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Pre-treating dentin with chlorhexadine and CPP-ACP: self-etching and universal adhesive systems

Ricardo Alves dos Santos, Eliane Alves de Lima, Marcos Antônio Japiassu Resende Montes and Rodivan Braz

Department of Dentistry, University of Pernambuco – Brazil, Recife, PE, Brazil

ABSTRACT

Objective: The aim of the present study was to compare the effect of pre-treating dentin with chlorhexidine, at concentrations of 0.2% and 2%, and remineralizing paste containing CPP-ACP (MI Paste – GC) on the bond strength of adhesive systems.
Material and methods: In total, 80 slides of dentin were used. These slides were 2 mm thick and were obtained from bovine incisors. Standard cavities were created using diamond bur number 3131. In the control groups, a Scotchbond Universal Adhesive (SUA) self-etching adhesive system of 3M ESPE and a Clearfil SE Bond (CSE) adhesive system of Kuraray were applied, following the manufacturer's instructions. In the other groups, dentin was pretreated with chlorhexidine (0.2% and 2%) for 1 min and with MI Paste for 3 min. The cavities were restored with Z350 XT resin (3M ESPE). After 24 h of storage, the push-out test was applied at a speed of 0.5 mm/min.
Results: The different dentin pretreatment techniques did not affect the intra-adhesive bond strength. There was a difference between treatment with MI Paste and chlorhexidine 0.2% in favor of the SUA, with values of 15.22 and 20.25 Mpa, respectively.
Conclusions: The different pretreatment methods did not alter the immediate bond strength to dentin. Differences were only recorded when comparing the adhesives.

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Chlorhexidine; dentin; remineralization

Introduction

The bond stability of adhesive restorations to dentin is a theme that permeates dentistry. Despite the advances made in this area, long-lasting bonds between adhesive systems and dentin are still not available. Problems are generally related to the composition of the adhesive systems, structural characteristics and the composition of dentin. Adhesive systems exhibit great hydrophilicity and generally use resin monomers with a high molecular weight, which hinders the penetration of demineralized dentin.[1,2] Dentin has a highly variable architectural structure due to factors such as age, pathological and physiological processes and the proximity to dental pulp. In addition, its highly organic and aqueous composition also affects adhesion.[3,4]

Despite the abovementioned factors, the key to determining the longevity of adhesive restorations seems to be collagen.[5] During the formation process of the hybrid layer, dentin demineralization is essential. This exposes the network of collagen fibers and is followed by the application of the adhesive systems, which should involve all of the exposed collagen.[6] However, it has been established that the complete involvement of collagen fibers in the hybrid layer does not occur.[7]

Conventional adhesive systems use phosphoric acid to demineralize dentin. These adhesives involve the exposure of a wide range of collagen fibers, which exceed the infiltration capacity of the adhesive systems.[8] In an attempt to understand these characteristics, self-etching adhesive systems were used. These self-etching adhesive systems use acidic monomers to condition dental tissues. In addition, they condition and infiltrate the collagen network simultaneously, thereby avoiding the formation of a strip of collagen that is not involved in the hybrid layer.[9,10] However, it is now known that self-etching adhesive systems cannot involve the entire exposed collagen network, despite the fact that they are more effective than conventional systems in this context.[11]

The network of collagen that is not involved in the hybrid layer is found at the base of the same layer and is susceptible to hydrolytic degradation and the action

CONTACT Ricardo Alves dos Santos ricardofop@gmail.com Department of Dentistry, University of Pernambuco Brazil, Pessoa De Melo Street, 178; Madalena, Recife, Pernambuco, 50610-220, Brazil

of collagenolytic enzymes. These enzymes belong to the family known as the matrix metalloproteinases (MMPs).[12,13] The degradation of collagen fibers causes the infiltration of restorations, recurring caries, sensitive teeth and the premature replacement of restorations, due to less clinical longevity.[14]

Among proposals found in the literature to determine and control the effects of the incomplete involvement of collagen fibers exposed by the hybrid layer, special attention must be paid to studies that used synthetic MMP inhibitors and those that sought to remineralize the exposed collagen.

MMP's belong to the family of zinc-calcium-dependent collagenolytic enzymes. They are involved in the formation of organs that contain collagen, including the teeth.[15] There are more than 20 types of MMP, although the most common forms, which are found in human and bovine dentin, are known as subtypes 2, 8 and 9. In their inactive form, they are produced by odontoblasts and their activation is controlled by inhibitors.[16] They can be activated by heat, the progression of caries and an acidic pH. Due to the technical procedures involved in using adhesive systems, including the use of phosphoric acid or formulations with a low pH, it has been established in the literature that all adhesive systems can activate MMP's and consequently, have a negative effect on clinical longevity.[17,18] Thus, pre-treating dentin with synthetic MMP inhibitors seems to be a valid option when seeking to improve bond stability.

Chlorhexidine has been highlighted as a synthetic inhibitor of MMP's. Chlorhexidine digluconate is a cationic antimicrobial that belongs to the group of quaternary ammonium compounds (QAC).[19] It was initially used in periodontics and its potential to heal damaged tissue has been previously demonstrated.[1] Gendron and collaborators (1999) used zymography to demonstrate the capacity of chlorhexidine to inhibit the MMP's present in injured periodontal tissues, including MMP's 2 and 9, which are also present in dentin. Therefore, based on an understanding of the association between MMP's and dental tissues, chlorhexidine has become more common in studies of the longevity of adhesive interfaces.[20] Chlorhexidine was originally considered a synthetic MMP inhibitor when used at a concentration of 2%. Recently, however, lower concentrations, such as 0.2%, have been associated with satisfactory bond strength results in mechanical trials and MEV assessments.[15]

The remineralization of exposed collagen is another theory that has been associated with the stability of adhesive bond interfaces. Based on the principle that collagen fibers protected by the hybrid layer and those surrounded by mineral tissue are inaccessible to MMPs, remineralization seems to be a valid option for attaining longer clinical longevity.[2]

It is important to take into consideration that remineralization is possible due to the capacity of the electrostatic attraction between collagen and calcium phosphate. In order to form mineral tissue, calcium and phosphate ions are essential.[19,21,22] A casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has the capacity to remineralize human and bovine dentin [23] and is commercially available in the form of MI Paste (GC Comp, Japan).

Recently, 'universal', 'multi-purpose' or 'multi-mode' adhesive systems were launched. These systems take into consideration the judgment of professionals in relation to the adhesive strategy and the number of clinical steps involved.[24] According to the manufacturers, these adhesives have the capacity to adhere to dental tissue through acid conditioning and self-etching techniques. They are also capable of adhering to substrates such as metal and dental ceramics. However, data about the efficiency of this new class of adhesive system remain scarce.[25,26]

Therefore, the aim of the present study was to assess the influence of different dentin pretreatment methods on the immediate bond strength of self-etching and universal adhesive systems. The recommended pretreatment of dentin used in the present study involved the application of paste containing CPP-ACP and chlorhexidine at concentrations of 0.2% and 2%. The hypothesis used was that the different methods of pre-treating dentin would not affect the immediate bond strength during inter and intra-adhesive system comparisons.

Materials and methods

The present study involved the use of 80 crowns of bovine incisors, which were worn on the vestibular and lingual surfaces, polishing equipment (Panabra-São Bernardo dos Campos, Brazil) and sandpaper (120, 180 and 240 granulation), until the dentin slide was 2 mm thick. Standardized conical cavities were prepared in the dentin using 3131 diamond points (KG-Soresen). The diamond burs were substituted after every five cavities.

The following adhesive systems were used in the control groups: Clearfil SE Bond (Kuraray- Okayama, Japan); and Scotchbond Universal Adhesive (3M ESPE- Neuss, Germany). Both were applied following the manufacturer's instructions. The universal adhesive system was only used for the self-etching strategy (Table 1).

In the study groups, dentin was pretreated with chlorhexidine, in concentrations of 0.2% and 2% (VICOFARMA- Recife, Brazil), and MI Paste (GC Comp, Japan), which contains CPP-ACP. Both concentrations of chlorhexidine were applied to the dentin for 1 min [18] using a disposable brush. The MI Paste was applied for 3 min, also using a disposable brush, as described by Borges et al. [27] The adhesive systems were then applied. The composition of the study materials is displayed in Table 2. Photopolymerization was conducted using LED Optilight Plus (Gnatus- São Paulo, Brazil) at 600mW/cm^2, measured by a radiometer. Thus, a total of eight groups, each containing 10 teeth, were obtained (Table 3).

The cavities were restored with Filtek Z350 XT composite resin (3M ESPE, St Paul, MN) using color A2 in a single increment. The resin was photopolymerized for 20 s at both extremities of the cavity. Once finished, the specimens were stored in distilled water in an organic greenhouse at a temperature of 37 °C for 24 h. After the storage period, the samples were ground in a polisher, using sandpaper of granulation 240, on both surfaces to remove any excess resin that could affect the bond strength results.

The push-out test was carried out using the Kratos universal trials machine (K2000, São Paulo, Brazil). A support was adapted to the machine with a central opening of 3 mm, where the samples were positioned to ensure that the larger base of the cavity, which had a conical shape, was positioned downwards, thereby enabling the extrusion of the composite resin restoration. The extrusion of the restoration was possible due to the adaptation of a metal pin, with a 1 mm thick tip, to the 200 N load cells. The pin exerted force on the lower base of the restoration at a speed of 0.5 mm/min until extrusion was attained. Once completed, the Kratos computer provided the value of force required to attain extrusion, thereby determining the bond strength.

Results

The following descriptive statistical measurements were obtained: mean; standard and deviation. Inferential statistical techniques were used based on the student's t-test and the F test (ANOVA). The margin of error used in the decisions of the statistical tests was 5.0%. The bond strength results are displayed in Table 4.

Discussion

Numerous factors contribute to the extreme sensitivity and limited clinical longevity of dentin, including

Table 3. Division of the study groups.

Groups	Treatment
Group1	Clearfil SE Bond (Control)
Group 2	Clearfil SE Bond + MI Paste
Group 3	Clearfil SE Bond + Chlorhexidine 2%
Group 4	Clearfil SE Bond + Chlorhexidine 0.2%
Group 5	Scotchbond Universal Adhesive (control)
Group 6	Scotchbond Universal Adhesive + MI Paste
Group 7	Scotchbond Universal Adhesive + Chlorhexidine 2%
Group 8	Scotchbond Universal Adhesive + Chlorhexidine 0.2%

Table 1. Instructions of use for the application of adhesive systems.

Adhesive system	Instructions of use
Clearfil SE Bond (Kuraray – Okayama, Japan)	Primer: 1. Actively apply the primer on the surface for 20 s; 2. Dry softly; Bond: 1. Apply the adhesive on the surface; 2. Dry softly with a jet of air; 3. Photopolymerize for 10 s
Scotchbond Universal Adhesive (3M ESPE- Neuss, Germany)	1. Actively apply the adhesive on the surface for 20 s; 2. Dry softly; 3. Photopolymerize for 10 s

Table 2. Composition of the study materials.

Material	Composition
Clearfil SE Bond (Kuraray – Okayama, Japan)	1-Primer: water, MDP, HEMA, camphorquinone, hydrophilic dimethacrylate; 2- Bond: MDP, Bis-GMA, camphorquinone, hydrophilic dimethacrylate, N, N-diethanol para-toluidine, colloidal silica
Scotchbond Universal Adhesive (3M ESPE- Neuss, Germany)	MDP, dimethacrylate resin, HEMA, polyalkenoic acid copolymer modified by methacrylate, load, silane, ethanol, water, primers
Filtek Z 350 XT (3 M ESPE/St Paul, MN-USA)	Ceramic treated with silane, Bis-GMA, Bis-EMA, silica treated with silane, zirconia-silica oxide treated with silane, TEG-DMA, BHT, pigments
MI Paste (GC Comp.- Japan)	Glirerol, CPP-ACP, D-sorbitol, propylene glycol, silica dioxide, titanium dioxide
Chlorhexidine 2% (VICOFARMA/Recife, PE- Brazil)	Chlorhexidine diglucanate 2%

Table 4. Mean microtensile bond strength in mpa for the different adhesive systems and pretreatment of dentin.

Application technique	Clearfil SE bond	Scotchbond universal
Control (Manufacturer)	14.51 (3.14) (A,a)	15.86 (5.70) (A,a)
MI Paste	11.89 (2.34) (A,b)	15.22 (3.44) (A,a)
Chlorhexidine 2%	15.11 (5.42) (A,a)	16.36 (3.74) (A,a)
Chlorhexidine 0.2%	11.49 (3.70) (A,b)	20.25 (4.28) (A,a)

Different small letters indicate significant differences between the lines, based on the student's *t*-test.
Different capital letters indicate significant differences between the columns, based on the *F* test (ANOVA).

hydrophilicity, a high organic content and the presence of collagenolytic enzymes. Thus, studies of these factors that seek to attain a longer clinical longevity are relevant. The present study compared the immediate bond strength result of two types of dentin pretreatment (chlorhexidine and CPP-ACP) to outline the limiting characteristics of dentin.

The results of the present study indicate that the different methods of pre-treating dentin did not affect the immediate bond strength for the Clearfil SE Bond and Scotchbond Universal adhesive systems. The objective of this pretreatment was to increase the stability of the adhesive-dentin interface, as observed in longitudinal studies. Thus, an increase in the immediate bond strength would be a surprise.

With regards to pretreatment with chlorhexidine, many studies have unanimously reported that the effects of this treatment are only visible after six months [16–28] and chlorhexidine does not affect bond strength in 24 h.[12–20] Given the premise that chlorhexidine is involved in the deactivation of MMP's, which do not degrade collagen fibers very quickly, it seems logical that this result cannot be quantified over 24 h. If this was the case, adhesive restorations would need to be replaced at a lower frequency, since there is always a strip of collagen exposed at the base of the hybrid layer.[29]

Nishitani et al. [18] confirmed that chlorhexidine, at concentrations ranging from 0.5% to 2%, did not affect the degree of conversion of resinous monomers in adhesive systems. Despite the fact that there were no differences in the immediate bond strength results at concentrations of 0.2% and 2% in the present study, Breschi et al. [15] demonstrated that the 0.2% concentration of chlorhexidine was more beneficial to bond strength, based on two years of monitoring. The abovementioned study of the degree of conversion did not use a concentration of 0.2% and instead used a range from 0.5% to 2%, reporting a difference, although not significant, in favor of lower concentrations. This leads to speculation about whether a concentration of 0.2% (or even lower) would be more beneficial to the degree of conversion of resinous monomers in adhesive systems.

The MI Paste did not alter the immediate bond strength based on intra-adhesive comparisons between this group and the control. The results for Clearfil SE Bond were similar to those reported by Borges et al., [27] who applied the same methodology as the present study. The literature contains no studies associating the Scotchbond Universal adhesive system with MI Paste up to and including the last review. However, Adebayo, Burrow and Tyas [30] indicated that the use of MI Paste with 1-step self-etching systems is not beneficial to bond strength. Therefore, the results of the present study were contrary to those found in the literature. This result could have been caused by the presence of the MDP monomer in both adhesive systems. According to Yoshida et al.,[31] the interaction of these monomers with calcium, including that found in CPP-ACP, generates a stable monomer-Ca salt, capable of high bond strength values to dentin.

In the present study, the fact that the MI Paste did not have a negative effect on the immediate bond strength could be indicated as a benefit, given that the paste contains a range of components that could affect adhesion, including the element of interest of the present study (CPP-ACP). Given that self-etching adhesive systems were used in the present study, these other components probably functioned as debris and did not interfere. These adhesive systems are capable of encompassing the smear layer and becoming a part of the hybrid layer.[32] Furthermore, this hybridized smear layer can increase the module of elasticity of the hybrid layer as a whole, favoring the bond strength of adhesive systems that exhibit less dentin conditioning power.[33]

Since the aim of the application of MI Paste is to remineralize the exposed collagen fibers, satisfactory results for the bond strength to dentin could only be achieved in longitudinal studies. It has been established in the scientific literature that the remineralization of collagen fibers is only evident after a period of 3–4 months.[2]

It is noteworthy that calcium phosphate, which is present in CPP-ACP, can increase the pH.[19] The literature indicates that smooth self-etching adhesive systems with a higher pH achieve better results for bond strength to dentin.[16] Therefore, this characteristic of CPP-ACP could have been beneficial in the groups in which dentin was pretreated with MI Paste, given that there were no differences between these groups and their respective control groups.

Based on the comparisons between the adhesives, there were no differences between the control groups and those pretreated with chlorhexidine 2% in terms of bond strength. This result is contrary to those reported in a number of studies of mechanical resistance.[24–34] In these studies, the Scotchbond Universal self-etching adhesive system exhibited lower values for bond strength.

The two adhesive systems used in the present study have a chemically similar composition. Both involve water and alcohol as solvents and methacryloxy decile phosphate (MDP) as the monomer responsible for the conditioning of dental structure.[8–34] However, the Scotchbond Universal adhesive system contains copolymers of glass ionomer, which negatively affect the adhesive process due to competition with MDP for the chemical bond to dentin.[24–31] However, these results were not observed in the present study. It is worth reasserting that we are comparing results obtained using different methodologies. In studies reporting that the Scotchbond Universal adhesive system exhibits lower bond strength results, the methodology commonly used is the micro-traction test, which involves excessive handling and stress for the specimens.[27] Thus, the results of these studies serve as a guide but cannot be extrapolated for a direct comparison with the results of the present study.

The bond of copolymers of glass ionomer to dentin is indicated as more fragile than that of MDP. However, according to Mena-Serrano et al.,[8] the chemical bond between copolymers of glass ionomer and dentin plays a crucial role in the bond mechanism of self-etching adhesive systems.

Muñoz et al. [24] compared the degree of conversion of monomers and the pH of both adhesive systems used in the present study and thus helped the understanding of our results. According to the cited study, the pH of the primer of Clearfil SE Bond is 2.1, whereas that of Scotchbond Universal Adhesive is 3.0. Smooth self-etching adhesive systems have been associated with better results for bond strength to dentin.[35] In the same cited study, the degree of conversion of monomers after 24 h of storage was significantly different in the two systems: Clearfil SE Bond = 87.7%; Scotchbond Universal Adhesive = 69.1%. However, based on the results of the present study, this factor was not significant to the point of altering the results in favor of Clearfil.

Despite exhibiting a lesser degree of monomer conversion when compared with Clearfil SE Bond, the Scotchbond Universal adhesive system exhibited a degree of conversion that was within the limits established in the literature for photo-activated materials: between 55 and 60% after 24 h.[36] In addition, Borges et al. [37] reported that an elevated degree of monomer conversion (above recommended levels) is not directly related to the increase in bond strength of adhesive systems when using the push-out test, which was also used in the present study. The lower degree of conversion found for the Scotchbond Universal adhesive system was within the recommended levels and enables the resinous monomers to interact with dentin for longer, which could have caused a possible increase in bond strength in a longitudinal study.

Based on a direct comparison of both adhesive systems, pretreatment of dentin with chlorhexidine (2%) did not alter the bond strength. This may have been due to the similar composition of the two adhesive systems, which provided a chemical bond to dentin due to the presence of MDP, as well as the degree of monomer conversion and pH which were adequate. With regards to chlorhexidine, the concentration of 2.0% did not alter the degree of monomer conversion and thus had no negative effect on the bond strength. There was also no positive effect as these would only be visible after at least six months of monitoring in a longitudinal study.[20]

When the adhesive systems were compared in terms of the pretreatment of dentin with MI Paste and chlorhexidine at a concentration of 0.2%, there was a difference in the immediate bond strength: both favored the Scotchbond Universal adhesive system. The result related to the MI Paste is understandable for two reasons. The Scotchbond Universal adhesive system has a higher pH than the Clearfil system, which could have had a summation effect on the capacity of calcium phosphate, present in CPP-ACP, making the environment less acidic. Therefore, the MI Paste could have made the Scotchbond Universal adhesive system less acidic and more adequate for adhesion to dentin.[19]

The second possible reason for the better results obtained by the Scotchbond Universal adhesive system with MI Paste, when compared with Clearfil, involves the probable interaction of the copolymers of ionomer with the calcium ions of the MI Paste, which may even have eliminated a competition effect between MDP and the copolymers of ionomer.

The most remarkable result of the present study was found for the Scotchbond Universal adhesive system associated with 0.2% chlorhexidine pretreatment. Despite the fact that there was no statistically significant difference between this method and the other groups for the same adhesive system, it exhibited better immediate bond strength than Clearfil SE Bond associated with chlorhexidine 0.2%. It was not possible to find a logical explanation for this result in the literature. It was not caused by an immediate deactivation of the MMP's, since the positive effect of chlorhexidine 0.2% was not found with the Clearfil adhesive system. Perhaps there was an effect caused by the cleaning of debris caused by the application of the low concentrations of chlorhexidine to dentin, since low concentrations contain more diluents. This cleaning would make the dentin surface more reactive. In addition, the resinous monomers, MDPs and copolymers of ionomer could have acted synergistically to promote a bond with the adhesive, thereby exerting the opposite effect of that found in the literature: competition for the dentin substrate.[31] It should also be noted that there were beneficial factors associated with the Scotchbond Universal adhesive system, including the degree of conversion of monomers and adequate pH values.

The bond strength results recorded in the present study need to be supported by studies involving longitudinal assessments. All of the pretreatment techniques employed sought stability in the adhesive interface, which could only be visualized and quantified in studies that involve monitoring for at least six months. However, the results can be classed as satisfactory since the different pretreatment techniques for dentin did not negatively affect the immediate bond strength in the push-out test. Satisfactory results are to be expected in longitudinal monitoring projects.

Conclusions

In an intra-adhesive comparison, the different methods of pre-treating dentin did not alter the immediate bond strength;

The pretreatment of dentin with MI Paste and chlorhexidine (0.2%) had a positive effect on the immediate Bond strength of the Scotchbond Universal adhesive system.

The proposed hypothesis was partially rejected.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Perdigão J, Reis A, Loguercio AD. Dentin adhesion and MMPs: a comprehensive review. J Esthet Restor Dent. 2013;25:219–241.

[2] Tjaderhane L, Nascimento FD, Breschi L, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater. 2013;29:116–135.

[3] Macari S, Gonçalves M, Nonaka T, et al. Scanning electron microscopy evaluation of the interface of three adhesive systems. Braz Dent J. 2002;13:33–38.

[4] Orellana N, Ramirez R, Roig M, et al. Comparative study of the microtensile bond strength of three different total etch adhesives with different solvents to wet and dry dentin (*in vitro* test). Acta Odontol Latinoam. 2009;22:47–56.

[5] Kenshima S, Reis A, Uceda-Gomes N, et al. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. J Adhes Dent. 2005;7:117–126.

[6] Spencer PYQ, Park J, Topp EM, et al. Adhesive/dentin interface: the weak link in the composite restoration. Ann Biomed Eng. 2010;38:1989–2003.

[7] Osorio R, Yamauti M, Ruiz-Requena ME, et al. MMPs activity and bond strength in deciduous dentine-resin bonded interfaces. J Dent. 2013;41:549–555.

[8] Mena-Serrano A, Kose C, De Paula EA, et al. A new universal simplified adhesive: 6-month clinical evaluation. J Esthet Restor Dent. 2013;25:55–69.

[9] Bortolatto JF, Takatsui F, Oliveira Junior OB, et al. Effect of additional hydrofobic layer on the microtensile bond strenght of all-in one adhesive systems. Rev Odontol UNESP. 2011;40:113–117.

[10] Felizardo KM, Lemos LVFM, Carvalho RV, et al. Bond strength of HEMA-containing versus HEMA-free self-etch adhesive systems to dentin. Braz Dent J. 2011;22:468–472.

[11] Tay FR, Pashley DH, Suh BI, et al. Single-step adhesives are permeable membranes. J Dent. 2002;30:371–382.

[12] Ricci HA, Sanabe ME, Souza-Costa CA, et al. Chlorhexidine increases the longevity of *in vivo* resin-dentin bonds. Eur J Oral Sci. 2010;118:411–416.

[13] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827–839.

[14] Osorio R, Yamauti M, Osorio E, et al. Zinc reduces collagen degradation in demineralized human dentin explants. J Dent. 2011;39:148–153.

[15] Breschi L, Mazzoni A, Nato F, et al. Chlorhexidine stabilizes the adhisive interface: a 2-year *in vitro* study. Dent Mater. 2010;26:320–325.

[16] Sabatini C. Effect of a chlorhexidine-containing adhesive on dentin bond strength stability. Oper Dent. 2013;38:609–617.

[17] Mazzoni A, Scaffa P, Carrilho M, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res. 2013;92:82–86.

[18] Nichitani Y, Yoshima M, Wadgaonkar B. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci. 2006;114:160–166.

[19] Imazato S, Ma S, Chen JH, et al. Therapeutic polymers for dental adhesives: loading resins with bioactive components. Dent Mater. 2014;30:97–104.

[20] Lenzi TL, Tedesco TK, Soares FZM, et al. Chlorhexidine does not increase immediate bond

strength of etch-and-rinse adhesive to caries-affected dentin of primary and permanent teeth. Braz Dent J. 2012;23:438–442.

[21] Melo MAS, Cheng L, Zhang K, et al. Novel dental adhesives containing nanoparticles of silver and amorphus calcium phosphate. Dent Mater. 2013;29:199–210.

[22] Wang Y, Azais T, Robin M, et al. The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite. Nat Mater. 2012;11:724–733.

[23] Rahiotis C, Vougiouklakis G. Effect of a CPP-ACP agent on the demineralization and remineralization of dentine *in vitro*. J Dent. 2007;35:695–698.

[24] Muñoz MA, Luque I, Hass V, et al. Immediate bonding properties of universal adhesives to dentine. J Dent. 2013;41:404–411.

[25] Hanabusa M, Mine A, KUboki T, et al. Bonding effectiveness of a new "multi-mode" adhesive to enamel and dentine. J Dent. 2012;40:475–484.

[26] Perdigão J, Sezinando A, Monteiro PC. Laboratory bonding ability of a multi-purpose dentin adhesive. Am J Dent. 2012;25:153–158.

[27] Borges BCD, Souza-Jr J, Costa GFA, et al. Effect of dentin pre-treatment with a casein phosphopeptide-amorphus calcium phosphate (CPP-ACP) paste on dentin bond strength in tridimensional cavities. Acta Odontol Scand. 2013;7:271–277.

[28] Kim J, Vaughn RM, Gu L, et al. Imperfect hybrid layers created by na aggressive one-step self-etch adhesive in primary dentin are amendable to biomimetic remineralization *in vitro*. J Biomed Mater Res. 2010;93:1225–1234.

[29] Oliveira SSA, Pugach MIK, Hilton JF, et al. The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch system. Dent Mater. 2003;19:758–767.

[30] Adebayo OA, Burrow MF, Tyas MJ. Resin-dentine interfacial morphology following CPP-ACP treatment. J Dent. 2010;38:96–105.

[31] Yoshida Y, Yoshihara K, Nagaoka N, et al. Self-assembled nano-layering at the adhesive interface. J Dent Res. 2012;91:376–381.

[32] Uekusa S, Tsubota K, Tonegawa M, et al. Microtensile bond strengths of single-step self-etch adhesive systems to bovine dentin. J Oral Sci. 2007;49:183–189.

[33] Vaidyanathan TK, Vaidyanathan J. Recent advances in the theory and mechanism of adhesive resin bonding to dentin: a critical review. J Biomed Mater Res Part B Appl Biomater. 2008;88:558–578.

[34] Iwai H, Fujita K, Ikemi T, et al. Development of MDP-based one-step self-etch adhesive-effect of additional 4-META on bonding performance. Dent Mater J. 2013;32:1–9.

[35] De Munck J, Van Landuyt K, Peumans M. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84:118–132.

[36] Alshali RZ, Silikas N, Satterthwaite JD. Degree of conversion of bulk-fill compared to conventional resin-composites at two time intervals. Dent Mater. 2013;29:213–217.

[37] Borges BCD, Vilela ARRC, Silva-Jr AC, et al. Influence of the preheating of bonding agents on the degree of conversion and bond durability in tridimensional dentin cavities. J Adhesion Sci Technol. 2013;27:1226–1235.