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Effect of Chlorhexidine Pretreatment on Bond Strength Durability of Caries–affected Dentin Over 2-Year Aging in Artificial Saliva and Under Simulated Intrapulpal Pressure

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Clinical Relevance

Pretreatment of caries-affected dentin with 5% chlorhexidine decelerates the bond strength loss over the long term.

SUMMARY

Objective: To evaluate the influence of 2% and 5% chlorhexidine (CHX) pretreatment on bond durability of a self-etching adhesive to normal (ND) and caries-affected (AD) dentin after 2-years of aging in artificial saliva and under simulated intrapulpal pressure (IPP).

Methods: One hundred twenty freshly extracted carious teeth were ground to expose ND and AD. Specimens were distributed into three equal groups (n=40) according to whether the dentin substrates were pretreated with 2% or 5% CHX or with water (control). Clearfil SE Bond (Kuraray) was applied to both substrates and composite cylinders (0.9 mm diameter and 0.7 mm height) were formed. Pretreatment and bonding were done while the specimens were subjected to 15 mm Hg IPP. After curing, specimens were aged in artificial saliva at 37°C and under IPP at 20 mm Hg until being tested after 24 hours or 2 years (n=20/group).

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Microshear bond strength was evaluated. Failure modes were determined using a scanning electron microscope (SEM) at 400× magnification. Data were statistically analyzed using three-way analysis of variance (ANOVA); one-way ANOVA tests, and *t*-test ($p<0.05$). Additional specimens (n=5/group) were prepared to evaluate interfacial silver precipitation.

Results: For the 24-hour groups, there were no significant differences among the ND groups and AD groups. For ND aged specimens, the 5% CHX group had the highest value followed by the 2% CHX and control groups, although the difference was statistically insignificant. For AD aged specimens, the 5% CHX group revealed statistically higher bond values compared to the 2% CHX and control groups. Fracture modes were predominately adhesive and mixed. Different interfacial silver depositions were recorded.

Conclusions: Two percent or 5% CHX pretreatment has no adverse effect on the 24-hour bonding to ND and AD. Five percent CHX was able to diminish the loss in bonding to AD after 2years of aging in artificial saliva and under simulated IPP.

INTRODUCTION

Self-etching adhesive systems were introduced in the market to overcome some of the problems encountered with their predecessors, etch and rinse adhesive systems, such as the discrepancy between the depth of demineralization and the resin infiltration.[1] However, it has been reported that even the self-etching adhesive systems are prone to leave some denuded demineralized dentin collagen without resin infiltration.[2] Because they have mild acidity, they provide a low pH environment that may activate latent endogenous matrix metalloproteinase (MMP) enzymes.[3]

Human dentin contains at least five types of MMPs including MMP-2, MMP-3, MMP-8, MMP-9, and MMP-20.[4-9] They are trapped within the mineralized dentin matrix during tooth development[5] and dentinal caries.[8] The majority of them are produced as latent zymogens (pro-MMPs). Disruption of MMP-collagen binding due to exposure to mild acids (pH=2.3-5)[8] converts the pro-MMPs to active MMPs through splitting the low-molecular-weight peptides.[10,11] Over time, auto activation of further pro-MMPs may result in increasing the enzymatic activity.[3] These MMPs are thought to be responsible for degrading most of the extracellular matrix components, including the native and denatured forms of collagen.[12,13] MMP enzymes also require water to hydrolyze peptide bonds in the collagen molecules at the adhesive-dentin interface.[14] Water infiltration into the adhesive-dentin interface occurs through dentinal tubules as well as through the dentin margin. Previous studies have indicated that both forms of dentin wetness affect the initial and delayed bonds to dentin[15-17]and nanoleakage.[18]

One strategy to prevent the host-derived proteases is the usage of MMP inhibitors. These include either specific ones such as galardin[19] or nonspecific ones like chemically-modified tetracyclines[20,21] and chlorhexidine (CHX).[14,22] The effectiveness of CHX, as an MMP inhibitor, on bond durability to normal dentin (ND) has been the most frequently investigated strategy compared to other MMP inhibitors.[14,22-26] For caries-affected dentin (AD), little research[27,28] is available despite it being the most relevant dentin structure for bonding and it being a challenging issue.

To the best of the author's knowledge, no study so far has studied the effect of CHX concentrations on nanoleakage and bond durability of adhesive resin to normal as well as to caries-affected dentin while aging was done in artificial saliva and under simulated intrapulpal pressure (IPP). Therefore, the current study evaluated the effect of 2% or 5% CHX on the durability of a two-step self-etching adhesive system to normal and caries-affected dentin after aging for 2 years in artificial saliva and under simulated IPP. The tested hypotheses were that 1) under simulated IPP, CHX concentrations (2% or 5%) do not cause a detrimental effect on the 24-hour bond strength to ND and AD; and 2) different CHX concentrations have no effect on preserving bond strengths to both dentin substrates.

MATERIALS AND METHODS

Specimen Preparation

Extracted human molars having occlusal caries extending approximately halfway into the dentin were used in the present study. Immediately after extraction, the teeth were thoroughly washed, scrubbed, and scaled to remove blood, mucus, shreds of periodontal ligament, plaque, and calculus. The teeth were stored in phosphate buffer solution with 0.2% sodium azide at 4°C to be used in less than 2 weeks.[29]

The occlusal enamel was trimmed using a rotary grinding machine under copious water coolant to provide a uniform flat dentin surface perpendicular to the long axis of the tooth. Specimens were prepared as described by Yoshiyama and others,[30,31] to test bonding to caries-affected dentin. After grinding of the occlusal dentin, 1% acid red dye in propylene glycol (Kuraray Medicine Inc, Tokyo, Japan) was applied.[32] Further finishing was performed, using wet 600-grit SiC paper for 20 seconds under running water, according to the combined criteria of hardness to the sharp excavator, visual examination, and staining with dye.[18] The discolored, harder dentin which stained pink was considered AD, while the surrounding yellow dentin was considered ND.[18] A flat surface was produced including both ND and AD with a standardized smear layer.[33] Then the roots were sectioned below the cementoenamel junction, thus creating direct communication with the pulp chamber.

To prepare the teeth (n=120) for intrapulpal pressure simulation, the crown segments were bonded to an acrylic circular plate and penetrated by a 19-gauge butterfly stainless steel needle (JMS Singapore Pte Ltd, Singapore, Singapore), using a cyanoacrylate adhesive (Rocket light, Dental Venture of America Inc, Corona, CA, USA). The teeth with their acrylic plates were embedded in polyester resin with the needle inserted in the previously drilled hole. The needle served to connect the pulp chamber to a plastic fluid barrel containing water that was adjusted to the desired pressure using the intrapulpal pressure assembly. The assembly was comprised of three interconnected units. The first unit consisted of a plastic fluid barrel connected by plastic tubing to the second unit. The second unit was formed with a T-shape connection assembly joining the tube ends of the 19-gauge butterfly needles to each other and to the third unit (sphygmomanometer). The sphygmomanometer (Wenzhou Hongshun Industries & Trade Co, Wenzhou/Zhejiang, China) was used to monitor the intrapulpal pressure of the specimens. The height of the fluid barrel was adjusted to produce an intrapulpal pressure of 20 mm Hg for 24 hours before and after the bonding procedures, but was reduced to produce 15 mm Hg during the bonding procedures.

Specimen Grouping and Restorative Procedures

Teeth (n=120) were divided into three equal groups (n=40) according to the dentin pretreatment used. One group acted as the control; 0.1 mL water instead of chlorhexidine pretreatment was applied before using the self-etching primer adhesive. The second and third groups received 2% and 5% chlorhexidine (Cairo Pharmaceutical and Chemical Industries, Cairo, Egypt) pretreatments, respectively. Half of the specimens within each group (n=20) were tested after 24 hours and the other half were tested after 2 years of aging in artificial saliva and under intrapulpal pressure of 20 mm Hg. Each tooth received 0.1 mL of the proposed chlorhexidine concentration for 60 seconds. Each specimen was then air-dried for 30 seconds.

Before the application of the adhesive, a double-faced adhesive tape with two holes, each punched 2.3 mm in diameter, was used for isolation of the dentin surface except for the areas to be bonded. One circular area of normal dentin and another area of caries-affected dentin[29] were isolated. The self-etch primer adhesive system Clearfil SE Bond (Kuraray Medicine Inc) was applied following manufacturer's instructions. Table 1 lists its chemical composition and mode of application. Then the adhesive layer was light-cured for 10 seconds following the manufacturer's instructions. A transparent polyethylene tube, with 0.9 mm internal diameter, was cut into small segments of approximately 0.7 mm in length to aid in resin composite packing. Resin composite (Filtek Supreme XT, 3M ESPE, St Paul, MN, USA) of shade A3 was applied and condensed in slight excess into the short tubes using a plastic instrument to ensure it was devoid of air bubbles. The two polyethylene segments were centered over the two punched holes in the double-faced adhesive tape. Each composite cylinder was light-cured for 40 seconds with a light-curing unit (Bluephase, Ivoclar Vivadent, Schaan, Lichtenstein). The light-curing unit intensity (450–500 mW/cm^2) was checked using a radiometer (Demetron LED Radiometer, SDS, Kerr, Orange, CA, USA). The double-sided adhesive

Table 1: *Chemical Composition and Application Mode of the Adhesive System (Clearfil SE Bond) Used*

	Composition (Batch Number Number)	Application Mode
Primer	10-MDP, HEMA, hydrophilic dimethacrylate, photo-initiator and water (00733A)	Apply primer and leave it undisturbed for 20 seconds, dry with mild air flow for 10 seconds
Adhesive	10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, and microfiller (01052A)	Apply adhesive; gently air-dry for 5 seconds, light-cure for 10 seconds

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol-glycidyl methacrylate.

tape and the polyethylene segments were then cautiously removed leaving the composite microcylinders bonded to the dentin surface. Each microcylinder was checked using a 6× magnifying glass lens (Bausch and Lomb Optics Co, Rochester, NY, USA) for any defect or air bubble, so that upon detection of any defect the specimen was discarded. The diameter of the microcylinders (0.86 mm) was checked using a digital caliper (Mitutoyo 500-171 0-6, Digimatic caliper, Mitutoyo Corp, Tokyo, Japan) and that bonded to caries-affected dentin was coded using an indelible marker. The specimens were then immersed in artificial saliva prepared in accordance with Pashley et al.,[14] but without protease inhibitors, for the proposed aging times at 37°C in a specially constructed large incubator, to accommodate the intrapulpal pressure assembly. Artificial saliva was composed of (mmol/L): $CaCl_2$ (0.7), $MgCl_2$ (0.6), H_2O (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20). Sodium azide was added to prevent bacterial growth.[27] This was confirmed through the maintenance of the clear color of artificial saliva during the study duration.

Microshear Bond Strength Testing

Each embedded tooth with its bonded resin composite microcylinders was secured with tightening screws to a specially designed attachment jig (Mobarak attachment)[29] to hold the specimens onto the testing machine. Load was applied *via* the testing machine at a crosshead speed of 0.5 mm/min until debonding occurred. The test was performed once for normal dentin followed by caries-affected dentin in each tooth. The microshear bond strength (μSBS) was calculated by dividing the load at failure by the bonded cross-sectional area.

Statistical Analysis

Data were presented as means and standard deviations. Data distribution was tested for normality using the Shapiro-Wilk W test; thus, parametric analysis was used. Three-way analysis of variance (ANOVA) was used to compare the effect of pretreatment (control, 2% CHX, and 5% CHX), dentin type (normal and caries-affected), time of testing (24 hours and 2 years), and their interaction. One-way ANOVA was used to test the effect of pretreatment on the bond strength values of each dentin type either at 24 hours or at 2 years. This was followed by the Bonferroni *post hoc* test for pairwise comparison. *T*-test was used to compare between 24 hour and 2 year mean values for each dentin type with each pretreatment condition (control, 2% CHX, and 5% CHX). Statistical significance was preset at α=0.05. All statistical calculations were done using the computer program SPSS version 15 for Microsoft Windows (SPSS, Chicago, IL, USA).

Failure Mode Analysis Using Scanning Electron Microscope

All fractured surfaces were investigated in a back scattered mode with an environmental scanning electron microscope (ESEM, Quanta 200, Philips-, Eindhoven, The Netherlands) at 100× and 400× magnification. Failure modes were classified as: 1) adhesive failure at the dentin side; 2) mixed adhesive at the dentin side and cohesive in adhesive resin; 3) cohesive in adhesive resin; 4) mixed adhesive at the dentin side and cohesive in dentin; and 5) mixed all.

Scanning Electron Microscope Interfacial Observation With Silver Tracer

Additional specimens (n=5/group) were vertically sectioned across the resin dentin interface into slabs of approximately 1 mm using a diamond saw (IsoMet, Buehler Ltd, Lake Bluff, IL, USA) under water coolant. Two slabs were obtained from each tooth forming a total of 10 slabs per group. Bonded slabs were coated with two layers of fast set nail varnish applied up to 1 mm of the bonded interface. To evaluate interfacial silver precipitation using SEM, bonded specimens were permeated with 50% (wt/v) ammoniacal silver nitrate solution for 24 hours in the dark. Ammonical silver nitrate was prepared by dissolution of 25 g of silver nitrate crystals (Sigma-Aldrich Corp, St Louis, MO, USA) in 25 mL of water. Concentrated (28%) ammonium hydroxide (Sigma-Aldrich) was used to titrate the black solution until it became clear as ammonium ions complexed the silver into diamine silver ions. This solution was diluted to 50 wt% solution with a pH of 9.5.[34] After silver staining, the specimens were rinsed thoroughly with distilled water and placed in photodeveloping solution for 8 hours under fluorescent light.[34] The slabs were then wet polished with SiC paper of ascending finesse (up to 4000 grit).[35] The slabs were immersed in 10% ethylene diamine tetraacetic acid solution for 5 seconds, rinsed with water for 3seconds, and then dried for 24 hours .[36] Following sputter coating with gold, the interfaces were analyzed with a scanning electron microscope (SEM, XL30, Philips, Eindhoven, The Netherlands). Images were obtained with backscattered signals. Micrographs were collected at magnifications up to 2000×.

Table 2:—Microshear Bond Strength Results (mean in MPa [SD])*

	24 hours		2 years	
	ND	AD	ND	AD
Control	24.33 (5.1)^{aA}	21.73 (6.0)^{aA}	9.46 (3.4)^{aB}	9.97 (3.5)^{aB}
2% CHX	23.79 (5.9)^{aA}	20.84 (6.2)^{aA}	8.74 (3.2)^{aB}	9.99 (3.4)^{aB}
5% CHX	25.94 (6.4)^{aA}	20.59 (5.1)^{aA}	10.98 (3.3)^{aB}	14.67 (4.5)^{bA}

* *Within columns, groups with same lowercase letters are not significantly different (p>0.05); groups with same uppercase letters within rows are not significantly different (p>0.05).*
Abbreviations: ND, normal dentin; AD, caries-affected dentin.

RESULTS

Microshear Bond Strength Results

The means and standard deviations (SD) from the μSBS test are shown in Table 2. Three-way ANOVA indicated that there were statistically significant differences for pretreatments ($p<0.001$) and aging time ($p<0.001$) but not for the type of dentin substrate ($p>0.05$). Also, there were significant interactions among the three variables (pretreatment, aging time, and dentin type, $p=0.02$). At 24 hours, one-way ANOVA demonstrated that there was no significant difference among the pretreatment groups for ND and AD ($p>0.05$). For ND 2-year aged specimens, the 5% CHX group had the highest μSBS mean value followed by the 2% CHX and control groups; the difference was not statistically significant ($p>0.05$). For AD aged specimens, the 5% CHX group revealed statistically higher bond values compared to the 2% CHX and control groups (Bonferroni test, $p<0.05$). Regarding the aging time, a significant change in bond strength was recorded between the 2-year groups (t-test, $p<0.05$) and the 24-hour groups except for AD groups pretreated with 5% CHX (t-test, $p>0.05$). In all groups, the major mode of failure was adhesive at the dentin side, then mixed adhesive at the dentin side, and cohesive in the adhesive. Cohesive failures in adhesive resin and in dentin were very few (Figure 1). Representative SEM micrographs for some modes of failure are presented in Figure 2.

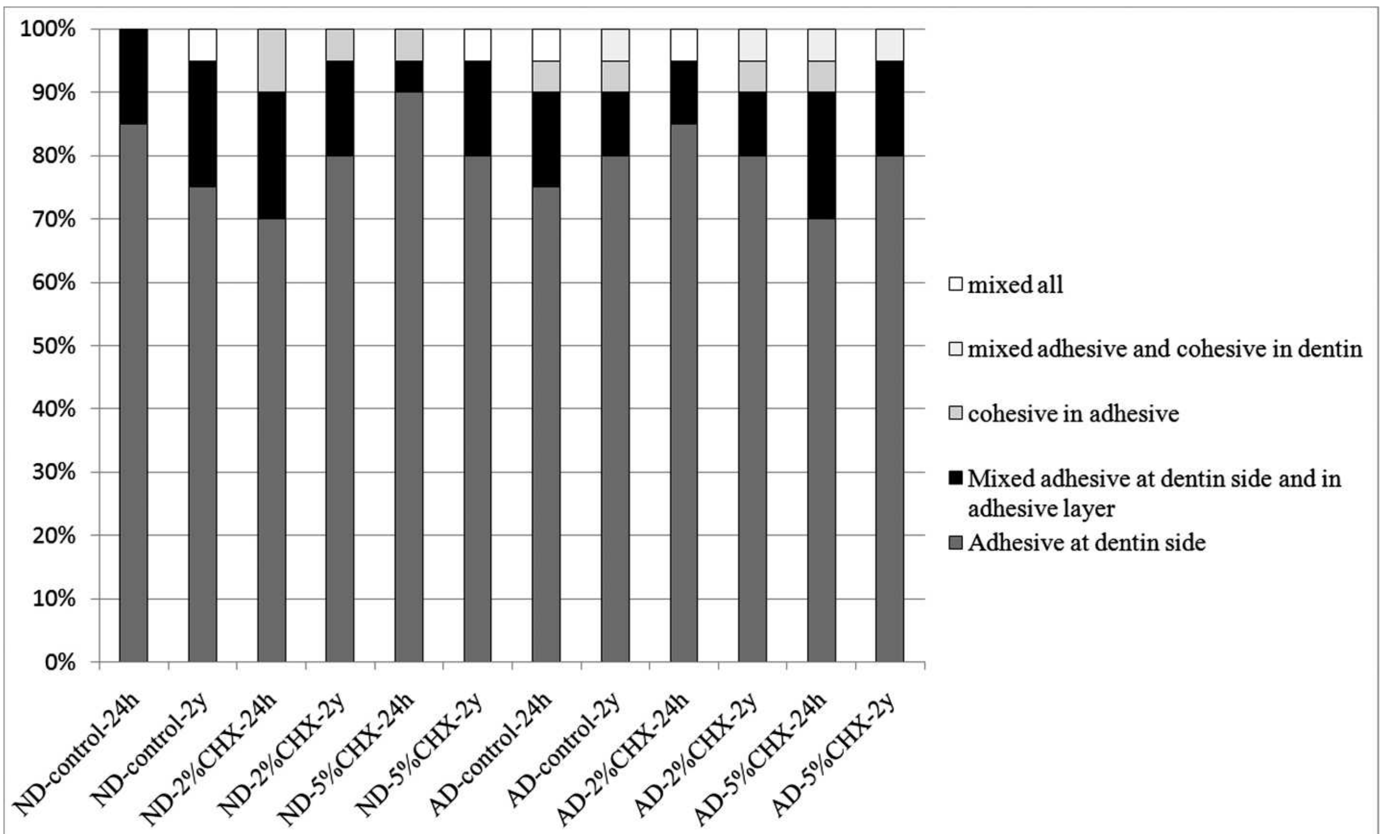

Figure 1. *Distribution of failure modes in percentages. (Abbreviations: ND, normal dentin; AD, caries-affected dentin; CHX, chlorhexidine)*

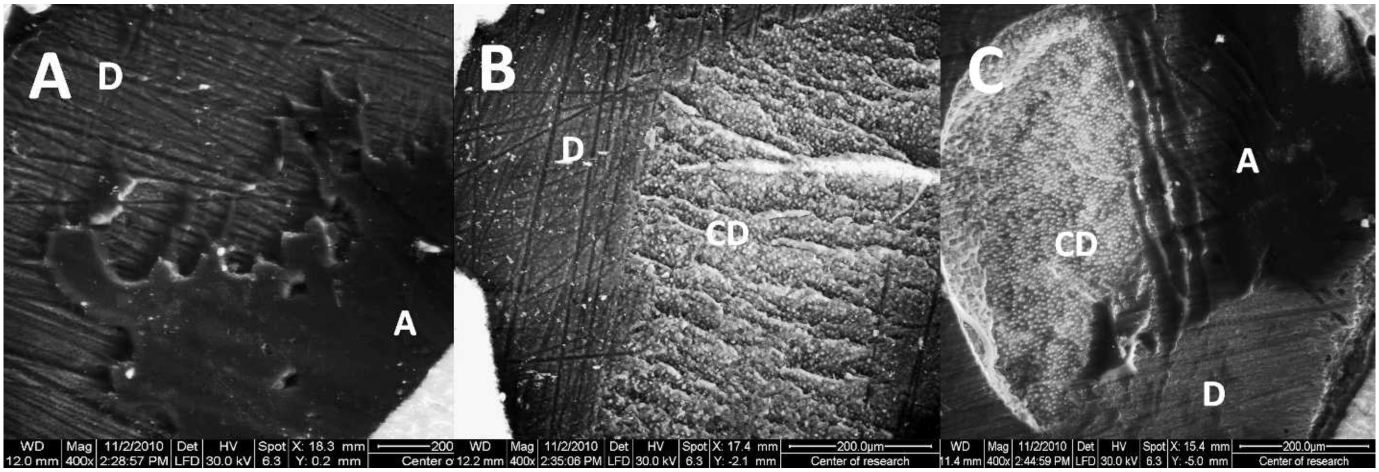

Figure 2. *Representative SEM micrographs of failure modes (400×). (A): Shows mixed adhesive at the dentin side and cohesive in the adhesive resin. (B): Shows mixed adhesive failure at the dentin side and cohesive failure in dentin. (C): Shows mixed all (adhesive at the dentin side and cohesive in adhesive resin and in dentin). (Abbreviations: D, dentin side; A, adhesive resin; CD, cohesive fracture in dentin)*

Interfacial Observation Results

Silver deposits were predominately restricted to the hybrid layer (HL) in both periods (Figures 3 through 5). No evident differences were observed in the silver deposition pattern among the 24-hour groups (control, 2% CHX, and 5% CHX) of both dentin substrates as shown in Figures 3 through 5, A,B). Limited silver deposition was recorded for ND, while no silver deposition was detected for AD. After 2 years, however, there was an increase in the intensity and continuity of silver deposition especially in the HL for control (Figure 3C,D) and 2% CHX groups (Figure 4C,D). For caries-affected dentin pretreated with 5% CHX, there was sporadic silver uptake after 2 years (Figure 5D). Underneath the hybrid layer, streak-like silver deposits were observed within the intertubular dentin rather than within the dentinal tubules for AD, which is opposite to that detected for ND (Figure 6).

DISCUSSION

In vitro studies examining the durability of bonding are useful, but they are not done under *in vivo* conditions which can result in a discrepancy between the *in vivo* and the *in vitro* findings. In the present study, the entire evaluation was carried out under simulated IPP, and the teeth were immersed in artificial saliva to simulate, to a certain extent, the clinical conditions. Dentin wetness and pulpal pressure are extremely important variables during dentin bonding procedures.[37] It was expected that seepage of water under pulpal pressure from the dentinal tubules might have a hydrolytic degradation effect that may adversely affect the durability of the resin dentin bonding.[38]

The results of the present study support the first hypothesis where neither 2% nor 5% CHX had a detrimental effect on the 24-hour bond strength of normal and caries-affected dentin, even when bonding was done under simulated IPP. With regard to 2% CHX, previous results support our findings that 2% CHX pretreatment did not adversely affect the bonding to ND compared to the control group.[27] However, no study has so far evaluated the effect of CHX concentrations on the bond strength durability and nanoleakage of resin dentin adhesives to caries-affected dentin after aging over 2 years under IPP and in artificial saliva.

The present study also showed that the 24-hour bond strength of SE Bond to ND and AD were similar. This finding corroborates our previous findings[29] and those of others.[39] The comparable finding of the present study and former ones, despite the fact that none of these previous studies were done under simulated IPP, may be attributed to the mild self-etching effect encountered with SE Bond on the smear layer. The smear plugs limited the water seepage to the bond interface over such a short period (24 hours).[40]

For the effect of CHX on the bond durability of SE Bond to ND, current study results suggest partial acceptance of the null hypothesis that neither CHX concentration was to preserve long-term bonding. Previous research studies reported controversial results. One study showed that 2% CHX was able to preserve the bonding of SE Bond over 6 months.[27] Another study revealed that SE Bond was not able to

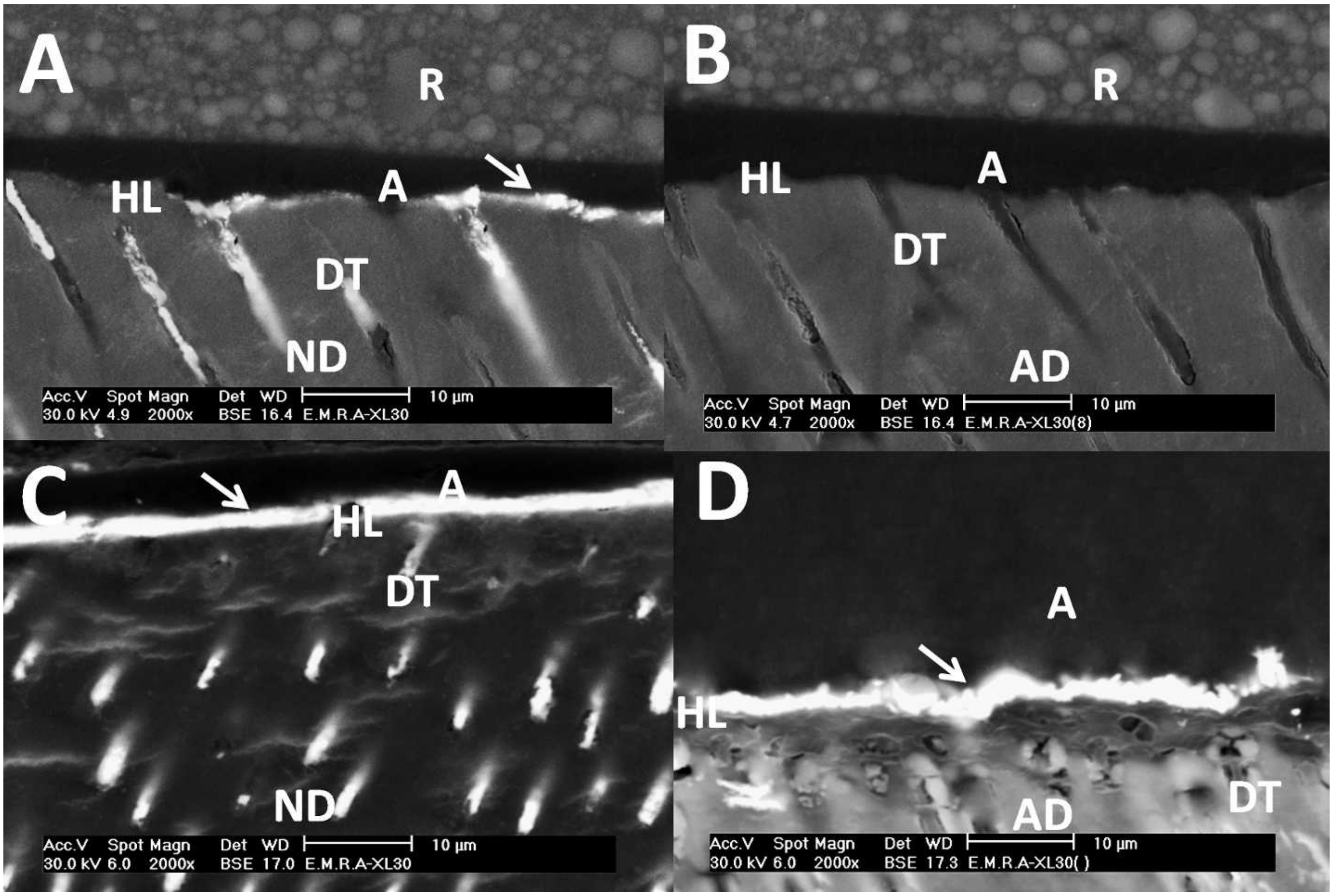

Figure 3. *Backscattered SEM micrograph of the interface of normal and caries-affected dentin bonded with SE Bond (2000×). In 24-hour control groups (A for ND and B for AD) there were very limited silver deposits found at the base of hybrid layer of ND and no silver deposits for AD. After 2 years, more intense and continuous silver deposits could be detected (white arrow) for both ND (C) and AD (D). (Abbreviations: ND, normal dentin; AD, caries-affected dentin; HL, hybrid layer; DT, dentinal tubules; A, adhesive layer; R, resin composite)*

activate MMP-2 and MMP-9; consequently, the CHX pretreatment, with a concentration of 0.05%, was not able to preserve the bond of SE Bond over the same 6-month period.[41] Such a discrepancy may be due to the difference in the CHX concentration tested in both studies. Another important issue is that the study by De Munck and others[41] tested the activation ability of the SE Bond on MMP-2 and MMP-9 only; however, dentin in fact contains MMP-3, MMP-20, and MMP-8. The role of MMP-3. MMP-8, and MMP-20 is still unclear. The current study results contrast previous research that reported the effectiveness of CHX pretreatment in preserving the normal dentin bond strength with concentrations lower than 2% which was used in the present study. However, the duration of these studies was limited to 6 months.[42] Another study reported that a higher CHX concentration (2%) was more effective than a lower CHX concentration (0.2%) in diminishing the bond strength loss over 6 months for self-etching adhesive, while for an etch-and-rinse adhesive the difference was not critical.[43] It seems that for a longer time period higher concentrations are required. In the present study, specimens were subjected to both aging conditions including artificial saliva and IPP over a long period of time (2 years). Both aging conditions may have a synergistic dilution effect on CHX even when used up to 5% concentration. Although it is not clear to which extent the MMPs influenced the bond strength, other causes for drop in bond strength, such as the resin hydrolysis over time, should not be overlooked.

Caries-affected dentin has been reported to exhibit increased collagenolytic activity when compared to intact normal dentin, which suggests faster bonding deterioration.[22,44] Accordingly, whether SE Bond was able to activate MMPs to a different degree as compared to normal dentin requires further research. Pretreatment with 2% CHX, similar to that

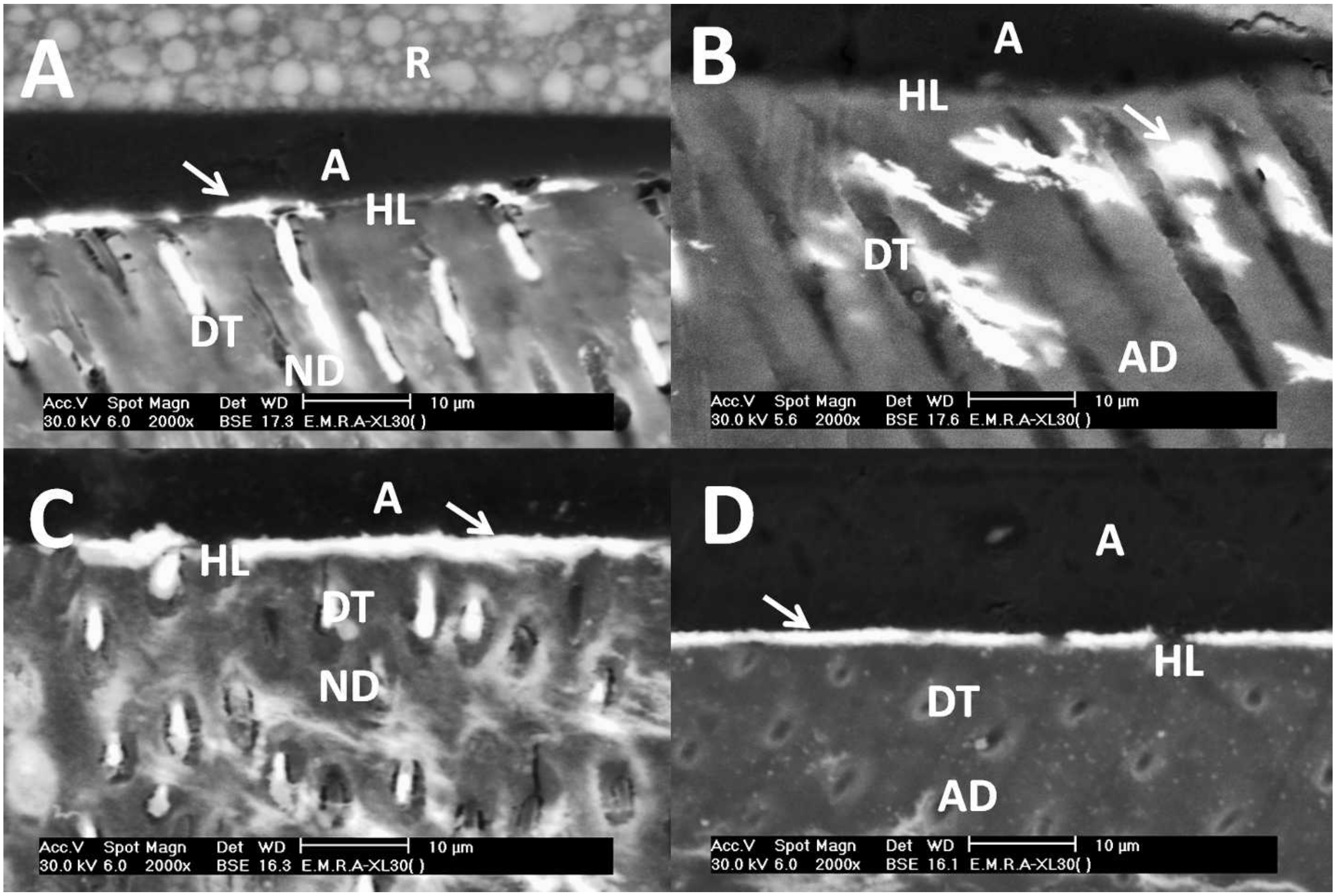

Figure 4. *Backscattered SEM micrograph of the interface of normal and caries-affected dentin bonded with SE Bond (2000×). In 24-hour 2% CHX groups (A for ND and B for AD) there were very limited silver deposits found at the base of hybrid layer of ND and no silver deposits for AD. After 2 years, more intense and continuous silver deposits could be detected (white arrows) for both ND (C) and AD (D). (Abbreviations: ND, normal dentin; AD, caries-affected dentin; HL, hybrid layer; DT, dentinal tubules; A, adhesive layer; R, resin composite; CHX, chlorhexidine)*

reported for ND, did not show an additional benefit in preserving bonding of SE Bond to caries-affected dentin over the 2 years of aging compared with the control group, while 5% CHX revealed a significant effect. Such a finding supports the fact that the substantivity of CHX is concentration dependent.[45] Previous study findings explained that at a higher concentration, the nature of the CHX interaction became reactive rather than adsorptive.[45] The reason behind the difference between normal dentin and caries-affected dentin in response to 5% CHX pretreatment may be attributed to the porous nature,[46] with more CHX uptake reported for caries-affected dentin than for normal dentin. Dilution of CHX due to dentinal tubule fluid was also more likely to occur in normal dentin rather than in caries-affected dentin as a result of the presence of calcium precipitates within the dentinal tubules of the caries-affected dentin.[47] Thus, the 5% CHX may serve as a reservoir, protecting the denuded collagen fibers from the effect of the MMPs for a longer period of time than normal dentin. At the same time, the lower dentinal tubule permeability in caries-affected dentin may impair the water hydrolytic effect on the resin.

It has been suggested that binding of CHX to collagen[26] and to calcium[42] may decrease the nanoleakage within the HL to which silver nitrate can deposit. However, this was not confirmed with the current study findings as the 24-hour groups showed comparable nanoleakage patterns. The low initial silver deposition for SE Bond even under IPP has also been reported by other studies.[48,49] This was explained due to its mild etching effect, which only modifies the smear layer rather than removing it, thus reducing the outward fluid flow. Also, the presence of 10-MDP may allow for the formation of more intense stable calcium salts with residual hydroxyl apatite, which further helps in preventing early nanoleakage. Additionally, SE Bond has a

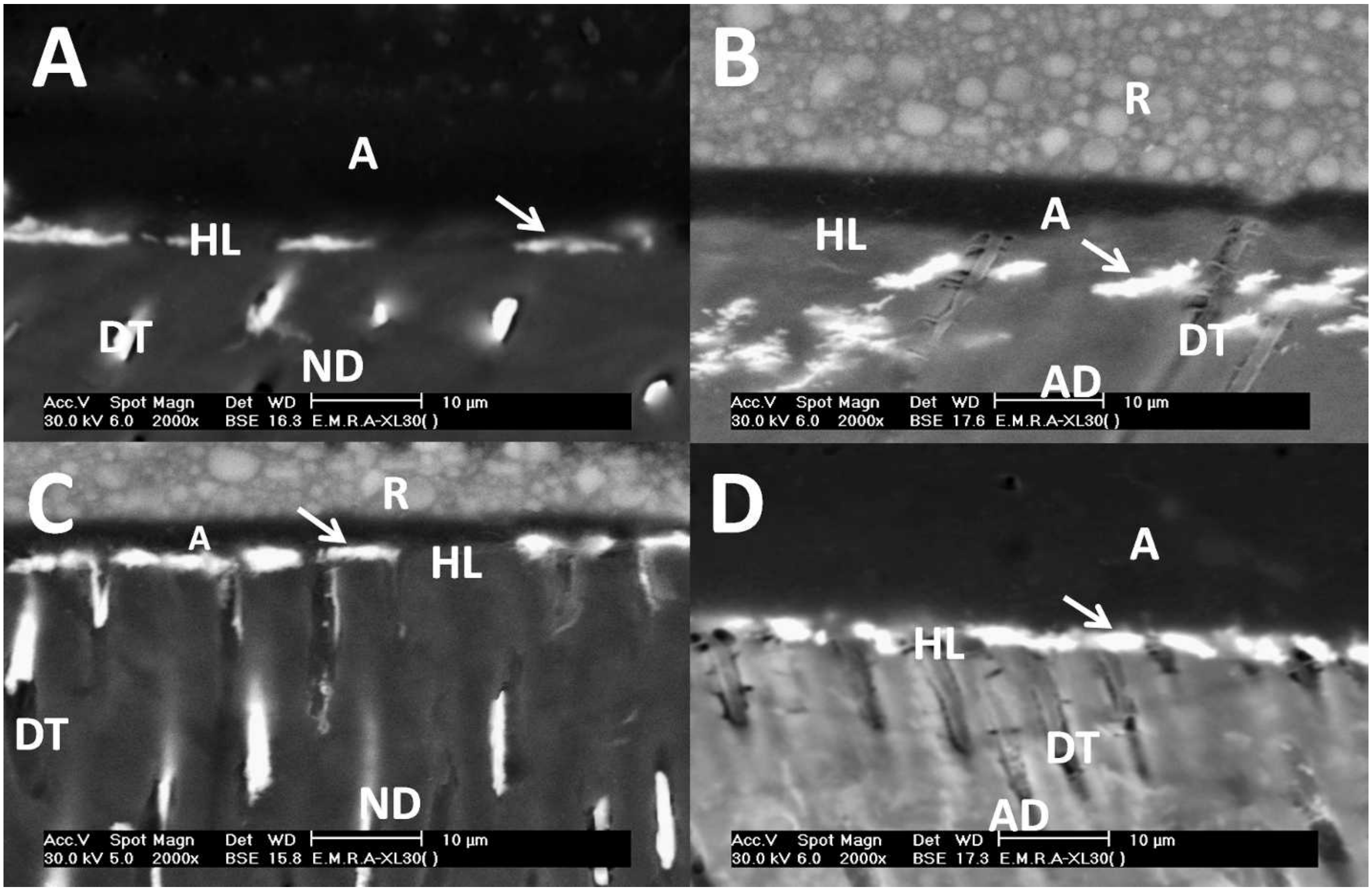

Figure 5. *Backscattered SEM micrograph of the interface of normal and caries-affected dentin bonded with SE Bond (2000×). In 24-hour 5% CHX groups (A for ND and B for AD), there were very limited silver deposits found at the base of the hybrid layer of ND along the dentinal tubules and no silver deposits for AD. After 2 years, more intense but less continuous silver deposits, compared to control and 2% CHX groups, could be detected (white arrow) for both ND (C) and AD (D). (Abbreviations: ND, normal dentin; AD, caries-affected dentin; HL, hybrid layer; DT, dentinal tubules; A, adhesive layer; R, resin composite; CHX, chlorhexidine)*

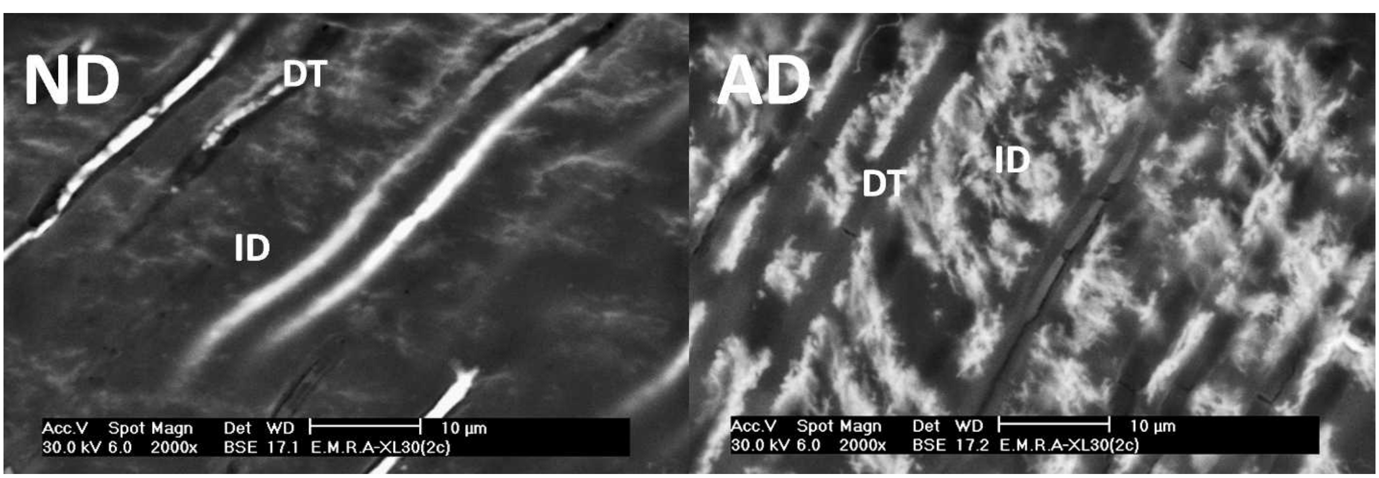

Figure 6. *Backscattered SEM micrograph of normal and caries-affected dentin (2000×). In normal dentin silver deposits were limited to dentinal tubules, while for caries-affected dentin silver deposits were found in the intertubular dentin (ID). (Abbreviations: ND, normal dentin; AD, caries-affected dentin; DT, dentinal tubules; ID, intertubular dentin)*

separate bonding step utilizing the hydrophobic bonding resin. This kind of application design was able to improve the hydrophobicity of the adhesive layer.[50] For caries-affected dentin, a drastic decrease in the water flux within the tubules is expected where the dentinal tubules are fully or partially blocked by mineral crystals.[47] This may explain the absence of nanoleakage for caries-affected dentin in 24-hour groups, which is in agreement with previous studies.[51,52] The streak-like deposits in the underlying caries–affected intertubular dentin as opposed to within the dentinal tubules may be due to the increased porosity of intertubular dentin in AD compared with the intertubular dentin in ND[46] and the occlusion of the dentinal tubules with mineral deposits.[47] The increase in silver deposition after 2 years of aging may be due to residual smear layer not being completely waterproof to prevent water seepage from the underlying dentin, even at a slow rate.

Although different nanoleakage patterns were observed for the 24-hour groups compared to the 2-year groups, failure modes did not differ among all groups. Such findings corroborate those of others[36,53] who tested the same adhesive. This supports the findings of others who reported no correlation between low bond strength and the increase in nanoleakage, indicating that silver stains were not always the site for generating crack initiation and propagation during bond testing.[54]

One of the reported concerns with the usage of CHX for dentin pretreatment is its toxicity. It has been reported that a concentration of 0.002% is not toxic to human periodontal cells[55] or to odontoblastic cells.[56] However, it is not yet known what concentration of CHX should be initially applied in order to reach this concentration (0.002%) at the pulp after the dilution effect of the dentinal tubule fluid through different dentin thicknesses permeated under IPP. This requires further investigation.

An interesting aspect in the present study is that even 5% CHX pretreatment and application of a relatively hydrophobic monomer were not able to completely resist the synergistic effect of extrinsic dentin wetness due to an exposed margin to artificial saliva and intrinsic wetness due to IPP over the long term (2 years). This emphasizes the fact that bond durability depends on both the resistance of adhesive resin and uncovered collagen fibers to degradation over time.[27] This also calls for the need to modify the adhesive monomers or innovation of new versions that are more resistant to hydrolytic degradation and are able to directly deactivate the MMPs without compromising the simplification achieved by the current adhesives.

CONCLUSIONS

1) 2% CHX and 5% CHX do not alter the 24-hour bond strength to either normal or caries-affected dentin under simulated IPP.
2) 2% CHX was not able to maintain the bond strength either to normal dentin or to caries-affected dentin over 2 years of aging.
3) 5% CHX was able to decelerate the regression in bond strength to caries-affected dentin over time.

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REFERENCES

1. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: methods and results *Journal of Dental Research* **84(2)** 118-132.

2. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG & Pashley DH (1995) Nanoleakage: leakage within the hybrid layer *Operative Dentistry* **20(1)** 18-25.

3. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, Carvalho RM, Tjaderhane L, Tay FR & Pashley DH (2006) Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives *European Journal of Oral Sciences* **114(2)** 160-166.

4. Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T & Tjaderhane L (2007) Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin *Archives of Oral Biology* **52(2)** 121-127.

5. Bourd-Boittin K, Fridman R, Fanchon S, Septier D, Goldberg M & Menashi S (2005) Matrix metalloproteinase inhibition impairs the processing, formation and mineralization of dental tissues during mouse molar development *Experimental Cell Research* **304(2)** 493-505.

6. Mazzoni A, Mannello F, Tay FR, Tonti GA, Papa S, Mazzotti G, Di Lenarda R, Pashley DH & Breschi L (2007) Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin *Journal of Dental Research* **86(5)** 436-440.

7. Sulkala M, Larmas M, Sorsa T, Salo T & Tjaderhane L (2002) The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth *Journal of Dental Research* **81(9)** 603-607.

8. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M & Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions *Journal of Dental Research* **77(8)** 1622-1629.

9. Boukpessi T, Menashi S, Camoin L, Tencate JM, Goldberg M & Chaussain-Miller C (2008) The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive

properties of restorative resins *Biomaterials* **29(33)** 4367-4373.

10. Davis GE (1991) Identification of an abundant latent 94-kDa gelatin-degrading metalloprotease in human saliva which is activated by acid exposure: implications for a role in digestion of collagenous proteins *Archives of Biochemistry and Biophysics* **286(2)** 551-554.

11. Gunja-Smith Z & Woessner JF Jr (1993) Activation of cartilage stromelysin-1 at acid pH and its relation to enzyme pH optimum and osteoarthritis *Agents Actions* **40(3–4)** 228-231.

12. Visse R & Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry *Circulation Research* **92(8)** 827-839.

13. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A & Engler JA (1993) Matrix metalloproteinases: a review *Critical Reviews in Oral Biology and Medicine* **4(2)** 197-250.

14. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM & Ito S (2004) Collagen degradation by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.

15. Belli R, Sartori N, Peruchi LD, Guimaraes JC, Araujo E, Monteiro S Jr, Baratieri LN & Lohbauer U (2010) Slow progression of dentin bond degradation during one-year water storage under simulated pulpal pressure *Journal of Dentistry* **38(10)** 802-810.

16. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH & Tay F (2007) Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media *Dental Materials* **23(7)** 885-892.

17. Armstrong SR, Vargas MA, Fang Q & Laffoon JE (2003) Microtensile bond strength of a total-etch 3-step, total-etch 2-step, self-etch 2-step, and a self-etch 1-step dentin bonding system through 15-month water storage *Journal of Adhesive Dentistry* **5(1)** 47-56.

18. Erhardt MC, Toledano M, Osorio R & Pimenta LA (2008) Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure *Dental Materials* **24(6)** 786-798.

19. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjaderhane L, Visintini E, Cadenaro M, Tay FR, De Stefano Dorigo E & Pashley DH (2010) Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer *Dental Materials* **26(6)** 571-578.

20. Tjaderhane L, Sulkala M, Sorsa T, Teronen O, Larmas M & Salo T (1999) The effect of MMP inhibitor metastat on fissure caries progression in rats *Annals of The New York Academy of Sciences* **878** 686-688.

21. Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T & Tjaderhane L (2001) The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats *Journal of Dental Research* **80(6)** 1545-1549.

22. Hebling J, Pashley DH, Tjaderhane L & Tay FR (2005) Chlorhexidine arrests subclinical degradation of dentin hybrid layers *in vivo Journal of Dental Research* **84(8)** 741-746.

23. Brackett WW, Tay FR, Brackett MG, Dib A, Sword RJ & Pashley DH (2007) The effect of chlorhexidine on dentin hybrid layers *in vivo Operative Dentistry* **32(2)** 107-111.

24. Breschi L, Cammelli F, Visintini E, Mazzoni A, Vita F, Carrilho M, Cadenaro M, Foulger S, Mazzoti G, Tay FR, Di Lenarda R & Pashley D (2009) Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: a 12-month *in vitro* study *Journal of Adhesive Dentistry* **11(3)** 191-198.

25. Carrilho MR, Carvalho RM, de Goes MF, di Hipolito V, Geraldeli S, Tay FR, Pashley DH & Tjaderhane L (2007) Chlorhexidine preserves dentin bond *in vitro Journal of Dental Research* **86(1)** 90-94.

26. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L & Pashley D (2007) *In vivo* preservation of the hybrid layer by chlorhexidine *Journal of Dental Research* **86(6)** 529-533.

27. Komori PC, Pashley DH, Tjaderhane L, Breschi L, Mazzoni A, de Goes MF, Wang L & Carrilho MR (2009) Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentin *Operative Dentistry* **34(2)** 157-165.

28. Ricci HA, Sanabe ME, de Souza Costa CA, Pashley DH & Hebling J (2010) Chlorhexidine increases the longevity of *in vivo* resin-dentin bonds *European Journal of Oral Sciences* **118(4)** 411-416.

29. Mobarak E, Korashy D & Pashley D (2010) Effect of chlorhexidine concentrations on micro-shear bond strength of self-etch adhesive to normal and caries-affected dentin *American Journal of Dentistry* **23(4)** 217-222.

30. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, Carvalho RM, Nakajima M & Pashley DH (2002) Bonding of self-etch and total-etch adhesives to carious dentin *Journal of Dental Research* **81(8)** 556-560.

31. Yoshiyama M, Urayama A, Kimochi T, Matsuo T & Pashley DH (2000) Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin *Operative Dentistry* **25(3)** 163-169.

32. Yokota H, Kubo S, Ohsawa M & Hayashi Y (2006) Effect of a caries-detecting solution on the tensile bond strength of four dentin adhesive systems *Dental Materials Journal* **25(1)** 66-74.

33. Shinohara MS, De Goes MF, Schneider LF, Ferracane JL, Pereira PN, Di Hipolito V & Nikaido T (2009) Fluoride-containing adhesive: durability on dentin bonding *Dental Materials* **25(11)** 1383-1391.

34. Tay FR, Pashley DH & Yoshiyama M (2002) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* **81(7)** 472-476.

35. Suppa P, Breschi L, Ruggeri A, Mazzotti G, Prati C, Chersoni S, Di Lenarda R, Pashley DH & Tay FR (2005) Nanoleakage within the hybrid layer: a correlative FEISEM/TEM investigation *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **73(1)** 7-14.

36. Hashimoto M, Fujita S, Nagano F, Ohno H & Endo K (2010) Ten-years degradation of resin-dentin bonds *European Journal of Oral Sciences* **18(4)** 404-410.

37. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M & Pashley DH (2004) Fluid movement across the resin-dentin interface during and after bonding *Journal of Dental Research* **83(11)** 843-848.

38. Bakry AS, Nakajima M, Otsuki M & Tagami J (2009) Effect of Er: YAG laser on dentin bonding durability under simulated pulpal pressure *Journal of Adhesive Dentistry* **11(5)** 361-368.

39. Scholtanus JD, Purwanta K, Dogan N, Kleverlaan CJ & Feilzer AJ (2010) Microtensile bond strength of three simplified adhesive systems to caries-affected dentin *Journal of Adhesive Dentistry* **12(4)** 273-278.

40. Tay FR, Pashley DH, Hiraishi N, Imazato S, Rueggeberg FA, Salz U, Zimmermann J & King NM (2005) Tubular occlusion prevents water-treeing and through-and-through fluid movement in a single-bottle, one-step self-etch adhesive model *Journal of Dental Research* **84(10)** 891-896.

41. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G & Van Meerbeek B (2009) Inhibition of enzymatic degradation of adhesive-dentin interfaces *Journal of Dental Research* **88(12)** 1101-1106.

42. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD & Reis A (2009) Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability *European Journal of Oral Sciences* **117(5)** 587-596.

43. Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzaga CC & Zielak JC (2009) Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing *Journal of Dentistry* **37(2)** 108-114.

44. Dayan D, Binderman I & Mechanic GL (1983) A preliminary study of activation of collagenase in carious human dentine matrix *Archives of Oral Biology* **28(2)** 185-187.

45. Carrilho MR, Carvalho RM, Sousa EN, Nicolau J, Breschi L, Mazzoni A, Tjaderhane L, Tay FR, Agee K & Pashley DH (2010) Substantivity of chlorhexidine to human dentin *Dental Materials* **26(8)** 779-785.

46. Silva NR, Carvalho RM, Pegoraro LF, Tay FR & Thompson VP (2006) Evaluation of a self-limiting concept in dentinal caries removal *Journal of Dental Research* **85(3)** 282-286.

47. Shimizu C, Yamashita Y, Ichijo T & Fusayama T (1981) Carious change of dentin observed on longspan ultrathin sections *Journal of Dental Research* **60(11)** 1826-1831.

48. Fernando de Goes M & Montes MA (2004) Evaluation of silver methenamine method for nanoleakage *Journal of Dentistry* **32(5)** 391-398.

49. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26(9)** 1035-1042.

50. Tay FR & Pashley DH (2003) Have dentin adhesives become too hydrophilic? *Journal of the Canadian Dental Association* **69(11)** 726-731.

51. Hosoya Y, Tay FR, García-Godoy F & Pashley DH (2008) Ultrastructural examination of one-step self-etch adhesive bonded primary sound and caries-affected dentin *American Journal of Dentistry* **21(6)** 368-372.

52. Hosoya Y, Tay FR, Miyakoshi S & Pashley DH (2008) Hardness and elasticity of caries-affected and sound primary tooth dentin bonded with 4-META one-step self-etch adhesives *American Journal of Dentistry* **21(4)** 223-228.

53. Ulker M, Ozcan M, Sengun A, Ozer F & Belli S (2010) Effect of artificial aging regimens on the performance of self-etching adhesives *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **93(1)** 175-184.

54. Hiraishi N, Yiu CK, King NM & Tay FR (2009) Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements *Journal of Dentistry* **37(6)** 440-448.

55. Chang YC, Huang FM, Tai KW & Chou MY (2001) The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **92(4)** 446-450.

56. de Souza LB, de Aquino SG, de Souza PP, Hebling J & Costa CA (2007) Cytotoxic effects of different concentrations of chlorhexidine *American Journal of Dentistry* **20(6)** 400-404.