Papain–Based Gel for Chemo-Mechanical Caries Removal: Influence on Microleakage and Microshear Bond Strength of Esthetic Restorative Materials

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Abstract: Aim: This study was conducted to throw light on the effect of papain –based gel (Papacarié) on microleakage and microshear bond strength of two esthetic restorative materials ; glass ionomer restoration (Fuji IX GP) and light cured hybrid composite resin (Valux Plus, 3M ESPE). **Methods:** Microleakage test: thirty primary molars with carious occlusal surfaces were randomly assigned into three groups after caries removal using the papain based gel_(Papacarié) according to restorative material used. Group I: Glass ionomer restoration. Group II: Composite resin with etch and bond. Group III: Composite resin without etching step; bonding only. Restored teeth were subjected to thermocycling for 500 cycles at 5-55^{o} C with dowel time 15 seconds. Then teeth were immersed in 2% methylene blue solution for 24 hours, after which teeth were sectioned and the extent of dye penetration was evaluated under stereomicroscope. Micro-shear bond strength: twenty caries free molars imbedded in cylindrical acrylic moulds were prepared into flat dentinal surfaces. Teeth were randomly assigned into two groups according to bonded restorative material; Group I: glass iomomer and Group 2; light cured hybrid composite resin. Both groups were further subdivided into two subgroups according to the addition or omission of Papacarié prior to bonding. Restorative materials were bonded in the form of micro cylinders which were subjected to shear force until failure occurred. Fracture mode was analyzed under stereomicroscope. **Results:** Microleakage; glass ionomer restoration (group I) showed statistically significant highest percent leakage (50.71± 20.96%) and the highest mean score (2.71± o.95). No significant difference was noted between groups II and III. Micro-shear bond strength of glass ionomer restoration to dentin surface showed no statistically significant difference among subgroups I A and I B (P>0.05). While, micro shear bond strength of composite restoration to dentin surface was significantly higher in surfaces treated with Papacarié (subgroup II B) than those of untreated dentin surfaces (P <0.000). Fracture mode of glass ionomer was mainly adhesive and that of composite was cohesive. **Conclusion**: Composite resin restoration exhibited less microleakage and better micro-shear bond strength than glass ionomer after the use of Papacarié gel. Application of Papain-based gel (Papacarié) to dentine surface improves the micro-shear bond strength of composite resin, but it has no influence on micro-shear bond strength of glass ionomer.

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1. Introduction

Restorative dentistry has seen a paradigm shift from the invasive surgical approach laid by G.V. Black "extension for prevention" to a minimally invasive approach with advancement in diagnostic system and revolution in adhesion technology [1,2].

The conventional method for caries removal is usually carried out with high speed hand piece to access the lesion and a low speed hand piece to remove caries. Although, this method is quick and efficient in caries removal, it may result in unnecessary removal of sound tooth structure. In addition, caries removal with the conventional method is usually associated with pain, annoying sound and possibility of producing thermal and mechanical injuries to dental pulp. Furthermore, in children and patients with anxiety the conventional technique is often associated with discomfort [3,4]. These disadvantages potentiated the development of alternative minimally invasive techniques for caries removal; among them is the chemo-mechanical caries removal. Chemo-mechanical method of caries removal was first introduced by **Habib et al, 1975**, using sodium hypochlorite (Na OCl) [5]. However NaOCl itself was too corrosive to be used on healthy tissues , subsequently, it was diluted and buffered with sodium hydroxide ,sodium chloride and glycine producing a solution commercially known as GK101 [6] . The GK101 was able to soften only infected layers of carious dentine by selective attack of degenerated collagen fibers. [7] Glycine in GK 101 was later replaced by aminobutyric acid which is more effective. The product composed of N-monochloro-D-2aminobutyrate was marketed in the

United States as "Caridex". The Caridex system was claimed to involve the chlorination and disruption of the partially degraded collagen [8] .The large volume required for removal, lengthy procedures, unpleasant taste , in addition to the delivery system which is no more commercially available; have limited its use for caries removal[9] .

Carisolv , a newer system for chemo-mechanical caries removal possessing the same mode of action as Caridex but utilizing three amino acids; glutamic acid , leucin and lycine was manufactured. It was marketed in two syringes one containing 0.5% sodium hypochlorite solution and the other contains the three amino acids together with carboxymethycellulose to make it viscous and erythrosine to make it visible. The gel has been reformulated with higher concentration of sodium hypochlorite and omission of the color agent (colorless Carisolv) [9]. Although, Carisolv has been proven to be efficient in removing carious dentine, it has many disadvantages including short shelf life, high cost and the necessity of special set of instruments [10, 11].

In 2003, a Brazilian formulation was introduced and commercially denominated Papacarié. Papacarié is basically composed of papain, chloramines, toluidine blue, salts, thickening vehicle and preservatives[12]. Papain is a proteolytic enzyme, similar to pepsin , it acts only on infected tissues which lack the α1-antitripsine plasmatic anti-protease that inhibit proteolysis in healthy tissues. Chloramines present in the product have the potential to dissolve carious dentine through chlorination of partially degraded collagen [13]. Since the release of the papain –based gel (Papacarié), most of the conducted studies evaluated its efficiency in caries removal or its effect on the surface topography of residual dentine [12-15]. **Corrêa et al, 2008** studied the residual dentine surface following caries removal with Papacarié and Carisolv compared to conventional rotary instrument, they found that chemo-mechanical methods of caries removal results in amorphous layer similar to smear layer with few exposed dentinal tubules, while conventional caries removal produced a smooth and regular dentinal surface [16]. **Bittencourt et al., 2010** conducted a study to quantify the mineral content removed from primary teeth after Papacarié application and they found that Papacarié affects only the carious component of teeth [17].

Since the outcome of bond strength between the tooth surface and the restorative material is dependent on the characteristics of the remaining dentine surface, the question remains whether chemo-mechanical caries removal using Papacarié could influence the bond strength to restorative materials. Micro-tensile bond strength of total- etch and self-etch adhesives to sound and carious dentine exposed to Papacarié gel was evaluated. The results revealed that Papacarié reduced micro-tensile bond strength to carious dentine [18]. However few studies were conducted in this area, therefore the current study was conducted to throw light on the effect of Papacarié gel on microleakage and microshear bond strength of glass ionomer and composite to dentin surface.

2. Materials and Methods:

Fifty extracted primary molars were collected from the outpatient clinic of the Pediatric and Community Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University. Selected teeth were extracted, because of over retention, from patients with an age range of 9-11 years. Thirty molars with occlusal caries not involving pulp exposure were assigned for the microleakage test and 20 caries free molars for the microshear bond strength test. All teeth were cleaned, disinfected in 0.5% chloramine T, and subsequently stored in 0.9% saline solution at room temperature for no longer than one month until the beginning of the experiment [19].

Caries removal:

The primary molars with occlusal carious lesions were filled with the Papacarié (F&A Labartôrio Farmacéutico Ltda, São Paulo , Brazil), after 30 seconds ,the gel immediately turned turbid with debris; the softened carious dentin was scraped away using the opposite side of a spoon excavator in a pendulum movement. The gel was re-applied many times until it reached an unchanged light color, (according to the manufacturer's instructions). The cavities were neither washed nor dried in between applications. After caries removal had been completed, as judged by the clinical criteria (probing and visual inspection), the gel was removed with a cotton pellet soaked with water. The cavities appeared vitreous in appearance.

Restoration:

Teeth were randomly assigned into 3 groups (10 each) according to restorative material used.

Group I: Glass ionomer restoration (Fuji IX GP capsule, Gc Crop, Tokyo). Priming and coating of restorative material was performed.

Group II: Light curing hybrid composite resin with etch and bond (Valux Plus TM Restorative, 3M ESPE). It was built in increments of 2mm thickness that cured for 40 sec/increment(Xl 3000,3M ESPE ,st. paul MN, USA, 450mw/cm2)

Group III: Composite resin restoration without etching step, bond only.

All materials were manipulated according to manufacturer's instructions.

Microleakage test:

A. Thermocycling regime:

All specimens were thermocycled for 500 cycles between 5°C and 55°C, with dwell time 15 seconds in each bath (Neslab FTC-350, GP200, USA) and 5 seconds interval between them in the distilled water [19].

B. Preparation prior to sectioning:

After thermocyc1ing the specimens were taken out, dab dry with a tissue paper and the root apex sealed with sticky wax. The teeth were coated with a two layer of nail varnish except for 1.0 mm of the restoration margins, were kept free of any coating. The coated teeth were then immersed in 2 % methylene blue solution dye for 24 hours. After removal from the dye, the coating was removed with acetone solution and the teeth were thoroughly washed under tap water for 10 minutes and dab dry with tissue paper .The specimens were transferred to specimen bottles containing distilled water until the time of sectioning.

C. Sectioning of specimens

The specimens were embedded in an acrylic resin block (Orthodontic Resin, Dentsply/Caulk). Then teeth were sectioned longitudinally in the mesio-distal plane at the mid line of the restoration. Sectioning of teeth was carried out using a diamond wheel saw.

D. Microleakage evaluation procedure

Specimens were viewed under a binocular stereomicroscope at 20X magnification (Leica S 8APO –Leica DFC 290 camera). The extent of dye penetration along the tooth-restoration interface was measured in mm at all areas of tooth restoration interface and the mean was calculated. Both sectioned specimens were scored and the average of both readings was recorded .All specimens were examined by one calibrated examiner.

E. Scoring of microleakage

The severity of dye penetration was analyzed in two ways:

1. Scoring system [19,20] with a scale ranging from 0 to 3 as follows:
   0: No dye penetration
   1: Dye penetration up to less than half the cavity depth
   2: Dye penetration up to more than half the cavity depth, but not extending to the axial wall
   3: Dye penetration up to the axial wall and beyond
2. Percent value: obtained by recording length of dye penetration along the tooth restoration interface to the whole total length of the enamel and dentine interface [21].

2. Microshear bond strength:

A. Preparation of teeth

Twenty caries free molars were utilized for the micro-shear bond strength test. Occlusal surfaces of these molars were removed and the dentine was ground using 600-grit SiC paper under running water to create a smooth flat dentine surface, then teeth were imbedded in cylindrical acrylic moulds.

B. Grouping

Teeth were randomly assigned into two groups according to bonded restorative material;

Group I: glass iomomer restoration

Group 2: light curing hybrid composite resin

Both groups were further subdivided into two subgroups;

Subgroup A: no treatment was performed before bonding of restorative material (control)

Subgroup B: Papacarié was applied to the dentin surface for 60 seconds, and then gel was removed with spoon excavator. The same procedure was repeated three times after which the dentine surface was cleaned with a wet cotton pellet and restorative material was applied.

C. Bonding procedure

Both materials were manipulated according to manufacturer instructions and bonded to dentine surfaces in a polyvinyl tube 1.8 mm in diameter and 2mm in height to form micro-cylinders of glass ionomer or composite resin. Three tubes were placed per tooth giving rise to15 samples in each subgroup.

D. Measurement of bond strength

Each acrylic embedded molar tooth with its own bonded glass ionomer or composite resin micro-cylinders was secured with tightening screws to the lower fixed compartment of the testing machine (Model LRX-plus; Lloyd Instruments Ltd, Fareham, UK) with a load cell of 5 KN. A loop prepared from orthodontic wire 0.014 inch in diameter was wrapped around the bonded micro-cylinder assembly as close as possible to the base of the micro cylinder and aligned with the loading axis of the upper movable compartment of the testing machine.

Shear forces were applied to the material – dentine interface at a cross head-speed of 0.5 mm

/min until failure occurred .The relatively slow crosshead speed was selected in order to produce a shearing force that resulted in debonding of the micro-cylinder along the substrate-adhesive interface. The load required to debonding was recorded in Newton. The load-deflection curves were recorded using computer software (Nexygen-MT Lloyd examined).

Microshear bond strength calculation;

- The load at failure was divided by bonding area to express the bond strength in MPa :

$\boldsymbol{\delta = P/ \pi r^2}$

Where; δ =bond strength (in MPa)

P =load at failure (in N)

π =3.14

r = radius of micro-cylinder (in mm)

Fracture analysis

The fractured surfaces were examined using stereomicroscope at X20 magnification, the failure modes were categorized into one of three types [22]:

1. Adhesive failure between the material and dentin surface
2. Cohesive failure in the restorative material
3. Mixed failure ; combination of both

Statistical analysis:

Analysis was carried out using SPSS program version17 (Software Package for Social Statistics). The test used was ANOVA test (one-way) followed by post hoc test (Bonferroni test) to differentiate between the Means. Pearson's correlation test was carried out to detect possible correlation between microleakage and microshear bond strength. Level of significance was set at $p<0.05$.

3. Results:

A. Microleakage

Group I (glass ionomer restoration) showed the highest statistically significant percentage of leakage (50.71± 20.96) and the high mean scores (2.71±0.95). Sixty percent of samples in group I, showed dye penetration up to the axial wall of the cavity or beyond (score 3), in 25% of them, the dye penetration reached more than halve of the cavity wall (score 2) . The remaining 15% showed dye penetration to less than halve of the cavity wall (score1) .While 57% of specimens in group II exhibited excellent marginal seal, dye penetration score (0) and 43% of them showed dye penetration score (1). When etch procedure was omitted in group III, the microleakage score 1 and 2 in 75% and 25% of specimens respectively. However, mean values for microleakage in group II and III (0.43 ± 0.53 and 1.25 ± 0.50 respectively) were not statistically significant ($p>0.05$) .Table 1, figure 1.

B. Microshear bond strength

Micro shear bond strength of glass ionomer restoration (Group I) to dentin surface in either those treated with Papacarié or non - treated samples (subgroups A & B) showed no statistical significant differences ($p>0.05$). On the other hand Micro shear bond strength of composite resin restoration to dentin surface treated with papacarie (Group II B) was significantly higher than that of untreated dentin surface (Group II A) $p<0.000$. (table2)

Micro shear bond strengths of composite resin restoration in both subgroups were significantly higher than micro shear bond strengths of glass ionomer subgroups (table2).

Moreover, Pearson's correlation test revealed that there was an inverse correlation between microleakage and microshear bond strength of the studied materials ($r = -0.769$ at $p<0.01$) figure 2

C. Fracture analysis

Adhesive and mixed failures were most frequently observed in Group I, with higher percentage of adhesive failure (66.6%) in subgroup IA compared to subgroup IB (46.6%).

Group II showed mostly a cohesive failure and few specimens showed mixed failure, higher percentages of cohesive failure were observed in subgroup II B (86.6%) compared to subgroup IIA (73.3%).

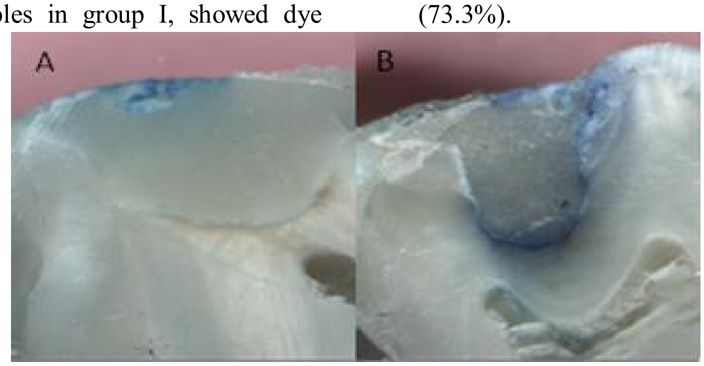

Figure 1: A; specimen restored with composite resin showing no micro-leakage (score 0), B: specimen restored with glass ionomer showing micro-leakage extending beyond the axial wall (score 3)

Table 1: Mean score values and percent of microleakage for the tested groups

	Percentage leakage			Mean score values of microleakage				
Groups	**Mean**	**S. D**	**Post hoc**	**Mean**	**S. D**	**Upper value**	**Lower value**	**Post hoc**
Group I	50.71	20.96	a	2.71	.951	3	1	**a**
Group II	2.86	5.06	b	0.43	.535	1	0	**b**
Group III	13.77	8.17	b	1.25	.500	2	1	**b**
p-value	0.000			0.000				

Group means with the same letters are not significantly different.

Table 2: Mean values for microshear bond strength (MPa)

Groups	Mean	S.D.	Post hoc
Group I A	2.364	1.204	c
Group I B	2.233	0.951	c
Group II A	10.829	1.594	b
Group II B	17.855	4.729	a
p-value	0.000		

Group means with the same letters are not significantly different than each other.
Group means with letter (a) are significantly higher than those with letter (b) or (c)

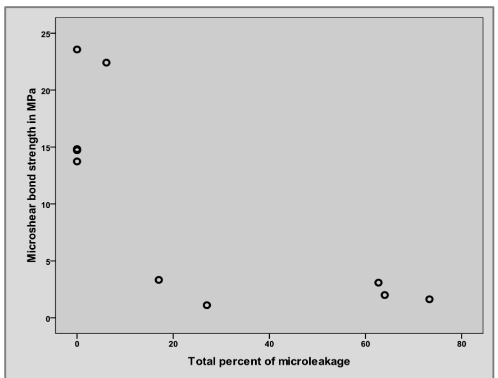

Figure 2: Correlation between microleakage and microshear bond strength of group I and group II

4. Discussion:

This study evaluated the influence of a papain based gel (Papacarié) as a chemo-mechanical caries removal agent on the microleakage and microshear bond strength of two esthetic restorative materials; glass ionomer and hybrid composite. These materials are selected for the investigation as being the most commonly used ones in restorative Pediatric Dentistry.

Since dye penetration is the most frequently used method for detecting microleakage (23) , it was employed in this study because it is simple, inexpensive and does not need sophisticated laboratory equipments. Prior to microleakage assessment, the investigated specimens were subjected to thermocycling to mimic intra-oral temperature variations. Temperature changes are responsible for the induced stresses at tooth restoration interface. There is no standard for thermocycling methodology in microleakage studies (24,25), and this permits contradictory discussions and results in various in vitro studies. In the current study temperature was standardized at 5ºC-55ºC and the dwell time was 15 s. These variables seem to be tolerated by the oral tissues and are suitable for clinical conditions (26)

Adequate marginal seal of a restorative material is attributed to proper adhesion capacity to tooth structure, low thermal coefficient and minimal setting shrinkage which are inherent properties in glass ionomer (27). Paradoxically, glass ionomer showed the highest significant microleakage scores (60% score 3), a finding that goes in accordance with several previous studies (28-30). **Ferriera et al.,2006** (31) obtained 94% of score 3 in teeth restored with Fuji IX and attributed his results to the reduced setting time of glass ionomer that does not allow proper flow of the material and consequently disrupts the marginal seal. An increased solubility and the porous nature of this material are important factors which might promote the potentiality to microleakage. The granulated texture of glass ionomer viewed under the stereomicroscope confirmed the previous suggestion and was in accordance with the study conducted by **Gupta et al., 2011**(24). Generally, discrepancies in microleakage results might be attributed to the numerical scoring system of leakage which is somewhat subjective and/or the applied thermocycling protocols that varies greatly among different studies. Therefore, results of the microleakage were assured through assessment of both scoring grade and percentage leakage to overcome lack of standardization in cavity size in tested groups.

However, obtained results contradicted those of **Castro and Feigal 2002** [21] who found that the improved conventional glass ionomer (Fuji IX) was similar to composite in terms of microleakage. Furthermore, they reported 24% mean percentage leakage in glass ionomer which was almost half of that obtained in the current study. This contradiction may be attributed to the difference in the dye used, way of scoring as well as the size and location of restored cavities.

Despite, the fact that glass ionomer exhibited varies degree of microleakage in-vitro, yet, the material performs better in clinical situations due to its fluoride releasing capacity and potential remineralization ability. Regardless of the method employed in caries removal, it can prevent recurrent caries which is one of the major causes of microleakage [32].

Polymerization shrinkage remains a major disadvantage for composite restorations due to contraction stresses at the tooth/adhesive interface, which could lead to gap formation and/or debonding. However, less filler content accompanied with more water sorption of microfilled composite would compensate for polymerization shrinkage. Besides, an expected improved viscosity and hence better marginal adaptability would be reasons to explain the lower statistical significance microleakage scores of composite resin groups [33].

The effectiveness of enamel acid etching technique in achievement of proper marginal sealing of the restorations was demonstrated by previous studies [33,34]. However, to signify the sealing ability of composite resin to dentin margins, the etching step was skipped in group III. The exhibited minimal dentinal leakage (25% score 2) agreed with a previous study which reported that adhesive systems increase sealing ability at restoration /dentin interface [35]. Additionally, the efficiency of Papain-based gel (Papacarié) in achieving good marginal seal instead of acid etchant was not questionable and could be extrapolated.

In this study the microshear bond test was selected for bond strength evaluation of both tested materials to dentin surface of primary teeth. This technique allocates stress distribution to be more concentrated at the interface allowing diminishing the cohesive failure of the restorative material [36] .In addition, it does not necessitate particular preparation that alters the bonding surface of the specimens as in the microtensile test [37].The mean micro-shear bond strength values of Fuji IX in the current study was (2.364 ± 1.204) approximating that obtained by **Banomyong et al., 2007**[38]. As regards the effect of papain-based gel (Papacarié) application, non-significant difference was recorded between microshear bond strengths of glass ionomer to either untreated or treated dentin surface by the gel. **Tanumiharja et al., 2000** [39] **and Banomyong et al., 2007**[38] reported that the chemical bonding to glass ionomer is not greatly influenced by conditioning of the dentin surface. A different adhesion strategy approach involves glass ionomer, in addition to chemical bonding interaction with the tooth surface. The less aggressive polyalkenoic acid of glass ionomer cleans the tooth surface, removes the smear layer only up to 0.5-1 µm depth and exposes collagen fibers without denuding hydroxyapatite [27]. Consequently, the effect of papain-based gel (Papacarié) could look a lot like a dentin conditioner in action and thus produced no effect on the bond strength of glass ionomer to tooth surface.

Several authors found diversity in the bond strength of restorative materials to sound and caries affected dentin. Reported bond strength to caries affected dentin was non-significantly higher than that of sound dentin but dependent on the adhesive type [40]. Yet, complete chemo-mechanical caries removal after Papacarié application in clinical situations possibly expose normal dentin with open tubules nearly similar to the sound dentin morphology as confirmed by SEM studies [41,42] . It was stated that chemo-mechanical caries removal by Papacarié resulted in less marked destruction of dentinal tubules [15]. Therefore, the results of the current study can be considered valid as in-vivo situations.

As regards the composite resin group, dentin surfaces treated with Papacarié recorded significantly higher bond strength values (17.855± 4.729) than those of untreated ones (10.829± 1.594). The quality of adhesion of this restorative material to tooth structure is affected by the smear layer produced by the excavation process, the mineral and organic content of the substrate and the hybrid layer formed due to the interaction between the bonding agent and the dentin [43]. Chemo-mechanical caries removal with Papacarié removes the smear layer completely and exposes dentinal tubules in similarity to total etch technique. [41,42,44]

Moreover, the papain-based gel (Papacarié) could induce micro-morphological changes to the collagen fibrils. A recent study by **Bertassoni and Marshall 2009** [45] showed that Papacarié promotes superficial degradation of collagen fibrils independent of a preceded partial degradation due to bacterial action. Therefore, the enhanced bond strength of composite resin to the tooth structure could be attributed to the clearly distinguishable hybrid layer which was found at the dentin resin interface after caries removal using Papacarié that allow achievement of utmost and durable adhesive bond [41,42,46].

In harmony with the concept of microshear test which reduces the possibility of cohesive failure of the material, mode of failure of glass ionomer was mainly adhesive. Mode of failure revealed differences between subgroups of glass ionomer. Partly in accordance with the results of **Banomyong et al 2007**[38] who reported differences in the mode of failure of conditioned and unconditioned dentin , in this previous study mixed failure was most frequently observed with Fuji IX glass ionomer. This might interpret for the mainly obtained adhesive failure which was in disagreement with cohesive failure of glass ionomer reported by previous studies.[39,47]

Regarding the composite resin group, the failure mode was mainly cohesive in the restoration itself. In contrast to the majority of previous studies which reported mostly adhesive failure. Generally, micromechanical interlocking of the restorative material to the dentin surface is enhanced after mechanical excavation [48]. Hence, a possible explanation is that the viscosity of the composite resin allows it to flow inside open dentinal tubules creating a micromechanical strong bond. The alteration in failure mode of both tested materials could refer to the reguosity of dentinal surfaces formed due to chemo-mechanical caries removal and/or the employed technique; microshear test. However, probability of achievement of stronger bond due to Papain- based gel (Papacarié) application cannot be overlooked.

In the current study a strong negative correlation could be established between micoleakage and microshear bond strength supporting the notion that failure in the interaction between adhesive system and tooth substrate yields poor marginal seal with consequent microleakage.

5. Conclusions:

According to the experimental design used in this study; the following conclusions could be drawn

1. Glass ionomer restoration (group I) recorded the maximum microleakage score compared to composite restoration (groups II and III).
2. Papain-based gel (Papacarié) increased the microshear bond strength of composite to dentin, whereas, it had no influence on that of glass ionomer.
3. Papain-based gel (Papacarié) enhanced the bond strength of composite resin restoration.

Recommendations:

Further researches are required to:

1. Investigate the effect of Papain –based gel (Papacarié) on dentin surface regarding topographical characteristics such as hardness and roughness and consequently adhesion.

2. Evaluate the clinical performance of esthetic restorations following chemo-mechanical caries removal using Papain –based gel (Papacarié).

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