



ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Long-term nanomechanical properties and gelatinolytic activity of titanium tetrafluoride-treated adhesive dentin interface

Enrico Coser Bridi^a, Ariene Arcas Leme-Kraus^b,
Roberta Tarkany Basting^{a,*}, Ana Karina Bedran-Russo^b

^a São Leopoldo Mandic Institute and Dental Research Center, Rua José Rocha Junqueira 13, Bairro Swift, Campinas, São Paulo, CEP: 13045-755, Brazil

^b Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, 801 South Paulina Street, Chicago, IL, 60612, USA

ARTICLE INFO

Article history:

Received 16 March 2019

Received in revised form

30 May 2019

Accepted 15 July 2019

Keywords:

Titanium tetrafluoride

Reduced modulus of elasticity

Hardness

Hybrid layer

In situ zymography

ABSTRACT

Objective. This study investigated the effects of dentin pretreatment with 2.5% titanium tetrafluoride (TiF₄) on nanomechanical properties, and the *in situ* gelatinolytic activity of the dentin–resin interface, for up to 6 months.

Methods. Twenty-four human teeth were prepared by exposing occlusal flat dentin surfaces, and were randomly assigned to experimental groups, according to application or non-application of a TiF₄ pretreatment, and to the adhesive systems (Clearfil SE Bond or Scotchbond Universal). Resin composite (Filtek Supreme Ultra) was built up incrementally on the teeth in all the groups. Then, the specimens were sectioned and randomly selected for evaluation at 24 h, 3 months and 6 months of storage time. The reduced modulus of elasticity (E_r) and the nanohardness of the underlying dentin, as well as the hybrid layer and the adhesive layer were measured using a nanoindenter. Gelatinolytic activity at the dentin–resin interfaces was assessed by *in situ* zymography using quenched fluorescein-conjugated gelatin at 24 h and 6 months. Statistical analyses were performed with ANOVA and Tukey's tests.

Results. There were no differences in E_r and nanohardness values between adhesives systems and pretreatment ($p = 0.1250$). *In situ* zymography showed significantly higher gelatinolytic activity after 6 months for all the experimental groups ($p = 0.0004$), but no differences between the adhesive systems ($p = 0.7708$) and the surface pretreatment ($p = 0.4877$). Significance: Dentin pretreatment with 2.5% TiF₄ followed by self-etching adhesive systems did not influence nanomechanical properties or gelatinolytic activity of the adhesive–dentin interface layers, over time.

© 2019 The Academy of Dental Materials. Published by Elsevier Inc. All rights reserved.

* Corresponding author at: Faculdade de Odontologia e Instituto de Pesquisas São Leopoldo Mandic, Departamento de Odontologia Restauradora - Dentística, Rua José Rocha Junqueira, 13. Bairro Swift, Campinas, SP, CEP: 13045-755, Brazil.

E-mail addresses: enricobridi@gmail.com (E.C. Bridi), leme@uic.edu (A.A. Leme-Kraus), roberta.hofling@slmandic.edu.br (R.T. Basting), bedran@uic.edu (A.K. Bedran-Russo).

<https://doi.org/10.1016/j.dental.2019.07.016>

0109-5641/© 2019 The Academy of Dental Materials. Published by Elsevier Inc. All rights reserved.

1. Introduction

Poor resin infiltration and incomplete enveloping of the dentin matrix leaves collagen exposed at the adhesive interface [1], thus increasing the vulnerability of the hybrid layer to proteases [2,3]. Different dentin pretreatment agents can minimize the *in vitro* degradation of the hybrid layer by inhibiting matrix metalloproteinase (MMP) activity and/or by biomodification of the collagenous dentin matrix [4–11]. Certain multi-functional chemical agents can increase the biomechanical properties of collagen-based tissues and decrease biodegradation rates [12].

Dentin pretreatment with an aqueous solution of titanium tetrafluoride (TiF₄) can be applied to prevent demineralization of the dentin underneath resin-composite restorations [13–15]. This solution does not affect hybrid layer formation by one- or two-step self-etching adhesive systems [16–19], and can increase the bond strength of a conventional two-step adhesive system [20]. Aqueous or varnish formulations of 0.1–4% TiF₄ have shown both anti-cariogenic and anti-erosive effects on enamel surfaces through the formation of a titanium- and fluoride-rich layer [21–26]. Recent studies have shown that the pretreatment of dentin with a 2.5% TiF₄ solution inhibited *in situ* secondary caries [14], and increased the immediate mechanical properties and resistance of the dentin matrix to enzymatic biodegradation [27].

The findings to date on TiF₄ are promising and warrant further mechanistic and performance studies, particularly in regard to its long-term effects. The present study proposes to investigate the individual mechanical contributions of the dentin-adhesive components using nanoindentation. Since enzymatic activity at the dentin–resin interface contributes to the degradation of interfacial components [28], the present study also assessed enzymatic activity at the dentin–resin interface using the *in situ* zymography method. Recent studies have shown that a pretreatment solution using TiF₄ could be effective in preventing secondary caries [14], and improving the mechanical properties and resistance of the dentin matrix to enzymatic biodegradation [27]; bearing this mind, the present study could contribute significantly to understanding the effects of TiF₄ on the components of the hybrid layer, as well as the process of biodegradation over time. Accordingly, the specific aims were to evaluate the effect of an aqueous formulation of 2.5% TiF₄ on the nanomechanical properties and the gelatinolytic activity of one- and two-step self-etching adhesive systems at the dentin–resin interface, in up to 6 months of storage. The null hypotheses tested were that 2.5% TiF₄ would not affect: (1) the long-term reduced modulus of elasticity, (2) the nanohardness, or (3) the gelatinolytic activity of dentin–resin interfaces created by two self-etching adhesive systems.

2. Materials and methods

2.1. Specimen preparation and restorative procedures

After approval by the Research Ethics Committee (CAAE no. 30721114.6.0000.5374), twenty-four recently extracted sound

human molars free of fractures and enamel malformations were selected for this study. All the extracted teeth were stored in a freezer for no longer than 6 months, and were then debrided with scalpel blades and periodontal curettes.

The roots were sectioned 2 mm below the cemento-enamel junction using a flexible diamond disk mounted on a precision electric saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The occlusal enamel portion was removed, and a flat dentin surface was obtained in the occlusal third, perpendicular to the long axis of the tooth, by using #400 and #600 grit silicon carbide (SiC) paper (Buehler, Lake Bluff, IL, USA).

The teeth were randomly assigned into four experimental groups to perform the nanomechanical measurements of the adhesive interface component ($n=3$ per group), and to determine the gelatinolytic activity ($n=6$ per group). The gelatinolytic activity at the dentin–resin interface was evaluated by cutting the teeth in half to maintain the control and the experimental group in the same tooth. The teeth were then assigned to the surface dentin pretreatments (control and TiF₄) and adhesive systems (Clearfil SE Bond/ Kuraray Medical Inc., Kurashiki, Okayama, Japan; or Scotchbond Universal/ 3M ESPE, St. Paul, MN, USA), which comprised the variables of the study ($n=6$). The application method, composition and product information for all the restorative materials are presented in Table 1.

TiF₄ P.A. (Pro-Analyses) was dissolved in distilled water to a concentration of 2.5% (w/v%; pH 1.4). TiF₄ was actively applied to the dentin surface 60 s prior to application of the adhesive systems, according to previous studies [16–19]. The excess water was removed from the dentin with absorbent paper prior to the pretreatment. After application and polymerization of the adhesive systems, resin composite was applied incrementally (Filtek Supreme Ultra/3M ESPE, St. Paul, MN, USA) to build a core about 5.0 mm high and 5.0 mm wide. Each increment was light-cured for 40 s with an intensity of 600 mw/cm² (Optilux 501, Kerr).

The dentin–resin specimens were sectioned (Isomet 1000, Buehler, Lake Bluff, IL, USA) after 24 h storage in distilled water at 37 °C, yielding three 1.5-mm-thick slabs to study nanoindentation, and several 1.0-mm-thick slabs to perform the *in situ* zymography assays. One slab was randomly selected for each evaluation time point: 24 h, 3 month and 6 months. Long-term specimens were stored in distilled water, which was changed every 2 weeks.

2.2. Interfacial nanomechanical properties

Dentin–resin slabs were embedded in cold cure epoxy resin (Buehler, Lake Bluff, IL, USA) at each evaluation timepoint, and allowed to cure for 8 h at room temperature. The specimens were high-gloss polished with #400, 600, 800 and 1200 grit SiC paper (Buehler, Lake Bluff, IL, USA), followed by 9, 6, 3, 1 μm diamond suspension and 0.05 μm alumina suspension polish (MasterPrep, Buehler, Lake Bluff, IL, USA). The specimens underwent ultrasonic cleaning between each suspension for 5 min. The polishing procedures were carried out immediately before the evaluations [29]. The reduced modulus of elasticity (E_r) and the nanohardness (H) of the bonded interface components were evaluated using a custom Ubi nanoindenter (Hysitron, Minneapolis, MN) and a Berkovich fluid cell tip with

Table 1 – Materials, manufacturer and lot numbers, composition and pH, and protocol for use.

Material	Product information	Composition/pH	Protocol for use
2.5% TiF ₄	Sigma Aldrich, Saint Louis, MO, USA. Lot #MKBQ5311	Titanium tetrafluoride P.A. + distilled water/pH 1.4	Active application with a microbrush for 60 s
Clearfil SE Bond	Kuraray Medical Inc., Kurashiki, Okayama, Japan Lot Primer: 01250A	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water	^a Application of primer for 20 s; gentle air; application of adhesive; gentle air; photopolymerization 10 s.
	Lot Bond: 01888A	Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photoinitiators, silanized colloidal silica/pH 2	
Scotchbond Universal	3M ESPE, St. Paul, MN, USA Lot: 564145	MDP, dimethacrylate, HEMA, copolymer of polyalkenoic acid, filler, ethanol, water, silane, photoinitiators/pH 2.7	^a Active application for 20 s, air-dry for solvent evaporation and photo-polymerization for 10 s.
Filtek Supreme Ultra	3M ESPE, St. Paul, MN, USA Lot: N697117	Bis-GMA, UDMA, TEGDMA, Bis-EMA(6), PEGDMA, silica, zirconia.	

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

^a Manufacturer's instructions.

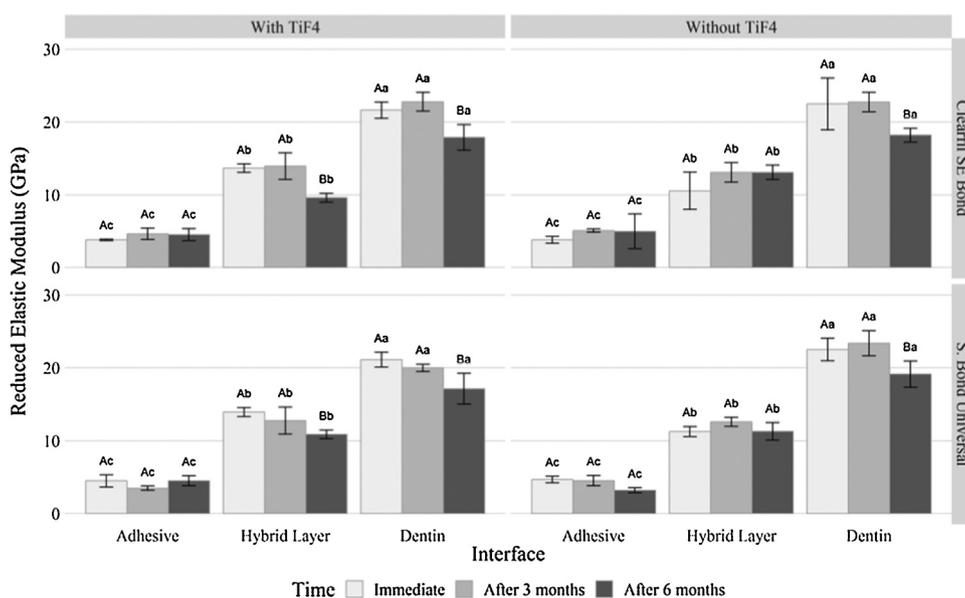


Fig. 1 – Results of reduced modulus of elasticity (GPa) of Clearfil SE Bond and Scotchbond Universal, according to the groups and aging timepoints. Distinct uppercase letters depict differences among timepoints within the same condition of the layer (TiF₄, type of adhesive system), and lowercase letters depict differences within each layer for each timepoint (i.e. effect of TiF₄, type of adhesive systems) ($p < 0.05$).

a 100-nm radius. A calibration function with a known modulus of elasticity ($E_r = 69.6$ GPa) and hardness ($H = 9.36$ GPa) was carried out on a standard quartz sample prior to testing. The specimens were attached to a metal disc using cyanoacrylate glue (Scotch, 3M, St. Paul, MN, USA), and positioned on the device. The indentations were performed under a standard trapezoidal load function with 5 s loading and unloading

times, to a maximum load of 1000 mN and a holding period of 2 s [29]. Three different areas were selected for indentation along the tooth-restoration interface. Forty-five indents were made in each specimen, fifteen in each component of the adhesive interface (Fig. 3): adhesive layer (A), hybrid layer (HL) and underlying dentin (D). Indentations were made with specimens fully immersed in HBSS (Hank's balanced salt solu-

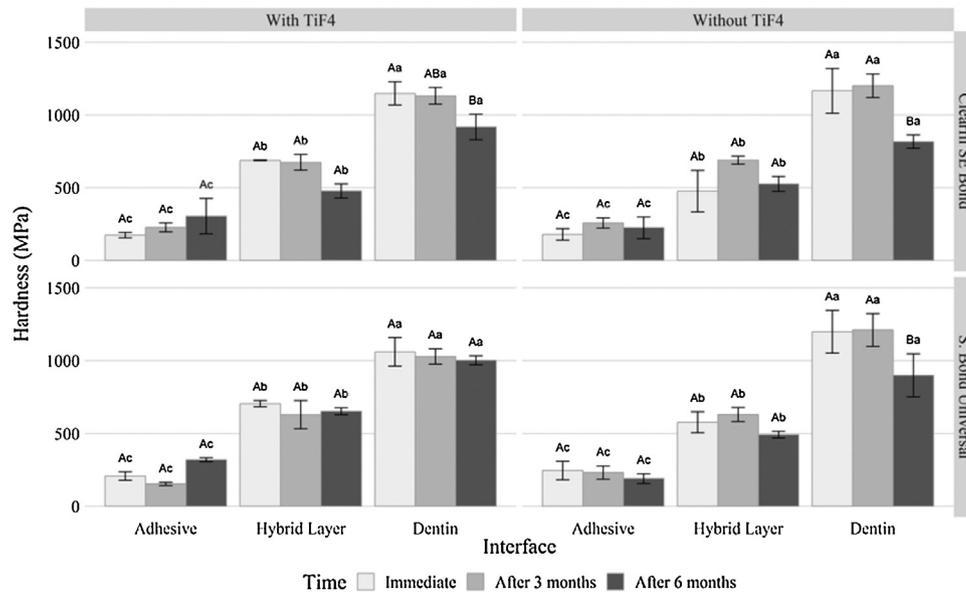


Fig. 2 – Results of nanohardness (MPa) of Clearfil SE Bond and Scotchbond Universal, according to the groups and storage timepoints. Distinct uppercase letters depict differences among the timepoints within the same condition of the layer (TiF₄, type of adhesive system), and lowercase letters depict differences within each layer for each timepoint (i.e. effect of TiF₄, type of adhesive systems) ($p < 0.05$).

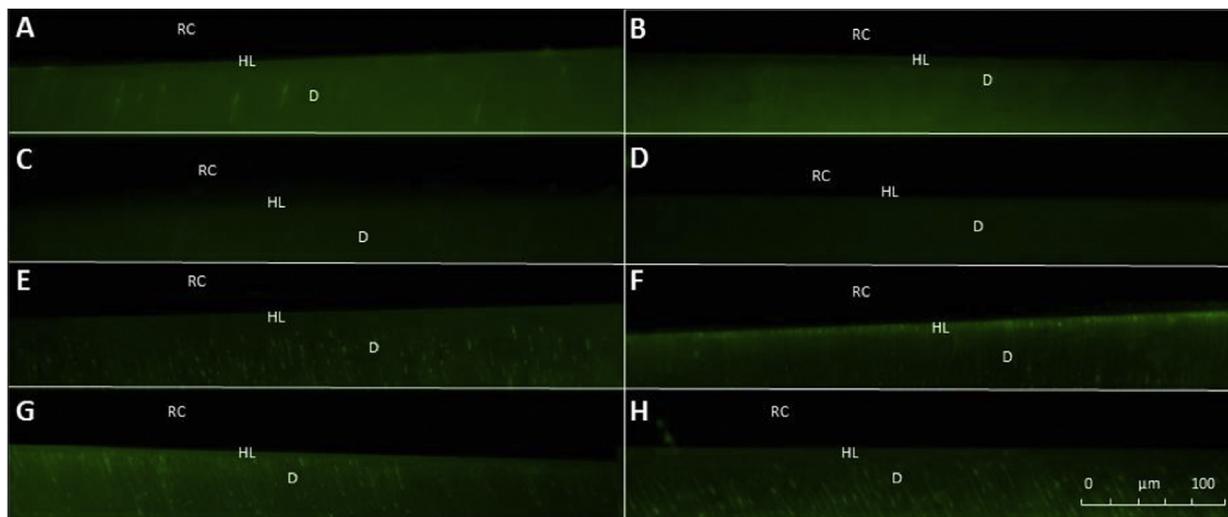


Fig. 3 – Representative fluorescent microscopy images of in situ zymography with quenched fluorescein-conjugated gelatin. Dentin–resin interface immediately after treatment: (A) Clearfil SE Bond; (B) TiF₄ followed by Clearfil SE Bond; (C) Scotchbond Universal; (D) TiF₄ followed by Scotchbond Universal. Dentin–resin interface after 6 months of storage: (E) Clearfil SE Bond; (F) TiF₄ followed by Clearfil SE Bond; (G) Scotchbond Universal; (H) TiF₄ followed by Scotchbond Universal (D = dentin; HL = hybrid layer; RC = composite resin).

tion, Lonza Group, Basel, Switzerland) at a distance of at least 15 μm between each other. E_r was calculated based on the load-displacement curves, as described previously [29]. Indentations were performed in the intertubular dentin.

2.3. Gelatin preparation and fluorescence microscopy analysis

The specimens were cut vertically into 0.6-mm-thick slices at each timepoint (24 h and 6 months) to expose their

adhesive interfaces, using a slow-speed saw under water cooling (Isomet 1000, Buehler, Lake Bluff, IL, USA), and further polished manually (Buehler) until obtaining 500- μm thickness. In situ zymography was performed with quenched fluorescein-conjugated gelatin as the MMP substrate (EnzChek Gelatinase/Collagenase Assay, Eugene, OR, USA), as previously reported [12]. In brief, a 1 mg/mL stock solution of fluorescein-labeled gelatin was prepared by suspending lyophilized substrate in distilled water. Then, the gelatin stock solution was diluted 1:8 with dilution buffer (NaCl 150 mM,

CaCl₂ 25 mM, Tris–HCl 50 mM, pH=8). An 80 µL aliquot of the fluorescent gelatin mixture was placed on top of each slab and covered with a coverslip (Microscope Cover Slips, Rochester Scientific, Rochester, NY, USA). Slides were light-protected and incubated in a humidified chamber at 37 °C for 2 h, and then observed under fluorescence microscopy (Leica DMI 6000, Buffalo Grove, IL, USA). Three images of each slab were acquired, after which all the images were analyzed and quantified in ImageJ (NIH, Frederick, MD, USA). The fluorescence emission of quenched gelatin at the different layers (adhesive layer, hybrid layer and dentin) was quantified using two 100-µm-long representative regions of each image.

2.4. Statistical analysis

The results of the reduced modulus of elasticity (E_r), nanoindentation hardness and in situ zymography were analyzed statistically with ANOVA in a sub-subdivided plot scheme. Multiple comparisons were analyzed by the Tukey test with a significance level of 5%, using SAS software (SAS Institute Inc., Cary, NC, USA, Release 9.2, 2010).

3. Results

The results of the reduced modulus of elasticity showed no effect of the triple interactions (adhesive system \times TiF₄ \times time: $p=0.1250$; adhesive system \times layer \times time: $p=0.7975$; TiF₄ \times layer \times time: $p=0.5810$; adhesive system \times TiF₄ \times layer: $p=0.1214$). The highest means in all the groups and timepoints were observed in the dentin layer, followed by the hybrid layer and the adhesive layer ($p<0.0001$). After 6 months, the values of both the hybrid and the dentin layers decreased significantly regardless of the adhesive systems and the pretreatment (TiF₄ \times layer: $p=0.0367$; TiF₄ \times time: $p=0.0501$; layer \times time: $p<0.0001$) (Figs. 1 and 2).

There were no interactions among the factors of adhesive system \times TiF₄ \times layer ($p=0.3297$) for H. However, the effects of the triple interactions of adhesive system \times TiF₄ \times timepoint ($p=0.0339$) and adhesive system \times layer \times timepoint ($p=0.0070$) were significant. There was a significant decrease in H after 6 months for different layers with Scotchbond without TiF₄ and for Clearfil SE Bond with or without TiF₄ application ($p<0.0001$). No significant differences were found between adhesive systems with or without TiF₄ ($p=0.6751$) (Fig. 3). The highest means were observed in the dentin layer, followed by the hybrid layer and the adhesive layer, in all groups and timepoints ($p<0.0001$).

No significant interactions were observed among the adhesive system \times TiF₄ \times timepoints for in situ zymography ($p=0.7708$). There was also no significant effect of the double interactions of the adhesive system \times TiF₄ ($p=0.9473$), adhesive \times timepoints ($p=0.4435$) and TiF₄ \times timepoints (0.9110), or between the adhesive systems themselves ($p=0.9887$). Moreover, the TiF₄ dentin pretreatment had no effect ($p=0.4877$), as shown in Table 2. The average green fluorescence increased significantly after 6 months for all the experimental groups ($p=0.0004$). Representative images of the fluorescence microscopy are presented in Fig. 3. A very low, and often absent fluorescence, was observed at the immediate time-

point (24 h), regardless of the adhesive system and the TiF₄ pretreatment. After 6 months, the fluorescence significantly increased, regardless of the adhesive system and TiF₄ dentin pretreatment.

4. Discussion

The degradation of the hybrid layer involves hydrolysis and leaching of the adhesive resin, and the proteolytic degradation of the exposed collagen matrix [2,3]. TiF₄ can be used to promote mechanical protection against degradation due to its chemical interaction with the mineral phase of dentin. This is because titanium has a strong tendency to couple with an oxygen atom of the phosphate group on the tooth surface, forming a glazed surface [21,22,30]. It is already known that TiF₄ promotes dentin demineralization when used as a dentin pretreatment, because of its very low pH. This demineralization seems to provide limited superficial modification at 1–5 µm of dentin depth [22]. However, the acidity of the self-etching adhesive systems allows demineralization of the modified superficial dentin layer, leading to hybrid layer formation, as observed in previous studies [16–19]. In this study, TiF₄ did not affect the surface H or the reduced modulus of elasticity of the adhesive layer, regardless of the time point or the adhesive system. However, the underlying dentin layer showed reduced E_r and H after 6 months of storage, regardless of the adhesive system and the TiF₄ pretreatment. Therefore, the first and second null hypotheses were rejected. The present findings indicate that the underlying dentin degraded faster than the adhesive layer, resulting in decreased nanomechanical properties, as also reported by other authors [29,31]. The long-term nanomechanical stability of the adhesive layer is corroborated by other authors [29,32].

The selected self-etching adhesive systems contain 10-methacryloyloxydecyl dihydrogen phosphate monomer (10-MDP) in their composition. This may have contributed to both systems behaving similarly. 10-MDP can chemically bridge to the mineral phase of dentin; thus, the adhesive interface does not rely solely on the micro-mechanical adhesion of the hybrid layers [33]. It is worthy of note that both adhesives systems contain acidic and hydrophilic functional monomers. The pH ranges between 2 (Clearfil SE Bond) and 2.7 (Scotchbond Universal), a level which could result in inefficient polymerization for both. The similarity in the hydrophilic composition of these adhesive systems may favor water sorption, and consequent hydrolytic degradation of the adhesive interface over time [34]. Even so, the hybrid layer is formed with these systems [35], and no difference was observed in this study between the two commercial brands.

There is evidence that titanium remains on the dentin surface after treatment with 2.5% TiF₄ [36]. However, no significant differences were observed in the nanomechanical properties of the underlying dentin adjacent to the hybrid layer after TiF₄ treatment, or after application of the self-etching adhesive systems immediately after the restorative procedure and after 3 months of aging. The decrease in the resulting nanomechanical values found in the underlying dentin after 6 months suggests biodegradation of the dentin-adhesive interface, since TiF₄ has a low pH, which accelerates

Table 2 – Results of the gelatinolytic activity expressed as fluorescence emission intensity (FEI) at the dentin–resin interface. Mean (standard deviation) of green fluorescent photons according to treatments and timepoints. Means followed by different letters in each row are different from each other ($p \leq 0.05$).

Interfacial gelatinolytic activity [mean (standard deviations)] TiF ₄	Adhesive system	Timepoint	
		24 h	6 months
With	Clearfil SE Bond	5.02 (2.79) B	13.76 (11.90) A
	Scotchbond Universal	5.82 (1.47) B	10.63 (5.71) A
Without	Clearfil SE Bond	6.07 (3.52) B	15.47 (12.68) A
	Scotchbond Universal	8.35 (1.51) B	13.68 (5.81) A

interfacial biodegradation. If TiF₄ were incorporated into the adhesive system, it would not come in direct contact with the underlying dentin, but would be restricted to the hybrid layer, since it would be applied simultaneously with the self-etching primer/adhesive system and the adhesive layer. The adverse effect of the very low pH of the TiF₄ aqueous solution in accelerating interfacial degradation could be countered if TiF₄ were incorporated into the adhesive systems, as was performed in the present study. Although the adverse effect of the very low pH of the TiF₄ aqueous solution in accelerating interfacial degradation is probably negligible for systems using acidic primers, the incorporation of TiF₄ in the self-etching adhesive system is an interesting approach for preventing underlying dentin degradation over time. This would make a worthy subject for further study.

The present study observed no differences in the in situ gelatinolytic activity between the adhesive systems and the dentin pretreatment with TiF₄. However, the third null hypothesis was rejected, since time had a significant effect on the interfacial gelatinolytic activity. The increase in collagenolytic and gelatinolytic activities of dentin is well-reported for both etch-and-rinse and self-etching bond agents [2,37–41].

In the present study, significant increase in gelatinolytic activity was detected after both adhesive systems underwent aging, and 2.5% TiF₄ pretreatment had no significant effect on this activity, regardless of aging of the adhesives. The lack of inhibition of endogenous proteases supports prior studies on the stability of dentin matrix treated with TiF₄ solutions, and was investigated [27] in a previous bond strength study [18]. As expected, the aging process increased the gelatinolytic activity. This is likely due to the proteolytic action of MMPs present in the underlying dentin [42,43], as well as the hydrolytic effect of water in the components of the adhesive systems, considering that water is known for its negative effect of degrading the dentin–resin interface over time [2,3,44,45].

This increased degradation rate after aging of the self-etching adhesive system interface was observed previously, when it was speculated that the presence of the 10-MDP monomer of the self-etching adhesive system may have partially covered the exposed collagen fibrils. In so doing, the monomer prevented access of the soluble gelatin substrate to the endogenous proteases, early after the restorative procedure began. However, after aging, the dentin–resin interface was solubilized, thus increasing the endogenous gelatinolytic activity [9]. This might explain why no differences were observed in the gelatinolytic activity in the present study for either adhesive system, since both contain 10-MDP.

The relatively rapid degradation of the nanomechanical properties of the underlying dentin, and the increase in gelatinolytic activity at the dentin–resin interface highlights the powerful role of the underlying dentin in maintaining the stability of adhesive resins. Long storage evaluation periods could reveal more significant results regarding nanomechanical and biodegradation tests, but it should be noted that the aim of the present study was to obtain preliminary information on the aging of an interface treated with TiF₄. For this reason, it is important for researchers to continue their investigation into the mechanisms and targeted strategies that can be used to stabilize the host-derived degradation of dentin.

5. Conclusion

Dentin pretreatment with 2.5% TiF₄ did not affect the nanomechanical properties of any dentin–resin interface component, regardless of the time and the adhesive system. After 6 months of storage time, only the underlying dentin showed significant degradation, as indicated by its reduced nanomechanical properties. Dentin pretreatment with 2.5% TiF₄ did not inhibit gelatinolytic activity at the adhesive–dentin interfaces over time. The gelatinolytic activity at the interfaces was higher after 6 month for all the groups.

Acknowledgement

This research was supported by the São Paulo State Foundation for Research Support FAPESP (BEPE grant number 2014/18848-9).

REFERENCES

- [1] Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/dentin interface: the weak link in the composite restoration. *Ann Biomed Eng* 2010;38:1989–2003.
- [2] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater* 2008;24:90–101.
- [3] Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability—a literature review. *Dent Mater* 2016;32:e41–53.
- [4] Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjäderhane L, et al. Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dent Mater* 2010;26:571–8.

- [5] dos Santos PH, Karol S, Bedran-Russo AK. Long-term nano-mechanical properties of biomodified dentin-resin interface components. *J Biomech* 2011;44:1691–4.
- [6] Bedran-Russo AK, Castellan CS, Shinohara MS, Hassan L, Antunes A. Characterization of biomodified dentin matrices for potential preventive and reparative therapies. *Acta Biomater* 2011;7:1735–41.
- [7] Castellan CS, Bedran-Russo AK, Karol S, Pereira PN. Long-term stability of dentin matrix following treatment with various natural collagen cross-linkers. *J Mech Behav Biomed Mater* 2011;4:1343–50.
- [8] Bedran-Russo AK, Vidal CM, Dos Santos PH, Castellan CS. Long-term effect of carbodiimide on dentin matrix and resin-dentin bonds. *J Biomed Mater Res B Appl Biomater* 2010;94:250–5.
- [9] Mazzoni A, Angeloni V, Sartori N, Duarte Jr S, Maravic T, Tjäderhane L, et al. Substantivity of carbodiimide inhibition on dentinal enzyme activity over time. *J Dent Res* 2017;96:902–8.
- [10] Yang H, Li K, Yan H, Liu S, Wang Y, Huang C. High-performance therapeutic quercetin-doped adhesive for adhesive-dentin interfaces. *Sci Rep* 2017;7:8189.
- [11] Liu Z, Li F, Zhang L, Yu H, Yu F, Chen J. The effect of active components from citrus fruits on dentin MMPs. *Arch Oral Biol* 2017;83:111–7.
- [12] Silva Sousa AB, Vidal CMP, Leme-Kraus AA, Pires-de-Souza FCP, Bedran-Russo AK. Experimental primers containing synthetic and natural compounds reduce enzymatic activity at the dentin-adhesive interface under cyclic loading. *Dent Mater* 2016;32:1248–55.
- [13] Bridi EC, do Amaral FL, França FM, Turssi CP, Basting RT. Inhibition of demineralization around the enamel-dentin/restoration interface after dentin pretreatment with TiF₄ and self-etching adhesive systems. *Clin Oral Investig* 2016;20:857–63.
- [14] Bridi EC, Amaral FLBD, França FMG, Turssi CP, Basting RT. Influence of dentin pretreatment with 2.5% titanium tetrafluoride on inhibiting caries at the tooth-restoration interface in situ. *Arch Oral Biol* 2018;86:51–7.
- [15] Abbatepaulo GL, Gangana TMMC, Martinez EF, Turssi CP, França FMG, Amaral FLB, et al. TiF₄ incorporated into a self-etching primer in different concentrations: antimicrobial properties and effects on demineralization inhibition around the enamel-dentin/restoration interface. *Oral Health Prev Dent* 2019;17(1):57–67.
- [16] Bridi EC, Amaral FL, França FM, Turssi CP, Basting RT. Influence of dentin pretreatment with titanium tetrafluoride and self-etching adhesive systems on microtensile bond strength. *Am J Dent* 2013;26:121–6.
- [17] Domingues LG, Real CM, Bridi EC, Amaral FLB, França FMG, Turssi CP, et al. Effects of dentin pretreatment with 2.5% titanium tetrafluoride on microtensile bond strength: influence of application method and degree of dentin mineralization. *Int J Adhes Adhes* 2014;54:159–64.
- [18] Basting RT, Basting RT, Velarde Barrientos S, Bridi EC, França FMG, Turssi CP, et al. Titanium tetrafluoride incorporated into a two-step self-etching adhesive system: physico-mechanical characterization and bonding stability. *J Mech Behav Biomed Mater* 2017;75:197–205.
- [19] Torres GB, da Silva TM, Basting RT, Bridi EC, França FMG, Turssi CP, et al. Resin-dentin bond stability and physical characterization of a two-step self-etching adhesive system associated with TiF₄. *Dent Mater* 2017;33:1157–70.
- [20] Tranquilin JB, Bridi EC, Amaral FL, França FM, Turssi CP, Basting RT. TiF₄ improves microtensile bond strength to dentin when using an adhesive system regardless of primer/bond application timing and method. *Clin Oral Investig* 2016;20:101–8.
- [21] Büyükyılmaz T, Ogaard B, Rølla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. *Eur J Oral Sci* 1997;105:473–7.
- [22] Sen BH, Büyükyılmaz T. The effect of 4% titanium tetrafluoride solution on root canal walls—a preliminary investigation. *J Endod* 1998;24:239–43.
- [23] Magalhães AC, Kato MT, Rios D, Wiegand A, Attin T, Buzalaf MA. The effect of an experimental 4% TiF₄ varnish compared to NaF varnishes and 4% TiF₄ solution on dental erosion in vitro. *Caries Res* 2008;42:269–74.
- [24] Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF₄) varnish on demineralisation and remineralisation of bovine enamel in vitro. *J Dent* 2008;36:158–62.
- [25] Comar LP, Souza BM, Al-Ahij LP, Martins J, Grizzo LT, Piasentim IS, et al. Mechanism of action of TiF₄ on dental enamel surface: SEM/EDX, KOH-soluble F, and X-ray diffraction analysis. *Caries Res* 2017;51:554–67.
- [26] Alexandria AK, Nassur C, Nóbrega CBC, Valença AMG, Rosalen PL, Maia LC. In situ effect of titanium tetrafluoride varnish on enamel demineralization. *Braz Oral Res* 2017;31:e86.
- [27] Bridi EC, Leme-Kraus AA, Aydin B, Basting RT, Bedran-Russo AK. Long-term evaluation of the stability of dentin matrix following treatments with aqueous solutions of titanium tetrafluoride at different concentrations. *Arch Oral Biol* 2018;91:51–6.
- [28] Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. *Dent Mater* 2018;34:78–96.
- [29] Leme AA, Vidal CM, Hassan LS, Bedran-Russo AK. Potential role of surface wettability on the long-term stability of dentin bonds after surface biomodification. *J Biomech* 2015;48:2067–71.
- [30] Tveit AB, Hals E, Isrenn R, Tøtdal B. Highly acid SnF₂ and TiF₄ solutions. Effect on and chemical reaction with root dentin in vitro. *Caries Res* 1983;17:412–8.
- [31] Anchieta RB, Machado LS, Martini AP, Santos PH, Giannini M, Janal M, et al. Effect of long-term storage on nanomechanical and morphological properties of dentin-adhesive interfaces. *Dent Mater* 2015;31:141–53.
- [32] Freitas PH, Giannini M, França R, Correr AB, Correr-Sobrinho L, Consani S. Correlation between bond strength and nanomechanical properties of adhesive interface. *Clin Oral Investig* 2016;21:1055–62.
- [33] Yoshihara K, Hayakawa S, Nagaoka N, Okihara T, Yoshida Y, Van Meerbeek B. Etching efficacy of self-etching functional monomers. *J Dent Res* 2018;97:1010–6.
- [34] Sofan E, Sofan A, Palaia G, Tenore G, Romeo U, Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type. *Ann Stomatol (Roma)* 2017;8:1–17.
- [35] Makishi P, André CB, Ayres A, Martins AL, Giannini M. Effect of storage time on bond strength and nanoleakage expression of universal adhesives bonded to dentin and etched enamel. *Oper Dent* 2016;41:305–17.
- [36] Basting RT, Leme AA, Bridi EC, Amaral FL, França FM, Turssi CP, et al. Nanomechanical properties, SEM, and EDS microanalysis of dentin treated with 2.5% titanium tetrafluoride, before and after an erosive challenge. *J Biomed Mater Res B Appl Biomater* 2015;103:783–9.
- [37] Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomaterials* 2006;27:4470–6.
- [38] Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, et al. Activation of

- gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. *Eur J Oral Sci* 2006;114:160–6.
- [39] Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjäderhane L, et al. MMP activity in the hybrid layer detected with in situ zymography. *J Dent Res* 2012;91:467–72.
- [40] Mazzoni A, Apolonio FM, Saboia VP, Santi S, Angeloni V, Checchi V, et al. Carbodiimide inactivation of MMPs and effect on dentin bonding. *J Dent Res* 2014;93:263–8.
- [41] Apolonio FM, Mazzoni A, Angeloni V, Scaffa PM, Santi S, Saboia VP, et al. Effect of a one-step self-etch adhesive on endogenous dentin matrix metalloproteinases. *Eur J Oral Sci* 2017;125:168–72.
- [42] Koshiro K, Inoue S, Sano H, De Munck J, Van Meerbeek B. In vivo degradation of resin-dentin bonds produced by a self-etch and an etch-and-rinse adhesive. *Eur J Oral Sci* 2005;113:341–8.
- [43] Osorio R, Yamauti M, Ruiz-Requena ME, Toledano M. MMPs activity and bond strength in deciduous dentine-resin bonded interfaces. *J Dent* 2013;41:549–55.
- [44] De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, et al. Inhibition of enzymatic degradation of adhesive-dentin interfaces. *J Dent Res* 2009;88:1101–6.
- [45] Sabatini C, Pashley DH. Mechanisms regulating the degradation of dentin matrices by endogenous dentin proteases and their role in dental adhesion. A review. *Am J Dent* 2014;27:203–14.