Effect of zinc-doping in physicochemical properties of dental adhesives

César Pomacóndor-Hernández[1], DDS, PhD, **Raquel Osorio[2]**, DDS, PhD, **Fátima S. Aguilera[2]**, DDS, PhD, **Inmaculada Cabello[2]**, DDS, PhD, **Mario de Goes[1]**, DDS, PhD, **Manuel Toledano[2]**, DDS, PhD

[1]Dental Materials Area, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas (UNICAMP), Piracicaba - SP, Brazil.

[2]Department of Dental Materials, School of Dentistry, University of Granada, Granada, Spain.

Short title: Physicochemical properties of Zn-doped adhesives.

Corresponding author details:

Manuel Toledano
Facultad de Odontología, Campus de Cartuja s/n, University of Granada, E-18071 Granada, Spain
Phone: +34 958 243788
Fax: +34 958 240908
E-mail address: toledano@ugr.es

Effect of zinc-doping in physicochemical properties of dental adhesives

ABSTRACT: Zinc addition to resin adhesives may exert an inhibitory effect on MMPs-mediated collagen degradation and promote dentin remineralization. ***Purpose:*** To evaluate changes in the physicochemical properties, water sorption (WS), solubility (SO), modulus of elasticity (E), ultimate tensile strength (UTS), and microhardness (MH) tests were undertaken in zinc-doped dental adhesives. ***Methods:*** Two bonding resins (Adper Single Bond Plus -SB- and Clearfil SE Bond -SEB-) were zinc-doped by mixing them with 5, 10 or 20wt% of ZnO powder, or with 1 or 2wt% $ZnCl_2$. Resin disks were made of each adhesive blend for the evaluation of WS, SO, and MH, and dumbbell-shaped specimens were prepared for E and UTS testing. ***Results:*** An increase in WS and SO was observed for adhesives doped with $ZnCl_2$. A reduction in WS was observed for the adhesive blends containing 10% or 20wt% ZnO, while the SO was not altered in any of the ZnO-doped adhesives. An increase in E values was observed only for the SB adhesive doped with $ZnCl_2$. For SEB-blends, the incorporation of zinc compounds did not alter the E values. UTS values decreased when SEB was doped with ZnO. SB-blends doped with 20wt% ZnO significantly increased their MH, and the addition of zinc to the SEB-blends augmented the MH values in all cases.

CLINICAL SIGNIFICANCE: The addition of 20wt% ZnO particles to adhesive blends is preferred as it decreased water sorption and increased microhardness of the tested adhesives. No deleterious effect was encountered on the other tested properties.

Introduction

The adhesion of contemporary resin-based restorative materials to dentin relies on the creation and stability of a microscopic interfacial structure composed of collagen fibrils reinforced by a resin matrix called the hybrid layer.[1] Although the hybrid layer is efficiently obtained using most dental adhesives, its stability can be compromised by the degradation of both of its resin and collagen components.[2] It is well-known that endogenous enzymes present in dentin may cause the breakdown of resin-sparse collagen fibrils in resin-dentin bonds.[3,4] Matrix metalloproteinases (MMPs) are a family of structurally related calcium- and zinc-dependent endopeptidases that contribute to the organization and mineralization of the dentin matrix, while also playing an important role in pathological processes such as caries progression.[5] Degradation of the collagen component of resin-dentin bonds attributed to MMPs is evidenced by the loss of integrity of hybrid layers[6-8] and reduction of bond strength *in vitro* and *in vivo* long-term studies.[9-11] Thus, the activity of MMPs can importantly affect the long-term bonding durability of esthetic restorations.

To promote adhesion to dentin, the mineral phase from the substrate has to be removed and the water-filled voids left by removal of mineral should be filled with the adhesive resin that undergoes complete *in situ* polymerization to form the hybrid layer.[1] Two main strategies are employed in creating dentin bonding. The first strategy involves the use of etch-and-rinse adhesives, which requires the treatment of dentin with phosphoric acid to remove the smear layer and to demineralize the underlying dentin, exposing a dense filigree of organic-matrix fibrils, followed by the application of a primer/bonding adhesive to form the hybrid layer.[12] The second strategy utilizes self-etch adhesives, which are based on polymerizable acidic monomers that simultaneously condition/prime and bond dentin.[13]

Even though the self-etch strategy seems to reduce depth of the non-resin covered collagen layer, both bonding strategies unprotected collagen may provide the sites for collagen hydrolysis by endogenous enzymes such as MMPs.[14-16]

Different approaches for inhibiting the enzymatic activity of MMPs have been studied in attempts to improve the stability of hybrid layers.[17] Recently, it has been reported that zinc is a potent inhibitor of MMPs in dentin collagen degradation.[18] The incorporation of zinc into an etch-and-rinse adhesive not only exerted a protective effect on MMPs-mediated collagen degradation, but also preserved bond strength, thus representing a promising and novel strategy for stabilizing resin-dentin bonds over time.[19,20] Although the mechanism of zinc inhibition of matrix-bound MMPs is not completely understood, it is possible that it involves coordination of the hydroxide moiety of $ZnOH^+$ to the catalytic zinc ions of MMPs. This mode of inhibition was demonstrated with carboxypeptidase A, a zinc metalloenzyme.[21]

The addition of zinc compounds (i.e. zinc chloride [$ZnCl_2$] or zinc oxide [ZnO] in different concentrations) into dental adhesives may affect mechanical properties of the resin-dentin interface. Mechanical properties are important for dental adhesives to compensate the stresses generated by the polymerization shrinkage of resin composites.[22] Additionally, occlusal loading stresses may easily deteriorate an adhesive interface with inadequate mechanical properties, leading to the failure of the restoration.[22] Properties related to resin degradation such as water sorption and solubility may also be altered in zinc-modified polymers, thereby influencing stabilization of the resin-dentin bond over time.[2] It is known that water sorption and solubility of dental adhesives causes both plasticization and hydrolysis of the polymers within the hybrid layer.[16] However, when

using zinc-doped adhesives, solubility may contribute to the effective release of zinc ions at the resin-dentin interface, thereby not only exerting an improved protective effect of collagen from MMPs, but also influencing signaling pathways and stimulating a metabolic effect in hard tissue mineralization, permitting nucleation of hydroxyapatite crystallites in collagen fibrils during remineralization.[20]

Analysis of the limited literature available pertaining to this innovative approach presents no information regarding the effect of zinc on the physicochemical properties of zinc-doped adhesives. Therefore, the aim of the present study was to evaluate water sorption, solubility, modulus of elasticity, ultimate tensile strength, and microhardness of zinc-doped dental adhesives. The null hypothesis tested was that the incorporation of $ZnCl_2$ or ZnO into dental adhesives does not affect their physicochemical properties.

Materials and Methods

Preparation of adhesive solutions

An etch-and-rinse adhesive system, Adper Single Bond Plus (SB)[a], and the adhesive component of the two-step self-etch adhesive Clearfil SE Bond (SEB)[b] were tested. Both adhesive resins were zinc-doped by mixing them with 5, 10 or 20wt% ZnO microparticles[c] (2 μm diameter), or 1 or 2wt% of $ZnCl_2$.[d] To achieve complete dissolution of $ZnCl_2$ and dispersion of ZnO particles, adhesive blends were vigorously shaken for 1 min in a tube agitator.[e] Preparation of the adhesives and zinc concentrations were based upon previous studies.[19,20,23] The complete process was performed in a darkroom. Descriptions of the adhesives are provided in Table 1.

Water sorption (WS) and solubility (SO)

The WS and SO were determined according to ISO specification 4049, except for the specimens' dimensions.[24] Resin disks (n = 10) of each adhesive blend were prepared using a silicon mold (6.0 mm-diameter and 1.0 mm-thick in order to fit the light output guide of the light curing unit). An acetate strip was placed on top of the adhesives, and then covered with a 1.0 mm-thick glass slide. Resin blends were light activated for 10 s with the light tip in contact with the glass slide, using a LED light curing unit[f] with light irradiance of 750 mW cm^{-2}. The light was tested for light output by means of a commercial radiometer.[g] The opposite surface of the specimens was submitted to the same light curing procedure. Then, disks were polished using 1200-grit SiC paper to obtain specimens of approximately 6.0 mm in diameter and 0.5 mm in thickness.[25] The polymerized specimens were stored at 37ºC in a desiccator containing silica gel. Disks were weighed after a 24 h

interval in an analytical balance until a constant mass (m_1) was obtained (mass loss of each disk was not greater than 0.1 mg in 24 h). Diameter and thickness were measured to calculate the exact volume (V) of each specimen. Then, they were individually immersed in one milliliter of distilled water at 37ºC. After 7 days of water storage, disks were wiped with absorbent paper and weighed again (m_2). Next, specimens were dried in a desiccator, as previously described, and weighed daily until a dried constant mass (m_3) was obtained. The WS and SO were calculated individually for each specimen over the 7 days of water immersion using the formula (1). The WS and SO values were expressed in $\mu g/mm^3$.

$$(1) \quad WS = m_2 - m_3 / V$$

$$SO = m_1 - m_3 / V$$

Modulus of elasticity (E)

Adhesive blends were poured into dumbbell-shaped (10 mm-long x 1.0 mm-thick) silicone molds with a gauge length of 5 mm. After 10 min of solvent evaporation, an acetate strip was placed on the top of the adhesive blends and covered with a 1.0 mm-thick glass slide. Adhesive blends were then light activated for 90 s; after that, the opposite surfaces received a similar light curing protocol. The number of specimens per group was 10 according to previous studies.[26,27]

Following light activation, the dumbbell-shaped specimens were stored for 24 h at 37ºC in dry condition and then subjected to a three-point flexural bending test in order to obtain the E values. The three-point flexural bending test was performed with a miniature three-point bending aluminum device, consisting of a supporting base with a 5 mm span

and a loading piston. Three-point flexure was measured by centrally loading the polymer specimen using a universal testing machine[h] at a displacement rate of 0.5 mm min^{-1}, sufficient to induce a 1% strain. The compressive force necessary to induce a 1% strain in the polymer specimens was measured using a 50 N load cell. Load-displacement values were converted to stress and strain. The width and thickness of all specimens were measured to ensure accuracy of the test. The E values were calculated as the slope of the linear portion of stress-strain curve using the following formula:

$$(2) \qquad E = FL^3 / 4Dbh^3$$

where F is the force (N), L is the span length (5.0 mm), D is the vertical deflection (mm) of the specimen, b is the width of test specimens (1.0 ± 0.1 mm), and h is the thickness (1.0 ± 0.1 mm). The E values were obtained in MPa and converted to GPa.

The strain (ε) in the three-point flexural bending test followed the formula:

$$(3) \qquad \varepsilon = 6hd / L^2$$

where h is the thickness of the beam (1.0 ± 0.1 mm), d is displacement of the beam (mm), and L is the span length of the beam between the supports (5 mm).

Ultimate tensile strength (UTS)

Dumbbell-shaped specimens (n = 20) with same dimensions and fabrication method used in three-point flexural test, were attached to the universal testing machine using a cyanoacrylate adhesive[i] for UTS determination.[26,27] A tensile load was applied at a cross-head speed of 0.5 mm min^{-1} until failure. The width and thickness of the specimens were measured at the fracture site and the UTS of the polymers was calculated with the formula:

$$(4) \quad UTS = F / A$$

where F is the tensile force at failure (N) and A is the cross-sectional area of the specimen (mm^2). The UTS (N mm^{-2}) was expressed in MPa.

Microhardness (MH)

Five disk-shaped specimens (6.0 mm in diameter; 2.0 mm in height) were prepared with each adhesive blend using a silicon mold. An acetate strip was placed on top of the adhesives, and then covered with a 1.0 mm-thick glass slide. Resin blends were light activated for 20 s and then polished using silicon carbide paper (up to 4000 grit). Microhardness was evaluated on the photoactivated surface by applying 50 g of load for 30 s using a Knoop diamond microindentor.[j] Ten indentations (0.5 mm of distance between them) were made in each specimen (n = 50). The dimension of each indentation was measured as the length of the longest diagonal of the indentation mark (800X). Lengths were converted to Knoop Hardness Number (KHN) using the following formula:

$$(5) \quad KHN = 14.229 \, P / L^2$$

where P is the applied load (50 g), and L is the longest diagonal of the indentation (µm).

Statistical analysis

Differences in the mean values of WS, SO, UTS, E, and MH were examined for each adhesive system (SB or SEB) using one-way analysis of variance (ANOVA). The Bonferroni test was used for post hoc multiple comparisons. In cases where equal variance tests failed, the data were analyzed using Tamhane's test. The statistic tests were applied at a significance level of 5%, using the software SigmaPlot 12.0.[k]

Results

The mean values and standard deviations of tested physicochemical properties in all experimental groups are displayed in Tables 2 (SB-based blends) and 3 (SEB-based blends).

Water sorption and solubility

An increase in WS and SO was observed for both SB and SEB when doped with 1% or 2% $ZnCl_2$. WS of the adhesives doped with 5% ZnO were not significantly different from the control groups. A significant reduction in WS was observed for the 10% and 20% ZnO-doped adhesive blends. The SO was not modified when ZnO was incorporated in the tested adhesives.

Modulus of elasticity

E values increased when SB was doped with $ZnCl_2$. There were no differences in E values between SB-control and ZnO-doped SB-blends. For the SEB-blends, the incorporation of zinc compounds did not modify E values.

Ultimate tensile strength

No significant differences were found in UTS values between SB-blends. When SEB adhesive was doped with ZnO, UTS values were decreased.

Microhardness

A significative increase in MH was only observed when SB was doped with 20wt% ZnO. For SEB-blends, the addition of zinc increased microhardness values in all cases. $ZnCl_2$-doped SEB-blends attained higher microhardness values than did ZnO-doped SEB ones.

Discussion

Two commercial dental adhesives were doped with two different zinc compounds and the physicochemical properties of the resulting adhesive blends were evaluated. The null hypothesis tested in the present study was rejected because the addition of zinc chloride ($ZnCl_2$) or zinc oxide (ZnO) to dental adhesives altered some of their physicochemical properties.

Incorporation of zinc compounds yielded similar variations in WS and SO for both adhesives evaluated (Tables 2 and 3). WS is a phenomenon related to adhesives formulation (i.e. the polarity of monomers, chain topology, and amount of solvent) that consists basically of water uptake into the polymer network.[28,29] As a result, the polymer is softened by swelling of the network and reduction of the frictional forces between the polymer chains, a process known as plasticization.[30] In this way, the higher the WS of an adhesive system, the larger is the reduction of mechanical properties when polymers are exposed to water.[26,31-32]

The SO consists of the elution of some inadequately polymerized monomers and other resin components as photoinitiators, photostabilizers, decomposition products, and resin-sparse inorganic filler particles.[33,34] WS, SO, and hydrolysis constitute a simultaneous and dynamic process, where hydrolysis and SO depend primarily on water uptake into the polymer network; SO is also significantly influenced by the existence of low-weight molecules (e.g. previously hydrolyzed or unreacted oligomers/monomers, and resin-sparse fillers) that can be leached out of the polymer network. Therefore, WS generally shows a direct association with SO.[25] In the present study, this association is clearly evidenced, since $ZnCl_2$-doped adhesives produced increased WS and SO values, whereas ZnO-doped

adhesives showed a tendency toward reduction of WS and SO with higher concentrations of ZnO. It is known that the presence of filler might provide the adhesives with improved mechanical properties and decreased water sorption.[34-36] It can be speculated that ZnO particles were not completely solubilized into the adhesive blends, thereby performing as fillers. As more zinc "fillers" were added, the E and MH values increased (Tables 1 and 2) because there was less resin in the mixture. Similarly, as the resin content fell with increases in zinc oxide, there was less resin to absorb water so WS decreased.

The gradual hydrolysis of the polymerized adhesive and the low-weight hydrolyzed molecules leaching out of the hybrid layer due to WS and SO permit some matrix-bound endogenous enzymes (e.g. MMPs) to enter and cleave unprotected collagen fibrils within the decalcified dentin.[19] Furthermore, the polymer degradation may also serve as a pathway for dentinal fluid from the pulp chamber charged with additional MMPs, which may increase the collagen proteolysis over time.[16] Although it is well-known that WS and SO processes may reduce the mechanical properties of the resin-bond interface,[26,31,32] it is important to note that in the zinc-doped adhesives, SO of the polymer are necessary for release of zinc ions into the hybrid layer and the underlying dentin.[19,20] In this way, zinc may not only act as an MMP inhibitor, but may also influence signaling pathways and stimulate a metabolic effect in hard tissue mineralization.[37,38] The precipitation of a calcium phosphate layer has been described for other zinc-doped biomaterials, and further formed hydroxyapatite has been shown to be less soluble, being in terms of solubility and quality strictly dependent on.[39] Moreover, zinc has also been shown to enhance the occlusion of dentinal tubules by crystal precipitation, and these crystals do not easily dissolve after acid exposure.[40] Many zinc containing/releasing materials have been widely employed in

Restorative Dentistry with high degrees of clinical success (e.g. silver amalgam, and a variety of zinc oxide-containing cements used as temporary fillings, cavity liners, and root canal filling materials).[41,42]

Mechanical properties such as UTS and E are important for dental adhesives to compensate the stresses generated by the polymerization shrinkage of resin composites.[22] Also, occlusal loading stresses deteriorate the adhesive interface over time, leading to the failure of the restoration.[22] For this reason, it was important to know if the addition of zinc compounds into dental adhesives could affect their mechanical properties. While addition of up to 20wt% ZnO reduced the amount of resin in the adhesive by up to 20%, adding of 2wt% $ZnCl_2$ would not be expected to produce any changes due to its "filler" effect.

It was observed that the addition of zinc compounds into SB did not modify UTS values. In the case of SEB, a slight reduction of UTS mean values was observed (approx. 10%) when the adhesive was doped with ZnO (Table 3), which may be related to zinc chelation by one of the main components in SEB (10-MDP), by inducing differences in coherence within the polymer matrix. However, when ZnO-doped SEB was bonded to dentin, bond strength was not affected, being similar to that of the zinc-free SEB.[19]

The expected decrease in the mechanical properties originated by WS and SO processes may be reduced when these Zn-doped adhesives are in contact with dentin, since the release of zinc ions within the hybrid layer inhibits MMPs-mediated collagen degradation and may induce crystals precipitation and hydroxyapatite formation,[23] thereby rendering the resin-dentin interface mechanically and chemically more resistant over time.[20]

E values of SB-blends significantly increased only with the addition of $ZnCl_2$ (Table 2). A tendency towards increased E values with the incorporation of ZnO was also observed, reaching a magnitude significantly similar to the $ZnCl_2$-doped SB-blends. The variation in E of Zn-doped SB-blends when compared to SB-control ranged from 24% to 48% (Table 2). On the other hand, E values of Zn-doped SEB-blends were not significantly different in comparison with zinc-free SEB (with a variation from -11% to 5%) (Table 3). Thus, in the present study it was demonstrated that the addition of zinc compounds to dental adhesives did not negatively affect tested mechanical properties.

Microhardness is usually considered as an indirect measurement of the degree of conversion of polymeric matrices that have the same composition.[43] As zinc-doped resin blends used in the present study have different compositions (different percentage of $ZnCl_2$ or ZnO), the MH test was interpreted only as a mechanical property to confirm the E and UTS results. It was observed that 20% ZnO-doped SB-blends and all ZnO-doped SEB-blends had MH values that were significantly higher than control resins. $ZnCl_2$-doped SEB-blends also increased MH values to levels that were higher than ZnO-doped SEB-blends. As the effect of zinc incorporation in E and microhardness was adhesive-dependent, it may be speculated that noticeable differences in adhesives formulations are the major reason for this finding. SB contains high rates of solvent (ethanol/water) and copolymer of acrylic acids that provide a hydrophilic nature to this adhesive. On the other hand, the adhesive component of SEB contains non-volatile solvents and has self-etching capacity due to the presence of a functional acidic 10-MDP monomer. Further studies are necessary to clearly understand how zinc might interact with 10-MDP.

In conclusion, addition of 20wt% ZnO to resin adhesives does not decrease their physicochemical properties but instead, reduces WS and increases MH. Evaluation of long-term water aging on the mechanical properties of zinc-doped adhesives is recommended to predict the durability of restorations treated with this novel approach. Other benefits of Zn-doping etch-and-rinse dentin adhesives might also be studied, e.g. possible antimicrobial properties.[44-46] Endodontic sealers, luting cements and caries preventive materials may also be benefited by this Zn-doping strategy.

a. 3M ESPE, St. Paul, MN, USA

b. Kuraray, Tokyo, Japan

c. Panreac Química, Barcelona, Spain

d. Sigma Aldrich, St. Louis, MO, USA

e. Vortex Wizard 51075; Velp Scientifica, Milan, Italy

f. Bluephase; Ivoclar Vivadent, Schaan, Liechtenstein

g. Model 100; Demetron Research Corporation, Danbury, CT, USA

h. Instron 4411; Instron, Canton, MA, USA

i. Zapit; Dental Venture of America, Corona, CA, USA

j. V-testor 402; Instron Wolper GmbH, Ludwigshafen, Germany

k. Systat Software Inc., San Jose, CA, USA

Disclosure statement

The authors have no financial affiliation or involvement with any commercial organization with direct financial interest in the materials discussed in this manuscript. Any other potential conflict of interest is disclosed. This investigation was supported by grants CICOM/FEDER MAT2014-52036P and AUIP-JA 2011.

References

1. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *J Biomed Mater Res* 1982;16:265-273.

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. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. *J Dent Res* 2004;83:216-221.

4. Osorio R, Yamauti M, Osorio E, Ruiz-Requena ME, Pashley D, Tay F, Toledano M. Effect of dentin etching and chlorhexidine application on metalloproteinase-mediated collagen degradation. *Eur J Oral Sci* 2011;119:79-85.

5. Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. *Acta Odontol Scand* 2007;65:1-13.

6. Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. *J Dent Res* 2005;84:741-746.

7. Brackett MG, Li N, Brackett WW, Sword RJ, Qi YP, Niu LN, Pucci CR, Dib A, Pashley DH, Tay FR. The critical barrier to progress in dentine bonding with the etch-and-rinse technique. *J Dent* 2011;39:238-248.

8. Brackett MG, Tay FR, Brackett WW, Dib A, Dipp FA, Mai S, Pashley DH. In vivo chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive. *Oper Dent* 2009;34:379-383.

9. Hashimoto M, Ohno H, Sano H, Kaga M, Oguchi H. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. *Biomaterials* 2003;24:3795-3803.

10. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. *J Dent Res* 2007;86:529-533.

11. Ricci HA, Sanabe ME, de Souza Costa CA, Pashley DH, Hebling J. Chlorhexidine increases the longevity of in vivo resin-dentin bonds. *Eur J Oral Sci* 2010;118:411-416.

12. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. *Dent Mater* 2011;27:1-16.

13. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater* 2011;27:17-28.

14. Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *J Adhes Dent* 2002;4:255-269.

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

16. Hashimoto M. A review - micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. *J Biomed Mater Res B Appl Biomater* 2010;92:268-280.

17. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res* 2011;90:953-968.

18. Osorio R, Yamauti M, Osorio E, Ruiz-Requena ME, Pashley DH, Tay FR, Toledano M. Zinc reduces collagen degradation in demineralized human dentin explants. *J Dent* 2011;39:148-153.

19. Osorio R, Yamauti M, Osorio E, Roman JS, Toledano M. Zinc-doped dentin adhesive for collagen protection at the hybrid layer. *Eur J Oral Sci* 2011;119:401-410.

20. Toledano M, Yamauti M, Ruiz-Requena ME, Osorio R. A ZnO-doped adhesive reduced collagen degradation favouring dentine remineralization. *J Dent* 2012;40:756-765.

21. Larsen KS, Auld DS. Carboxypeptidase A: mechanism of zinc inhibition. *Biochemistry* 1989;28:9620-9625.

22. Ausiello P, Apicella A, Davidson CL. Effect of adhesive layer properties on stress distribution in composite restorations--a 3D finite element analysis. *Dent Mater* 2002;18:295-303.

23. Toledano M, Sauro S, Cabello I, Watson T, Osorio R. A Zn-doped etch-and-rinse adhesive may improve the mechanical properties and the integrity at the bonded-dentin interface. *Dent Mater* 2013;29:e142-e152.

24. International Organization for Standardization. ISO 4049:2009. *Dentistry - Polymer-based restorative materials.* Geneva, Switzerland, 2009.

25. Nishitani Y, Yoshiyama M, Hosaka K, Tagami J, Donnelly A, Carrilho M, Tay FR, Pashley DH. Use of Hoy's solubility parameters to predict water sorption/solubility of experimental primers and adhesives. *Eur J Oral Sci* 2007;115:81-86.

26. Hosaka K, Tagami J, Nishitani Y, Yoshiyama M, Carrilho M, Tay FR, Agee KA, Pashley DH. Effect of wet vs. dry testing on the mechanical properties of hydrophilic self-etching primer polymers. *Eur J Oral Sci* 2007;115:239-245.

27. Hosaka K, Nakajima M, Takahashi M, Itoh S, Ikeda M, Tagami J, Pashley DH. Relationship between mechanical properties of one-step self-etch adhesives and water sorption. *Dent Mater* 2010;26:360-367.

28. Soles CL, Chang FT, Gidley DW, Yee AF. Contributions of the nanovoid structure to the kinetics of moisture transport in epoxy resins. *J Polym Sci Part B: Polym Phys* 2000;38:776-791.

29. Soles CL, Yee AF. A discussion of the molecular mechanisms of moisture transport in epoxy resins. *J Polym Sci Part B: Polym Phys* 2000;38:792-802.

30. Ferracane JL, Berge HX, Condon JR. In vitro aging of dental composites in water - effect of degree of conversion, filler volume, and filler/matrix coupling. *J Biomed Mater Res* 1998;42:465-472.

31. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, Tay FR. Effect of resin hydrophilicity and water storage on resin strength. *Biomaterials* 2004;25:5789-5796.

32. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. *Biomaterials* 2005;26:6449-6459.

33. Durner J, Spahl W, Zaspel J, Schweikl H, Hickel R, Reichl FX. Eluted substances from unpolymerized and polymerized dental restorative materials and their Nernst partition coefficient. *Dent Mate*r 2010;26:91-99.

34. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med* 2001;12:136-151.

35. Tay FR, Moulding KM, Pashley DH. Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin. *J Adhes Dent* 1999;1:103-117.

36. Tay FR, Pashley DH, Yiu C, Cheong C, Hashimoto M, Itou K, Yoshiyama M, King NM. Nanoleakage types and potential implications: evidence from unfilled and filled adhesives with the same resin composition. *Am J Dent* 2004;17:182-190.

37. Hoppe A, Güldal NS, Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. *Biomaterials* 2011;32:2757-2774.

38. Liu Y, Mai S, Li N, Yiu CK, Mao J, Pashley DH, Tay FR. Differences between top-down and bottom-up approaches in mineralizing thick, partially demineralized collagen scaffolds. *Acta Biomater* 2011;7:1742-1751.

39. Oudadesse H, Dietrich E, Gal YL, Pellen P, Bureau B, Mostafa AA, Cathelineau M. Apatite forming ability and cytocompatibility of pure and Zn-doped bioactive glasses. *Biomed Mater* 2011;6:035006.

40. Gu H, Ling J, LeGeros JP, LeGeros RZ. Calcium phosphate-based solutions promote dentin tubule occlusions less susceptible to acid dissolution. *Am J Dent* 2011;24:169-175.

41. Souza AP, Gerlach RF, Line SR. Inhibition of human gelatinases by metals released from dental amalgam. *Biomaterials* 2001;22:2025-2030.

42. Santos MC, de Souza AP, Gerlach RF, Trevilatto PC, Scarel-Caminaga RM, Line SR. Inhibition of human pulpal gelatinases (MMP-2 and MMP-9) by zinc oxide cements. *J Oral Rehabil* 2004;31:660-664.

43. Braga RR, Cesar PF, Gonzaga CC. Mechanical properties of resin cements with different activation modes. *J Oral Rehabil* 2002;29:257-262.

44. Spencer CG, Campbell PM, Buschang PH, Cai J, Honeyman AL. Antimicrobial effects of zinc oxide in an orthodontic bonding agent. *Angle Orthod* 2009;79:317-322.

45. Aydin Sevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. *J Biomed Mater Res B Appl Biomater* 2010;94:22-31.

46. Kasraei S, Sami L, Hendi S, Alikhani MY, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. *Restor Dent Endod* 2014;39:109-114.

Table 1. Description of the adhesive resins used in the study.

Adhesive and manufacturer	Basic formulation
Adper Single Bond Plus (SB)[a]	Bis-GMA, HEMA, glycerol 1,3-dimethacrylate, UDMA, ethyl alcohol, water, silane treated silica, copolymer of acrylic and itaconic acids
Clearfil SE Bond - Bonding component (SEB)[b]	Bond: MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, dl-camphorquinone, diethanol-*p*-toluidine, colloidal silica, non-volatile solvents

Bis-GMA, bisphenol A diglycidyl ether methacrylate; HEMA, 2-hydroxyethyl methacrylate; UDMA, diurethane dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate.

Table 2. Physicochemical properties of Zn-doped Adper Single Bond Plus blends.

Adhesive blends	WS ($\mu g/mm^3$)	SO ($\mu g/mm^3$)	E (GPa)	UTS (MPa)	MH (KHN)
SB	256.5 (13.7)^c	84.5 (11.0)^a	1.82 (0.48)^a	20.68 (3.6)^a	22.8 (4.0)^a
SB+ZnCl$_2$ (1%)	279.3 (14.3)^d	116.4 (9.1)^b	2.59 (0.32)^b	19.23 (3.7)^a	24.6 (5.0)^{ab}
SB+ZnCl$_2$ (2%)	280.3 (7.3)^d	142.3 (5.0)^c	2.70 (0.35)^b	21.64 (2.4)^a	25.9 (6.9)^{ab}
SB+ZnO (5%)	250.6 (13.9)^{bc}	84.2 (4.3)^a	2.49 (0.57)^{ab}	19.86 (4.2)^a	26.0 (6.7)^{ab}
SB+ZnO (10%)	227.3 (16.5)^{ab}	77.5 (7.2)^a	2.42 (0.43)^{ab}	20.24 (4.4)^a	26.3 (5.2)^{ab}
SB+ZnO (20%)	213.1 (11.4)^a	73.9 (7.2)^a	2.26 (0.29)^{ab}	21.34 (4.5)^a	28.8 (5.8)^b
P values	< 0.001	< 0.001	0.004	0.411	< 0.001

Values are means and standard deviations. Different superscript lower case letters indicate significant differences within columns ($p < 0.05$).
WS = water sorption; SO = solubility; E = modulus of elasticity; UTS = ultimate tensile strength; MH = microhardness.

Table 3. Physicochemical properties of Zn-doped Clearfil SE Bond blends.

Adhesive blends	WS ($\mu g/mm^3$)	SO ($\mu g/mm^3$)	E (GPa)	UTS (MPa)	MH (KHN)
SEB	83.4 (6.5)[b]	8.8 (5.2)[ab]	3.00 (0.21)[ab]	50.45 (7.2)[b]	27.2 (3.4)[a]
SEB+ZnCl$_2$ 1%)	102.8 (3.3)[c]	22.1 (4.6)[c]	2.67 (0.33)[a]	51.65 (4.8)[b]	37.5 (2.9)[d]
SEB+ZnCl$_2$ (2%)	118.0 (5.8)[d]	24.1 (6.5)[c]	2.76 (0.30)[ab]	52.89 (5.4)[b]	38.5 (4.5)[d]
SEB+ZnO (5%)	87.9 (2.4)[b]	16.5 (3.6)[bc]	2.95 (0.30)[ab]	47.01 (5.6)[a]	33.8 (3.6)[bc]
SEB+ZnO (10%)	74.8 (4.0)[a]	6.1 (2.7)[a]	2.89 (0.34)[ab]	45.74 (7.54)[a]	31.4 (3.5)[b]
SEB+ZnO (20%)	76.2 (2.7)[a]	4.6 (3.8)[a]	3.15 (0.30)[b]	44.80 (6.9)[a]	34.4 (5.3)[c]
P values	< 0.001	< 0.001	0.014	<0.001	< 0.001

Values are means and standard deviations. Different superscript lower case letters indicate significant differences within columns ($p < 0.05$).
WS = water sorption; SO = solubility; E = modulus of elasticity; UTS = ultimate tensile strength; MH = microhardness.