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Microleakage Evaluation of Adhesive Systems Reinforced with Fluoroapatite or Calcium Fluoride

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Abstract: ***Objectives**: to evaluate the effect of ideal ratio addition of Fluoroapatite or calcium fluoride to the Total etch and Self etch adhesive systems on the microleakage along tooth/ restoration interface. **Methods**: total etch adhesive system (Tetric® N-Bond) and Self etch adhesive system (Tetric® N-Bond Self-Etch) were used with ratio addition of Fluoroapatite or calcium fluoride that didn't affect the shear bond strength of adhesive systems.Class V cavities were prepared on the buccal and lingual surfaces of sixty extracted human lower third molars. Teeth were then divided randomly into six groups according to the adhesives used. All cavities were restored with composite filling materials and subjected to thermocycling (5-55°C for 500 cycles) and axial load cycling (50N for 50000 cycles). Microleakage was evaluated using a stereomicroscope with a reflected light under magnification of 45xby measuring the linear penetration of the dye using Image analysis (Image J) software. Data were statistically-analyzed using ANOVA, LSD and T-tests. **Results**: The result showed that, there were a significant difference between occlusal and gingival linear dye penetration depthof all groups at P≤0.001 level (occlusal is less microleakage). **Conclusions**: The addition of CaF_2 5% to TE and 7% of CaF_2 to SE decrease the linear dye penetration depth gingivally.*

1. Introduction

Tight sealing between the restoration and dental substrate must be provided by the adhesive systems to prevent leakage along restoration margins and to resist mechanical forces that includes stresses of polymerization shrinkage from the resin composite.However, despite resin improvements, when bonding fails, there will be a possibility of microleakage happening (that is: the passage of bacteria, fluids, chemical substances, molecules and ions between the tooth and restoration), which jeopardize the longevity of the restoration, contributing to staining, recurrent caries and adverse pulp response, in addition to post-operative sensitivity. It should be considered that all dental restorative materialshaveinherent short coming which ismarginal leakage **(Van Landuyt et al., 2007)**. A great effort has been made to diminish the microleakage around the restorations including the development of adhesive system to improves the adhesion of composite restoration to the tooth substances especially to dentin(**Torii et al, 2001**).

Torii et al. in 2001 reported that it is important to add an inhibitory effect against secondary caries to the restorative materials because the difficulty of having perfect seal at the cervical margins.

In some studies, it has been concluded that secondary caries formation inhibition may be influenced by the fluoride containing adhesive systems because the fluoride ions released in the hybrid layer that will penetrate into the dentin **(Shinohara et al., 2006).**

In this study the best ratio addition (that didn't affect the shear bond strength) of Fluoroapatite or Calcium fluoride to Tetric® N-Bond and Tetric® N-Bond Self-Etch adhesive which was 3% of Fluoroapatite or 5% of Calcium fluoride to Tetric® N-Bond adhesive, while the best ratio addition for Tetric® N-Bond Self-Etch adhesive were 1% of Fluoroapatite or 7% of Calcium fluoride were done to study their effect on microleakage of composite filling restoration.

2. Materials and Methods

Sixty sounds, human lower third molar teeth were selected for this study. Dehydration of teeth during all stages of the study was avoided by storage of teeth in distilled water **(Silva et al., 2012).**

To enhnce the handling and control of the samples, the acrylic block construction was performed by using a specially made cubic rubber mold, with an internal hole dimensions of 1.5 cm depth, length and width. the teeth were mounted in theacrylic resin to within 2 mm apical to the (CEJ).

The teeth randomly were divided into six main groups (ten each and twenty class V in each group):

Group I: The teeth were received composite filling using Total EtchTetric® N-Bond Adhesive (TE).

Group II: The teeth were received composite filling using Fluoridated Total EtchTetric® N-Bond Adhesive (TE + FA 3%).

Group III: The teeth were received composite filling using Fluoridated Total Etch Tetric® N-Bond Adhesive (TE + CaF_2 5%).

Group IV: The teeth were received composite filling using Tetric® N-Bond Self-Etch Adhesive (SE).

Group V: The teeth were received composite filling using Fluoridated Tetric® N-Bond Self-Etch Adhesive (SE + FA 1%).

GroupVI: The teeth were received composite filling using Fluoridated Tetric® N-Bond Self-Etch Adhesive (SE + CaF_2 7%).

On the buccal and lingual surfaces, preparation of a standardized class V cavities were done (3mm height, 3mm width, 2mm depth) in which the margins of the cavities were located about 1 mm occlusal to the cemento-enamel junction.

In groups using of Tetric® N-Bond as a dentine bonding adhesive (**groups I, II and III)** , the Class V cavities were etched using the total etch technique with 37% phosphoric acid gel for 15 seconds as manufacturer's instructions, then using water for rinsing the bonding surface for 5seconds until there was no trace of etchant gel could be seen on the surface, excess water was removed by applying gentle stream of air for 5 seconds at a distance of approximately one cm **(Stanislawczuk et al., 2009).** A thick layer of Tetric® N-Bond (TE, TE+FA 3% or TE +CaF_2 5% according to each group) was applied on the cavity surfaces by using of a disposable brush tip, the material was brushed gently into the dentin for 10 seconds, excess material and the solvent was removed by a gentle stream of air using of triple syringe as recommended by manufacturer's instruction. The LED light curing unit (SDI, Australia) was used to light-cure the adhesives for 10 seconds according to the manufacturer's instruction.

In groups utilizing of Tetric® N-Bond Self-Etch as a dentine bonding adhesive, (**Groups IV, V and VI**), a dry operating field was ensured. Subsequently, a thick layer of Tetric® N-Bond Self-Etch (SE, SE+FA 1% or SE +CaF_2 7% according to each group) on all cavity surfaces was applied and brush in for at least 30 seconds (the total reaction time was no shorter than 30 seconds), then a strong stream of air was utilized to disperse excess amounts of Tetric® N-Bond Self-Etch until there was no longer any movement of the material as recommended by manufacturer's instruction. The LED light curing unit (SDI, Australia) was used to light cure the adhesives for 10 seconds according to the manufacturer's instruction.

For all groups of this study the Tetric® N-Ceram(shade A2) composite was applied by the aid of plastic instrument according to the manufacturer's instruction. The application of restorative material (Tetric® N-Ceram) was done in a single increment slightly more than the height of the cavity (2 mm). By using of a suitable size of transparent-cervical-anatomically shaped matrices standardized for class V restorations for molars that selected and placed it on the restoration. Then the excess of composite material was removed by using dental explorer and the composite was cured by using of light curing device for 20 seconds according to the manufacturer′s instructions. Optrapol finishing burs was used for finishing the restoration. A stereo microscope was used to check the restored teeth to confirm that no excess of composite material was remained along the margins of the restorations.

All specimens were exposedto thermal changes cycles utilizing custom made thermocycling device. The procedure performed by cycling the teeth in a two custom made water baths; one of the water baths kept at 5°± 0.5°C while the other at 55°± 0.5°C, with a dwell time of 30 seconds. The cycles number were 500 cycles as per the International Organization for Standardization (ISO TR 11405) (**Pazinatto et al., 2003; Loguercioel al., 2004).**

Each sample was subjectedtoan axial mechanical load cycling (50N for 50000 cycles)by a custom-made apparatus in trying to mimic the clinical situation **(Paula et al., 2008)**.

The teeth were coated with two layers of nail varnish except for the restoration and 1 mm of peripheral area. This was done to prevent dye penetration from anywhere except via the margins of the restoration. **(Webber et al., 2014; Hajizadeh et al., 2015).**

The teeth were then stored in container containing 2% Methylene Blue for 24 hours at room temperature. After removal from the dye solution, teeth were rinsed under running tap water and left at room temperature for 2 hours for dryness and dye fixation **(AL-Fatlawi, 2004).**

The specimens were separated in bucco- lingual direction at the middle of the restorations. All specimens were segmentedinto two equal halves by the sectioning machine with water cooling. The extent of dye penetration was measured for each restoration on both the gingival and occlusal restoration-tooth interfaces using a stereomicroscope under a magnification of 45x.

Image analysis (Image J) software was used for the estimation of the linear leakage occurred at the gingival and occlusal margin areas.

3. Results

The descriptive statistics (mean values, standard deviation, standard error, maximum and minimum values) at the occlusal and gingival regions for all groups are represented below.

Gingivally; the TE+CaF_2 5% group shows the least linear dye penetration depth between the Total Etch groups while TE+FA 3% group shows the highest values. While in the Self-Etch groups, the SE group shows the highest linear dye penetration depth while the SE+CaF_2 7% shows the lowest values.

Occlusally, the TE group shows the least linear dye penetration depth between the Total Etch groups while TE+FA 3% group shows the highest values. In the Self-Etch groups, the SE group shows the lowest linear dye penetration depth while the SE+CaF_2 7% shows the highest linear dye penetration depth values.

To compare among the materials at each region, ANOVA test was performed to analyze the presence of statistically significant difference for the linear dye penetration depth (μm) at gingival region and occlusal region.

For the Total Etch groups gingivally, ANOVA test shows that there was a highly significant difference at $P<0.01$ level while for the Self-Etch groups, ANOVA test shows that there was a significant difference at $P<0.01$ level.

For occlusal region, ANOVA test for the Total Etch groups shows that there was a highly significant difference at $P<0.01$ level while for the Self-Etch groups, ANOVA test shows that there was a significant difference at $P<0.01$ level.

LSD test was performed for gingival regions; the results for Total Etch groups showed that, there was statistical highly significant difference when comparing TE with TE+FA 3% (TE is less in microleakage) and when comparing TE+FA 3% with TE+CaF_2 5% (TE+CaF_2 5% is less in microleakage), and a significant difference when comparing TE with TE+CaF_2 5% (TE+CaF_2 5% is less in microleakage). while for Self-Etch groups, the results showed that there was a significant difference when comparing SE with SE+CaF_2 7% (SE+CaF_2 7% is less in microleakage) and when comparing SE+FA 1% with SE+CaF_2 7%(SE+CaF_2 7% is less in microleakage)and a non-significant difference when comparing SE with SE+FA 1%.

For occlusal regions LSD test was performed; the results for Total Etch groups showed that, there was a highly significant difference when comparing TE with TE+FA 3% (TEis less in microleakage) and when comparing TE+FA 3% with TE+CaF_2 5% (TE+CaF_2 5% is less in microleakage), and a non-significant difference when comparing TE with TE+CaF_2 5%. while for Self-Etch groups the results showed that there was a significant difference when comparing SE with SE+CaF_2 7%(SEis less in microleakage) and a non-significant difference when comparing SE with SE+FA 1%and a non-significant difference when comparing SE+FA 1% withSE+CaF_2 7%.

T-test was performed to identify the presence of statistically significant differences for the linear dye penetration depth(μm) between control groups and also between Fluoroapatite addition groups and also between Calcium Fluoride addition groups at gingival and occlusal regions. The result showed that, in the gingival region there was a non-significant difference between control groups and a non-significant difference between Calcium Fluoride addition groups while between Fluoroapatite addition groups there was a highly significant difference at $P<0.01$ level (SE+FA 1% is less microleakage thanTE+FA 3%).

In the occlusal region, there was a highly significant difference between control groups (TE is less microleakage) and a highly significant difference between Calcium Fluoride addition groups (TE+CaF2 5% is less microleakage than SE+CaF_2 7%) while between Fluoroapatite addition groups there was a significant difference at $P<0.01$ level (SE+FA 1% is less microleakage).

T-test was performed to identify the presence of statistically significant differences for the linear dye penetration depth(μm) between gingival and occlusal regions of each group. The result showed that, there were a highly significant difference between occlusal and gingival linear dye penetration depthof all groups unless between TE+CaF_2 5% group there was a significant difference at $P<0.01$ level(occlusal is less microleakage).

4. Discussion

One of the most important inherent shortcoming of dental composite is the marginal leakage which leads to the gap between the restoration and tooth structure and subsequent problems due to this gap. The important cause of this leakage is the polymerization shrinkage of dental composite which have different thermal linear expansion than the thermal linear expansion of enamel and dentin. One of rules to prevent the marginal leakage is the used of dentin adhesive agent to ensure the sealing ability to prevent leakage and ensure longevity of restoration. Reinforcement of adhesive system with Fluoroapatite or Calcium Fluoride to ensure the release of Fluoride which is of great benefit in the gap formation and microleakage which cannot be prevented in any restoration in addition to the chemical reaction between this Fluoroapatite or Calcium Fluoride with the tooth structure that enhance the reduction of leakage in the restoration-tooth interface.

One of the methods to predict the clinical performance of restorative materials is the in vitro dye penetration study. For linear dye penetration study , restoration of class V cavity preparation was used because most of the in vitro studies using this cavity preparation to study the performance of adhesive bonding system because the flowing of composite resin during the polymerization shrinkage affected by the C factor which leads to increase the stress across the bonding interface, in addition to that this class V cavity design includes gingival margins in the dentin or a thin enamel thickness **(Santiago et al., 2010)**.

Thermal cycling was applied to induce maximum stress that the restored tooth can be exposed in the oral cavity (**Asaka et al., 2007)**.

To simulate the clinical situationin which teeth are subjected to mechanical stresses, an accelerated aging test in vitro by using mechanical load cycling was performed by using 50000 cycles which was applied on the center of the occlusal surface of the tooth , these forces promotes deformation of tooth and creates stresses on the margin of restoration which will increase the existing gaps or develop another gaps and this comes in agreement with others who used the same methods and mechanical load cycling **Gonzalez et al., 2006 andWang et al., 2011.**

In this study none of the adhesive systems whether fluoridated or non-fluoridated, occlusal or gingival, Total Etch or Self Etch systems showed perfect sealing at the tooth-restoration interface and this comes in agreement with **Dörfer et al.,2000**; **Li et al.,2002**.

The overall results in this study showed that the leakage gingivally for all adhesive systems whether fluoridated or non-fluoridated were more than the leakage occlusally and this can be attributed to the adequate thickness of enamel and the orientation of enamel prisms and dentinal tubules at this region which are aligned generally in perpendicular direction to both dentinoenamel junction and tooth surface **(Owens et al.,2006)**.

The overall result showed that the linear dye penetration weather occlusally or gingivally were confined to enamel and didn't reach the dentin and this may be due to chemical reaction between fluoridated adhesive and dentin in addition to the micromechanical interlocking.

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Table: Means, standard deviations, standard error, minimum and maximum values for linear dye penetration depth (µm) at the gingival region

	TE	TE+FA 3%	TE+CaF 5%	SE	SE+FA 1%	SE+CaF 7%
Mean	353	812.5	177.9	338.3	309.45	189.65
SD	257.751	282.85	316.29	191.24	153.43	81.439
SE	57.662	63.278	70.759	42.784	34.325	18.219
Min	100	471	13	92	79	61
Max	857	1435	1081	673	664	320

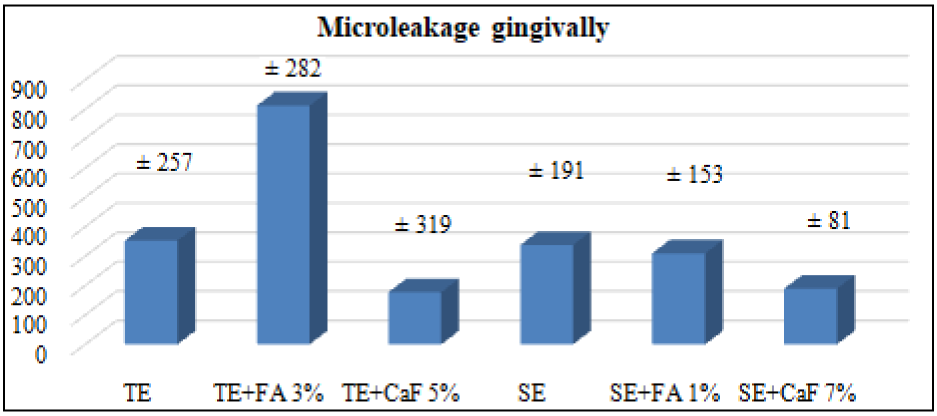

Figure: Bar chart showing the mean values for the linear dye penetration depth (µm) at gingival region.

Table: Means, standard deviations, standard error, minimum and maximum values for linear dye penetration depth (µm) at the occlusal region

10	TE	TE+FA 3%	TE+CaF 5%	SE	SE+FA 1%	SE+CaF 7%
Mean	45.05	173.25	50	71.05	89.25	122.2
SD	39.949	165.525	61.29	62.567	64.929	123.74
SE	8.9371	37.03	13.71	13.997	14.525	27.682
Min	0	9	7	9	8	9
Max	119	581	219	180	195	363

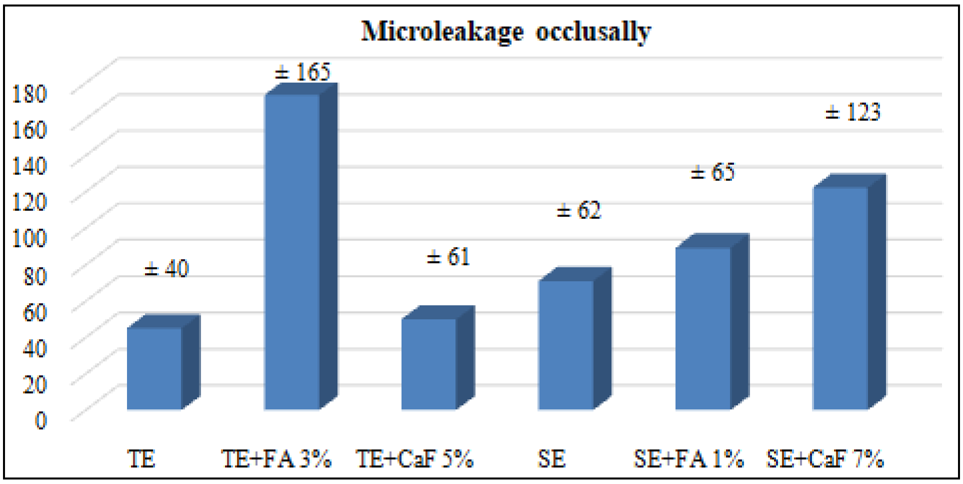

Figure: Bar chart showing the mean values for the linear dye penetration depth(µm) at occlusal region

Table: ANOVA test for the linear dye penetration depth(µm) among the Total Tech groups at gingival region.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4296782.800	2	2148391.400	26.483	.000
Within Groups	4683224.800	57	82161.839		
Total	8980007.600	59			

Table: ANOVA test for the linear dye penetration depth(µm) among the Self-Tech groups at gingival region.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	248541.233	2	124270.617	5.585	.006
Within Groups	1268255.700	57	22250.100		
Total	1516796.933	59			

Table: ANOVA test for the linear dye penetration depth(µm) among the Total Tech groups at occlusal region.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	211002.033	2	105501.017	9.664	.000
Within Groups	622270.700	57	10917.030		
Total	833272.733	59			

Table: ANOVA test for the linear dye penetration depth(µm) among the Self-Tech groups at occlusal region.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26888.433	2	13444.217	2.020	.049
Within Groups	445407.900	57	7814.174		
Total	472296.333	59			

Table: LSD test for the linear dye penetration depth(µm) among the Total Etch groups at gingival region.

	P-value	Sig
TE& TE+FA 3%	P<0.01	HS
TE& TE+CaF2 5%	0.045	S
TE+FA 3%& TE+CaF2 5%	P<0.01	HS

Table: LSD test for the linear dye penetration depth (µm) among the Self-Etch groups at gingival region.

	P-value	Sig
SE& SE+FA 1%	0.543	NS
SE& SE+CaF2 7%	0.003	S
SE+FA 1%& SE+CaF2 7%	0.014	S

Table: LSD test for the linear dye penetration depth (µm) among the Total Etch groups at occlusal region.

	P-value	Sig
TE& TE+FA 3%	P<0.01	HS
TE& TE+CaF2 5%	0.881	NS
TE+FA 3%& TE+CaF2 5%	P<0.01	HS

Table: LSD test for the linear dye penetration depth (µm) among the Self-Etch groups at occlusal region.

	P-value	Sig
SE& SE+FA 1%	0.518	NS
SE& SE+CaF2 7%	0.048	S
SE+FA 1%& SE+CaF2 7%	0.243	NS

Table: t-test for comparison of linear dye penetration depth(µm) between same addition groups at gingival region.

	t-test	P-value	Sig
TE& SE	.595	.559	NS
TE+FA 3%& SE+FA 1%	15.982	.000	HS
TE+CaF2 5%& SE+CaF2 7%	-.196	.847	NS

Table: t-test for comparison of linear dye penetration depth (µm) between same addition groups at occlusal region.

	t-test	P-value	Sig
TE& SE	4.962	.000	HS
TE+FA 3%& SE+FA 1%	3.468	.003	S
TE+CaF2 5%& SE+CaF2 7%	4.209	.000	HS

Table: t-test for comparison of linear dye penetration depth (μm) between gingival and occlusal regions of each group.

Gingival&Occlusal	t-test	P-value	Sig
TE& TE	6.267	.000	HS
TE+FA 3%& TE+FA 3%	22.145	.000	HS
TE+CaF2 5%& TE+CaF2 5%	2.216	.039	S
SE& SE	8.957	.000	HS
SE+FA 1%& SE+FA 1%	10.030	.000	HS
SE+CaF2 7%& SE+CaF2 7%	5.071	.000	HS