

Microleakage Evaluation of Composite Restorations Following Papain-Based Chemo-Mechanical Caries Removal in Primary Teeth

Marwa Abdel Hafez / Mona Elkateb / Sonia El Shabrawy / Amel Mahmoud / Omar El Meligy

Aim: To evaluate the microleakage of composite restorations following Papain-based chemo-mechanical caries removal compared to the conventional drilling method. The characteristic of the hybrid layer was also studied using scanning electron microscopy. **Study design:** The sample included thirty freshly extracted and exfoliated primary molars with open proximal carious dentin lesions. Teeth were divided into two equal groups, according to method of caries removal. Following caries removal, cavity preparations were restored with composite resin. After thermocycling, teeth were sealed apically and coated with nail polish except the surface of restorations and the surrounding Imm. Teeth were immersed in basic fuschin dye solution, then they were sectioned mesiodistally. The extent of dye penetration was detected using a light stereomicroscope. After microleakage test, the resin/dentin interface was examined using scanning electron microscopy. **Results:** There was no significant difference in the degree of leakage between both groups. In the Papacarie group, longer and numerous resin tags were observed with statistically significant thicker hybrid layer than those following the drilling method. However, there was no significant difference between the diameters of resin tags of both groups. **Conclusions:** Papacarie does not adversely affect the microleakage of composite restorations and provides a suitable surface for bonding.

Key words: Chemo-mechanical Caries Removal, Primary Teeth, Papain gel.

* Marwa Abdel Hafez, BDS, MSc, Pediatric Dentist, Ministry of Health, Alexandria, Egypt

** Mona Elkateb, BDS, MSc, PhD, Professor of Pediatric Dentistry, Faculty of Dentistry, Alexandria University, Egypt and Professor of Pediatric Dentistry, College of Dentistry, Princess Nora bint Abdulrahman University, Saudi Arabia

*** Sonia El Shabrawy, BDS, MSc, PhD, Professor of Dental Biomaterials, Faculty of Dentistry, Alexandria University, Egypt

**** Amel Mahmoud, BDS, MSc, PhD, Lecturer of Pediatric Dentistry, Faculty of Dentistry, Alexandria University, Egypt

***** Omar El Meligy, BDS, MSc, PhD, Professor of Pediatric Dentistry, Faculty of Dentistry, King Abdulaziz University, Saudi Arabia and Professor of Pediatric Dentistry, Faculty of Dentistry, Alexandria University, Egypt

Send all correspondence to:

Omar El Meligy

Department of Pediatric Dentistry

Faculty of Dentistry

King Abdulaziz University

P.O.Box: 80209

Jeddah 21589, Kingdom of Saudi Arabia

Phone: 0122871660

Mobile: 00966557521584

E-mail: omeligy@kau.edu.sa

INTRODUCTION

In view of the need for adopting conservative treatment to preserve sound tooth structure in teeth affected by carious lesions, conventional caries removal may result in excessive loss of sound structure^{1,2} and often induces pain and discomfort³.

Several methods of caries removal have been proposed and developed as an alternative to the conventional drilling method⁴⁻⁸. Chemo-mechanical caries removal (CMCR) has so far been a promising method in pediatric dentistry, especially for anxious or medically compromised patients⁹. It helps to reduce the patient's stress¹⁰, minimize the need of local anesthesia⁹ as well as allowing a more selective removal of carious tooth structure¹¹.

The CMCR involves the chemical softening of carious dentin, followed by its removal with gentle excavation. Its main objective is to eliminate the outer infected layer, leaving behind the affected demineralized dentin that can be remineralized and repaired¹¹.

Studies have been carried out on the nature of the dentin surface remaining after complete caries removal by CMCR "Carisolv". They noted that the dentin surface left after the CMCR is rough, highly irregular and well suited to bonding with composite or glass ionomer^{9,12}.

Scanning electron microscopic analysis has also shown that “Carisolv” removes the smear layer leaving opened dentinal tubules^{13, 14}. Consequently, the dentin becomes more permeable to the penetration of the adhesive system and, the bonding of resin restoration to tooth structure will be enhanced¹⁵.

A stable adhesion between resin restorations and dental structure is fundamental to the clinical success of restorations because an adhesion failure yields poor marginal seal with consequent microleakage¹⁶. This allows the infiltration of bacteria and oral fluids that may lead to the development of a secondary carious lesion¹⁷. The results of several studies revealed no significant difference in microleakage of bonded restorations between the conventional and Carisolv CMCR method^{18, 19}.

Papacarie is a CMCR product consisting basically of papain and chloramine²⁰, which together are responsible for the Papacarie’s bactericide, bacteriostatic and anti-inflammatory characteristics²¹. Papain, the main component of the gel, is a proteolytic enzyme similar to the human pepsin.

Researchers concluded that Papacarie was effective in removing infected dentin, while preserving the deeper layer of affected dentin²²⁻²⁶. In addition, Papacarie was easy to manipulate, did not require neither special instruments nor local anesthesia, cheap and comfortable to the patient^{20, 27}. Moreover, the results of several studies indicated that the papain-based gel did not interfere with the bond strength of the adhesive restorative materials to sound and demineralized dentin²⁸⁻³⁰.

Few studies are available in the literature concerning the effect of papain-based chemo-mechanical system on marginal seal of the adhesive restorations in primary teeth³¹. More investigations are still needed to verify if this method would provide a tooth surface suitable for bonding and marginal seal of resin restorations.

The aim of this study was to evaluate and compare the microleakage of composite restoration following caries removal using a new chemo-mechanical agent (Papacarie) versus the conventional drilling method. In addition, study the characteristic of resin /dentin interface using scanning electron microscope (SEM) following both caries removal methods.

MATERIALS AND METHOD

The Ethical Committee at the Faculty of Dentistry, Alexandria University approved the research protocol.

Thirty exfoliated human primary molars and freshly extracted at time of shedding with proximal carious dentin lesions were selected. All teeth were cleaned from debris and blood stains and kept in distilled water at room temperature, before the restorative and testing procedure.

The teeth were randomly divided using the random bowl technique³² into two equal groups of fifteen teeth each, according to the method of caries removal. Group I: the experimental group, in which the carious lesions were removed using CMCR agent, Papacarie (Laboratorio Farmaceutico Ltda, Sao Paulo (SP)–Brazil). Group II: the control group, in which the carious lesions were removed using conventional drilling method.

Microleakage Test

For group I using the chemo-mechanical method²³: The Papacarie was applied on the carious lesion for 60 seconds. The softened decayed dentin was scraped away in a pendulum motion with a blunt excavator (Martin. Gebruder Tuttlingen. Germany). The gel was reapplied whenever it appeared cloudy. The procedure was repeated until the gel appeared clear and reached an unchanged light color. The remaining gel was removed with a cotton-pellet soaked in water.

For group II using the conventional drilling method: Cavity preparation has been done using a high speed hand piece under water irrigation with a # 330 bur (SS White Burs, New Jersey) and caries were removed using a sharp excavator. The cavity outline followed the guidelines of conventional cavity preparation for resin restorations³³. The gingival margin was beveled to enhance resin adaptation and seal. The cavities were checked for remaining caries by visual and tactile sensation using an explorer.

Restorative Procedure³⁴

After caries removal by either method, cavities of the two groups were rinsed and dried with oil free compressed air. The wall of the preparation was etched with 35% phosphoric acid (3M Dental Products, St. Paul, MN, USA) for 15 seconds. Then it was rinsed with water spray for 10 seconds and dried using oil-free compressed air for 15 seconds. Two consecutive coats of 3M single bond adhesive (3M Dental Products, St. Paul, MN, USA) were applied to etched enamel and dentin for 15 seconds with gentle agitation using fully saturated brush, followed by gentle air thinning for 5 seconds to evaporate solvents. Visible light (Halogen Light Unit, mega-physik Rastatt/Germany) curing for 10 seconds using a high intensity visible light source was done. 3M light activated composite Filtek Z250 (3M Dental Products, St. Paul, MN, USA) was applied in successive laminated increments to prevent excessive polymerization shrinkage^{35, 36}. The first increment, no thicker than 1mm was placed against the gingival floor, using small plastic filling instrument³⁷. After complete adaptation, it was light cured. Subsequent increments were placed in thickness no greater than 2 mm in an oblique layering technique. The restoration was cured from the facial and lingual aspects to ensure adequate polymerization throughout the entire restoration³⁸. Finishing was delayed for 3 minutes to allow approximately 70% of maximal polymerization to occur during the dark-curing phase following application of the curing light. This is because early finishing of composite resin after placement has been shown to significantly increase microleakage³⁹. After that, shaping of occlusal surfaces was accomplished with fine diamonds finishing burs to provide the smoothest surface and minimize trauma-induced microleakage^{40, 41}. A final high polish was accomplished using a rubber prophylaxis cup with aluminum oxide polishing pastes. Before rebonding, etching was performed by applying 35% phosphoric acid to all the restoration margins for 10 seconds, rinsed off and then thoroughly dried. The single bond adhesive was applied, thinned with a blotted brush and light cured for 40 seconds⁴².

All teeth were stored ⁴³in distilled water at 37°C inside an incubator unit for 24 hours.

After storage, the teeth were thermocycled ⁴⁴ in a water bath for 500 cycles using the thermocycling machine, alternating between 5^o and 45^o C with a dwell time 20 seconds.

The pulp chamber, root apices and furcations were sealed with intermediate restorative material “IRM” (Dolla Soom, Egypt) and sticky wax (Dentsply International, Milford, USA). Teeth surfaces were coated with three layers of nail polish except the surface of restorations and the surrounding 1mm. Teeth were immersed in 0.5% basic fuchsin solution for 24 hours, then they were removed from the dye solution and washed under running water for half an hour. Teeth were sectioned mesiodistally using a diamond saw through the center of the restoration. Each tooth produced two specimens and the extent of dye penetration was detected using a light stereomicroscope (Olympus stereomicroscope SZ11, Tokyo, Japan)⁴⁵.

The following scoring criteria have been used according to Milleding ⁴⁶:

- 0=No microleakage
- 1=Microleakage along the enamel
- 2=Microleakage extending beyond the amelodentinal junction
- 3=Microleakage along the floor of the cavity
- 4=Microleakage reaching the pulp.

The samples were evaluated for the degree of dye penetration by two independent examiners. The examiners were faculty from Departments of Pediatric Dentistry and Dental Biomaterials, Alexandria University. The examiners scored the degree of dye penetration on an ordinal scale ranging from 0 to 4.

Scanning Electron Microscopic Study

After the microleakage test, seven specimens were selected from each group of different microleakage score.

Materials and Equipments for SEM:

1. For sample preparation: (a) Ethyl alcohol, acetone and amyl acetate; (b) Vacuum desiccator; (c) Jeol coating apparatus (JFC-110 Sputtering Device, Jeol-Japan).
2. For studying the sample: Jeol SEM (JSM-5300 Scanning Microscope, Jeol-Japan).

Specimens were soaked in 6 mol/l Hydrochloric acid (HCl) for 30 seconds to dissolve the mineral component of dentin, followed by immersion in 5% Sodium hypochlorite (NaOCl) for 5 minutes to remove collagen that was not resin protected. Specimens were immersed in ethyl alcohol of serial concentrations, 30%, 50%, 70%, and 90%, 10 minutes each, then in absolute alcohol for one hour for dehydration of the specimens. Then specimens were passed in acetone for 30 minutes for neutralization of ethyl alcohol and complete dehydration. After that, the specimens were placed in amyl acetate for 30 minutes for absorption of acetone and complete dehydration. The specimens were kept in vacuum desiccator containing silica gel to avoid rehydration. The specimens

were then mounted on a copper stub using a silver paint. They were placed in the glass-bell jar of the Jeol coating apparatus for 3 minutes to be covered with a thin layer of gold palladium at 200 Å⁰ (Å⁰ = Angstrom = 1x10⁻⁶ mm) that was enough for the scanning procedure without affecting the surface details of the specimens. Then the specimens were placed in the chamber of the Jeol SEM operating at 25 KV to be scanned and photographed. The resin / dentin interface were then examined at magnification (X 3500) and at higher magnification (X 5000) to evaluate the adaptation of hybrid layer, resin-tag formation and the lateral branching. The thickness of hybrid layer (tags' penetration) and the diameter of resin tags were measured using computer calibration linked to the SEM ⁴⁷.

Statistical Analysis

I. Microleakage Test:

Descriptive statistics for microleakage scores were displayed.

Comparison of microleakage scores at the occlusal and cervical locations in the same group as well as between group I (experimental group) and group II (control group) was done using Fisher exact test.

II. Scanning Electron Microscopic Study:

Descriptive statistics for the thickness of hybrid layer as well as the diameter of resin tags were displayed as mean and standard deviation. Comparison of mean values between the experimental group and control group was done using t test.

Significance level was set at 5% level. Statistical Package for Social Sciences Computer Software (SPSS) version 15.0 was used for the statistical analysis.

RESULTS

Microleakage Test

In the present study, 30 exfoliated human primary molars and freshly extracted at time of shedding with proximal carious dentin lesions were used for the microleakage test. After longitudinal sectioning, each tooth produced 2 specimens. The scoring was done according to Milleding scoring criteria ⁴⁶.

Table 1 shows a comparison of microleakage scores between occlusal and cervical margins in group I (Papacarie group). Statistical analysis was carried out utilizing Fisher exact test. At the occlusal margin, all specimens (100%) treated with Papacarie gel showed score 0 microleakage. Regarding the cervical margin, score 0 was noted in 93.3% of the specimens, while 6.7% (2 out of 30 specimens) revealed score 1 microleakage. There was no statistically significant difference between microleakage scores at the occlusal and cervical margins in group I (Papacarie) with P value = 0.49 NS set at 5% level.

Regarding group II (conventional group); there was no significant difference between occlusal and cervical margins in microleakage scores, which were listed in table 2. No microleakage was observed in group II specimens prepared by the conventional drilling method (100% of the specimens).

Table 3 shows a comparison of microleakage scores between group I (Papacarie) and group II (conventional). Score 0 represents 93.3% of total specimens treated with Papacarie gel. Score 1 microleakage was only detected in 6.7% (2 out of 30 specimens), while no specimens showed scores 2, 3 or 4. Regarding group II (conventional), score 0 microleakage was observed in 100% of specimens. Statistical analysis of the data using Fisher exact test showed no statistically significant difference between group I and group II with P value = 0.49 NS.

Scanning Electron Microscopic Study

The scanning electron microscopic study was done to study the characteristic of the resin/dentin interface (hybrid layer). After microleakage test, 7 specimens were selected from each group according to microleakage scores.

Table 4 shows the thickness of hybrid layer of group I (Papacarie) and group II (conventional method). Table 5 shows the mean and standard deviations of the thickness of the hybrid layer of the two groups. The mean thickness of the hybrid layer in group I (Papacarie group) was 55.35 ± 12.10 microns, while it was 11.04 ± 1.83 in group II (conventional method). The statistical analysis of data using t test showed a significant difference between the two groups with P value < 0.0001.

Table 6 shows the diameter of resin tags in microns in group I (Papacarie) and group II (conventional) specimens.

Table 7 shows the mean and standard deviations of the diameter of resin tags between the two groups. The mean diameter of resin tags in group I (Papacarie) was 0.79 ± 0.11 microns, while it was 0.80 ± 0.11 in group II (conventional method). There was no statistically significant difference between the two groups using t test with P value = 0.79 NS.

In group I, where the teeth have been prepared with Papacarie gel, a well defined hybrid layer and resin tags formation was observed, with intimate adaptation between resin and dentin (figure 1). Multiple lateral branching of resin tags were evident. The thickness of hybrid layer was approximately 43.8-68.6 microns in most specimens presented with score 0 microleakage (figure 2), also multiple long resin tags were seen.

However, the specimens with score 1 microleakage revealed a thinner hybrid layer, the thickness were approximately 42.5 microns (figure 3). Intimate adaptation was noted but fracture of dentin and gaps occurred in some parts of the specimens. The resin tags appeared broken and shorter than those of score 0 microleakage.

SEM of resin/ dentin interface of specimens prepared with conventional bur (group II) revealed thinner hybrid layer than that of group I. It ranged from 9.31 to 14.15 microns (figure 4). Intimate adaptation of hybrid layer and resin tags formation were also seen (figure 5). The lateral branching of resin tags were evident but to a lesser extent than those of group I.

Table 1. Comparison of microleakage scores between occlusal and cervical margins in group I (Papacarie).

Microleakage scores	Occlusal N (%)	Cervical N (%)
Score 0	30 (100)	28 (93.3)
Score 1	0	2 (6.7)
Score 2	0	0
Score 3	0	0
Score 4	0	0
P value of Fisher exact test	0.49 NS	

NS: Not statistically significant

Table 2. Comparison of microleakage scores between occlusal and cervical margins in group II (conventional method).

Microleakage scores	Occlusal N (%)	Cervical N (%)
Score 0	30 (100)	30 (100)
Score 1	0	0
Score 2	0	0
Score 3	0	0
Score 4	0	0
Test of significance	-	

Table 3. Comparison of microleakage scores between group I (Papacarie) and group II (conventional method).

Microleakage scores	Group I Papacarie N (%)	Group II Conventional N (%)
Score 0	28 (93.3)	30 (100)
Score 1	2 (6.7)	0
Score 2	0	0
Score 3	0	0
Score 4	0	0
P value of Fisher exact test	0.49 NS	

NS: Not statistically significant

Table 4. Thickness of the hybrid layer in microns of group I (Papacarie) and group II (conventional method).

Specimen number	Group I (Papacarie)	Group II (conventional)
1	42.50 microns	12.80 microns
2	53.46 microns	10.47 microns
3	66.27 microns	11.30 microns
4	43.80 microns	9.47 microns
5	68.60 microns	9.80 microns
6	44.60 microns	9.31 microns
7	68.21 microns	14.15 microns

Table 5. Comparison of mean hybrid layer thickness in microns between the Papacarie and the conventional method of caries removal.

	Thickness in microns	
	Group I Papacarie	Group II Conventional
Min- max	42.50- 68.60	9.31- 14.15
Mean ± SD	55.35 ± 12.10	11.04 ± 1.83
t test		9.58
P value		<0.0001*

*: Statistically significant at P≤0.05

Table 6. Diameter of resin tags in microns of group I (Papacarie) and group II (conventional method).

Specimen number	Group I (Papacarie)	Group II (conventional)
1	0.59 microns	0.64 microns
2	0.74 microns	0.71 microns
3	0.74 microns	0.75 microns
4	0.81 microns	0.83 microns
5	0.87 microns	0.86 microns
6	0.88 microns	0.91 microns
7	0.88 microns	0.92 microns

Table 7. Comparison of mean resin tags diameter in microns between the Papacarie and the conventional method of caries removal.

	Diameter in microns	
	Group I Papacarie	Group II Conventional
Min- max	0.59- 0.88	0.64- 0.92
Mean ± SD	0.79 ± 0.11	0.80 ± 0.11
t test		0.28
P value		0.79 NS

NS: Not statistically significant

Figure 1. A SEM image of the hybrid layer in group I specimen (Papacarie group) of score 0 microleakage showing hybrid layer formation and resin tags (arrows). Note the intimate adaptation between resin and dentin (X 3500). Bar = 5 microns. C = Composite, D = Dentin.

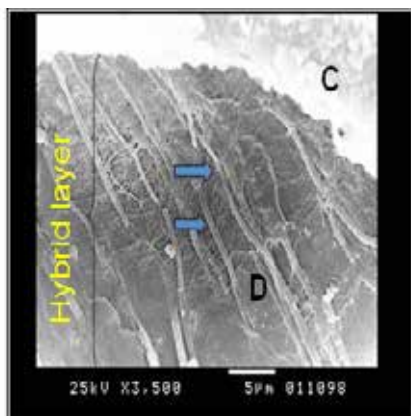


Figure 2. Another SEM image of the hybrid layer in group I specimen (Papacarie group) of score 0 microleakage showing multiple resin tags (arrows). Note the thickness of hybrid layer (X 3500). Bar = 5 microns. C = Composite, D = Dentin.

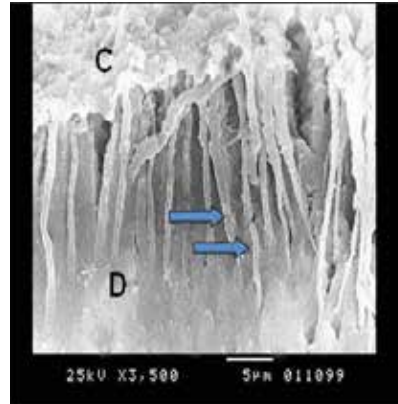


Figure 3. A SEM image of the hybrid layer in group I specimen (Papacarie group) of score 1 microleakage. Note the thinner hybrid layer than that of score 0 specimen. The arrows showing fracture in dentin in some parts of the specimen (X 3500). Bar = 5 microns. C = Composite, D = Dentin.

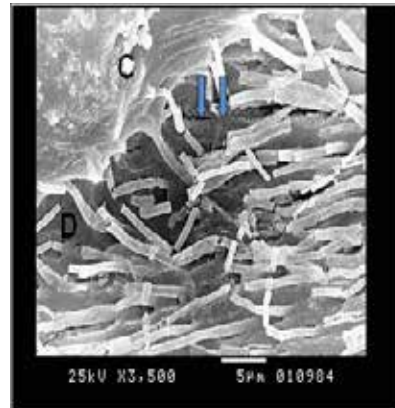


Figure 4. A SEM image of the hybrid layer in group II specimen (conventional method) of score 0 microleakage showing a well-defined hybrid layer. Note the thinner hybrid layer than that of group I specimens (X 3500). Bar = 5 microns. C = Composite, D = Dentin.

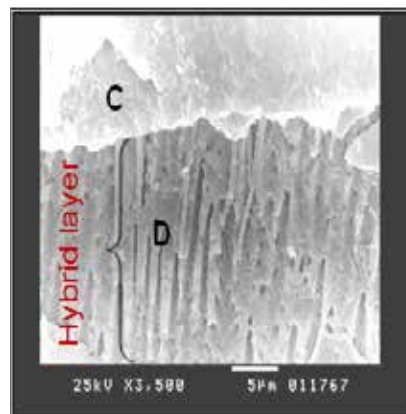
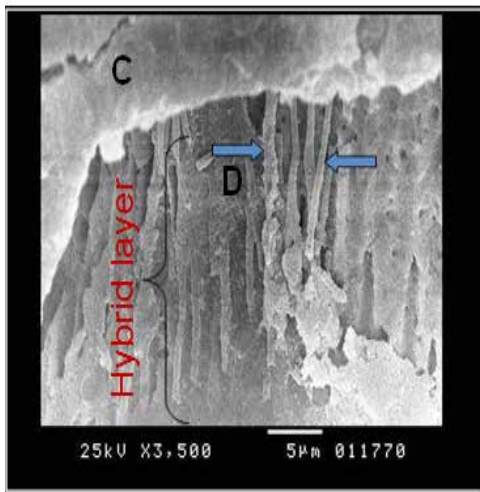


Figure 5. A SEM image of the hybrid layer in group II specimen (conventional method) of score 0 microleakage showing intimate adaptation between dentin and resin. Resin tags (arrows) are evident but to a lesser extent than those of group I (X 3500). Bar = 5 microns. C = Composite, D = Dentin.



DISCUSSION

Microleakage Test

Marginal seal plays a major role in the success of dental restorations. Additionally, proper adhesion between the restorative material and the cavity walls results in good marginal sealing with less microleakage and a longer life of the restoration⁴⁸.

The statistical analysis in this present study indicated that there was no statistically significant difference between the microleakage in the two methods of caries removal. These results were in accordance with several previous studies who reported that using the CMCR method appeared to decrease the microleakage^{19, 49, 50}.

On the contrary, Araujo *et al*³¹ suggested that the chemo-mechanical method of caries removal using Papacarie gel compromised the marginal seal of the adhesive restorative material. In addition, they showed that there was no statistically significant difference in marginal leakage at the cervical wall between the Papacarie group and the conventional drilling method. However, there was a statistically significant difference at the occlusal wall between the two groups regarding the degree of microleakage.

In the present study, 93.3% of group I (Papacarie) specimens and 100% of group II (conventional) showed no microleakage (score 0). This may be attributed to the technique followed during cavity preparation and restorative procedures. Also a perfect seal along the resin / dentin interface can be established within demineralized collagen matrix when it is completely infiltrated by adhesive resins⁵¹. In group II (conventional method), a bevel was done at the gingival margins to enhance the resin adaptation and seal³⁴. According to Sturdevant *et al*⁵², it has been recorded that the bevel is potential to increase the retention and the marginal seal, as bevels in enamel provide more surface area for acid etching and

bonding. During restoration, incremental technique was used in both groups, which was suggested to decrease the marginal leakage⁵³. Studies suggested that the incremental technique prevent excessive polymerization shrinkage because the thin increments ensured proper light irradiation^{35, 36}.

Additionally, the manner of curing used from the facial and lingual aspects might improve the marginal integrity, because this allows the polymerization shrinkage to be directed toward the facial or lingual proximal walls and margins^{54, 55}. Also the rebonding has been shown to improve the marginal integrity of composite resin restoration in vitro⁵⁶. Eick and Welch⁵⁷ and Lutz *et al*⁵⁸ suggested that the finishing procedures are destructive to the composite resin, which may exacerbate the marginal gaps formed during polymerization. So all restoration margins should be rebonded because the low viscosity of the rebonding resin facilitates its penetration in the interfacial gaps and microcracks⁵⁹.

In the present study, score 1 microleakage was detected in only two specimens (6.7%) from a single tooth in group I prepared with Papacarie gel. The leakage was observed at the cervical margin in that tooth. This may be attributed to the deep location of the cervical margin gingivally with thin enamel wall. During sample selection, it was difficult to find primary molars with proximal caries without extensive lesions and pulp involvement.

The same results were obtained by Toledano *et al*⁶⁰, who found that the microleakage in occlusal margins is less than gingival margins because the enamel interfaces show a better resistance against polymerization shrinkage forces. This resistance will lead to crazing in enamel margins. If shrinkage forces overcome the dentin bond strength, it will produce marginal gap that is usually seen in the gingival margins.

These findings also accord the results of Mousavinasab and Jafary⁴³, who reported a more considerable degree of leakage in the gingival than the occlusal margin either in conventional or chemo-mechanical method. Additionally, this was in agreement with Casagrande *et al*⁶¹ who found that cervical enamel-dentin / composite resin interface is more vulnerable to microleakage than other sites of tooth / resin interface. According to Herrin and Berry⁶², and Fuks *et al*⁶³, leakage at the gingival margin was contributed to a combination of factors such as thin enamel, poor adherence of the material at the cervical margin and the difficulty of condensation of the composite to the gingival wall.

Scanning Electron Microscopic Study

The formation of a uniform hybrid layer in all cavity walls becomes fundamental to allow a hermetic sealing of the tooth / restoration interface⁶⁴.

In the present study, to assess the characteristic of the hybrid layer after CMCR using the Papacarie gel versus the conventional drilling method, the resin / dentin interfaces were treated with 0.5 N HCl followed by 5% NaOCl to examine the features of the hybrid layer. The hybrid layer was treated with HCl to remove the surrounding mineral allowing its visualization without affecting the acid resistant hybrid layer or resin. NaOCl was used to remove any exposed

collagen that was not resin protected⁶⁵. The hybrid layer can be revealed from either sound dentin or resin⁶⁶.

In the present study, a significant difference in the thicknesses of the hybrid layer was observed between the Papacarie group (experimental group) and the conventional group (control group). In group I (Papacarie group), the hybrid layer was thicker than that of group II in most specimens, and ranging from 42.5 – 68.6 microns and many evident long resin tags with lateral branching were seen.

Regarding the conventional drilling group, the thickness of the hybrid layer was lower than that of group I ranging from 9.31 – 14.15 microns with few lateral branchings. The two groups showed a well defined hybrid layer and intimate adaptation between resin and dentin with resin tags formation. The results of Kotb *et al*²⁵ could explain the deeper resin penetration in group I. Following Papacarie treatment; the dentin surface was generally free of smear layer with patent dentinal tubules. A possible explanation of the absence of the smear layer is the presence of chloramines, which tend to chlorinate and dissolve the denaturated organic components leaving opened dentinal tubules²³. Consequently, the quality of bonding to adhesive material would be increased which in turn decreased the degree of microleakage⁶⁷.

The intimate adaptation in group II, could be attributed to the acid etch which removes the smear layer and smear plugs, opening the dentinal tubules, thus increasing dentinal permeability as well as enhancing the quality of bonding between resin and dentin⁶⁸. These results were in accordance with previous studies^{51,66}. Therefore, etching procedures need to be reviewed to ensure optimum adhesion of resin – based materials to dentin.

Also the result of the present study accords with Cehreli *et al*⁶⁹, who evaluated a single – bottle adhesive to caries affected human dentin after four different caries removal techniques. They demonstrated that CMCR using Carisolv system revealed the thickest hybrid layer. Moreover, the hybrid layer formed when affected dentin was bonded with the single – bottle adhesive systems was almost twice as thick compared with normal dentin, and this difference in thickness may be due to the reduction or absence of smear layer after treatment with Carisolv⁷⁰. On the other hand, the bur removal of dentinal caries produces a considerable smear layer on the dentinal surface with occluded tubules⁴⁹.

In 2005, Hosoya *et al*⁷¹ carried out another study comparing the characteristic of hybrid layer in primary dentin and permanent dentin. It has been demonstrated that the primary dentin had a thicker hybrid layer than permanent dentin after treatment with a CMCR agent (Carisolv).

In the present study, only one specimen with score 1 microleakage at the gingival margin was used to study the characteristic of resin /dentin interface. Intimate adaptation between resin and dentin was observed with fracture in dentin and gaps in some parts of the specimen close to the gingival wall. The resin tags appeared broken and to some extent shorter than those of the other specimens with score 0 microleakage among the Papacarie group. This may be related to the bonding of residual dentin after caries removal at the gingival margin that might contribute to the little micro-mechanical

retention⁷². This finding accords with another study done to analyze the voids in Class II composite resin restorations at the axial and gingival walls using SEM. It has been noted that more voids along the tooth / resin interface at gingival wall than the axial wall and this voids resulted in stress concentration and incomplete adhesion to dentin⁷³.

In the current study, measuring of the diameter of resin tags revealed no statistically significant difference between the experimental group (Papacarie group) and the control group (conventional group). This finding accords with Ceballos *et al*⁷⁴ who found that the resin bonding would form resin tags with a diameter similar to that of the dentinal tubules lumen. Brajdic *et al*⁷⁵ also reported a similar finding when studying the number, diameter, surface area of exposed dentinal tubules of the human coronal dentin and its influence on dentin hybridization.

On considering the multiple advantages of chemo-mechanical methods for dental caries removal, regarding the patient acceptance and comfort, the adhesive bonding, and the biocompatibility with both soft tissues and restorative materials^{76,77}, several studies concluded that the chemo-mechanical approach seems to be a valuable alternative for caries removal especially in pediatric patients⁷⁸⁻⁸¹.

So, it appears from the results of the present study that the utilization of Papacarie as a mean for caries removal in opened dentinal lesions has encouraging outcomes. This is a consequence of the marginal adaptation, which supports the clinical results of previous studies^{22, 23, 25, 82, 83}. Therefore, this method of caries removal might also be an alternative treatment to realize the new strategies of minimal invasive techniques.

CONCLUSIONS

From the results of the present study, it was concluded that:

1. The use of Papacarie for caries removal does not adversely affect the microleakage of composite restorations.
2. Papacarie treatment influences the characteristics of the hybrid layer (resin/dentin interface) and provides a suitable surface for bonding.
3. Papacarie treatment produces longer and numerous resin tags with thicker hybrid layer than those following the conventional drilling method.
4. The use of Papacarie gel does not affect the diameter of resin tags.

RECOMMENDATIONS

1. Since Papacarie does not affect the microleakage in class II resin restoration, it can be used for caries removal in primary teeth.
2. Further studies are needed to determine the shear bond strength of adhesive restorations following caries removal using Papacarie gel.
3. The effect of acid etching following Papacarie treatment on surface topography and depth of demineralization of dentin has to be determined.

REFERENCES

1. McNierny HD and Petruzillo MA. A gentle approach to operative dentistry: The Caridex caries removal system. *Gen Dent*, 34: 282-4, 1986.
2. Gwinnett JA and Barkmeier WW. Morphology of dentin surfaces in chemo-mechanically prepared cavities. *Am J Dent*, 1: 101-4, 1988.
3. Cederlund A, Lindskog S and Blomlof J. Efficacy of Carisolv-assisted caries excavation. *Int J Periodont Res Dent*, 19: 465-9, 1999.
4. Horiguchi S, Yamada T, Inokoshi S and Tagami J. Selective caries removal with air abrasion. *Oper Dent*, 23: 236-43, 1998.
5. Nielsen AG, Richards JR and Wolcott RB. Ultrasonic dental cutting instrument. *J Am Dent Assoc*, 50: 392-9, 1995.
6. Bassi G, Chawla S and Patel M. The Nd: YAG laser in caries removal. *Br Dent J*, 177: 248-50, 1994.
7. Miller CC, Decup F, Domejean-Orliaguet S, Gillet D, Guigand M, Kaleka R, Laboux O, Lafont J, Medioni E, Serfaty R, Toumelin-Chemla F, Tubiana J and Lasfargues JJ. Clinical evaluation of Carisolv chemo-mechanical caries removal technique according to the site/stage concept, a revised caries classification system. *Clin Oral Invest*, 7: 32-7, 2003.
8. Frencken JE, Pibt T, Sengpaisan Y, Phantum and Vanit P. Atraumatic restorative treatment (ART): Rational, technique, and development. *J Public Health Dent*, 56: 135-40, 1996.
9. Beeley JA, Yip HK and Stevenson AG. Chemo-mechanical caries removal: a review of techniques and latest developments. *Br Dent J*, 188: 427-30, 2000.
10. Kavvadia K, Karagianni V, Polychronopoulou A and Papagiannouli L. Primary teeth caries removal using the Carisolv chemo-mechanical method: a clinical trial. *Pediatr Dent*, 26: 23-8, 2004.
11. Ericson D, Zimmerman M, Raber H, Gotrick B, Bornstein R and Thorell J. Clinical evaluation of efficacy and safety of a new method for chemo-mechanical removal of caries. *Caries Res*, 33: 171-7, 1999.
12. Correa FNP, Filho LER and Rodrigues C. Evaluation of residual dentin after conventional and chemo-mechanical caries removal using SEM. *J Clin Pediatr Dent*, 32: 115-20, 2007.
13. Beely JA, Yip HK and Stevenson AG. Mineral content of the dentin remaining after chemo-mechanical caries removal. *Caries Res*, 29: 111-7, 1995.
14. Banerjee A, Kidd EA and Watson TF. Scanning electron microscope observations of human dentin after chemo-mechanical caries excavation. *J Dent*, 28: 176-86, 2000.
15. Hosoya Y, Shinkawa H and Marshall GW. Influence of Carisolv on resin adhesion for two different adhesive systems to sound human primary dentin and young permanent dentin. *J Dent*, 33: 283-91, 2004.
16. Yasuhiro T, Kousuke I, Yoshihiro N, Shikawa K and Kazuoni S. Effect of phosphoric acid etching prior to self-etching primer application on adhesion of resin composite to enamel and dentin. *Am J Dent*, 15: 305-8, 2002.
17. Dean JA, Avery DR and McDonald RE. McDonald and Avery's Dentistry for the child and adolescent. 9th ed. St. Louis, Missouri: Mosby; 297, 2011.
18. Mousavinasab SM and Jafary M. Microleakage of composite restorations following chemo-mechanical and conventional caries removal. *J Dent TUMS*, 1: 12-7, 2004.
19. Okida RC, Martins TM and Briso AL. In vitro evaluation of marginal leakage in bonded restorations, with mechanical or chemical-mechanical (Carisolv) removal of carious tissue. *Braz Oral Res*, 21: 176-81, 2007.
20. Bussadori SK, Martins MD, Fernandes KF, Guedes CC, Motta LJ, Reda SH and Santos EM. Evaluation of in vitro biocompatibility of a new material for the chemo-mechanical removal of caries-Papacarie. *Pesq Bras Odontoped Clin Integr*, Joao Pessoa, 5: 253-9, 2005.
21. Osata JA, Santiago LA, Remo GM, Cuadra MS and Mori A. Antimicrobial and antioxidant activities of unripe papaya. *Life Sci*, 53: 1383-9, 1999.
22. Silva LR, Motta LJ, Reda SH, Facanha RA and Bussadori SK. Papacarie: A new system for chemo-mechanical caries removal: A case report. *Rev Paul Odontol*, 26: 4-8, 2004.
23. Bussadori SK, Castro LC and Galvao AC. Papain gel: A new chemo-mechanical caries removal agent. *J Clin Pediatr Dent*, 30: 115-9, 2005.
24. Bussadori SK, Guedes CC, Martins MD, Fernandes KP and Santos EM. Papain gel: a new alternative for chemo-mechanical caries removal. *Acta Odont*, 3: 35-9, 2006.
25. Kotb RM, Abdella AA, El Kateb MA and Ahmed AM. Clinical evaluation of Papacarie in primary teeth. *J Clin Pediatr Dent*, 34: 117-23, 2009.
26. Bussadori SK, Godoy CH, Alfaya TA, Fernandes KP, Mesquita-Ferrari RA and Motta LJ. Chemo-mechanical caries removal with Papacarie: case series with 84 reports and 12 months of follow-up. *J Contemp Dent Pract*, 15: 250-3, 2014.
27. Motta LJ, Bussadori SK, Campanelli AP, Silva AL, Alfaya TA, Godoy CH and Navarro MF. Randomized controlled clinical trial of long-term chemo-mechanical caries removal using Papacarie gel. *J Appl Oral Sci*, 22: 307-13, 2014.
28. Lopes MC, Mascarini RC, Silva BRC, Florio FM and Basting RT. Effect of a Papain-based gel for chemo-mechanical caries removal on dentin shear bond strength. *J Dent Child*, 74: 93-7, 2007.
29. Gianini RJ, do Amaral FL, Flório FM and Basting RT. Microtensile bond strength of etch-and-rinse and self-etch adhesive systems to demineralized dentin after the use of a papain-based chemomechanical method. *Am J Dent*, 23: 23-8, 2010.
30. Botelho Amaral FL, Martão Florio F, Bovi Ambrosano GM and Basting RT. Morphology and microtensile bond strength of adhesive systems to in situ-formed caries-affected dentin after the use of a papain-based chemo-mechanical gel method. *Am J Dent*, 24: 13-9, 2011.
31. Araujo NC, Oliveira APB, Rodrigues VM and Andrade PMM. Evaluation of the marginal sealing of adhesive restorations after use of papain gel. *Pesq Bras Odontoped Clin Integr*, Joao Pessoa, 7: 67-73, 2007.
32. Bland M. An introduction to medical statistics. 3rd ed. Oxford University Press; 29-31, 2000.
33. Casamassimo PS, Fields HW, McTigue DJ and Nowak AJ. Pediatric dentistry; Infancy through adolescence. 5th ed. St. Louis, Missouri: Elsevier Saunders; 309-18, 2013.
34. Summit JB, Robbins JW and Schwartz RS. Fundamentals of Operative Dentistry. 2nd ed. Chicago: Quintessence; 260-98, 2001.
35. Crim AG. Microleakage of three resin placement techniques. *Am J Dent*, 4: 69-72, 1991.
36. Donly KJ, Wild TW, Browen RL and Jensen ME. An in vitro investigation of the effects of glass inserts on the effective composite resin polymerization shrinkage. *J Dent Res*, 68: 1234-7, 1989.
37. Bryant RW. Direct posterior composite resin restorations: a review 2. Clinical technique. *Aust Dent*, 37: 161-71, 1992.
38. Tjan AHL, Bergh BH and Linder C. Effect of various incremental techniques on the marginal adaptation of Class II composite resin restorations. *J Prosthet Dent*, 67: 62-6, 1992.
39. Fusayama A and Kohno A. Marginal closure of composite restorations with the gingival wall in cementum / dentin. *J Prosthet Dent*, 61: 293, 1989.
40. Ashe MJ, Tripp GA and Eichmiller FC. Surface roughness of glass-ceramic insert composite restorations: assessing several polishing techniques. *J Am Dent Assoc*, 127: 1495-500, 1996.
41. Kaplan BA, Goldstein GR, Vijayaraghavan TV and Nelson IK. The effect of three polishing systems on the surface roughness of four hybrid composites: a profilometric and scanning electron microscopic study. *J Prosthet Dent*, 76: 34-8, 1996.
42. Garcia-Godoy F and Malone WF. Microleakage of composite restorations after rebonding. *Compendium*, 8: 606-9, 1987.
43. Mousavinasab SM and Jafary M. Microleakage of composite restorations following chemo-mechanical and conventional caries removal. *J Dent TUMS*, 1: 12-7, 2004.
44. Virmanis, Tandon S and Roa N. Cuspal fracture resistance and microleakage of glass ionomer cement in primary molars. *J Clin Pediatr Dent*, 22: 55-8, 1997.
45. Chan DCN, Summit JB, Garcia-Godoy F, Hilton TJ and Chung KH. Evaluation of different methods for cleaning and preparing occlusal fissures. *Oper Dent*, 24: 331-6, 1999.
46. Milleding P. Microleakage of indirect composite inlays. An in vitro comparison with the direct technique. *Acta Odont Scand*, 50: 295-301, 1992.
47. Ishioka S and Caputo AA. Interaction between the dentinal smear layer and composite bond strengths. *J Prosthet Dent*, 61: 180-5, 1989.
48. Taylor MJ, Lynch E. Review microleakage. *J Dent*, 20: 3-10, 1992.

49. El kashlan H. Clinical and laboratory evaluation of a chemo-mechanical method of caries removal in primary teeth. PhD thesis, Faculty of Dentistry, Alexandria University, 2003.
50. Yamada Y, Kimura Y, Hossain M, Kinoshita J, Shimizu Y and Matsumoto K. Caries removal with carisolv system: criteria evaluation and microleakage test. *J Clin Pediatr Dent*, 30: 121-6, 2005.
51. Sakoolnamarka R, Burrow MF, Kubo S and Tyas MJ. Morphological study of demineralized dentin after caries removal using two-different methods. *Aust Dent J*, 47: 116-22, 2002.
52. Sturdevant CM, Roberson TM, Heymann HO and Sturdevant JR. *The Art and Science of Operative Dentistry*. 5th ed. St. Louis, Missouri: Mosby; 700-4, 2006.
53. Leclaire CC, Blank LW, Hargrave LW and Pellen GB. A two-stage composite resin fill technique and microleakage below the cemento-enamel junction. *J Dent Res*, 65: Abstr 799, 1986.
54. Tjan AHL, Bergh BH and Linder C. Effect of various incremental techniques on the marginal adaptation of Class II composite resin restorations. *J Prosthet Dent*, 67: 62-6, 1992.
55. Dickinson GL, Gerbo LR and Leinfelder KF. Clinical evaluation of highly wear resistant composite. *Am J Dent*, 6: 85-7, 1993.
56. Sturdevant CM, Roberson TM, Heymann HO and Sturdevant JR. *The Art and Science of Operative Dentistry*. 5th ed. St. Louis, Missouri: Mosby; 534-86, 2006.
57. Eick JD and Welch FH. Polymerization shrinkage of posterior composite resins and its influence on post operative sensitivity. *Quint Inter*, 17: 103-11, 1986.
58. Lutz F, Krejci I and Barbakow F. Quality and durability of marginal adaptation in bonded composite restorations. *Dent Mater*, 7: 107-13, 1991.
59. Reid JS, Saunders WP and Chen YY. The effect of bonding agent and fissure sealant on microleakage of composite resin restorations. *Quint Inter*, 22: 295-8, 1991.
60. Toledano M, Perdiago J and Osorio E. Effect of dentin deproteinization on microleakage of Class V composite restorations. *Oper Dent*, 25: 497-504, 2002.
61. Casagrande L, Brayner R, Brata JS and Araujo FB. Cervical microleakage in composite restorations of primary teeth. In vitro study. *J Dent*, 33: 627-32, 2005.
62. Herrin HK and Berry EA. Variables affecting the microgap of the enamel-composite interface. *J Dent Res*, 65: Abstr 777, 1986.
63. Fuks AB, Chosack A and Eidelman E. Assessment of marginal leakage around Class II composite restorations in retrieved primary molars. *Pediatr Dent*, 12: 24-7, 1990.
64. Patric C, Chersoni S, Acquaviva GL, Breschi L, Suppa P and Pashely DH. Permeability of marginal hybrid layers in composite restorations. *Clin Oral Invest*, 9: 1-7, 2005.
65. Correa FNP, Filho LER and Rodrigues C. Evaluation of residual dentin after conventional and chemo-mechanical caries removal using SEM. *J Clin Pediatr Dent*, 32: 115-20, 2007.
66. Hosoya Y, Kawashita Y, Marshall GW and Goto G. Influence of Carisolv for resin adhesion to sound human primary dentin and young permanent dentin. *J Dent*, 29: 163-71, 2001.
67. Perdiago J, Ericksson S and Rosa BT. Effect of calcium removal on dentin bond strengths. *Quint Inter*, 32: 142-6, 2001.
68. Nor JE, Feigal RJ, Dennison JB and Edwards CA. Dentin bonding: SEM comparison of the dentin surface in primary and permanent teeth. *Pediatr Dent*, 19: 246-57, 1997.
69. Cehreli ZC, Yazici AR, Akca T and Ozgünlaltay G. A morphological and micro-tensile bond strength evaluation of a single-bottle adhesive to caries-affected human dentine after four different caries removal techniques. *J Dent*, 31: 429-35, 2003.
70. Hoak R, Wicht MJ and Noack MJ. Does chemo-mechanical caries removal affect dentin adhesion? *Eur J Oral Sci*, 108: 449-55, 2000.
71. Hosoya Y, Shinkawa H and Marshall GW. Influence of Carisolv on resin adhesion for two different systems to sound human primary dentin and young permanent dentin. *J Dent*, 33: 283-91, 2005.
72. Tay FR, Gwinnett AJ, Pang KM and Wei SH. Variability in microleakage observed in a total-etch-wet-bonding technique under different handling conditions. *J Dent Res*, 74: 1168-78, 1995.
73. Purk JH, Dusevich V, Glaros A and Eick JD. Adhesive analysis of voids in Class II composite resin restorations at the axial and gingival cavity walls restored under in vivo versus in vitro conditions. *Dent Mater*, 23: 871-7, 2007.
74. Ceballos L, Toledano M, Osorio R, Tay FR and Marshall GW. Bonding to Er-YAG-laser-treated dentin. *J Dent Res*, 81: 119-22, 2002.
75. Brajdic D, Krznaric OM, Azinovic Z, Macan D and Baranovic M. Biological bases of dentin hybridization. *Coll Antropol*, 3: 901-6, 2008.
76. Martins MD, Fernandes KP, Motta LJ, Santos EM, Pavesi VC and Bussadori SK. Biocompatibility analysis of chemomechanical caries removal material Papacarie on cultured fibroblasts and subcutaneous tissue. *J Dent Child (Chic)*, 76: 123-9, 2009.
77. Garcia-Contreras R, Scougall-Vilchis RJ, Contreras-Bulnes R, Kanda Y, Nakajima H and Sakagami H. Cytotoxicity and pro-inflammatory action of chemo-mechanical caries-removal agents against oral cells. *In Vivo*, 28: 549-56, 2014.
78. El Kholany NR, Abdelaziz KM, Zaghoul NM and Aboulenine N. Chemo-mechanical method: A valuable alternative for caries removal. *J Min Int Dent*, 2: 248-60, 2009.
79. Bussadori SK, Guedes CC, Bachiega JC, Santis TO and Motta LJ. Clinical and radiographic study of chemical-mechanical removal of caries using Papacarie: 24-month follow up. *J Clin Pediatr Dent*, 35: 251-4, 2011.
80. Gupta S, Singh C, Yeluri R, Chaudhry K and Munshi AK. Clinical and microbiological evaluation of the carious dentin before and after application of Papacarie gel. *J Clin Pediatr Dent*, 38: 133-8, 2013.
81. Motta LJ, Bussadori SK, Campanelli AP, Silva AL, Alfaya TA, Godoy CH and Navarro MF. Efficacy of Papacarie in reduction of residual bacteria in deciduous teeth: a randomized, controlled clinical trial. *Clinics (Sao Paulo)*, 69: 319-22, 2014.
82. El-Kimary EIS. Comparison between the effectiveness of two chemo-mechanical caries removal methods on the residual bacteria in dentin of primary teeth. MSc thesis, Faculty of Dentistry, Alexandria University, 2009.
83. Kumar J, Nayak M, Prasad KL and Gupta N. A comparative study of the clinical efficiency of chemomechanical caries removal using Carisolv and Papacarie-a papain gel. *Indian J Dent Res*, 23: 697, 2012.