©Operative Dentistry, 2015, **40-1,** 80-86

Effect of Different Matrix Metalloproteinase Inhibitors on Microtensile Bond Strength of an Etch-and-Rinse and a Self-etching Adhesive to Dentin

P Zheng • M Zaruba • T Attin
A Wiegand

Clinical Relevance

Matrix metalloproteinase (MMP) inhibitors prevented bond strength loss of an etch-and-rinse adhesive over time. Bond strength of a self-etching adhesive was not significantly reduced with 9 months of aging; thus, MMP inhibitors did not show a significant effect.

SUMMARY

Aim: This study aimed to analyze the effect of different matrix metalloproteinase (MMP) inhibitors on the microtensile bond strength (microTBS) of an etch-and-rinse and a self-etching adhesive after 9 months of aging.

Methods and Materials: Flat human dentin surfaces were bonded either with an etch-and-rinse adhesive (Optibond FL) or a self-etching adhesive (Clearfil SE Bond). Dentin surfaces were left untreated or were pretreated with MMP inhibitors (2% chlorhexidine digluconate [CHX], 0.05% green tea extract, 1 mM ferrous sulfate, or 0.2 mM galardin) prior to application of the adhesive. Composite buildups were made incrementally. Pretreated groups were tested after 9 months of storage in artificial saliva (37°C) and compared with untreated groups, which were tested immediately (initial microTBS) and upon aging (9-month microTBS). Data were analyzed by linear mixed-model regression. Failure mode analysis was performed microscopically and statistically analyzed by repeated-measures analysis of variance ($p<0.05$).

Pei Zheng, Medical College, Zhejiang University, Conservative Dentistry and Periodontics, Affiliated Hospital of Stomatology, Zhejiang, China

Markus Zaruba, Dr Med Dent, University of Zurich, Preventive Dentistry, Periodontology and Cariology, Zurich, Switzerland

Thomas Attin, Prof Dr Med Dent, Center for Dental Medicine, University of Zurich, Department of Preventive Dentistry, Periodontology and Cariology, Zurich, Switzerland

*Annette Wiegand, Prof Dr Med Dent, University of Göttingen, Preventive Dentistry, Periodontology and Cariology, Göttingen, Germany

*Corresponding author: Robert-Koch-Strasse 40, Göttingen, 37073, Germany; e-mail: annette.wiegand@med.uni-goettingen.de

DOI: 10.2341/13-162-L

Results: MicroTBS of the etch-and-rinse adhesive but not of the self-etching adhesive was significantly decreased by aging. For Optibond FL, pretreatment with 2% CHX, 0.05% green tea extract, and 0.2 mM galardin revealed bond strength values (MPa) similar to the initial microTBS (32.1±14.8) and significantly higher compared with the microTBS (20.3±13.6) of aged untreated dentin. No significant differences were observed between groups bonded with Clearfil SE Bond (initial microTBS: 28.3±12.4; 9-month microTBS: 25.3±11.8). Application of the MMP inhibitors decreased the number of adhesive failures compared with untreated controls after 9 months of aging, but this effect was not significant.

Conclusion: The MMP inhibitors prevented the decrease in microTBS upon aging of the etch-and-rinse but not of the self-etching adhesive.

INTRODUCTION

Matrix metalloproteinases (MMPs) and cysteine cathepsins in dentin and dentinal fluid contribute to the enzymatic degradation of the adhesive hybrid layer and thus to the reduction of bond durability over time. pH changes caused by acid etching or acidic monomers and the adhesive resin monomers themselves can modulate the activation and expression of MMPs and cysteine cathepsins, resulting in an increased digestion of collagen within the hybrid layer.[1,2]

Different strategies have aimed to improve the bond durability by applying enzyme inhibitors as a pretreatment before resin infiltration or by admixing enzyme inhibitors to primers. It has been shown that chlorhexidine digluconate (CHX) can inhibit[3] MMP-2, −8, and −9 and of cysteine cathepsins,[4] preserving bond strength of etch-and-rinse and self-etching adhesives over time.[5-7]

As the application of 2% CHX on phosphoric acid-etched dentin is the only application procedure that has been tested clinically and shown to prevent bond strength loss *in vivo*,[8-10] this procedure can be considered as a kind of gold standard for maintaining hybrid layer stability. However, in recent studies, other potential MMP inhibitors, such as galardin, metal ions, or green tea catechins, were tested, which were suggested to be as or even more effective than CHX.[11,12] Similar to CHX, galardin as a synthetic inhibitor of MMP-1, −2, −3, −8, and −9 is acting as a zinc chelator.[13] Green tea catechins, mainly epigallocatechin-gallate (EGCG), change the secondary structure of collagenases by hydrogen bonding and hydrophobic interactions.[14] As well, binding of metal ions might cause conformational changes that inactivate their catalytic function.[15]

However, to date, the efficacy of these MMP inhibitors to prevent dentin bond strength loss over time has not been determined. Thus, the aim of the present study was to investigate the microtensile bond strength (microTBS) of an etch-and-rinse (Optibond FL) and a self-etching adhesive (Clearfil SE Bond), both of which have been shown repeatedly to be excellent adhesives in their respective class, after pretreatment with different MMP inhibitors.

This study tested the null hypothesis that the microTBS of an etch-and-rinse and a self-etching adhesive after 9 months of storage in artificial saliva is not affected by pretreatment with water-based solutions containing 2% CHX, 0.05% green tea extract, 1 mM $FeSO_4$, or 0.2 mM galardin.

METHODS AND MATERIALS

Specimen Preparation

Forty extracted, sound human molars were selected for this study. The teeth were stored in 0.01% (w/v) thymol at 4°C and were used within four weeks after extraction. Extracted teeth were collected as anonymous by-products of regular therapy. Because of that, our Medical Ethical Board stated that the performed research did not fall under the regulations of the "Act on Medical Research Involving Human Subjects" (METc 2009.305).

The teeth were embedded in circular molds with self-curing acrylic resin (Paladur, Heraeus Kulzer, Germany). A flat, mid-coronal dentin surface was prepared by means of a water-cooled, low-speed diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL). Polishing with wet 600-grit SiC paper (Water Proof Silicon Carbide Paper, Stuers, Erkrat, Germany) created a standardized smear layer. The dentin surfaces were verified for the absence of pulp chamber exposition using a stereomicroscope (Stemi 2000, Carl Zeiss, Feldbach, Switzerland).

The teeth were allocated randomly to test (each n=3) and control (n=5) groups of the two adhesives.

Bonding Procedure

Bonding of dentin surfaces was performed using an etch-and-rinse (Optibond FL, Kerr, Scafati, Italy) or a self-etching adhesive (Clearfil SE Bond, Kuraray, Tokyo, Japan). The two adhesives were applied

Table 1: *Manufacturer, Composition, and Application Procedure of the Adhesive Systems*

Adhesive/Manufacturer	Batch Number/Composition	Application Procedure
Optibond FL, Kerr, Scafati, Italy	Primer (LOT: 3490336): HEMA, ethanol, GPDM, MMEP, water, CQ, BHT Adhesive (LOT: 3502324): Bis-GMA, HEMA, GDMA, CQ, ODMAB, approximately 48wt% filled	Dentin conditioning: 37% H_3PO_4 (15 s) Water rinsing (15 s) Gentle air drying (5 s) Test groups only: MMP inhibitor solution (60 s) Excess removal Adhesive application: Primer (15 s) Gentle air drying (5 s) Adhesive (15 s) Light curing (20 s)
Clearfil SE Bond, Kuraray, Okayama, Japan	Primer (LOT: 00997A): HEMA, 10-MDP, hydrophilic aliphatic dimethacrylate, CQ, water, accelerators, dyes Bond (LOT: 011482A): Bis-GMA, HEMA, 10-MDP, hydrophobic aliphatic methacrylate, colloidal silica, QC, initiators, accelerators	Test groups only: MMP inhibitor solution (60 s) Excess removal Adhesive application: Primer (20 s) Gentle stream (evaporation) Adhesive (20 s) Light curing (10 s)

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; BHT, butylhydroxytoluene; Bis-GMA, bisphenol A diglycidyl methacrylate; CQ, camphorquinone; GDMA, glycerol dimethacrylate; GPDM, glycerol phosphate dimethacrylate; HEMA, 2-hydroxyl methacrylate; MMEP, mono-2-methacryloyloxyethyl phthalate; MMP, matrix metalloproteinase; ODMAB, 2-(ethylhexyl)-4-(dimethylamino)benzoate.

according to the manufacturer's instructions (Table 1) with or without the additional application of one of the different MMP inhibitor solutions: 1) CHX (2%), 2) green tea extract (0.05%), ferrous sulfate (1 mM), or 4) galardin (0.2 mM; Table 2).

To analyze the initial microTBS and the bond strength after aging (microTBS after 9 months), two groups of each adhesive were not treated with any MMP inhibitor. The remaining groups were treated with one of the MMP inhibitors and tested only after aging, as the immediate bond strength between the untreated controls and test groups was shown to be not different.[11,20]

The application of the MMP inhibitor solutions was performed with a microbrush for 60 seconds under a slight rubbing motion. The excess was removed using an absorbing paper, leaving the dentin surface moist.

After light-curing of the bonding, a composite buildup (CeramX Mono, A3, Dentsply, Konstanz, Germany) was made in five 1 mm increments, each light-cured for 40 seconds at 800 mW/cm^2 (bluephase, IvoclarVivadent, Schaan, Liechtenstein).

MicroTBS Test

After 24-hour storage in water at 37°C, the teeth were sectioned perpendicular to the interface with a water-cooled diamond saw (Struers-Accutom 50, Struers, Denmark, and MOD 10, Struers, Denmark) to obtain rectangular beams of approximately 1 mm^2. The interfaces were precisely checked under a stereomicroscope to examine whether enamel remained. Sticks with remaining enamel were discarded, while all other sticks from one tooth were used. The beams of the control group were tested immediately (initial microTBS), while the beams of test groups were stored in artificial saliva[21] at 37°C for 9 months. Thereby, all sticks from one tooth were stored in 3 mL of artificial saliva.

Table 2: *Manufacturers and Concentrations of the Water-Based Matrix Metalloproteinase (MMP) Inhibitor Solutions*[a]

MMP Inhibitor/Manufacturer	Concentration of MMP Inhibitor Solution	Reference
Chlorhexidine digluconate, Kantonsapotheke, Zurich, Switzerland	2 wt%	5,16
Green tea extract, OM24, Omnimedica, Zurich, Switzerland	0.05 wt%	14,17
$FeSO_4$, Merck, Darmstadt, Germany	1 mM	12,18
Galardin, Merck, Darmstadt, Germany	0.2 mM	11

[a] *MMP inhibitors at the respective concentrations were chosen according to studies demonstrating a reduction in dentin degradation[12,16] or MMP-2 and/or MMP-9 activity.[5,11,18,19]*

Table 3: *Microtensile Bond Strength (MPa, mean±standard deviation), Number of Specimens Tested (n), Pretesting Failures (pf), and Failure Distribution (%) in the Different Groups*[a]

Adhesive	Group	Aging	MicroTBS, MPa	n	pf	Failure Distribution, %			
						A	M	CD	CC
Optibond FL	Control	No (initial testing)	32.1 ± 14.8*	68	15	22.1	64.7	4.4	8.8
	No treatment	9 mo	20.3 ± 13.6	40	2	42.5	52.5	0	5.0
	CHX	9 mo	32.9 ± 11.3*	35	0	31.4	51.4	5.7	11.4
	GTE	9 mo	33.2 ± 14.0*	41	0	31.7	53.7	7.3	7.3
	$FeSO_4$	9 mo	25.3 ± 10.5	32	0	43.8	43.8	6.2	6.2
	Galardin	9 mo	33.6 ± 10.5*	35	0	42.9	45.7	2.8	8.6
Clearfil SE Bond	Control	No (initial testing)	28.3 ± 12.4	69	12	13.0	75.4	4.3	7.2
	No treatment	9 mo	25.3 ± 11.8	37	0	46.0	43.2	5.3	5.3
	CHX	9 mo	32.9 ± 11.3	41	1	36.6	53.7	2.4	7.3
	GTE	9 mo	26.1 ± 14.2	38	1	26.3	57.9	5.3	10.5
	$FeSO_4$	9 mo	25.3 ± 10.5	30	1	40.0	50.0	0	10.0
	Galardin	9 mo	33.6 ± 14.1	36	1	38.9	55.6	2.8	2.8

Abbreviations: A, adhesive; CC, cohesive in composite; CD, cohesive in dentin; CHX, chlorhexidine digluconate; GTE, green tea extract; M, mixed.
[a] *Groups marked by an asterisk were significantly different from the group without pretreatment.*

The artificial saliva contained 0.7 mmol/L $CaCl_2$, 0.2 mmol/L $MgCl_2 \cdot 6H_2O$, 4.0 mmol/L KH_2PO_4, 30 mmol/L KCl, 0.3 mmol/L NaN_3, and HEPES buffer (all reagents purchased by Merck, Darmstadt, Germany) and was renewed weekly.

The tensile load of the beams was tested in a universal testing machine (Z010, Zwick/Roell, Ulm, Germany) with a 200 N load cell (KAF-TC, A.S.T., Dresden, Germany) at a crosshead speed of 0.15 mm/min. Therefore, the nontrimmed beams were fixed to a sandblasted microtensile bond jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Tochigi, Japan) and tested under tensile force in a top-bottom manner described previously.[22] The dimensions of the beams were measured with a digital caliper to calculate the exact cross-sectional area.

The bond strength values (MPa) were determined by dividing the imposed force (N) at the time of fracture by the bond area (mm^2).

In addition, each beam was observed under a stereomicroscope (Wild M8, WILD HEERBRUGG, Heerbrugg, Switzerland) at 50× magnification to determine the mode of failure, classified as adhesive (A), cohesive in dentin (CD), composite (CC), or mixed (M).

The number of sticks and pretest failures in each group is given in Table 3.

Statistical Analysis

The mean microTBS (±standard deviation) for each group was computed. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to check the assumption of normality. Normal distribution was found in all groups, and data were further analyzed by linear mixed-model regression, separately for each adhesive, to account for the fact that multiple beams were gained from the same tooth and, therefore, were not independent from each other.

Relative frequencies of adhesive, mixed, and cohesive failures were calculated and analyzed by repeated-measures analysis of variance (ANOVA) followed by Greenhouse-Geyser correction using the adhesive and the test groups as independent covariables. As no significant differences with respect to the adhesives were found, differences between the test groups were statistically analyzed by one-way ANOVAs and Scheffé post hoc tests separately for each failure type.

The level of significance was set at $p<0.05$.

RESULTS

The microTBS of the etch-and-rinse adhesive was significantly decreased after 9 months of storage in artificial saliva ($p=0.01$). Pretreatment with all MMP inhibitors except $FeSO_4$ revealed bond strength values similar to the initial microTBS and significantly higher compared with the aged group without pretreatment (9-month microTBS, $p<0.013$).

The microTBS of the self-etching adhesive declined only slightly but not significantly over time ($p=0.49$). Bond strength values after pretreatment with the different MMP inhibitors were not different

from the initial microTBS nor from the 9-month microTBS data ($p>0.359$; Table 3).

The failure mode analyses revealed significant differences between the test groups for adhesive ($p=0.003$) and mixed ($p=0.04$) but not for cohesive failures (dentin: $p=0.749$; composite: $p=0.528$). Overall, the frequency of adhesive failures in groups without MMP inhibitors was significantly ($p=0.008$) increased by aging. Application of the MMP inhibitors decreased the number of adhesive failures, but this difference was not significant with respect to the untreated groups after 9 months of aging ($p>0.34$). However, the relative frequencies of adhesive failures after pretreatment with CHX and green tea extract were not significantly ($p>0.07$) different from the untreated groups tested immediately. Post hoc comparisons of mixed failures showed no significant differences between the test groups ($p>0.061$).

DISCUSSION

The null hypothesis that the microTBS after 9 months of storage in artificial saliva is not affected by pretreatment with different MMP inhibitors was corroborated for the self-etch adhesive but not for the etch-and-rinse adhesive. Pretreatment with all MMP inhibitors except $FeSO_4$ prevented microTBS loss of Optibond FL significantly and equally effectively.

In the present study, the MMP inhibitor solutions were applied in a separate step in addition to the three-step or two-step application procedure of the etch-and-rinse or the self-etching adhesive, respectively. This approach might increase the overall chair time for a composite restoration and might be less feasible for self-etching adhesives because of the synchronous demineralization and infiltration during treatment. However, the application time of the MMP inhibitor solutions was limited to 60 seconds, which seems realizable under clinical conditions. Moreover, the incorporation of MMP inhibitors into dental adhesives might affect the mechanical properties of the products by decreasing the degree of conversion and the E-modulus.[23]

The initial microTBS found for Optibond FL and Clearfil SE Bond correlates with the results found previously.[24,25] Both adhesives have consistently shown favorable bonding performance in different protocols for microTBS measurement. As the bonding performance of Optibond FL and Clearfil SE Bond was shown to be hardly affected by the cavity configuration[26] and dentin location,[25] the setup of the present study was simplified by using flat dentin surfaces. Flat dentin surfaces allowed for the use of multiple sticks per tooth; thus, the overall sample size could be increased. The sticks were aged by direct exposure to artificial saliva, which is a quicker aging strategy than aging intact bonded teeth.[27]

Both adhesives exhibited lower microTBS values after aging, but this effect was significant only for the etch-and-rinse adhesive. Moreover, the fracture analysis revealed predominantly mixed adhesive-cohesive failure patterns with a clear tendency to fail more at the interface after 9 months of storage in the case in which no MMP inhibitor was applied. These results are in accordance with previous studies indicating a more rapid destruction of hybrid layers for three-step etch-and-rinse adhesives compared with mild two-step self-etching adhesives.[20,25] Although adhesive hydrophilicity and water sorption of adhesive interfaces are still considered the principal mechanisms of the resin-bond degradation, enzymatic degradation of the hybrid layer by MMPs contributes to the degradation process and loss of bond strength over time. The higher levels of MMP-2 and MMP-9 activity demonstrated for etch-and-rinse compared with self-etching adhesives[2,20] might therefore partly explain why the bond durability of Optibond FL is affected to a higher extent than that of the Clearfil SE Bond. However, it is likely that with longer aging times, the effect of enzymatic degradation might become more apparent for Clearfil SE Bond as well.[20]

For both adhesives, application of the MMP inhibitors resulted in microTBS values similar to the initial bond strength. It is likely that this effect is related to the reduction of enzymatic dentin degradation. In a previous study, the degradation of demineralized dentin after application of 0.012% CHX, 400 μM EGCG, and 1 mM $FeSO_4$ was directly determined by assaying hydroxyproline. All test agents reduced enzymatic collagen degradation distinctly but were not significantly different from each other.[12]

As the catechin concentration in the green tea extract solution is about 30%,[28] the inhibitory doses reported for EGCG and ECG on MMP-2 and MMP-9 were distinctly exceeded.[14] EGCG concentrations between 0.02% and 0.5% were shown to prevent bond strength loss of an etch-and-rinse adhesive over 6 months,[29] thereby being as effective as a 2% chlorhexidine solution. Because of the low toxicity and the anti-inflammatory potential, polyphenol catechins can be considered as very biocompatible,

with the MMP inhibitor as a possible rewetting agent.

Pretreatment with the galardin solution was as effective as chlorhexidine in preserving bond strength over time, which supports the results of a previous study, in which 0.2 mM galardin solution was used as rewetting agent for an etch-and-rinse adhesive.[11] In contrast to these results, galardin-modified adhesives at lower concentration were shown to be unable to prevent bond strength loss after three months of storage time.[30]

Although ferrous sulfate was applied in a concentration previously shown to inhibit MMP-2 and MMP-9 activity and to reduce erosive dentin loss probably by inhibiting enzymatic degradation of the collagenous dentin matrix,[18] it was unable to inhibit bond strength loss significantly. The inhibitory action of ferrous sulfate on MMPs might be partly abrogated by the fact that precipitates formed on the dentin surface and in the dentin tubules[18,31] act as a mechanical barrier hampering penetration of the adhesive. Moreover, ferric sulfate might induce some dentin discoloration,[18] which might affect the color of a composite restoration negatively.

CONCLUSIONS

The MMP inhibitors chlorhexidine, green tea extract, and galardin preserved the microTBS of Optibond FL but not of Clearfil SE Bond significantly when aged for 9 months in artificial saliva. In the case of ferric sulfate application, bond strength was not affected negatively over time. It remains to be determined whether these MMP inhibitors will prevent bond strength loss in long-term experiments.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 3 December 2013)

REFERENCES

1. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, & Pashley DH (2013) Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins *Dental Materials* **29(1)** 116-135.
2. Mazzoni A, Scaffa P, Carrilho M, Tjäderhane L, Di Lenarda R, Polimeni A, Tezvergil-Mutluay A, Tay FR, Pashley DH, & Breschi L (2013) Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9 *Journal of Dental Research* **92(1)** 82-86.
3. Gendron R, Grenier D, Sorsa T, & Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8 and 9 by chlorhexidine *Clinical and Diagnostic Laboratory Immunology* **6(3)** 437-439.
4. Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, Pashley DH, Tjäderhane L, Tersariol IL, Nascimento FD, & Carrilho MR (2012) Chlorhexidine inhibits the activity of dental cysteine cathepsins *Journal of Dental Research* **91(4)** 420-425.
5. Breschi L, Mazzoni A, Nato F, Carrilho M, Visitini E, Tjaderhane L, Ruggeri A Jr, Tay FR, Dorigo Ede S, & Pashley DH (2010) Chlorhexidine stabilizes the adhesive interface: a 2-year *in vitro* study *Dental Materials* **26(4)** 320-325.
6. Yiu CK, Hirashi N, Tay FR, & King NM (2012) Effect of chlorhexidine incorporation into dental adhesive resin on durability of resin-dentin bond *Journal of Adhesive Dentistry* **14(4)** 355-362.
7. Carrilho MR, Carvalho RM, De Goes MF, di Hipolito V, Geraldeli S, Tay FR, Pashley DH, & Tjäderhane L (2007) Chlorhexidine preserves dentin bond *in vitro Journal of Dental Research* **86(1)** 90-94.
8. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, Mai S, & Pashley DH (2009) *In vivo* chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive *Operative Dentistry* **34(4)** 379-383.
9. Hebling J, Pashley DH, Tjäderhane L, & Tay FR (2005) Chlorhexidine arrests subclinical degradation of dentin hybrid layers *in vivo Journal of Dental Research* **84(8)** 741-746.
10. Carrilho MR, Geraldeli S, Tay F, De Goes MF, Carvalho RM, Tjäderhane 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.
11. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjaderhane L, Visitini 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.
12. Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhaes AC, Pereira JC, & Buzalaf MAR (2012) Impact of protease inhibitors on dentin matrix degradation by collagenase *Journal of Dental Research* **91(12)** 1119-1123.
13. Augé F, Hornebeck W, Dacarme M, & Laronze JY (2003) Improved gelatinase a selectivity by novel zinc binding groups containing galardin derivatives *Bioorganic & Medicinal Chemistry Letters* **13(10)** 1783-1786.
14. Demeule M, Brossard M, Page M, Gingras D, & Beliveau R (2000) Matrix metalloproteinase inhibition by green tea catechins *Biochimica et Biophysica Acta* **1478(1)** 51-60.
15. Buzalaf MAR, Kato MT, & Hannas AR (2012) The role of matrix metalloproteinases in dental erosion *Advances in Dental Research* **24(2)** 72-76.
16. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjaderhane L, Mazzoni A, Breschi L, Foulger S, & Pashley DH

(2009) Host-derived loss of dentin matrix stiffness associated with solubilization of collagen *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **90(1)** 373-380.

17. Park JW, Hong JS, Lee KS, Kim HY, Lee JJ, & Lee SR (2010) Green tea polyphenol (-)-epigallocatechin gallate reduces matrix metalloproteinase-9 activity following transient focal cerebral ischemia *Journal of Nutritional Chemistry* **21(11)** 1038-1044.
18. Kato MT, Leite AL, Hannas AR, Oliveira RC, Pereira JC, Tjaderhane L, & Buzalaf MAR (2010) Effect of iron on matrix metalloproteinase inhibition and on the prevention of dentine erosion *Caries Research* **44(3)** 309-316.
19. Roomi MW, Monterrey JC, Kalinovsky T, Rath M, & Niedzwiecki A (2010) Comparative effects of EGCG, green tea and a nutrient mixture on the patterns of MMP-2 and MMP-9 expression in cancer cell lines *Oncology Reports* **24(3)** 747-757.
20. 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.
21. 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.
22. Lu S, Zhao SJ, Wang WG, Gao Y, Zhang Y, Duo Q, & Chen JH (2013) A new fixation method for stick-shaped specimens in microtensile tests: laboratory tests and FEA *Journal of Adhesive Dentistry* **15(6)** 511-518.
23. Cadenaro M, Pashley DH, Marchesi G, Carrilho M, Antoniolli F, Mazzoni A, Tay FR, Di Lenarda R, & Breschi L (2009) Influence of chlorhexidine on the degree of conversion and E-modulus of experimental adhesive blends *Dental Materials* **25(10)** 1269-1274.
24. Scherrer SS, Cesar PF, & Swain MV (2010) Direct comparison of the bond strength results of the different test methods: a critical literature review *Dental Materials* **26(2)** e78-e93.
25. De Munck J, Mine A, Vivan Cardoso M, De Almeida Neves A, Van Landuyt K, Poitevin A, & Van Meerbeek B (2011) Effect of dentin location and long-term water storage on bonding effectiveness of dentin adhesives *Dental Materials Journal* **30(1)** 7-13.
26. Shirai K, De Munck J, Yoshida Y, Inoue S, Lambrechts P, Suzuki K, Shintani H, & Van Meerbeek B (2005) Effect of cavity configuration and aging on the bonding effectiveness of six adhesives to dentin *Dental Materials* **21(2)** 110-124.
27. Feitosa VP, Leme AA, Sauro S, Correr-Sobrinho L, Watson TF, Sinhoreti MA, & Correr AB (2012) Hydrolytic degradation of the resin-dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing *Journal of Dentistry* **40(12)** 1134-1143.
28. Magalhaes AC, Wiegand A, Rios D, Hannas A, Attin T, & Buzalaf MA (2009) Chlorhexidine and green tea extract reduce dentin erosion and abrasion *in situ Journal of Dentistry* **37(12)** 994-998.
29. Santiago SL, Osorio R, Neri JR, Carvalho RM, & Toledano M (2013) Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength *Journal of Adhesive Dentistry* **15(6)** 535-540.
30. Almahdy A, Koller G, Sauro S, Bartsch JW, Sheriff M, Watson TF, & Banerjee A (2012) Effects of MMP inhibitors incorporated within dental adhesives *Journal of Dental Research* **91(6)** 605-611.
31. Sales-Peres SH, Reinato JV, Sales-Peres Ade C, & Marsicano JA (2011) Effect of iron gel on dentin permeability *Brazilian Dental Journal* **22(3)** 198-202.