size nears the molecular scale, meaning < 5 nm. These unique properties are in the focus when research starts its innovative work to achieve materials with greatest efficiencies.

In 2007, a new generation of RMGI was introduced. Ketac Nano (3M ESPE) is described by manufacturers as a nano- ionomer. It is based on a simplified dispensing and mixing system (paste/paste) that requires the use of a priming step, without separate conditioning step. Its primary curing mechanism is by light activation, and no redox or self curing occurs during setting.[15] Killian and Croll[16] showed that nano-ionomer and its improved properties make it an effective alternative for restoring primary and permanent teeth. Manufacturers claimed that Ketac Nano physical properties exceed those of other popular RMGI restoratives. The manufacturer reports that the nano filler and nanofiller clusters comprise approximately 60% of the glass component of Ketac Nano and are responsible for higher filling contents and accompanying enhancement in physical properties. It has better polishability than other RMGI restorative cements and fluoride ion dynamics comparable to other glass ionomer. The manufacturer's technical profile also states that in vitro tests have shown that Ketac Nano has the ability to act as fluoride reservoir and recharge the fluoride release after application of a topical fluoride source.[16]

Polymer nano -composite is another new class of material with unique internal structure and properties and contain nano fillers that are 0.005 to 0.01 micron in size. Dabanoglu et al[17] found that a high filler degree combined with small particle dimensions reduced abrasion by up to 50% compared to composites of lower filler degree or those with organic (pre-polymerized) filler. Furthermore, Mitra *et al.,*[18] measured nano composite properties, in vitro, in comparison with several existing composites (hybrids, microhybrids, and microfill).Nano-composite showed high translucency, high polish ability and retention similar to those of microfill while maintaining physical properties and wear resistance equivalent to

Apart from the obvious improvements in mechanical properties and user-friendliness, it is not clear how the addition of nano-fillers will influence the adhesiveness of resin modified glass ionomer and composite in primary teeth. Therefore, the aim of this study was to evaluate and compare marginal adaptation of class v in primary teeth restored with:

1. Nano–resin modified glass ionomer (Nano-RMGI)
2. Nano-composite
3. Nano-composite in contact with nano resin-modified glass ionomer liner (Nano RMGI/nano composite sandwich technique)
4. Conventional resin modified glass ionomer. (Conv-RMGI)
5. Conventional composite (Conv-composite)

Materials and methods

Selection and Preparation of Teeth:

A total of 75 extracted primary molars, due to caries or orthodontic reasons were collected for this study. The selected teeth needed to have at least three sound walls and one half to two thirds of root length. The teeth were debrided and stored in distilled water at room temperature.

Cavity preparation:

Standardized class V cavity preparations were prepared in the middle third of each tooth on the buccal or the lingual surface, care was taken that cavity margins were surrounded by enamel. The cavity was prepared with # 330 carbide bur on a high-speed hand piece with water spray. The cavity preparation was oval in shape with dimensions approximately 3 mm mesiodistal width, 2 mm occlusogingival height and 1.5 mm axial depth (length of bur was used as guide for cavity depth)[19]. Each bur was replaced after five preparations. The dimensions were standardized by having the outline dimension cut on a matrix band figure (1). The cavo-surface walls finished to a butt joint.

Restorative Materials

Table 1: Materials used. Group

Materials	Product	Manufacturer
Nano- resin modified glass ionomer	- Ketac™ N100 (nanoRMGI)	3M ESPE Seefield, Germany
-Nano composite	- Ketac™ supreme XT universal Restorative	3M ESPE Seefiled, Germany
-Conventional resin-modified glass ionomer	PhotacFil Seefield,	3M ESPE (Aplicap) Germany
-Conventional composite resin	-Z-100 St. Paul,	3M Dental products Minn U.S.A.

Cavity restoration

The teeth were randomly selected and assigned to one of the five experimental groups according to the restoration type (15 per group). Group I restored by nano- resin modified glass ionomer (Nano RMGI), group II restored by nano composite, group III restored by nano RMGI/nano composite sandwich technique, group IV restored by conventional resin-modified glass ionomer and group V conventional composite resin. Restoration of prepared teeth were done according to manufacturer instructions and cured by the same light-curing unit (POLYlux II, KaVoDental Gmbh, KG, and Germany). All teeth were thermocycled for 500 cycles between 5° and 55°C with a dwell time of 30 seconds.[20]

Assessment of gap surface area

The gap surface area of each restoration were assessed. Photomicrograph was taken by CCD digital camera (Olympus-Japan) attached to zoom stereo microscope (Olympus SZ-PT-Japan) at magnification X15. A binary threshold of the desired area of gap was done. Surface area of the gap (measured by μm^2)at the restoration / tooth interface along the entire circumference of the restoration outline was automatically calculated using the image analysis software (Imageware, Image 1.3-1b, USA). The surface area fraction of the gap surface area to the entire surface area of the restoration was calculated. All the recorded data were collected, tabulated and statistically analyzed.

Assessment of Microleakage

- Assessment of linear dye penetration

After detection of the gap surface area, the teeth were covered with yellow sticky wax to occlude all the openings and the teeth received 3 coats of a colorless nail varnish, except for a 1-mm window around restoration margins. Then specimens were immersed in 0.5 % basic fuchsin solution for 24 hours at room temperature. Samples were rinsed with distilled water to remove excess dye, and then sectioned in a buccolingual direction through the center of each restoration with a water cooled diamond disc to avoid overheating and cracking of restorations.

The area of the restoration was captured by a CCD digital camera (DP10, Olympus, Japan) mounted on Zoom stereo microscope (Olympus SZ-PT-Japan) at a magnification 30x. Digital images were then transferred to a computer system.They were analyzed using the image analysis software (ImageJ, 1.31b, USA).

The linear dye penetration is measured in microns of each section from the outer surface into enamel or/and dentin was automatically calculated using the same software for both the occlusal and the gingival margins. Moreover, the percentage of the linear dye penetration (occlusally and gingivally) to the entire length of occlusal or gingival margins was calculated.[21]

Assessment of Microleakage scores

Microleakage was assessed also by scoring the degree of linear dye penetration in the tooth / restoration interface. The degree of dye penetration was identified according to ISO specification 11 405:2003,[22].

0=no dye penetration.

1= dye penetration to the enamel aspect of preparation wall.

2= dye penetration to the dentin aspect of the preparation wall, but not including the pulpal floor.

3= dye penetration including the pulpal floor of the preparation

Both sections of each restoration were scored and the section with the greatest amount of microleakage was recorded as the score of that restoration. Microleakage scores were recorded for both the occlusal and the gingival margins. Two investigators examined the teeth independently. If the scores were different discussion took place till agreement.

Statistical analysis:

Descriptive statistics were displayed as means and standard deviations for quantitative variables and frequencies and percents for qualitative variables. Mean gap surface area and mean gap surface area fraction were checked for normality and found to be normally distributed. Means were compared among groups using analysis of variance (ANOVA).

Percentage of linear dye penetration was checked and found to be non normally distributed. Comparison of mean die penetration and microleakage scores among the study groups was done using Kruskal Wallis test whereas comparison in the same group between the occlusal and the gingival aspects was done using Wilcoxon signed ranks test.

Significance level was set at $P < 0.05$.

3. Results

This study was carried out on 75 human clinically sound a naturally exfoliated primary molars. Standardized class V cavity preparations were prepared in the cervical third of each tooth on the buccal or lingual surface surrounded by enamel. The prepared teeth were classified into five equal groups, 15 specimens each, according to the type of restoration used.

Assessment of gap surface area at restoration / tooth interface in $microns^2$, linear dye penetration in microns and microleakage scores were done. All recorded data were tabulated and statistically analyzed.

Assessment of Gap Surface Area

For each restoration a photo micrograph was taken by CCD digital camera attached to zoom stereomicroscope at magnification x15. A binary threshold of the desired area of gap was done figure (2). A surface area of gap in μm^2 at restoration / tooth interface along the entire circumference of the restoration outline was calculated using image analysis software.The gap surface area fraction of the gap surface area to the entire surface area of the restoration was also calculated.

Table (2) figures (2, 3) show the comparative analysis of the mean gap surface area(μm^2) and the mean gap surface area fraction for the tested restorative materials.

The mean gap surface area of nano- RMGI (group I)was (71355.54±36161.06), nano composite (group II) was (57674.73± 43554.24), group(III) Nano RMGI /Nano composite sandwich technique was (42269.87± 11978.07), group (IV) Conv-RMGI was (74649.46± 44308.64) and for Conv-composite was (85124.46± 45875.51). The results revealed that there was statistically significant difference between the five tested groups(P.=0.015) regarding the mean gap surface area, at a significance level of (P <0.05).

For the mean gap surface area fraction of Nano-RMGI, Nano composite, Nano RMGI /Nano composite sandwich technique, Conv-RMGI and Conv-composite were (0.536± 0.3973), (0.422± 0.356), (0.390± 0.337), (0.618 ± 0.421)and (0.827± 0.408) respectively.Also, there was statistically significant difference between the five tested groups (P.= 0.021).

It is clear that nano RMGI /nano composite sandwich technique group, had the least the mean gap surface area and the mean gap surface area fraction followed by nano composite group, while conv-composite group, had the highest one.

Table (2):Comparative analysis of the mean gap surface area and the mean gap surface area fraction among the tested restorative materials

Dependent Variable	Nano-RMGI	Nano composite	Nano RMGI /Nano composite	Conv-RMGI	Conv-composite	ANOVA P value
Number of teeth	15	15	15	15	15	
Gap surface area (μm^2)	71355.54 ±36161.06	57674.73 ± 43554.24	42269.87 ± 11978.07	74649.46 ± 44308.64	85124.46 ± 45875.51	0.016*
Gap surface area fraction	0.536 ± 0.3973	0.422 ± 0.356	0.390 ± 0.337	0.618 ± 0.421	0.8267 ± 0.408	0.021*

** Statistically significant at P < 0.05*

Assessment of the linear dye penetration

Table (3), figures (4,5,6,7,8) demonstrates the comparative analysis for the mean of the linear dye penetration percentage both occlusally and gingivally among the tested restorative materials.

It is clear that, nano RMGI/nano Composite sandwich technique group showed the least degree of microleakage as they had the least mean linear dye penetration percentage both occlusally and gingivally restorations (12.84±8.00), (20.87±10.92) followed by nano composite group (14.62± 4.94), (21.83± 13.10), while conv-composite group were the highest where the mean of linear dye penetration percentage occlusally and gingivally were (20.63± 21.22), (29.51± 24.65) respectively. For Nano RMGI group the mean of linear dye penetration percentage occlusally and gingivally were (16.55± 7.42), (27.32± 10.31) and for Conv-RMGI were (16.28± 6.10), (29.24± 11.63) respectively.

Although there were differences among the five groups of restorative materials for the mean occlusal and gingival linear dye penetration percentage, that differences were not statistically significant as they were (P.=0.42) (P.=0.35) at the significance level P<0.05.

Also, the results revealed that there is statistically significant differences of the mean linear dye penetration percentage between the occlusal and gingival margins for some tested groups as in the nano RMGI/nano composite sandwich technique group (P.=0.03), nano RMGI group (P.=0.03) and conv-RMGI group (P.=0.001) at the significance level P <0.05.

Table (3): Comparative analysis of the mean linear dye penetration percentage both occlusaly and gingivally between the tested restorative materials.

Dependent Variable	Nano RMGI	Nano composite	Nano RMGI /Nano composite	Conv-RMGI	Conv-Composite	*P*. value
Mean Linear Occlusal Dye Penetration Percentage	16.55 ± 7.42	14.62 ± 4.94	12.84 ±8.00	16.28 ± 6.10	20.63 ± 21.22	0.42
Mean Linear Gingival dye penetration percentage	27.32 ± 10.31	21.83 ± 13.10	20.87 ±10.92	29.24 ± 11.63	29.51 ± 24.65	0.35
P value	0.003*	0.06	0.03*	0.001*	0.30	

** Statistically significant at P < 0.05*

Assessment of the microleakage scores

The comparative analysis of the microleakage scores at both occlusal and gingival margins were shown in table (4) (figures 4,5,6,7,8). None of the restorative materials showed occlusal or gingival dye penetration along the pulpal (axial) wall, score (3) microleakage. It was observed that (Nano-RMGI / nano composite) sandwich technique group showed the least degree of microleakage scores, followed by nano-composite group, meanwhile the conv–composite group showed the highest microleakage scores. There was no significant difference (*P*.=0.12) in the amount of microleakage scores at occlusal or gingival margins for nano-RMGI / nano composite sandwich technique group, nano-composite group or conv-composite group. However, the difference between the occlusal and gingival margins showed a statistically significant difference of nano–RMGI group (*P*=0.04), and conv-RMGI group (*P*=0.003).

Table (4): Comparative analysis of the microleakage scores among the tested restorative groups at the occlusal and gingival margins

Aspects		Nano-RMGI	Nano-composite	Nano RMGI / nano-composite	Conv-RMGI	Conv-Composite	*P*. value
Occlusal	Score	1 (6.7%)	2 (13.3%)	5 (33.3%)	1 (6.7%)	3 (20%)	0.12
	Score	13 (86.7%)	13 (86.7%)	9 (60%)	14	9 (60%)	
	Score	1 (6.7%)	0	1 (6.7%)	0	3 (20%)	
	Score	0	0	0	0	0	
Gingival	Score	1 (6.7%)	1 (6.7%)	1 (6.7%)	0	1 (6.7%)	0.56
	Score	7 (46.7%)	11 (73.3%)	11 (73.3%)	7 (46.7%)	9 (60%)	
	Score	7 (46.7%)	3 (20%)	3 (20%)	8 (53.3%)	5 (33.3%)	
	Score	0	0	0	0	0	
	P.	0.04*	0.17	0.15	.003*	0.47	

** Statistically significant at P < 0.05*

4. Discussion

There have been more changes and developments in dentistry over the past decade. In the current age of adhesive dentistry or microdentistry, conservation of tooth structure is paramount. Rather than using extension for prevention as a treatment guideline, emphasis now is placed on restriction with conviction.

Microleakag is the major problem in clinical dentistry. The longevity of the restoration is largely determined by marginal sealing of the cavity. Achieving a micromechanical and biomechanical bond between the restoration and tooth is considered effective and a standard procedure in clinical practice. The ability of a restoration to minimize the extension of microleakge at the tooth/ restoration interface is important in predicting its clinical success. Numerous investigations have used a variety of research tools to evaluate the extent of microleakage and the marginal integrity of restorations. The use of dye diffusion is one of the most commonly used methods. [23 -25]

In the present study, the evaluation and the comparison of the microleakage of the restorative materials were done *in vitro*. We choose class V preparations to study the behavior of the tested restorative materials in a high C-Factor design (preparations with high ratio of bonded "flow-inactive" to free "flow-active" surfaces). Moreover, we selected the butt joint enamel margin to comply with traditional enamel cavity margin designs advocated for most posterior restorations.

Thermocycling has been used in this study to simulate oral conditions. It is a method widely used in dental research, particularly when testing the performance of adhesive materials. It aims at thermally stressing the adhesive joint at the tooth/restoration interface by subjecting the restored teeth to extreme temperatures compatible with temperatures encountered intraorally. This process may highlight the mismatch in thermal expansion between the restoration and tooth structure, resulting in different volumetric changes during temperature changes and causing fatigue of the adhesive joint with subsequent microleakage. This is in agreement with other researches which stated that, thermo-cycling mimic intra-oral temperature variations and. subjecting the restorations on the tooth to temperature extremes compatible with oral cavity. In the absence of a definite recommendation for number of cycles needed to simulate oral conditions 500 cycles was applied in this study. The number of cycles reported in previous studies range from 300 to 5000.[20,26-28]

The results of the present study revealed that there was statistically significant difference between the five groups regarding gap surface area and gap surface fraction (*P*.=0.016,0.021). Nano-RMGI /nano composite sandwich technique group exhibited the lowest value followed by nano composite group while conv-composite showed the highest value.

Also analysis of the obtained data of this *in vitro* study concluded that, none of the tested restorative materials completely eliminate microleakage at the occlusal or gingival margins. Assessment of the linear dye penetration fraction and the microleakage scores showed that Nano -RMGI /nano composite sandwich technique group had the best results that they had the least linear dye penetration and the best control of microleakage, the second one was nano composite group. In contrast, conv-composite showed the greatest linear dye penetration and microleakage measures compared with other restorative groups in the present study. This can explained by that conv-composites shrink more than nan-ocomposite as they have lesser filler loading and a greater proportion of resin matrix. The difference in coefficient of thermal expansion and elastic modulus between the composite and dentin causes stress in the interfacial gaps contributing to microleakage. This is in agreement with Tolidos *et al.,*[14] who found that resin modified glass ionomer liner significantly reduce volumetric polymerization contraction for all the light-curing composite resin restorative materials tested. Croll and Cavanaugh[29]reported that properties of light–hardened glass ionomer cements makes them ideal dentin replacement and properties of composite makes them ideal enamel replacement. When used together, the properties of each material are maximized and the resulting restoration simulates the tooth form and function. In addition, Dabanoglu *et al.,*[17] has suggested that nano- composite has high filler degree and spherical nano particles that reduces organic matrix content and gave a hard surface compared to conventional composite. This quality would also improve its wear and abrasion resistance and enhance the marginal seal to enamel.[30] Hedge *et al.,*[31] compared microleakage in three different composite resins (nano- composite, nano- ceramic composite and nano-hybrid composite) in class V in permanent teeth. Although there was no statistical difference between the three groups, nano-composite showed the least mean leakage values.

The results of the present study showed that conv–RMGI group also showed low leakage level occlusally than nano–RMGI group. This finding is in consistence with Coutinho *et al.,*[32]who showed that nano-RMGI bonded less effectively than conv-RMGI.

With respect to the cavity margins evaluated in this study, there was no significant difference between the occlusal and gingival margins for nano composite and conv - composite. A suitable explanation for such results may be the fact that gingival wall was located in enamel. The use of etch-bond technique improved the composite bond to enamel gingivally. This was in agreement with Fahmy and Farag[33]who evaluated gingival microleakage in class II cavities in primary molars restored with nano hybride composite using three different techniques (total bonding, closed or open sandwich technique). The best gingival marginal seal was obtained with the total bonding technique.

On the other hand there were significant differences between the occlusal and gingival margins for nano-RMGI, nano-RMGI / composite sandwich technique and conv -RMGI. According to Croll and Cavanaugh [29]. RMGI including nano–RMGI bond to enamel and dentin through both chemical and micromechanical bonding mechanism. RMGI due to lack of an additional conditioning step might show more gingival leakage because of the superficial mechanical interlocking.[30] Moreover, Fakhri *et al.,*[34] support the conclusion that the difference in mineral content and a prismatic layer thickness may account for the difference in microleakage between the occlusal and gingival margins in primary teeth.

Conclusion

Within the experimental results of this in vitro study, the following conclusions were drawn:

- Nano- RMGI /nano composite sandwich technique exhibiting the best control of marginal leakage followed by nano composite. The conv–Composite, however, showed the highest microleakage values.
- For (nano –RMG), (nano- RMGI / nano composite) and (conv–RMGI), microleakage was higher at the gingival margin.
- Statistical significant differences were found between the five restorative groups
- regarding gap surface area and gap surface area fraction while no statistical significant differences were found in degree of microleakage between the five groups regarding the linear dye penetration fraction or microleakage scores.
- The results of this study potentiate the importance of using liners under nano- composite restorations.

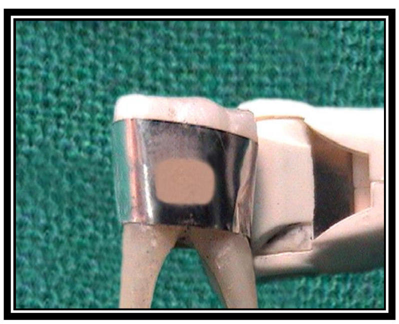

Fig (1): Standardized Class V Cavitypreparation.

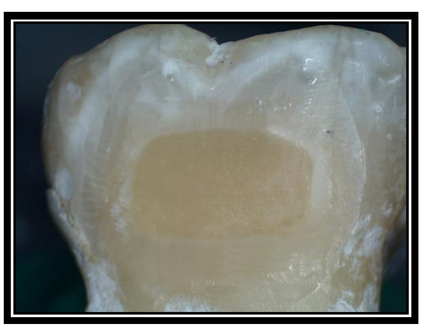

Fig (2): The photomicrograph shows a specimen of Nano Composite group before sectioning with magnification X15.

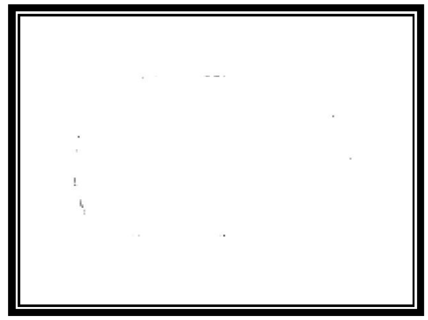

Fig (3): The previous photomicrograph after binary thresholding of the area of gap at the restoration/tooth interface. This area is then automatically calculated.

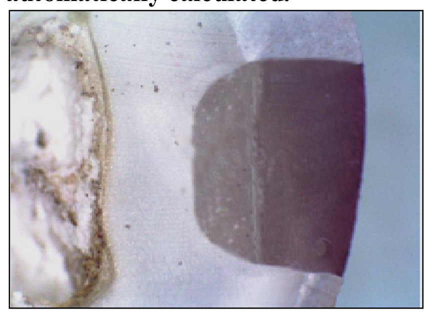

Fig (4): A Photomicrograph of Nano RMGI /Nano Composite sandwich restoration showing score 0 occlusally and score 0 Gingivally.

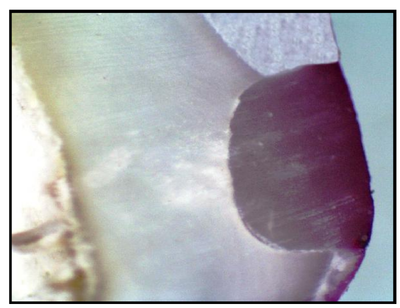

Fig.(5): A Photomicrograph of Nano Composite restoration showing score 1 occlusally and score 1 Gingivally.

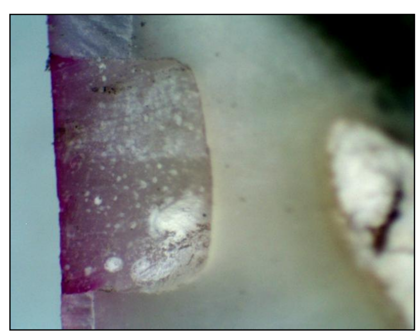

Fig.(6): A Photomicrograph of Nano RMGI restoration showing score 1 occlusally and score 1 Gingivally

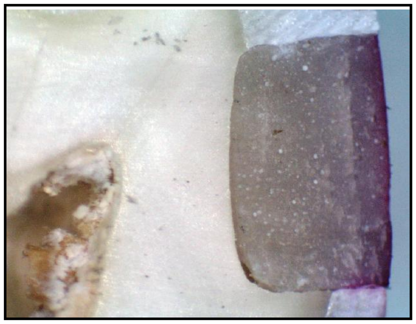

Fig.(7): A Photomicrograph of Conv-RMGI restoration showing score 0 occlusally and score 2 Gingivally.

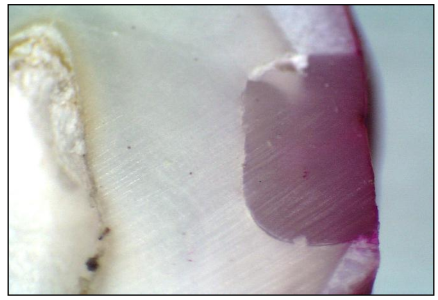

Fig.(8): A Photomicrograph of Conv-composite restoration showing score 1 occlusally and score 2 Gingivally

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