

Evaluation of Bond Strength (μ TBS) of Conventional Adhesives in Substrate Deproteinized - A Study *In Vitro*

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Abstract

Objective: Evaluate, *in vitro*, the bond strength of conventional adhesive systems to the deproteinized human dentin substrate.

Materials and Methods: 12 healthy human molars were subdivided randomly into 6 groups. Phosphoric acid was applied in the positive control groups (G1, G3, G5) and the adhesive systems All Bond 3 - AB3 (Bisco), All Bond 2 - AB2 (Bisco) and Adper Scotchbond Multi-Purpose - ASBMP (3M / ESPE), respectively. The negative control groups (G2, G4 and G6) were demineralized and deproteinized with Sodium Hypochlorite 10% for 01 minute and then the adhesive AB3, ASBMP and AB2 were applied, respectively. The mechanical microtensile test was performed in a Universal testing machine (KRATOS) at a test speed of 0.5 mm/min, followed by the evaluation of the dentin surface on Scanning Electron Microscope (SEM). The results were analyzed statistically by ANOVA and Tukey test ($p < 0.05$).

Results: The average bond strength ranged from 3.43 MPa to $12.18 \pm 33.63 \pm 10.53$ MPa, being higher for the G5 (33.63 ± 10.53 MPa), followed by G2 (32.53 ± 11.90 MPa) and G4 (31.74 ± 11.39 MPa), one may verify that there was statistical difference between the groups.

Conclusion: The adhesive system All Bond 3 (Bisco) established higher values of bond strength after performing deproteinization. The use of deproteinizing agents changed ultra-morphology and chemical composition of the dentin substrate.

Keywords: Bond Strength; Adhesive; Microtensile; Deproteinizing Agents; Sodium Hypochlorite

Abbreviations

μ TBS: Microtensile Bond Strength; NaClO: Sodium Hypochlorite; AB2: All Bond 2; AB3: All Bond 3; ASBMP: Adper Scotchbond Multi-Purpose; ICF: Informed Consent Form; ISO: International Organization for Standardization; SEM: Scanning Electron Microscope.

Introduction

The constant studies on dentin bond strength have been justified by variations of this substrate and the search for techniques that clinically ensure the stability of the adhesive bond, without injury to underlying structures, providing the longevity of the restorative procedure [1].

There is evidence that the primer and the adhesive cannot penetrate fully into the collagen layer of the demineralized dentin. The discrepancy between the depth of the demineralized dentin with the resin infiltration allows the formation of micropores under or within

the hybrid layer, which can be detected with silver nitrate [2] and become a pathway for the degradation of collagen fibers and resin polymerized [3,4].

The combined of the resin and the dentin collagen degradation may increase the water content in the adhesive interface, compromising the longevity of the bond. In fact, the water has been referred to as the major cause of collagen degradation [1,5,6].

The deproteinization transforms the rich-collagen demineralized dentin in a porous structure with multiple irregularities (secondary tubules and anastomosis) on the peri and intertubular dentin. The diameter of the tubule deproteinized substrate becomes greater and has the funnelled configuration [7], which may promote an increase in the bond strength values of the adhesive system applied over it [8].

In vitro studies have recommended the use of sodium hypochlorite [9] in order to remove the collagen network, exposed by acid etching [10], and allow that the resin penetrate into the spaces previously occupied by these fibers. Although the effects of the deproteinized agents on dentin bond strength/resin have been evaluated, little is known about the effects of such solutions on the mechanical properties of the dentin substrate [11].

The deproteinization, after acid etching, can generate the formation of “reverse hybrid layer”, responsible for resin micro-retaining [12], contributing to the achievement of a differentiated dentin substrate, with similar characteristics to the dental enamel, due to the high content of apatite [13].

It is suspected that the use of sodium hypochlorite (NaOCl) is one of the possible strategies for the dentin bonding improvement [14], since the removal of collagen fibers increase the adhesive bond strength [15], due to the increased wetting ability [16]. It results in a more reactive surface, since the hydroxyapatite is a high surface energy substrate, which does not happen with a high collagen content substrate [17].

Some authors have reported that the deproteinized dentin seems to be more compatible with the hydrophobic adhesives [18-21], they also reinforce that the high bond strength values are in dependence on this type of adhesive solvent. The acetone containing systems would produce higher bond strength values when the NaOCl is used after acid etching, suggesting that the adhesive interacts strongly with the surface containing a high mineral content [22].

For these reasons, this study aimed to evaluate *in vitro* the bond strength of conventional adhesive systems to the deproteinized dentin substrate using microtensile test, followed by assessment of the dentin surface by Scanning Electron Microscopy - SEM.

Materials and Methods

In this study we used 03 conventional adhesives (All Bond 2 [AB2] All Bond and 3 [AB3] - Bisco; Adper Scotchbond Multi-Purpose [ASBMP] - 3M/ESPE). This study was approved by the Research Ethics Committee of the State University of Paraíba (under the registration number CAAE 0275.0.133.000-08). 12 healthy human third molars were used, extracted by therapeutic indication after signing of the informed consent form (ICF).

The dental elements were divided into three groups with six subgroups, according to the type of adhesive system and surface treatment used, as follows:

Preparation of the specimens

The occlusal enamel was completely removed using a diamond blade in low rotating speed (200 rpm) with constant irrigation, to expose the outer dentin (ISO/TS 11405).

Dental elements were divided into six subgroups, according to the type of adhesive system, surface treatment and application time.

The dentin surface was abraded with silicon carbide sandpaper with decreasing granulations 180, 240, 320 and 600 in order to produce a standardized smear layer (Pashley, *et al.* 1988).

Restorative procedure

The positive control group received conventional treatment surface, through demineralization, application of the adhesive system and restorative procedure, following the rules of each manufacturer. The surface treatment of the experimental groups consisted of demineralization with 37% phosphoric acid, followed by deproteinization with 10% sodium hypochlorite for 1 minute. After applying the adhesive systems, the blocks with composite resin (Filtek Z350 - 3M/ESPE) were made on the dentin surface according to the manufacturer’s recommendations.

Microtensile bond strength (μ TBS)

Then, the specimens were stored in distilled water (37°C) for 24 hours. They were then brought to a serial cutting machine to obtain the specimens in the form of “sticks”, with transversal area to test for approximately 0.8 mm² ± 0.2, thereafter they were adapted in the universal testing machine (KRATOS K2000) and subjected at a speed of 0.5 mm/min.

Statistical Analysis

The results were subjected to analysis of variance - ANOVA with significance level of 5% (p < 0.05).

SEM Analysis

To perform the scanning electron microscopy, the specimens were immersed in 2.5% glutaraldehyde (Type I) in 0.1M Sodium Phosphate Buffer pH 7.2 for 12h at 4°C. After fixation, they were washed with 15 ml of 0.2M Sodium Phosphate Buffer pH 7.2 for one hour in 3 baths of 20 minutes each and subsequently, dehydrated in acetone ascending concentrations of 25%, 50% and 75% for 20 minutes each, 95% for 30 minutes and 100% for 60 minutes.

After plating, the samples were viewed by scanning electron microscopy (Quanta 200F - FEI Company) with the voltage acceleration of 10KV, under increased 570 - 8000 times under high vacuum, environmental mode, using the secondary electron imaging technique.

The data were analyzed using the statistical software SPSS (Statistical Package for Social Science) version 13.0, the significance level used in the decision of the statistical tests was 5.0%.

Adhesive system	Deproteinizing agent
All Bond 3	No deproteinizing (G1)
	10% NaOCl 60s (G2)
All Bond 2	No deproteinizing (G3)
	10% NaOCl 60s (G4)
Adper Scotchbond Multi-Purpose	No deproteinizing (G5)
	10% NaOCl 60s (G6)

Table 1: Adhesive systems and groups No deproteinizing and agent deproteinizing (NaOCl 10%).

Results and Discussion

In table 2, the comparative results of the microtensile bond strength are shown, between : the adhesives for each condition of the deproteinizing agent.

Deproteinizing Agent	All Bond 3 Average \pm SD	All Bond 2 Average \pm SD	ASBMP Average \pm SD	p value
Without NaOCl 10%	22.56 \pm 7.41	12.18 \pm 3.43	33.63 \pm 10.53	p ⁽¹⁾ 0.001*
10% NaOCl	32.53 \pm 11.90	31.74 \pm 11.39	21.41 \pm 7, 53	p ⁽¹⁾ = 0.005 *

Table 2: Microtensile bond strength (μ TBS) values (means \pm standard deviations) of the experimental groups, according to deproteinizing agent, antioxidant and adhesive system.

After analysis of scanning electron microscopy, the presence of smear layer obliterating the dentinal tubules after the polishing of healthy tooth specimens can be visualised (Figure 1).

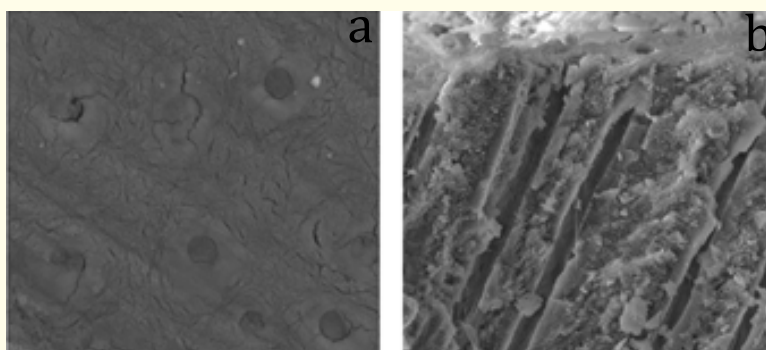


Figure 1: a) Dentin substrate smear layer; b) Obliterated dentinal tubules.

The process of dentin demineralization favoured the opening of its tubules, through the removal of the smear layer and smear plugs, it also created microporosity in the intertubular dentin and exposed its collagen fibers (Figure 2).

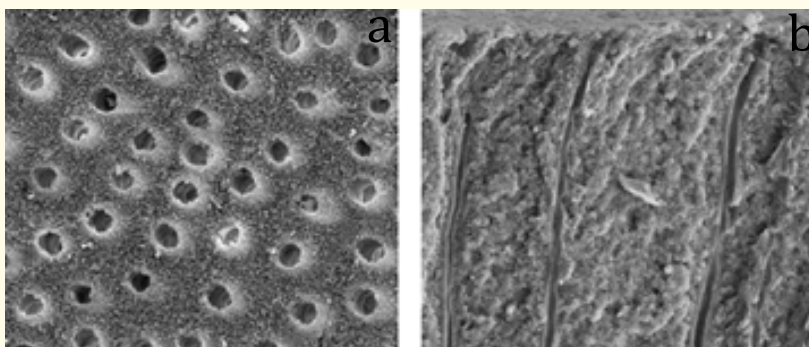


Figure 2: a) Demineralized dentin substrate with 37% phosphoric acid; b) Open dentinal tubules.

After hybridization with the adhesive system All Bond 3, it was observed the presence of failure in the bonding interface, achieving this interface a thickness of 27.48 μ M, where there was a formation of hybrid layer (Figure 3 and 4).

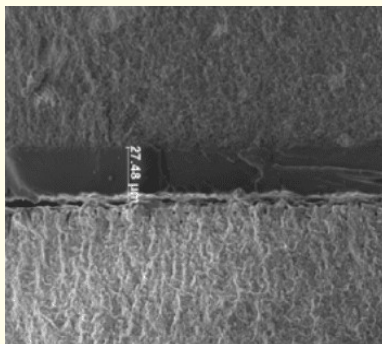


Figure 3: Bonding interface thickness of the adhesive system AB3 applied on the demineralized dentin substrate.

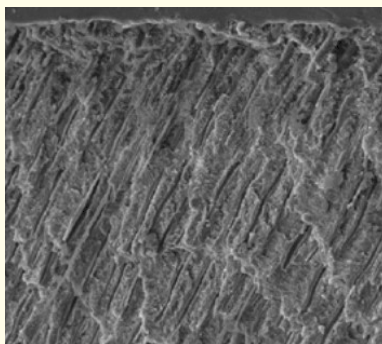


Figure 4: Bonding interface of the adhesive system AB3 applied on the demineralized dentin substrate.

The formation of the hybrid layer of the All Bond 2 was also visualised, reaching a thickness of 260.65 μ m, probably due the application of 5 layers of the adhesive system as recommendation of the manufacturer (Figure 5 and 6).

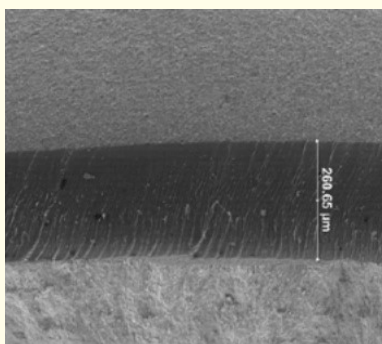


Figure 5: Bonding interface thickness of the adhesive system AB2 applied on the demineralized dentin substrate.

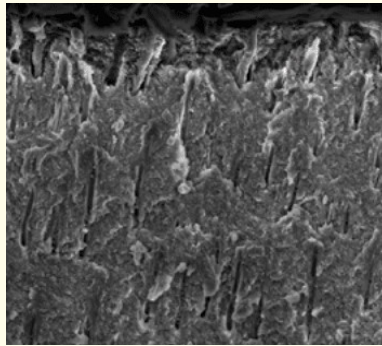


Figure 6: Bonding interface of the adhesive system AB2 applied on the demineralized dentin substrate.

The adhesive system Adper Scotchbond Multi-Purpose provided the formation of the hybrid layer having an adhesive interface 117.59 μ m and it was possible to observe the presence of collagen fibers on this substrate (Figure 7 and 8).

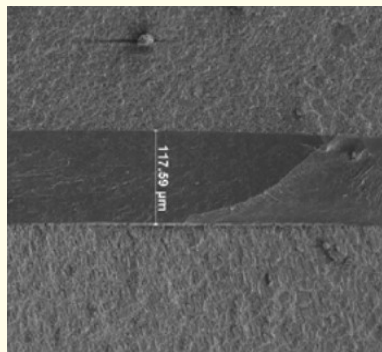


Figure 7: Bonding interface thickness of the adhesive system ASBMP applied on the demineralized dentin substrate.

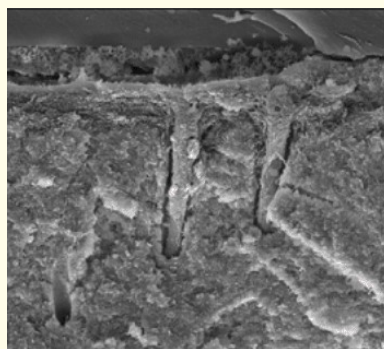


Figure 8: Bonding interface of the adhesive system ASBMP applied on the demineralized dentin substrate.

Followed by the deproteinization procedure, it was visualised the increasing of the dentinal tubules diameter, similar to a funnel, the microporosities, the presence of secondary tubules, anastomosis, however, it was possible to observe that there was a partial removal of the collagen fibers in the intertubular dentin (Figure 9).

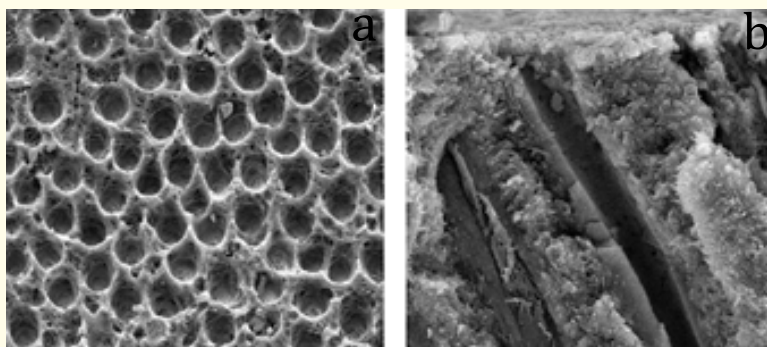


Figure 9: a) Deproteinized dentin substrate with 10% sodium hypochlorite; b) Tapered dentinal tubules.

After deproteinization, there was an increase in the thickness of the adhesive interface of the adhesive system All Bond 3, a better interlocking of resin tags and absence of the hybrid layer (Figure 10 and 11).

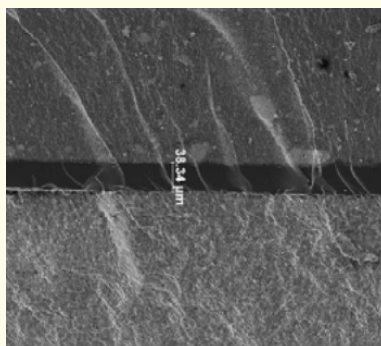


Figure 10: Bonding interface thickness of the adhesive system.

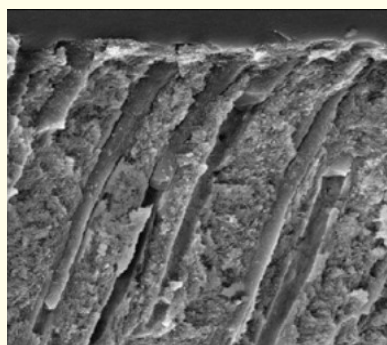


Figure 11: Bonding interface of the adhesive system AB3 applied on the demineralized dentin substrate.

The deproteinization favoured the formation of a adhesive interface with 129.47 μ m of the adhesive system All Bond 2 (Figure 12).

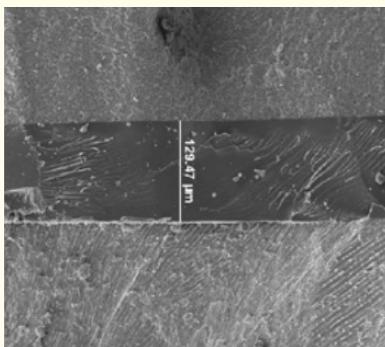


Figure 12: Bonding interface thickness of the adhesive system AB2 applied on the demineralized dentin substrate.

It was observed the presence of a gap with rupture of tags in the adhesive interface and absence of the hybrid layer (Figure 13).

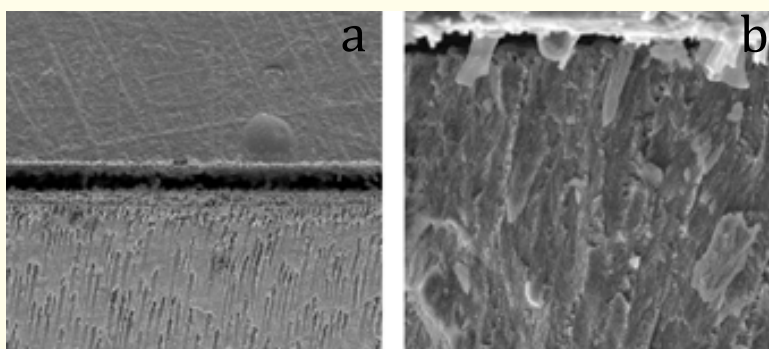


Figure 13: Bonding interface of the adhesive system AB2 applied on the deproteinized dentin substrate; b) Gap on bond interface.

However, it was noticed a decrease of the thickness and mechanical interlocking of the tags in the dentinal tubules after the removal of collagen and application of the adhesive system Adper Scotchbond Multi-Purpose (Figure 14).

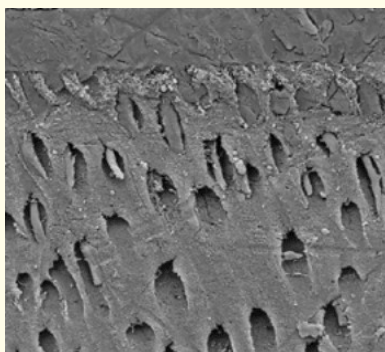


Figure 14: Bonding interface of the adhesive system ASBMP applied on the deproteinized dentin substrate.

Organic solvents with high vapor pressure, such as acetone and ethanol, are used to carry monomers into intimate contact with the dense filigree collagen fibers, resulting in a resin nanoflow within the spaces formed by the closeness of adjacent fibers [23] and this resin interlacing with collagen and hydroxyapatite crystals have been termed hybrid layer or interdiffusion zone resin/dentin [24,25].

The dentine inherent histological characteristics, in addition to the difficulties of wet bonding technique, can lead to a incomplete penetration of the adhesive in the plot of the demineralized collagen [26]. This failure could produce a weak and porous collagen demineralized zone and not infiltrated by fluid resin [27]. The subsequent hydrolysis of this plot would lead to degradation of the bonding process, resulting in strength decreased, as well as in an increased microleakage over time [28].

However, even in the absence of gaps in the restoration, found that silver ions could penetrate nano-sized spaces within the hybrid layer [29], noting the inefficiency of the diffusion of resin monomers through the demineralized collagen network, this phenomenon they termed nanoleakage, justifying the need of collagen removal with 10% sodium hypochlorite, which could prevent this phenomenon [30].

The proteolytic etching utilizes sodium hypochlorite to produce a higher porosity in demineralized dentin surface by increasing the opening of the tubules [1], favouring better diffusion of monomers by increasing their permeability, roughness, wettability and exposure of hydroxyapatite crystals, which could result in a long-term stable interface to be essentially mineral. Depending on the concentration, time and manner of application, the hypochlorite may remove different quantities of collagen, being dependent on the active chlorine concentration and superoxide radicals, providing the appearance of different bond strength values, may be indifferent [8], high or decrease, because the influence of this method on the resistance is dependent on the composition of each adhesive system.

To evaluate the bond strength was used the microtensile test, in which the samples used were prepared with an area of about 1 mm², which, theoretically, produces a more uniform distribution of stress and more accurate results than the tensile tests and shear, that have utilised an area of 7 to 12 mm² [29]. A large variability in bond strength values could be attributed to the degree of knowledge and familiarity of the different operators with the procedures and materials employed, due this, to find a statistical similarity of the bond strength values of this study, the surface preparation and dentin bonding procedures were performed by a single operator, previously trained for the use of all materials employed.

As for the influence of the adhesive systems used in deproteinization technical, the results of this study revealed a significant increase in bond strength for the bonding systems All Bond 3 and All Bond 2 after deproteinization, although there was no difference between them. The opposite occurred with the Adper Scotchbond Multi-Purpose adhesive, which could also be demonstrated by scanning electron microscopy, because it was noticed the decrease of the resin tags after deproteinization, favouring a smaller mechanical interlock. With regard to the morphology of the adhesive interface, it was observed different thicknesses, which are justified on account of various factors, such as, viscosity, presence or absence of charge in the adhesive system, the number of layers variation and application technique according to each manufacturer. The adhesive system All Bond 3 application in a deproteinized dentin substrate increased the thickness of the bonding interface from 27,48 to 38,34 μ m. For the All Bond 2 adhesive, was from 260.65 to 129.47, and in the ASBMP, there was a range of 117.59 to 39.88.

By virtue of these findings, it was observed that the thickness of the bonding interface did not influence the bond strength values, which disagreed [29,30] as the hybrid layer does not have to be thick to be resistant, but it must be continuous and uniform [31]. The All Bond 2 provided the greater thickness, because the manufacturer recommends the application of 5 layers, however, reported that a greater thickness of the hybrid layer contribute to greater nanoleakage, although [32] have affirmed that the application of NaOCl avoids this phenomenon.

These variable results may be justified by the sodium hypochlorite be a non-specific proteolytic agent, which has the ability to remove the organic material at room temperature, and may even reach to changes in mineral content, by obtaining a differentiated dentin substrate, high content of apatite, with high surface energy which makes it acquire a similar characteristic to the enamel promoting face-

to-face contact between the adhesive and the dentin since collagen has been designated as a fragile component of the bonding interface, can be hydrolyzed by bacteria or endogenous enzymes and have low surface energy which favours a greater contact angle of the adhesive system [33].

In this study, it could be visualised by SEM that the process of dentin demineralization favoured the opening of its tubules, by removing the smear layer and smear plugs, also created micro-porosities in the intertubular dentin and exposed its collagen fibers. It was found that after application of sodium hypochlorite, the ultramorphology of the demineralized dentin substrate changed, it was visualised the increase on diameter of the dentinal tubules, resembling a funnel, the microporosity, presence of secondary tubules, anastomosis, due to the loss of peritubular and reduce of intertubular dentin, which was also confirmed by [34].

However, the complete removal of the collagen fibers in the intratubular dentin could not be visualised, according to [35], since- said that the total removal of collagen fibers occur only after the application of 12% NaOCl for 45 min, which is a clinically unacceptable time. The dentin demineralization achieved by acid etching can leave residual apatite crystallites within the collagen matrix and a residual intrafibrillar mineral in the unchanged collagen fibers [36], suggesting that these minerals could have the role of protect the collagen fibers during the deproteinization with NaOCl [37].

Found through micromorphological analysis that the adhesive bonding interface did not contain the hybrid layer after deproteinization and showed numerous tags with few and shorts microtags [38]. In several areas between the tags were visualised projections similar to fibers, which appeared to be mineralized collagen fibers that were incorporated by the adhesive system, and these findings were confirmed in this study when using the ASBMP.

The complete removal of the collagen fiber network should be achieved to obtain the advantages of the deproteinization technique. Remnants of modified collagen fibers could lead to unexpected and deleterious effects on the interaction between the adhesive system and the dentin substrate [39]. However, different results may be reported on different presentations of the hypochlorite, gel or solution, passive or active application form, chloride share (solution age) and the substrate type (deep dentine with organic content), according, and can also be said that the behavior of this technique is dependent of the adhesive.

In this study, it was used the 10% NaOCl solution instead of the gel, because although it offer ease of application, studies have shown conflicting results when using the gel as reported high bond strength values to tensile [40], while [41] reported a progressive decrease of these values through mechanical shear test, suggesting that when NaOCl gel is applied, the collagen fibers would not be completely removed [3,8,19], which agreed to [3] which said that the NaOCl is unstable and the variation of their concentration in the gel could range results. However, different authors also argued that the effect of the deproteinization decrease the values of the bond strength resin-dentin [11] due to reduction of mechanical properties of the infiltrated resin, the use of 5.25% NaOCl as irrigating solution in intracanal preparation associated with resin cements in the presence residual glycosaminoglycan components in the organic matrix, which are resistant to hypochlorite, as well as in the disruption of the cross-linking of pyridinoline that occurs in type I collagen of the dentin, the formation of chloramines and intermediate radicals derived from, because the presence of these residual reactive free radicals could compete with the vinyl radicals generated in the photo-activation process of the bonding agent, resulting in premature failure of the chain termination and incomplete polymerisation [42].

Before the divergences of results between authors, the behavior of the various adhesive systems on deproteinized dentin is still not fully comprehended. In addition to a probable increase in of adhesive strength, the possibility of applying the adhesive in the dry dentin substrate, facilitate the standardization of adhesion. This would suggest further comparative studies, in order to identify the real responsible factor for the increase or decrease of the bond strength when the sodium hypochlorite is applied to dentin after his etching.

Conclusion

The influence of dentin treatment with NaOCl on the bond strength was dependent on the formulation of the adhesive system and its interaction with the substrate. The deproteinization improved the bond strength of the adhesive systems All Bond 3, All Bond 2, and interfered negatively on the Adper Scotchbond Multi-Purpose. We can conclude that the use of deproteinizing agents changed the ultramorphology and the chemical composition of the dentin substrate.

Bibliography

1. Breschi L., *et al.* "Dental adhesion review: Aging and stability of the bonded interface". *Dental Materials* 24.1 (2008): 90-101.
2. Sano H., *et al.* "Nanoleakage: Leakage Within the hybrid layer". *Operative Dentistry* 20.1 (1995): 18-25.
3. Bedran Castro AKB., *et al.* "Effect of sodium hypochlorite on gel Bond shear strength the receiver-bottle adhesive systems". *Brazilian Journal of Oral Sciences* 3.9 (2004): 465-469.
4. Tay FR., *et al.* "Two modes of expressions in nanoleakage SINGLESTEP adhesives". *Journal of Dental Research* 81.7 (2002): 472-476.
5. Hashimoto M., *et al.* "In vivo degradation of resin-dentin bonds in humans over 1 to 3 years". *Journal of Dental Research* 79.6 (2000): 1385-1391.
6. Sano H., *et al.* "Long-term durability of dentin bonds made with a self-etching primer, *in vivo*". *Journal of Dental Research* 78.4 (1999): 906-911.
7. Correr GM., *et al.* "Effect of sodium hypochlorite on primary dentin - The scanning electron microscopy (SEM) evaluation". *Journal of Dentistry* 34.7 (2006): 454-59.
8. Arias VG., *et al.* "Effects of sodium hypochlorite and sodium hypochlorite gel solution on dentin bond strength". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 72.2 (2005): 339-344.
9. Perdigão J., *et al.* "Effect of calcium removal on dentin bond strengths". *Quintessence International* 32.2 (2001): 142-146.
10. Toledano M., *et al.* "Effect of acid etching and collagen removal on dentin wettability and roughness". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 47.2 (1999): 198-203.
11. Fuentes V., *et al.* "Tensile strength and microhardness of dentin treated human". *Dental Materials* 20.6 (2004): 522-529.
12. Prati C. *et al.* "Effect of removal of surface collagen fibrils resin-dentin bonding". *Dental Materials* 15.5 (1999): 323-331.
13. Souza FB. *et al.* "Relación de la deproteinized dentin con el proceso adhesivo". *Acta Odontologica Venezolana* 43.2 (2005).
14. Castro AKB., *et al.* "Influence of collagen on removal of shear bond strength, one bottle in dentin adhesive systems". *Journal of Adhesive Dentistry* 2.4 (2000): 271-277.
15. Phrukkanon S., *et al.* "The influence of the modification of etched on bovine dentin bond strengths". *Dental Materials* 16.4 (2000): 255-265.
16. Hills MAJR., *et al.* "The effect of the removal and collagen use of a low-viscosity resin liner on marginal adaptation of composite resin restorations with margins in dentin". *Operative Dentistry* 28.4 (2003): 378-387.
17. Savoie VPA., *et al.* "Effect of collagen removal on microleakage of composite resin restorations". *Operative Dentistry* 27.1 (2002): 38-43.
18. Cederlund A., *et al.* "From intact collagen fibers Increase dentin bond strength?" *Swedish Dental Journal* 26.4 (2002): 159-166.
19. Pepper LSF., *et al.* "Stability of dentin bond strengths using different bonding techniques after 12 months: total-etch, deproteinization and self-etching". *Operative Dentistry* 29.5 (2004): 592-598.
20. Inai N., *et al.* "Adhesion between collagen depleted dentin and dentin adhesives". *American Journal of Dentistry* 11.3 (1998): 123-127.

21. Nakabayashi N., *et al.* "Identification of resin-dentin hybrid layer in vital human dentin created in vivo: durable bonding to vital dentin". *Quintessence International* 23.2 (1992): 135-141.
22. Nakabayashi N., *et al.* "The promotion of adhesion by infiltration of monomers into tooth substrate". *Journal of Biomedical Materials Research* 16.3 (1982): 265-273.
23. Van Meerbeek B., *et al.* "Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems". *Journal of Dental Research* 71.8 (1992): 1530-1540.
24. Vargas M A., *et al.* "Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer". *Operative Dentistry* 22.4 (1997): 159-166.
25. Bedran-Russo AKB., *et al.* "Stiffness of demineralized dentin after application of collagen crosslinkers". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 86.2 (2008): 330-334.
26. Sano H. "Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds". *Journal of Dental Research* 85.1 (2006): 11-14.
27. Sano H., *et al.* "Relationship between surface area for adhesion and tensile bond strength – Evaluation of a micro-tensile bond test". *Dental Materials* 10.4 (1994): 236-240.
28. Goes MF and Montes MAJR. "Evaluation of silver methenamine method for Nanoleakage". *Journal of Dentistry* 32.5 (2004): 391-398.
29. Hashimoto M., *et al.* "In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM". *Biomaterials* 25.25 (2004): 5565-5574.
30. D'Arcangelo C., *et al.* "The influence of adhesive thickness on the microtensile bond strength of three adhesive systems". *Journal of Adhesive Dentistry* 11.2 (2009): 109-115.
31. Erhardt MCG., *et al.* "Influence of dentin acid-etching and NaOCl-treatment on bond strengths of self-etch adhesives". *American Journal of Dentistry* 21.2 (2008): 44-48.
32. Pioch T., *et al.* "The effect of NaOCl dentin treatment on nanoleakage formation". *Journal of Biomedical Materials Research* 56.4 (2001): 578-583.
33. Sato H., *et al.* "Influence of NaOCl treatment of etched and dried dentin surface on bond strength and resin infiltration". *Operative Dentistry* 30.3 (2005): 353-358.
34. Monticelli F., *et al.* "Sealing effectiveness of etch and-rinse vs self-etching adhesives after water aging: Influence of acid etching and NaOCl pretreatment". *Journal of Adhesive Dentistry* 10.3 (2008): 183-188.
35. Sauro S., *et al.* "Deproteinization effects of NaOCl on acid-etched dentin in clinically-relevant vs prolonged periods of application. A confocal and environmental scanning electron microscopy study". *Operative Dentistry* 34.2 (2009): 166-173.
36. Habelitz S., *et al.* "In situ atomic force microscopy of partially demineralized human dentin collagen fibrils". *Journal of Structural Biology* 138.3 (2002): 227-236.
37. Inaba D., *et al.* "The effects of a sodium hypochlorite treatment on demineralized root dentin". *European Journal of Oral Sciences* 103.6 (1995): 368-374.
38. Salim DA., *et al.* "Micromorphological analysis of the interaction between a one-bottle adhesive and mineralized primary dentine after superficial deproteinization". *Biomaterials* 25.19 (2004): 4521-4527.

39. Osorio R., *et al.* "Effect of sodium hypochlorite on dentin bonding with a polyalkenoic acid-containing adhesive system". *Journal of Biomedical Materials Research* 60.2 (2002): 316-324.
40. Wakabayashi Y., *et al.* "Effect of dissolution of collagen on adhesion to dentin". *International Journal of Prosthodontics* 7.4 (1994): 302-306.
41. Perdigão J., *et al.* "Effect of a sodium hypochlorite gel on dentin bonding". *Dental Materials* 16.5 (2000): 311-323.
42. Yiu CKY., *et al.* "A nanoleakage perspective on bonding to oxidized dentin". *Journal of Dental Research* 81.9 (2002): 628-632.

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