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DOES ACID-ETCHING JEOPARDIZE DENTIN BONDING DURABILITY?

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ABSTRACT

Purpose: The purpose of the study was to evaluate the impact of the etching process on the durability of normal versus sclerotic dentin bonded interfaces.

Materials & Methods: Micro-tensile bond strengths and interfacial nanoleakage, within bonded normal or sclerotic dentin interfaces created by Adper Single Bond 2, Optibond XTR, Single Bond Universal, Ketac Molar (glass ionomer) or Photac Fil (resin-modified glass ionomer), were evaluated after 24 h, 6 m and 12 m of water storage.

Results: Adper Single Bond 2, Optibond XTR and Single Bond Universal exhibited higher immediate bond strengths and nanoleakage than did Ketac Molar or Photac fil. Normal dentin exhibited higher immediate bond strength and lower immediate nanoleakage when compared to sclerotic dentin, using Adper Single Bond 2, Optibond XTR and Single Bond Universal. Twelve months of water storage resulted in a significant decrease in micro-tensile bond strength in both normal and sclerotic dentin bonded by these three adhesives. However, there was no significance difference between the bond strengths and nanoleakage created by Ketac Molar and Photac Fil, used with normal or sclerotic dentin at the three storage periods.

Conclusion: Compared to resin-based etching restoratives, mineral-based non-etching restoratives (Ketac Molar and Photac Fil) provided much more durable bonds, to both normal and sclerotic dentin, over a period of 12 months. It is obvious that the acid-etching procedure is the main cause of instability of resin-dentin bonded interfaces. Sclerotic dentin did not act as a bonding impediment with mineral-based non-etching restoratives as it did with resin-based etching adhesives.

Clinical Significance: Although recently introduced adhesive resins have attempted to improve resin-dentin bond durability, biodegradation of resin-dentin bonds over time continues to jeopardize the durability of resin composite restorations.

KEY WORDS: Sclerotic dentin, Microtensile bond strength, Dental adhesives, Glass ionomers.

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INTRODUCTION

Several studies have shown that resin-dentin bonds, created by current etch-and-rinse and self-etch adhesives, deteriorate over time due to rapid loss of dentin bond strength [1-4]. This problem does not occur in resin–enamel bonds as they are very stable over time [5].

Dentin collagen degradation by some matrix metalloproteinases (MMPs) and cysteine cathepsin K is one of the main causes of the destruction of resin-dentin bonded interfaces [1,6]. The mechanism of proteolytic degradation of resin-dentin interfaces has drawn more attention nowadays [7]. Several approaches have been introduced to prevent such enzymatic activity, aiming to improve dentin bond durability and consequently prolong the life span of adhesive restorations.

Human dentin contains at least MMP-2, -9 (gelatinases) [8-11], -8 (collagenase) [12], -3 (stromelysin) [13,14], and -20 (enamelysin) [15]. Since endogenous dentin MMPs and cysteine cathepsins are bound to collagen fibrils in mineralized dentin [10], acid-etching causes exposure of these enzymes, and activates their enzyme activity [16-20].

Thus, preserving the collagen fibrils within hybrid layers is essential for the stability of resin-dentin bonds over time [2]. Many attempts have been made to improve bond durability by inhibiting or inactivating MMPs and cysteine cathepsins. These maneuvers include application of EDTA, MDPB, and more recently chlorhexidine (CHX) as MMPs inhibitors. CHX, proved as an efficient inhibitor of endogenous collagenolytic/gelatinolytic activity of matrix metalloproteinases, preserves the bonding integrity [21-24]. However, some doubts were raised regarding their long-term effect, as it was mentioned that over time they leach out from the bonded interface, leaving it highly susceptible to degradation. This disadvantage may be overcome by using 1-2 wt% chlorhexidine methacrylate, where the inhibitor becomes covalently bonded to methacrylate [25].

Furthermore, dentin collagen network strengthening using different cross-linking agents[2,26-28] has been advocated to increase resistance of collagen to degradation by MMPs and cysteine cathepsins. Collagen cross-linking can be done either by chemical methods, where different cross-linking solutions such as glutaraldehyde, formaldehyde, transglutaminase, carbodiimide, genepin, and proanthocyanidin are used[26-32]; or by a physical method (also called photo-oxidative) that uses light exposure, especially ultraviolet radiation [33-35].

Most of the earlier studies were performed using sound teeth as a bonding substrate, even though most bonded teeth often contain sclerotic dentin. Usually sclerotic dentin is observed adjacent to caries, cervical defects and exposed root surfaces. Sclerotic dentin differs from normal dentin, in that the dentin tubules are partially or completely obliterated with inorganic deposits, reducing dentin permeability [36-42]. Micro-tensile bond strength of the dentin adhesive to sclerotic dentin was lower than to normal dentin [36-42]. Such a finding suggested that acid etching of sclerotic dentin was limited and, therefore, may not expose or activate the MMP activity of acid-etched sclerotic dentin, and thus may increase the durability of sclerotic dentin compared to normal dentin. Moreover, in seeking a long lasting bonding, an alternative non-etching bonding mechanism had to be used as a control. Thus, the aim of the current study was to evaluate the impact of the etching process on the durability of normal versus sclerotic dentin bonded interfaces. The null hypotheses tested were that (1) the bond strength and interfacial nanoleakage are not affected by the type of dentin and (2) the etching process does not affect the bond strength and interfacial nanoleakage expression after 1 year.

MATERIALS AND METHODS

Specimen preparation for micro-tensile bond strength (μTBS) test

A total of 120 extracted human teeth were used in this study. They were obtained from patients with informed consent using a protocol approved by the Institutional Review Board of King Abdulaziz University, Jeddah, Saudi Arabia. Sixty extracted non-carious third molars, obtained from young patients, were selected to provide normal dentin, while the other 60 non-carious molars were obtained from geriatric patients with occlusal attrition to provide sclerotic dentin. All teeth were stored in 0.5% chloramine T solution at 4ºC for not more than one month. Occlusal enamel and superficial dentin were removed perpendicular to the long axis of each tooth by a low speed diamond saw under water irrigation (Micromet AG, Munich, Germany). A standardized smear layer was created on the exposed flat middle/deep coronal dentin with 320 grit wet silicon carbide paper.

Sclerotic dentin was identified by visual examination according to the dentin sclerosis [40, 43] scale (Table 1). The teeth used for sclerotic dentin were classified as being in category 4, and the teeth used for normal dentin were classified as being in category 1. The quality of the acid-etched dentin in categories 1 and 4 are shown in Fig 1.

TABLE (1) Dentin sclerosis scale

Category	Criteria
1	No sclerosis present. Dentin is light yellow, opaque, with little translucency or transparency.
2	More than category 1 but < 50% of way between categories 1 and 4
3	Less than category 4 but > 50% of way between categories 1 and 4
4	Significant sclerosis present. Dentin has whitish, glassy appearance, with significant translucency or transparency evident.

Based on scale developed by Dr. Steven E. Duke of the University of Texas Health Science Center at San Antonio, and modified by the Department of Operative Dentistry at the University of North Carolina School of Dentistry [40] then modified by the Department of Biomaterials, Faculty of Oral and Dental Medicine, Cairo University [43].

The 120 teeth were divided into 2 main groups according to type of dentin; 60 normal and 60 sclerotic. These 2 large groups were each divided equally and randomly into 5 material groups of 12 teeth each (Table 2, Fig 2); 1) Adper Single Bond 2, 2) Optibond XTR, 3) Single Bond Universal, 4) Ketac Molar (Glass ionomer- GI) and 5) Photac Fil (Resin-modified glass ionomer- RMGI). These materials were applied to dentin according to manufacturers' instructions (Table 3). Then, five 1-mm thick layers (three 1-mm thick layers in case of Ketac molar and Photac fil groups) of nanohybrid

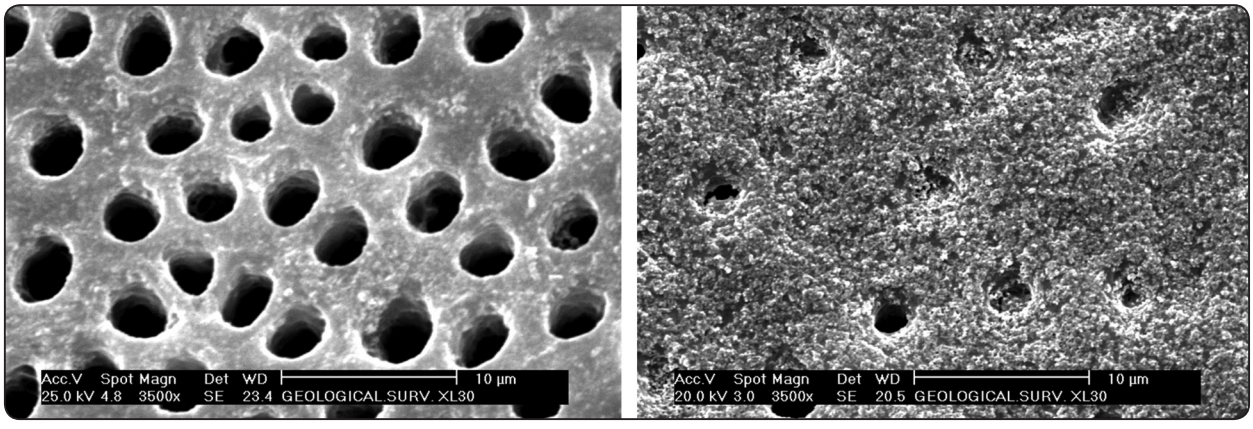

Fig (1) SEM photomicrograph image at 3500x showing : a) Category 1 tooth; etched normal dentin, b) Category 4 tooth; etched sclerotic dentin.

resin composite (Filtek Z 350 XT, 3M ESPE) were incrementally placed over the bonded dentin surface and individually polymerized for 20 s (Light Emitting Diode curing unit, 3M ESPE Elipar, Germany, 1200mW/cm^2, 430-480 nm). After soaking in 37ºC water for 24 h, each bonded tooth was cut into 16 sticks (1 mm x 1 mm ±0.1mm thick) using the non-trimming technique [44]. Sixteen sticks from each tooth were stored in separate containers (Table 2, Fig 2 and 3) in distilled water at 37ºC. The teeth of each of the 5 material groups were randomly assigned to three storage subgroups (N= 4 teeth) that were tested after storage for 24 h or 6 m or 12 m. One stick from each tooth was used to evaluate nanoleakage and the remaining 15 sticks were used to evaluate micro-tensile bond strength (Table 2, Fig 2 and 3).

TABLE (2): Flow chart of experimental design, number of teeth vs. sticks for microtensile bond strengths and nanoleakage.

			Micro-tensile Bond Strength	Nanoleakage
Adper Single Bond 2 + Normal Dentin	Teeth/Sticks	μTBS	Mean μTBS	
Time – 24 h	# 1-16	15	Tooth #1 mean μTBS	1 stick
	# 2-16	15	Tooth #2 mean μTBS	1 stick
	# 3-16	15	Tooth #3 mean μTBS	1 stick
	# 4-16	15	Tooth #4 mean μTBS	1 stick
	4 teeth x 16 sticks/ tooth= 64 sticks	60 sticks used for μTBS	Adper Single Bond 2, 24h: Grand mean ± SD	4 sticks used for 24 h nanoleakage*
Time – 6 m	# 5-16	15	Tooth#5 mean μTBS	1 stick
	# 6-16	15	Tooth#6 mean μTBS	1 stick
	#7-16	15	Tooth#7 mean μTBS	1 stick
	# 8-16	15	Tooth#8 mean μTBS	1 stick
	4 teeth x 16 sticks/ tooth= 64 sticks	60 sticks used for μTBS	Adper Single Bond 2, 6m: Grand mean ± SD	4 sticks used for 6 m nanoleakage
Time – 12 m				
	# 9-16	15	Tooth#9 mean μTBS	1 stick
	# 10-16	15	Tooth#10 mean μTBS	1 stick
	#11-16	15	Tooth#11 mean μTBS	1 stick
	# 12-16	15	Tooth#12 mean μTBS	1 stick
	4 teeth x 16 sticks/ tooth= 64 sticks	60 sticks used for μTBS	Adper Single Bond 2, 12m: Grand mean ± SD	4 sticks used for 12 m nanoleakage

Thus, each subgroup contained 4 teeth or 60 sticks for bond strength and 4 sticks for nanoleakage. There were three subgroups (24 h, 6 m, 12 m), and 2 types of substrates (normal and sclerotic dentin) so 24 teeth per material (12 teeth for normal dentin and 12 teeth for sclerotic dentin). The mean value for µTBS for 24 hr = the grand mean of 15 sticks per tooth. All 4 teeth were averaged to obtain a grand mean ± SD.

****Nanoleakage scores were averaged. The nanoleakage of the 4 sticks per subgroup were averaged to give a mean nanoleakage score for each time period in each material subgroup for each substrate.***

Table 2 could be extended to include Adper Single Bond 2 with sclerotic dentin at 24 h, 6 m, 12 m and the other 4 tested materials with normal or sclerotic dentin at 24 h, 6 m, 12 m, but these were not included to save space. The expanded table would show 24 more teeth for each of the 4 remaining materials. The total number of teeth is 24 x 5 (materials) = 120 teeth.

TABLE (3) Materials used in this study and their modes of application.

Material	Mode of application
Adper Single Bond 2 (2-step etch-and-rinse adhesive) 3M ESPE, St. Paul, MN, USA.	*Etching*: Apply Scotchbond™ Etchant to dentin. Wait 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. The surface should appear glistening without pooling of water. *Adhesive*: Immediately after blotting, apply 2-3 consecutive coats of adhesive to etched enamel and dentin for 15 seconds with gentle agitation using a fully saturated applicator. Gently air-thin for five seconds to evaporate solvents. Light cure for 10 seconds.
Optibond XTR (2-step self-etch adhesive) Kerr Corporation, Orange, CA, USA	Apply primer to dentin using scrubbing action for 20 sec. Air-thin with medium air pressure for 5 sec. Apply adhesive using light brushing motion for 15 sec. Air- thin with medium air pressure and then strong air for at least 5 sec. Light cure for 10 sec.
Single Bond Universal (1-step self-etch adhesive) 3M ESPE, St. Paul, MN, USA.	Apply adhesive to dentin by scrubbing action for 20 sec. Dry the adhesive for 5 sec and light cure for 10 sec.
Ketac Molar (Glass ionomer) 3M ESPE, St. Paul, MN, USA.	Mix the capsule at approx. 4,300 rpm in a high frequency mixing device for 10 sec. Apply the material to dentin forming a layer of 2 mm.
Photac Fil (Resin-modified glass ionomer) 3M ESPE, St. Paul, MN, USA.	Mix the capsule at approx. 4,300 rpm in a high frequency mixing device for 10 sec. Apply the material to dentin forming a layer of 2 mm. Light cure for 20 sec.

TABLE (4) Microtensile Bond Strengths (MPa) of the five material groups bonded to normal vs. sclerotic dentin after 24 h, 6m or 12 m of water storage.

	Normal Dentin			**Sclerotic Dentin**			**p-value**
	24 h	6 m	12 m	24 h	6 m	12m	
Adper Single Bond 2 (2- step E & R)	44.7 ± 2.6 Aa	34.5± 2.0 Cb	29.8± 2.6 Db	36.3± 2.3 Ba	34.8± 2.0 BCa	31.5± 2.5 Da	<0.001*
Optibond XTR (2- step SE)	46.4± 2.8 Aa	39.3± 1.8 Ba	34.7± 2.2 Ca	30.4± 2.4 Db	29.5± 1.9 Db	25.7± 2.2 Eb	<0.001*
Single Bond universal (1- step SE)	32.2± 2.2 Ab	31.7± 2.3 Ab	25.5± 2.3 Bc	21.5± 1.6 Cc	19.8± 2.2 Cd	15.2± 2.3 Dd	<0.001*
Ketac Molar Quick (GI)	20.6± 2.3 c	21.3± 2.0 c	20.8± 2.7 d	22.9± 2.3 c	24.5± 2.5 c	22.3± 2.5 c	0.175
Photac Fil Quick (RMGI)	18.8± 2.8 c	19.6± 2.4 c	18.1± 2.6 d	19.2± 2.5 c	21.5± 2.8 d	19.7± 2.5 c	0.416
p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	

Upper case letters are used for comparison between the groups within each horizontal row, while lower case letters are used for comparison between the groups within same vertical column (values are means ± SD in MPa, n=4). Four teeth contributed 15 sticks per tooth or 60 sticks per material/time group as described in Table 2. The statistical units were teeth, not sticks.

Table (5) Fracture mode analysis (%) of the five material groups bonded to normal vs. sclerotic dentin after 24 h, 6m or 12 m of water storage.

Material / Fracture Mode	Adper Single Bond 2 (2-step E & R)						Optibond XTR (2-step SE)						Single Bond universal (1-step SE)						Ketac Molar Quick (GI)						Photac Fil Quick (RMGI)						P-value
	Normal Dentin			Sclerotic Dentin			Normal Dentin			Sclerotic Dentin			Normal Dentin			Sclerotic Dentin			Normal Dentin			Sclerotic Dentin			Normal Dentin			Sclerotic Dentin			
	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	24 h	6 m	12 m	
Adhesive Failure	33	40	33	40	33	26	27	40	33	40	33	33	33	40	47	47	53	53	33	27	27	27	20	13	47	40	33	33	40	27	
Cohesive in material	7	0	7	0	7	7	0	7	7	0	7	0	7	13	6	0	7	0	20	20	26	13	20	20	13	7	13	7	13	13	
Cohesive in Dentin	0	0	7	0	7	7	13	13	7	13	7	0	7	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.001*
Mixed	60	60	53	60	53	60	60	40	53	47	53	67	53	40	47	53	40	47	47	53	47	60	60	67	40	53	54	60	47	60	

Four teeth contributed 15 sticks per tooth or 60 sticks per material/time group as described in Table 2. The fracture mode, whether adhesive or cohesive or mixed, of the 60 sticks from each group was examined following micro-tensile bond strength testing. The value was then expressed in % ($p<0.05$).

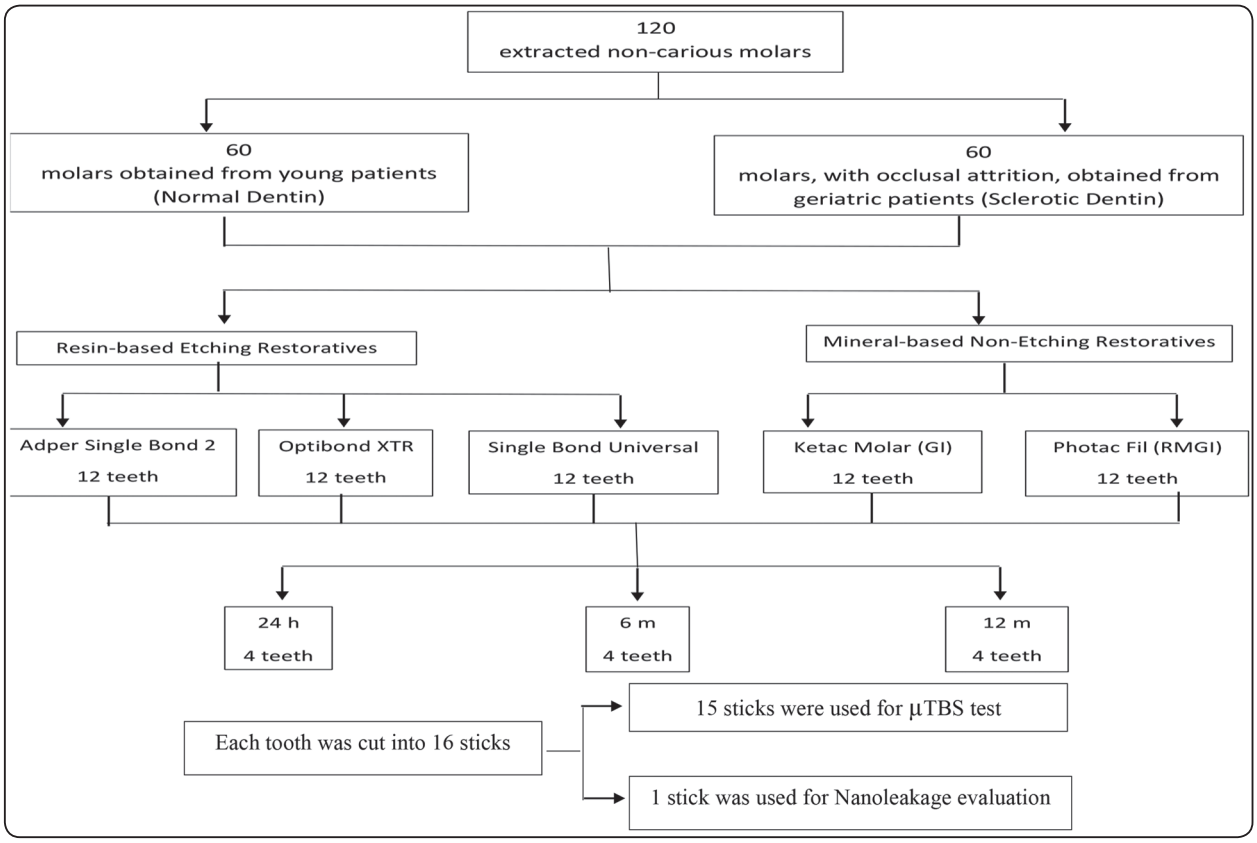

Fig. (2) Flow chart presenting the experimental design of the current study.

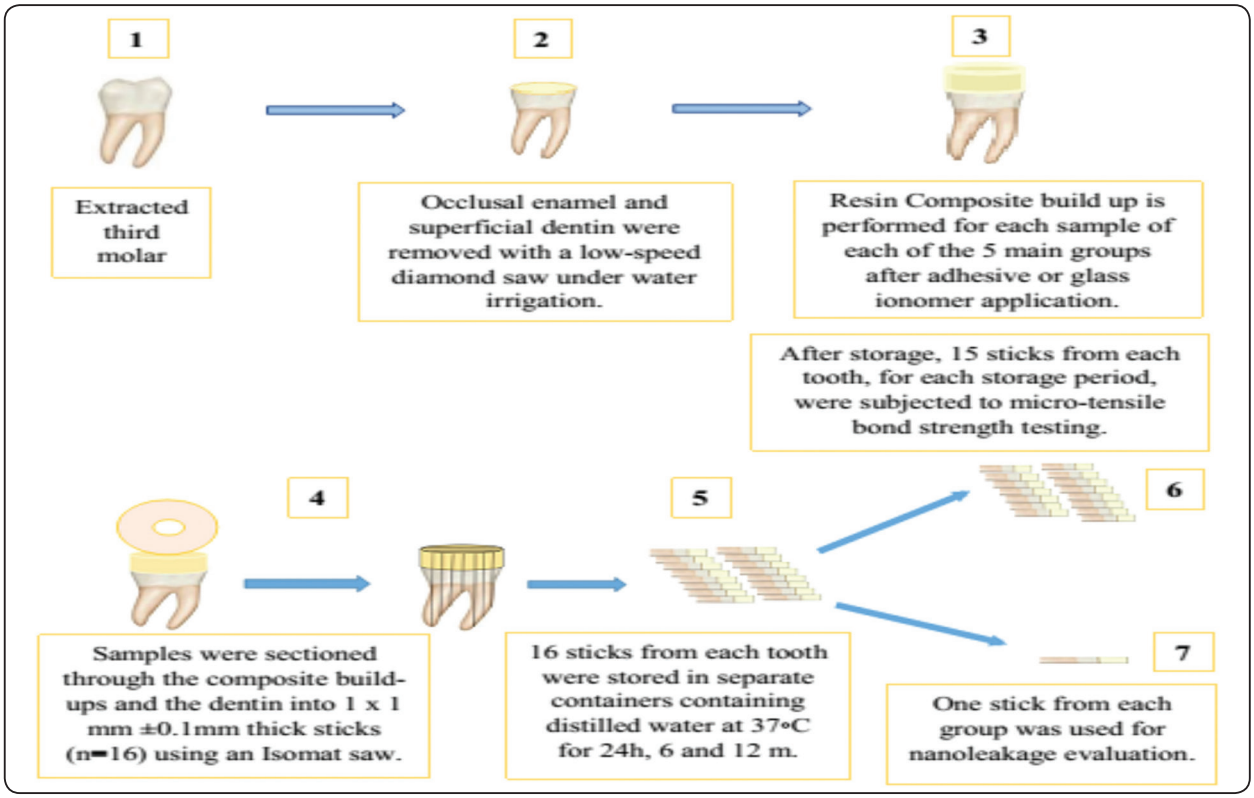

Fig. (3) Schematic illustration of sample preparation for microtensile bond strength test and nanoleakage evaluation.

TABLE (6) Silver nitrate nanoleakage (%) in the five material groups bonded to normal vs. sclerotic dentin after 24 h, 6 m or 12 m of water storage.

	Normal Dentin			**Sclerotic Dentin**			**p-value**
	24 h	6 m	12 m	24 h	6 m	12m	
Adper Single Bond 2 (2-step E & R)	51± 3.6 Da	62.7± 5.0 CDa	84± 3.4 Aa	61± 4.2 Ca	64± 4.3 Ca	73.5± 4.1 Ba	<0.001*
Optibond XTR (2-step SE)	48± 5.9 Da	58± 5.9Da	68.3± 3.1 Ca	75± 5.4 Ba	79± 3.4 Ba	86± 3.8 Aa	<0.001*
Single Bond Universal (1-step SE)	70.4± 4.5 Ba	74± 3.0 Ba	85± 6.9 Aa	87± 3.9 Aa	88.5± 3.1 Aa	92± 2.7 Aa	<0.001*
Ketac Molar (GI)	26.4± 10.4 b	26.9± 10.2 b	27.5± 8.6 b	23± 7.6 b	24.4± 7.9 b	23.5± 9.1 b	0.475
Photac Fil (RMGI)	27.8± 7.9 b	27.2± 8.3 b	28± 5.1 b	24.6± 9.2 b	25.7± 4.8 b	26.8± 7.9 b	0.215
p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	

Upper case letters are used for comparison between the groups within each horizontal row, while lower case letters are used for comparison between the groups within same vertical column (values are means ± SD in %, n=4 as described in table 2).

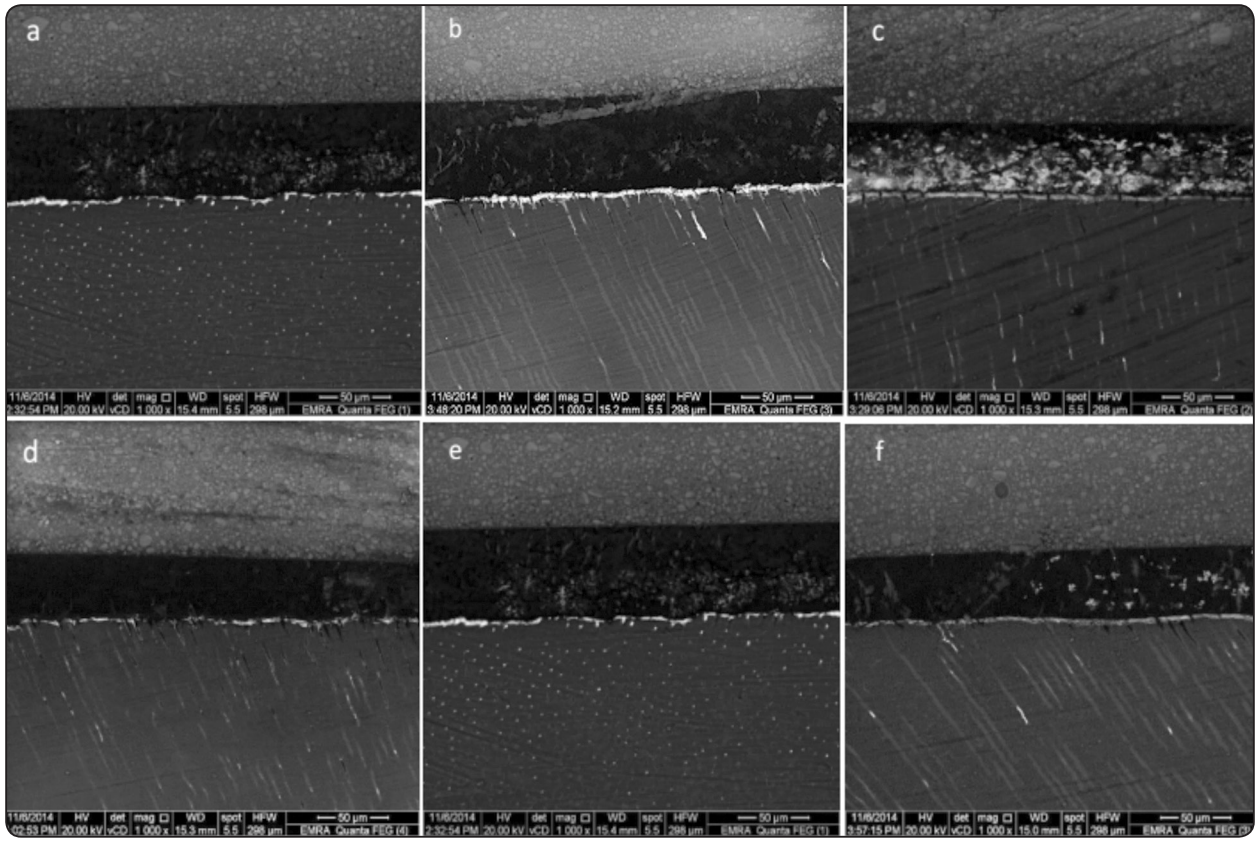

Fig 4: Adper Single Bond 2 group was used as a representative group to show nanoleakage within resin-dentin interfaces (due to significance difference between some of its subgroups). Fig 4 (a,b, c) show nanoleakage in normal dentin after 24 h, 6 and 12 m respectively, while fig 4 (d, e, f) show nanoleakage in sclerotic dentin after 24 h, 6 and 12 m respectively.

Micro-tensile bond strength evaluation (μTBS)

After storage, the dimensions of each stick were measured using a digital caliper to the nearest 0.01mm and were recorded to calculate the bond strength. Then, each stick was stressed under tension until failure using a simplified universal testing machine at a crosshead speed of 1 mm/min (Bisco Inc., Schaumburg, IL, USA). The number of prematurely debonded specimens was also recorded, but not included in the statistical analysis as all premature failures occurred during the cutting procedure (zero time). Thus, inclusion of these data in the statistical analysis was not considered since their bond strengths were unknown at the zero time (24 h) group. Furthermore, there were no premature failures in either 6 m or 12 m groups. After micro-tensile bond strength testing, all debonded surfaces were observed at 50X using a stereomicroscope (Meiji Techno Co. Ltd, Tokyo, Japan) to determine the modes of failure which were classified as adhesive, cohesive, or mixed failures.

Nanoleakage evaluation

One stick from each tooth of the four teeth in each bonding group (Table 2, Fig 2) was used for interfacial nanoleakage evaluation (N= 4/group i.e. four sticks from each bonding group at each time point). The resin composite bonded dentin specimens were coated with two layers of nail varnish, leaving only 1mm free at the dentin-bonded interface, and were then immediately immersed in a 50 wt % ammoniacal silver nitrate (AgNO3)

solution (pH = 9.5). After immersion in the tracer solution for 24 hours, specimens were removed, rinsed with water for 5 minutes and placed in a photo-developing solution (Eastman Kodak Co., Rochester, NY, USA) for 8 hours under fluorescent light, to reduce the diamine silver ions ([Ag(NH3)2]+) into metallic silver grains. The silver-impregnated specimens were then polished with SiC paper of increasing fineness (600 to 1200 grit) followed by soft polishing cloth with 0.05 μm alumina particles suspension (Buehler, Lake Bluff, IL, USA), and ultrasonically cleaned in distilled water for 30 minutes (Ultrasonic Cleaning System 2014, L&R Manufacturing, Kearny, NJ, USA). Bonded dentin interfaces were analyzed in an environmental scanning electron microscope (Quanta 200 ESEM, FEI France, Mérignac, France) operated in the backscattered electron mode at 1000X magnification. Quantitative analysis of the amount of silver nitrate that penetrated into the bonded interface was performed by measuring the percent distribution of silver within the dentin-bonded interface using image analysis software (NIH Image, Scion Corp. Fredrick MD, USA) [45]. Nanoleakage into the adhesive layer or the glass ionomer layer was not measured due to interferences of glass filler particles and to the wide variations in these layers' thickness.

Statistical Analysis

Numerical data were examined for normality by checking their distribution, calculating the mean and median values, and by using Kolmogorov-Smirnov and Shapiro-Wilk tests. Micro-tensile bond strength data showed parametric distribution, while nanoleakage % data showed non-parametric distribution. Data were represented by mean, standard deviation (SD), median, range and 95% Confidence Interval (95% CI) values. For parametric data; three-way ANOVA test was used to study the effect of material, substrate, time and their interactions on micro-tensile bond strength. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA tests were significant. For non-parametric data, Kruskal-Wallis test was used to compare the materials as well as the three time periods. Mann-Whitney U test with Bonferroni's adjustment was used for pair-wise comparisons when the Kruskal-Wallis test was significant. Mann-Whitney U test was also used to compare between normal and sclerotic dentin.

Qualitative data were represented by frequencies and percentages. Fisher's Exact test was used to compare between fracture modes of the different tested groups.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Table 4 summarizes the results of changes in micro-tensile bond strengths to normal and sclerotic dentin using Adper Single Bond 2, Optibond XTR, Single Bond Universal, Ketac Molar and Photac Fil, immediately (24 h) and after 6 and 12 months of water storage at 37°C.

The 24 h bond strengths to normal dentin were not different ($p > 0.001$) between Adper Single Bond 2 and Optibond XTR. However, both adhesives gave the highest mean micro-tensile bond strengths. Single Bond Universal showed significantly lower bond strengths than both Adper Single Bond 2 and Optibond XTR adhesives. There was no significant difference between bond strengths for bonds made with Ketac Molar (GI) and Photac Fil (RMGI); both showed the lowest mean micro-tensile bond strength.

Regarding sclerotic dentin, there was a significant decrease in the immediate bond strength values using Adper Single Bond 2, Optibond XTR and Single Bond Universal, when sclerotic bonds

were compared to their normal dentin values. With Ketac Molar (GI) and Photac Fil (RMGI), there was no significant difference in immediate bond strengths values between normal and sclerotic dentin (Table 4).

After 6 months of aging in distilled water at 37ºC, there was a significant decrease in bond strength to normal dentin using Adper Single Bond 2 and Optibond XTR. However, in sclerotic dentin, there was no significant difference between immediate micro-tensile bond strengths and 6 month-values using both adhesives. Using Single Bond Universal with normal as well as sclerotic dentin, there was no significant difference between immediate and 6 month- micro-tensile bond strengths (Table 4).

After 12 months of aging in distilled water at 37ºC, there was a significant reduction in the bond strength to both normal and sclerotic dentin using Adper Single Bond 2, Optibond XTR and Single Bond Universal (Table 4).

Ketac Molar and Photac Fil bonded to normal and sclerotic dentin showed no statistically significant difference in bond strength between the three time periods, as well as no significance difference between normal and sclerotic dentin at each time interval (Table 4).

To summarize, Adper Single Bond 2 bonds made to normal dentin fell 33% in 12 months but only fell 13.2% in sclerotic dentin. Single Bond Universal, used in the self-etch mode, only fell 20% in 12 months in normal dentin but 30% in sclerotic dentin. Optibond XTR only fell 11.7% in normal dentin and only 4.7% in sclerotic dentin. Bonds made using Ketac Molar or Photac Fil did not fall over 12 months of storage in either normal or sclerotic dentin.

Table 6 summarizes the results of changes in nanoleakage % within bonded interfaces in either normal or sclerotic dentin using Adper Single Bond 2, Optibond XTR, Single Bond Universal, Ketac Molar and Photac Fil, immediately (24 h) and after 6 and 12 months of water storage at 37ºC. The 24 h silver nanoleakage within both normal and sclerotic dentin were not different ($p > 0.05$) between Adper Single Bond 2, Optibond XTR and Single Bond Universal, although these three adhesives showed the highest mean nanoleakage %. There was also no significant difference between Ketac Molar (GI) and Photac Fil (RMGI); both showed significantly lower mean silver nanoleakage than the resin-based adhesives.

Regarding sclerotic dentin, there was a significant increase in the immediate nanoleakage values when bonding with Adper Single Bond 2, Optibond XTR or Single Bond Universal, when compared to their normal dentin values. Bonds made with Ketac Molar (GI) and Photac Fil (RMGI) showed no significant difference in immediate nanoleakage values between normal and sclerotic dentin (Table 6).

After 6 months of aging in distilled water at 37ºC, there was no significant difference in the silver nanoleakage within both normal and sclerotic using all tested groups. However, after 12 months of aging, a significant increase in the nanoleakage in normal dentin was seen using Adper Single Bond 2, Optibond XTR, Single Bond Universal. There was no significance difference in nanoleakage when normal dentin was bonded using Ketac Molar and Photac Fil between 6 and 12 month-storage periods. Twelve months of storage resulted in a significant increase in nanoleakage within sclerotic dentin using Adper Single Bond 2 and Optibond XTR; however, there were no significance differences between Single Bond Universal, Ketac molar and Photac Phil 6 and 12 month nanoleakage values (Table 6).

The fracture modes for all materials used for bonding are shown in Table 5. There was a significant difference between failure modes in the different tested groups. For Adper Single Bond 2

and Optibond XTR, most of the failures were mixed and varied between 40 and 67%. For Single Bond Universal, the mixed failures were slightly lower 40-53% due to a higher number of adhesive failures (33-53%). The adhesive failures for Adper Single Bond 2 and Optibond XTR varied from 26-40%. There was a tendency for more adhesive failures in sclerotic dentin compared to normal dentin.

The fracture modes for Ketac Molar(GI) and Photac Fil (RMGI) were generally mixed failures ranging from 40-67% (Table 5). Their adhesive failures were significantly lower than those seen in the resin-based adhesives, varying from 13-47% ($p < 0.001$). The highest cohesive failures in the study were seen with Ketac Molar (GI) and Photac Fil, that varied from 7-26% ($p < 0.001$). This was due to the lower cohesive strengths of these two materials.

DISCUSSION

During the last two decades, dentin adhesive systems have been well developed and have provided high initial bond strengths. However, hybrid layer degradation has compromised the bonding stability of resin-dentin interfaces [2,46]. Many factors were reported to be responsible for such degradation [1,2,47], including the degree of conversion [48,49] and hydrophilicity [50] of adhesive resins, as well as the host-derived MMPs and cysteine cathepsins. Moreover, the quality of hybrid layer may be highly affected by changing the dentin substrate itself. This should be also taken into consideration.

Hybrid layer degradation can occur due to ageing of one or more of its components, namely, dentin organic matrix, hydroxyapatite crystals, or resin polymers. Resin and collagen present in hybrid layers suffer from hydrolysis, increasing the water content at the interface, which adversely affect the longevity of the bond [1]. Thus, there is a great relation between the degree of hydrophilicity of the adhesive [50], its water sorption capacity [51], and the subsequent degradation. Whatever the adhesive is an etch-and-rinse or a self-etch one, presence of hydrophilic monomers leads to the formation of highly permeable hybrid layers[52], even after adhesive polymerization. This allows continuous passage of water from the underlying dentin with subsequent increased nanoleakage and degradation of bonded interfaces. This phenomenon is very clear when using simplified adhesives, as they have a high percentage of hydrophilic monomers [51, 53].

Nanoleakage is a phenomenon referring to nano-spaces that occur within the hybrid layer, even in absence of interfacial gaps [54, 55]. Nanoleakage may result from improper adhesive resin penetration into the collagen network, incomplete solvent evaporation, unpolymerized monomers, or hydrolytic degradation of collagen and/or resin. An inverse correlation between bond strength and nanoleakage is expected since nanoleakage represents interfacial degradation which causes a decrease in the bond strength [56, 57].

Stability of dentin bonding is crucial for improving the lifetime of adhesive restorations. Thus, the effect of dentin substrates on the bonding stability should be investigated. Most in-vitro studies of bonding to tooth structure are conducted on recently cut and polished normal substrate, although, such substrate with "normal" characteristics is not frequently found in clinical situations. A cavity may present various substrates, such as superficial and deep dentin, bur-prepared dentin, carious and non-carious sclerotic dentin [36-38, 40, 41].

Dentin is a dynamic substrate subjected to continuous physiological and pathological changes of composition and microstructure [58]. Consequently, the dentin found in clinical situations can be greatly different from normal unaltered dentin, which is commonly used in in-vitro bond strength tests. Sclerotic dentin, for instance, is a common clinical substrate. Besides physiological sclerosis occurring with age, a reactive sclerosis occurs in response to superficial injuries such as: abrasion, erosion, attrition, and caries [59]. In this situation, dentinal tubules are partly or totally obliterated with sclerotic

casts, and remain so even after acid-etching. Dentin permeability is therefore reduced, impairing or even preventing resin tag penetration [39]. In addition, the hybrid layer formed in this substrate is narrower in comparison to normal dentin, possibly due to the hypermineralization of this tissue making it more resistant to demineralization by acid etching [38-40].

According to the results of the current study, Adper Single Bond 2, Optibond XTR, and Single Bond Universal exhibited higher immediate bond strength to normal dentin than did to sclerotic dentin. This may be attributed to the hypermineralized nature of sclerotic dentin which makes it more resistant to the acid-etching process [42]. Bond strengths results were supported by nanoleakage results which showed higher silver deposition in sclerotic dentin bonded interfaces with the three resin adhesives. This idea was supported by Tay and Pashley [41] who stated that, bonding to pathologically altered substrates such as sclerotic dentin, using either etch-and-rinse or self-etch adhesives, resulted in compromised bonding.

Microscopically evaluating the effect of acid-etching on the dentin of non-carious lesions, Sakoolnamarka et al [59] found a difference in the appearance of demineralized sclerotic dentin of non-carious cervical lesions and that of normal dentin. In the sclerotic region, the intertubular dentin was still saturated with minerals, with little exposure of the intertubular collagen fiber network into which the adhesive might penetrate [59]. However, in the current study, SEM image (fig 1) shows partial or complete obliteration of dentinal tubules in sclerotic dentin after etching. Thus, although the acid-etching demineralization capacity may not be an important factor in obtaining high bond strength to normal dentin, it seems to be critical in bonding to sclerotic non-carious dentin [41, 42].

Storage for 12 months resulted in decreases of the bond strength and increases in silver nanoleakage within bonded interfaces in both types of dentin using the three resin adhesives. This may be attributed to many factors that may synergistically affect the integrity of each component of the hybrid layer. Water sorption, elution of unreacted monomers, plasticization of polymer chains, and water-mediated hydrolysis are some of the aging phenomena that can severely affect the mechanical and morphological integrity of the resinous component of hybrid layers. Increasing incorporation of high concentrations of ionic and polar resin monomers to current simplified adhesives seems to be the keystone in the lack of stability of these materials [3, 50, 51]. Nevertheless, degradation may also affect the other component of the hybrid layer, that is, its collagen matrix. If collagen fibrils are unprotected by hydrophobic resin coating, they may be vulnerable to degradation due to the activation of endogenous dentin MMPs [16, 17].

Since Adper Single Bond 2 bonds made to normal dentin fell 33% in 12 months but only fell 13.2% in sclerotic dentin, while Optibond XTR fell 11.7% in normal dentin and only 4.7% in sclerotic dentin, this indicates that the rate of resin-dentin bonds degradation is higher in normal dentin than in sclerotic dentin. This can be attributed to MMPs activity as MMPs become activated by acid- etching, etching of normal dentin demineralizes the dentin exposing the collagen which becomes gradually degraded by the active MMPs. In case of sclerotic dentin, acid-etching was not able to completely expose the collagen fibrils, thus there was less activation of matrix-bound MMPs and hence less collagen degradation.

Furthermore, the hypermineralized surface layer of sclerotic dentin may have less collagen content. This was supported by previous studies [60, 61] who stated that normal dentin is 48% by volume collagen and 45% by volume mineral. For significant hypermineralization to occur, there has to be a replacement of collagen by mineral. On the other hand, if denatured collagen microfibrils swell into the spaces previously occupied by the interfibrillar spaces, there would be a reduction in the protein content per unit volume. This may result in the

higher mineral/protein ratio reported by Mixson et al [61] in cervical sclerotic lesions.

Regarding Single Bond Universal, used in the self-etch mode, it provided significantly lower immediate bond strength than Adper Single Bond 2 and Optibond XTR, and the values fell 20% in 12 months in normal dentin, and 30% in sclerotic dentin. This may be due to inability of the adhesive to sufficiently etch the dentin, especially sclerotic dentin, resulting in low bond strength values. Nanoleakage results of the current study supported such assumption, as there was no significant difference between the 24h, 6m and 12 m nanoleakage within sclerotic dentin bonded with Single Bond Universal and the 12 m normal dentin group, suggesting poor bonding to sclerotic dentin from the start.

In seeking a long lasting bonding, an alternative bonding mechanism had to be used as a control. Thus, this study evaluated the influence of aging on the durability of glass ionomer (GI) and resin-modified glass ionomer (RMGI) materials bonded to normal and sclerotic dentin. Since the acid- etching procedure is accused of being the main cause of MMPs activation and resin-bonded interfaces degradation, GI and RMGI materials were used without the pre-conditioning step. Our results of stable bonds over 12 months with these materials is supported by multiple in-vivo and in-vitro studies which have shown that using a conditioner does not necessarily improve the adhesion of glass ionomers to the natural tooth structure. The literature shows that application of a conditioner prior to placement of these materials is successful in removing the "smeared" layer. However, this step is not necessary, possibly because there is adequate free acid in glass ionomers to dissolve the smear layer at the time of the restoration placement [62-67].

The immediate bond strengths exhibited by glass ionomer and RMGI were relatively low. However, the slight insignificant difference between both values was not in agreement with previous studies[66, 67] that showed that RMGI provided higher bond strengths. The authors of those studies explained their results that RMGI can bond to tooth structure via two mechanisms: chemically through ionic bonding of the carboxyl group to the calcium ions of the tooth substrate, and the resin part can interlock with the conditioned tooth surface via a 'micro-mechanical interlocking mechanism'. However, this was not the case in the current study, as we did not use a pre-conditioning step. Consequently, the bonding mechanism was dependent only on the chemical bonding which was probably a major contributor of bonding by conventional glass ionomers.

There was no significant difference between the bond strengths of GI or RMGI in either normal or sclerotic dentin. This indicates that the hypermineralized structure of sclerotic dentin did not adversely affect bonding using these materials compared to resin-based adhesives. Furthermore, there was no significant difference between different storage periods for both GI and RMGI in either types of dentin which suggest that these materials may provide more durable bonded interfaces than resin bonded ones.

The results of the current study support partial rejection of the first null hypothesis as when using resin-based etching restoratives, bond strength was higher and nanoleakage was lower with normal dentin when compared to sclerotic dentin. On the other hand, the type of dentin did not affect the bond strength or nanoleakage when mineral-based non-etching restoratives were used.

The second null hypothesis must be rejected as the resin-based etching restoratives exhibited a drop in bond strength and an increase in nanoleakage expression over a period of 1 year which was not the case with the mineral-based non-etching restoratives. Therefore, the etching process may be responsible for the degradation of resin-dentin bonded interfaces.

In conclusion, compared to resin-based etching restoratives, mineral-based non-etching restoratives

(Ketac Molar and Photac Fil) exhibited lower immediate dentin bond strengths, but provided much more stable bonds, to both normal and sclerotic dentin, over a period of 12 months and therefore prolong the life span of esthetic restorations. It is obvious that the acid etching procedure maybe the main cause of instability of resin-dentin bonded interfaces. Sclerotic dentin did not act as a bonding impediment with mineral-based non-etching restoratives as did with resin-based etching adhesives. Moreover, normal dentin showed more resin-dentin bond degradation than did sclerotic dentin. This may relate to the possible low level of MMPs activities in sclerotic dentin. Further studies are needed to measure the MMP activity in bonded interfaces in sclerotic dentin using mineral-based non-etching and resin-based etching restoratives.

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