Effect of Antioxidants on the Dentin Interface Bond Stability of Adhesives Exposed to Hydrolytic Degradation

Valéria B. Gotti[a] / Victor P. Feitosa[b] / Salvatore Sauro[c] / Lourenço Correr-Sobrinho[d] / Fernanda B. Leal[e] / Jeffrey W. Stansbury[f] / Américo B. Correr[g]

Purpose: This study assessed the effect of antioxidants vitamin C (Vit. C), vitamin E (Vit. E) and quercetin (Querc) on the dentin bonding performance, degree of conversion, and rate of polymerization of three commercial adhesive systems (Adper Single Bond 2 [SB], Clearfil SE Bond [CSE], Adper Easy Bond [EB]).

Materials and Methods: Human premolars were restored using antioxidant-doped adhesives. The samples were stored for 24 h in distilled water or 6 months under simulated pulpal pressure. Teeth were cut into sticks and the microtensile bond strength (μTBS) to dentin was tested in a universal testing machine. Qualitative nanoleakage analysis was performed from a central stick of each restored tooth. Degree of conversion and rate of polymerization of adhesive systems were evaluated in triplicate using real-time FT-IR.

Results: Although the inclusion of the antioxidants negatively affected the μTBS over 24 h, the antioxidant-doped adhesives maintained (SB-Vit. C, SB-Vit. E, CSE-Vit. C, EB-Querc) or increased (SB-Querc, CSE-Vit. E, CSE-Querc, EB-Vit. E, and EB-Vit. C) their μTBS during 6 months of storage. Only the μTBS of Adper Single Bond 2 dropped significantly after 6 months among the control groups. Slight changes in the nanoleakage pattern after aging were observed in all groups, except for the EB-control group, which showed a noteworthy increase in nanoleakage after 6 months, and for EB-Vit. C, which presented a remarkable decrease. A lower degree of conversion was obtained with all antioxidants in SB and EB, except for the EB-Vit. E group. Similar degrees of conversion were attained in control and experimental groups for CSE. The rate of polymerization was reduced in antioxidant-doped adhesives.

Conclusion: The performance of antioxidants changed according to the adhesive system to which they were added, and antioxidant-doped adhesives appear to have a positive effect on the adhesive interface durability, since their bond strength obtained after 24 h was maintained or increased over time.

Keywords: polymers, free radicals, hydrolysis, dentin adhesives.

J Adhes Dent 2015; 17: 35–44.
doi: 10.3290/j.jad.a33515

Submitted for publication: 06.08.14; accepted for publication: 13.01.15

Current dental adhesive systems perform satisfactorily in terms of bond-strength tests under controlled in vitro conditions, in particular if tested immediately after application or following short-term aging (< 3 months).[40] Nevertheless, the initial bond strength may be reduced in the long term due to exposure of the bonded interface to temperature changes,[6] chewing load,[9] and chemical biodegradation induced by enzymes and/or dietary or bacterial acids,[6,23] which may result in drastic effects on tooth/composite stability.[30] Therefore, it is important to

[a] *PhD Student, Department of Restorative Dentistry, Division of Dental Materials, Piracicaba Dental School, State University of Campinas, UNICAMP, Piracicaba, Brazil. Performed microtensile testing, interfacial nanoleakage, and optical/SEM analysis of failure mode, wrote first draft of the manuscript.*

[b] *Research Associate, Department of Restorative Dentistry, School of Dentistry, Federal University of Ceará, Fortaleza, Brazil. Contributed to experimental design, proofread the manuscript, contributed substantially to the discussion/conclusion.*

[c] *Professor, Dental Biomaterials and Minimally Invasive Dentistry, Facultad de Odontología, Universidad CEU Cardenal Herrera, Valencia, Spain. Proofread the manuscript, contributed substantially to the discussion/conclusion, revised the English text.*

[d] *Professor, Department of Restorative Dentistry, Division of Dental Materials, Piracicaba Dental School, State University of Campinas, UNICAMP, Piracicaba, Brazil. Performed statistical analysis.*

[e] *PhD Student, Department of Restorative Dentistry, Federal University of Pelotas, Pelotas, Brazil. Performed FT-IR analysis.*

[f] *Senior Associate Dean for Research, Professor and Vice-Chair, Department of Craniofacial Biology, University of Colorado School of Dental Medicine, Aurora, CO, USA. Proofread the manuscript, contributed substantially to the discussion/conclusion, revised the English text.*

[g] *Professor, Department of Restorative Dentistry, Division of Dental Materials, Piracicaba Dental School, State University of Campinas, UNICAMP, Piracicaba, Brazil. Supervised the entire project, proofread the manuscript.*

Correspondence: *Dr. Valéria Bisinoto Gotti, Division of Dental Materials, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil 13414-903. Tel: +55-19-2106-5345. e-mail: val_bisinoto@hotmail.com*

measure durability of the bonds and to apply artificial aging procedures (water storage, thermocycling, NaOCl storage, and pH cycling).[6,40]

Incomplete resin infiltration may result in areas rich in denuded collagen prone to enzymatic degradation.[23] Further degradation mechanisms, such as polymer hydrolysis and filler debonding,[38] may also occur simultaneously, jeopardizing the stability of the resin/dentin interface. The latter processes are particularly correlated to the highly hydrophilic adhesive systems,[30,34] and may be enhanced in the presence of free radicals from polymer breakage.[13]

Several substances, such as chlorhexidine[5] and crosslinking agents,[2,32] have been advocated to reduce collagen degradation. However, there are very few alternatives to avoid the hydrolytic degradation of dentin bonds.[4,29] The incorporation of antioxidants appears to reduce the potential degradation effects of free radicals and hydrolysis,[10] and the solubility characteristics of antioxidants might influence the reaction of polymerization of the adhesives according to phase separation. Phase separation of the adhesive systems is characterized by the presence of hydrophobic regions dispersed in a predominantly hydrophilic matrix.[28,36] After polymerization, the components of hydrophilic matrix have a low crosslinking density; the resulting material is unstable in aqueous environments and more prone to degradation over time.[28,36] Furthermore, depending on their characteristics, the photoinitiators of adhesive systems may be allocated to the hydrophilic or hydrophobic domains formed during phase separation, which may interfere in the polymerization process.[41] The use of antioxidants with different solubility characteristics as proposed here intended to test the influence of these substances in the domains formed by phase separation. This factor has not been studied previously.

Thus, the aim of this study was to assess the effect of antioxidants on the dentin bonding performance, degree of conversion, and rate of polymerization of three commercial adhesive systems. The null hypotheses to be tested were that the addition of antioxidants to adhesives does not: 1) interfere with dentin bond strengths, 2) affect the nanoleakage, or 3) influence the degree of conversion and rate of polymerization.

MATERIALS AND METHODS

Adhesive Systems

A two-step etch-and-rinse system (Adper Single Bond 2, 3M ESPE; St Paul, MN, USA), and two self-etching adhesives – a more hydrophobic two-step system (Clearfil SE Bond, Kuraray Medical; Tokyo, Japan) and a more hydrophilic one-step system (Adper Easy Bond, 3M ESPE) – were used in this study. The antioxidants vitamin C (l-ascorbic acid; hydrophilic), vitamin E (α-tocopherol; hydrophobic) and quercetin (amphiphilic) (Sigma Aldrich; St Louis, MO, USA) were chosen according to their solubility characteristics, which limited the incorporation to 5 wt% into the solutions of Adper Single Bond 2 and Adper Easy Bond and in Clearfil SE primer. The concentration was chosen such that there were no significant changes in preliminary tests of polymerization and pH and the amount of antioxidant was able to be solubilized in the adhesive systems. The adhesives were stored in the dark at 4°C for no longer than 3 months.

Microtensile Bond Strength (μTBS)

One hundred forty-four extracted human premolars were obtained under approval of the institutional ethics committee (protocol 11/2011) and stored in 0.5% chloramine/water solution at 4°C no longer than 4 months.

The roots were removed 2 mm beneath the cemento-enamel junction (CEJ), while the occlusal enamel was cut 2 mm above the CEJ using a slow-speed water-cooled diamond saw, exposing a flat surface of dentin (Isomet, Buehler; Lake Bluff, IL, USA). When necessary, the surface was abraded with 600-grit SiC paper to obtain a purely dentin surface. The pulpal tissue was carefully removed with small tweezers, avoiding scratching the pulpal walls. The exposed dentin surface was abraded before bonding with a 600-grit SiC paper for 60 s under running water to create a standardized smear layer.

Prepared teeth were randomly divided into 24 groups (n = 6) according to adhesive systems (Adper Single Bond 2, Clearfil SE Bond, and Adper Easy Bond), antioxidant incorporation (control, vitamin C, vitamin E and quercetin), and storage period (24 h and 6 months). All adhesive systems were applied following the instructions for use (Table 1).[18] Light activation was performed for 10 s using a halogen lamp XL-2500 lamp (600 mW/cm², 3M ESPE). The irradiance was controlled by a power meter (Ophir Optronics, Har-Hotzvim; Jerusalem, Israel). Composite build-ups were constructed using Filtek Z350-XT (3M ESPE) in 3 layers (each layer 2 mm thick). Aging for 6 months was performed under simulated pulpal pressure as described by Feitosa et al.[12] Two coats of nail varnish were applied on the enamel-composite border of specimens. The specimens were fixed to the inner part of the cylindrical receptacle's lid by pushing them sideways into the wax on the lid, without obstructing the pulpal chamber opening. The cylindrical container with distilled water was filled to reach 20 cm height, and the container was closed with samples attached to the lid. The container was turned upside down to submit the samples to 20 cm H_2O pulpal pressure.[12]

Approximately 12 matchsticks (bonding area 0.8 x 0.8 mm²) were obtained from each premolar by sectioning the bonded teeth. Only those from the central area were evaluated; the matchsticks from the periphery showing remaining enamel were excluded.

The matchsticks were fixed to a jig using a cyanoacrylate gel and tested to failure in the universal testing machine EZ-test (Shimadzu; Kyoto, Japan) at 1.0 mm/min. The exact cross-sectional area was measured after failure with a digital caliper. The μTBS of the sticks from the same bonded tooth were averaged and used for the statistical analysis as the statistical unit. Means and standard deviations were calculated and expressed in MPa. The μTBS data were statistically analyzed by equal variance and Kolmogorov-Smirnov normality tests and, after proving the normality of data and homogeneous variance, the results were

Table 1 List of materials used in this study

Material	Composition	Instructions for use
Adper Single Bond 2 (3M ESPE; St Paul, MN, USA)	Ethyl alcohol, bisphenol A diglycidyl ether dimethacrylate, silane treated silica, 2-hydroxyethyl methacrylate, copolymer of acrylic and itaconic acids, glycerol 1,3-dimethacrylate, water, diurethane dimethacrylate, diphenyliodonium hexafluorophosphate, ethyl 4-dimethyl aminobenzoate	1. Apply Scotchbond Etchant to enamel and dentin. Wait 15 s. 2. Rinse for 10 s. Blot excess water using a cotton pellet or mini-sponge. Do not air dry. 3. Immediately after blotting, apply 2–3 consecutive coats of adhesive for 15 s with gentle agitation using a fully saturated applicator. 4. Gently air thin for 5 s to evaporate solvent. Light cure for 10 s.
Clearfil SE Bond (Kuraray Noritake Dental; Tokyo, Japan)	Primer: 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water, accelerators, dyes, others Bond: bisphenol A diglycidylmethacrylate, 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, hydrophobic aliphatic methacrylate, colloidal silica, dl-camphorquinone, initiators, accelerators, others	1. Apply Clearfil SE Bond Primer and leave for 20 s. Dry thoroughly with mild air flow. 2. Apply Clearfil SE Bond Bond. Air flow gently. 3. Light cure for 10 s.
Adper Easy Bond (3M ESPE)	Bisphenol-A-diglycidyl ether dimethacrylate, 2-hydroxyethyl methacrylate, ethyl alcohol, water, phosphoric acid-6-methacryloxy-hexylesters, silane treated silica, 1,6-hexanediol dimethacrylate, copolymer of acrylic and itaconic acid, (dimethylamino) ethyl methacrylate, camphorquinone, 2,4,6-trimethylbenzoyldiphenylphosphine oxide	1. Apply the adhesive with the disposable applicator to all surfaces of the cavity and rub it in for 20 s. 2. Subsequently blow a gentle stream of air over the liquid for approx. 5 s. 3. Cure the adhesive with a commonly used curing light for 10 s.
Filtek Z350 XT (3M ESPE)	Silane treated ceramic, silane treated silica, diurethane dimethacrylate, bisphenol-a-polyethylene glycol, diether dimethacrylate, bisphenol-A-diglycidyl ether dimethacrylate, silane treated zirconia, polyethylene glycol dimethacrylate, triethylene glycol dimethacrylate, 2,6-di-tert-butyl-p-cresol	A layer of Filtek Z350 XT Universal Restorative should be applied and light cured for 20 s.

Composition based on the MSDS (Material Safety Data Sheet) of the material. Adper Single Bond 2 is also known as Adper Single Bond Plus or Adper Scotchbond 1XT. Filtek Z350 XT is known as Filtek Supreme Ultra or Filtek Supreme XTE.

analyzed using two-way ANOVA (antioxidants and storage period) and Tukey's test ($p < 0.05$) for each adhesive system separately. Sticks that failed prematurely were included in the statistical analysis as 0 MPa.[40]

Failure Mode

The fractured specimens were imaged using a stereomicroscopy at 60X magnification. Representative specimens exhibiting the most frequent failure pattern in each group and the μTBS close to the mean were processed for observation in an SEM. In brief, the selected specimens were paired and mounted on aluminum stubs and subsequently dehydrated in silica gel. Finally the specimens were gold-sputter coated and examined using SEM JSM-5600LV (JEOL; Tokyo, Japan), operated at 15 kV. The fractures were classified as adhesive, cohesive, or mixed.

Interfacial Nanoleakage

One central bonded-stick from each tooth was selected and submitted to the nanoleakage survey as described by Tay et al[31] using 50 wt% ammoniacal silver nitrate solution [$Ag(NH_3)_2NO_3(aq)$]. In brief, the specimens were immersed in the tracer solution for 24 h and then immersed in photo-developing solution for 8 h under a fluorescent light. Thereafter, the specimens were rinsed thoroughly in distilled water, embedded in epoxy resin and polished using wet #600, #1200, #2000 SiC papers and 3-, 1-, and 0.25-μm diamond pastes (Buehler) with polishing cloths. They were ultrasonically cleaned (5 min) after each polishing step. The specimens were coated with carbon and observed in SEM by means of backscattered electron mode.

Degree of Conversion and Polymerization Rate

The degree of conversion and rate of polymerization of adhesive systems were evaluated in triplicate using real-time FT-IR as described by Ely et al.[8] In brief, each solution (3 ml) was directly dispensed onto the emission plate of a Fourier-transform infrared spectrophotometer (FTIR-ATR Prestige 21, Shimadzu; Kyoto, Japan) equipped with an ATR crystal with a 45-degree mirror angle (PIKE Technologies; Madison, WI, USA) with the light-curing unit positioned at a standardized distance of 5 mm. The IR-Solution software (Shimadzu; Columbia, MD, USA) was used to control the scan mode. One scan was acquired every second for 60 s after the start of light curing which was also performed during the entire 60 s of evaluation.

All spectra were obtained in a range of 1800 to 1500 cm^{-1}, with 12 scans at 4 cm^{-1} resolution in trans-

Table 2 Mean (standard deviation) of microtensile bond strength (MPa) to Adper Single Bond 2 adhesive system

Antioxidant	Storage period	
	24 h	6 months
Control	63.7 (4.8)^{aA}	50.6 (6.2)^{aB}
Vitamin C	40.3 (5.3)^{bA}	39.6 (4.7)^{bA}
Vitamin E	56.8 (9.4)^{aA}	55.9 (7.0)^{aA}
Quercetin	31.2 (3.3)^{bB}	38.3 (5.7)^{bA}

Mean values with the same uppercase letters (row) and lowercase letters (column) are not significantly different ($p > 0.05$; Tukey's post-hoc test).

Table 3 Mean (standard deviation) of microtensile bond strength (MPa) to Clearfil SE Bond adhesive system

Antioxidant	Storage period	
	24 h	6 months
Control	69.0 (4.1)^{aA}	69.2 (4.8)^{aA}
Vitamin C	56.2 (6.5)^{bA}	61.0 (7.2)^{abA}
Vitamin E	41.2 (3.4)^{cB}	57.5 (7.2)^{bA}
Quercetin	40.7 (8.0)^{cB}	69.8 (5.8)^{aA}

Mean values with the same uppercase letters (row) and lowercase letters (column) are not significantly different ($p > 0.05$; Tukey's post-hoc test).

Table 4 Mean (standard deviation) of microtensile bond strength (MPa) to Adper Easy Bond adhesive system

Antioxidant	Storage period	
	24 h	6 months
Control	48.3 (6.6)^{aA}	51.1 (5.1)^{abA}
Vitamin C	14.9 (2.2)^{bB}	30.1 (7.5)^{cA}
Vitamin E	41.6 (5.6)^{aB}	58.8 (6.5)^{aA}
Quercetin	46.8 (4.3)^{aA}	44.2 (5.8)^{bA}

Mean values with the same uppercase letters (row) and lowercase letters (column) are not significantly different ($p > 0.05$; Tukey's post-hoc test).

mission mode and 2.8 mm/s mirror speed. The degree of conversion was calculated based on the intensity of the C=C stretching vibrations (peak height) at 1635 cm^{-1} and using the symmetric ring stretching at 1608 cm^{-1} from the polymerized and non-polymerized samples as an internal standard. The coefficient of determination was greater than 0.98 for all curves. Data were plotted and curve fitting was applied using logistic non-linear regression.

RESULTS

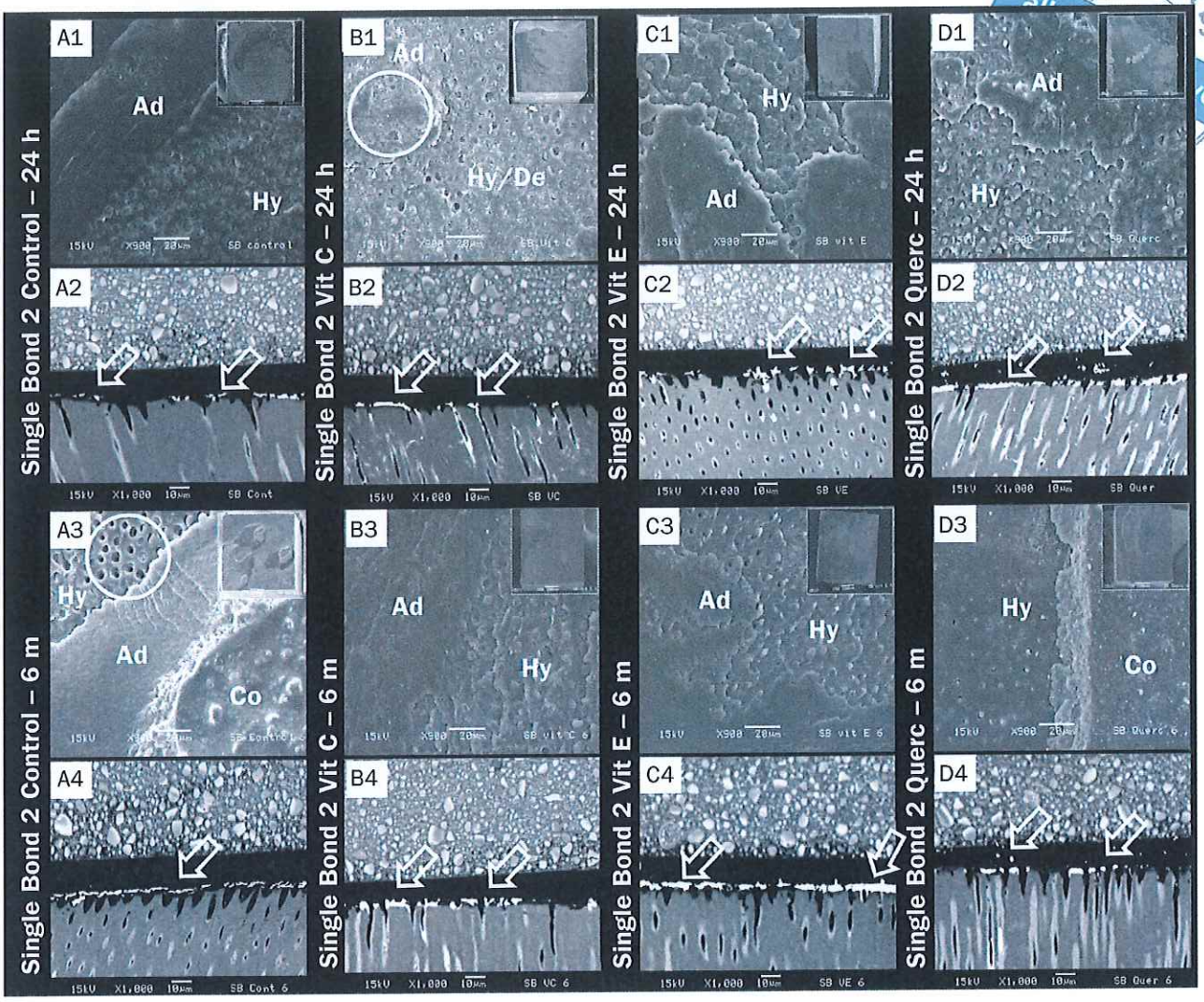
The bond strength outcomes are depicted in Tables 2 to 4. All data analyzed showed normality of data and homogeneous variance ($p > 0.05$). For all adhesive systems, each statistical factor presented significant difference ($p < 0.01$), and the interaction (antioxidant vs storage time) was statistically significant ($p = 0.002$, $p < 0.001$ and $p < 0.001$ for Adper Single Bond 2, Clearfil SE Bond and Adper Easy Bond, respectively).

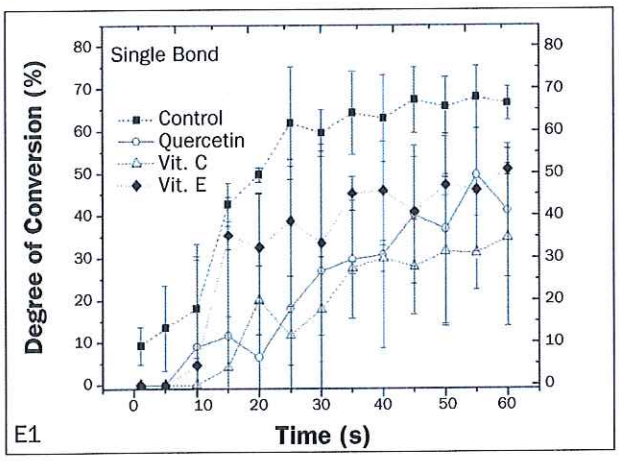
For Adper Single Bond 2 (Table 2) in both storage periods (24 h and 6 months), SB control and SB vitamin E groups exhibited bond strengths that were significantly higher than SB vitamin C and SB quercetin groups ($p < 0.05$). The bond strength of SB control dropped significantly after 6 months ($p < 0.05$), whereas for the SB quercetin group, the bond strength increased significantly in this period ($p < 0.05$). SB control presented several signs of degradation, such as patent dentinal tubules (Fig 1, A3). The SB vitamin C and SB vitamin E groups maintained their bond strength in 6 months of storage. The failure patterns (Fig 1) were predominantly mixed for SB control, SB vitamin C and SB vitamin E in both aging periods. For SB quercetin, there was a predominance of adhesive failures after 24 h and mixed failures after 6 months.

For Clearfil SE Bond (Table 3), the inclusion of antioxidants reduced bond strength after 24 h for all groups (vitamin C, vitamin E and quercetin) ($p < 0.05$). After 6 months, the groups CSE control and CSE quercetin showed significantly higher bond strength than did the CSE vitamin E group ($p < 0.05$). After 6 months under pulpal pressure, the bond strength of CSE vitamin E and CSE quercetin increased significantly ($p < 0.05$) and the failure mode (Fig 2) was predominantly adhesive at 24 h and mixed after 6 months for both groups. The CSE control and CSE vitamin C groups maintained their bond strength during 6 months of storage. For CSE control, the failure pattern changed from mixed to cohesive in dentin after storage, and the CSE vitamin C group maintained the predominance of mixed failures after 24 h and 6 months.

Adper Easy Bond (Table 4) vitamin C showed the lowest bond strength among the groups for both storage periods (24 h and 6 months) ($p < 0.05$). After 6 months, the EB vitamin C and EB vitamin E groups demonstrated an increase in bond strength ($p < 0.05$), whereas EB control and EB quercetin maintained their bond strength over the 6 months of storage. An adhesive layer with crystal-like voids was observed in EB quercetin, likely due to the presence of insoluble quercetin crystals, which were probably released or degraded during storage (Fig 3, D1 and D3). Failure pattern analysis (Fig 3) revealed a predominance of mixed fractures for EB control, EB vitamin E and EB quercetin, while adhesive/mixed modes were most common for EB vitamin C.

Slight changes in the nanoleakage pattern after aging were observed in all groups, except for the EB control group, which showed a noteworthy increase in nanoleak-





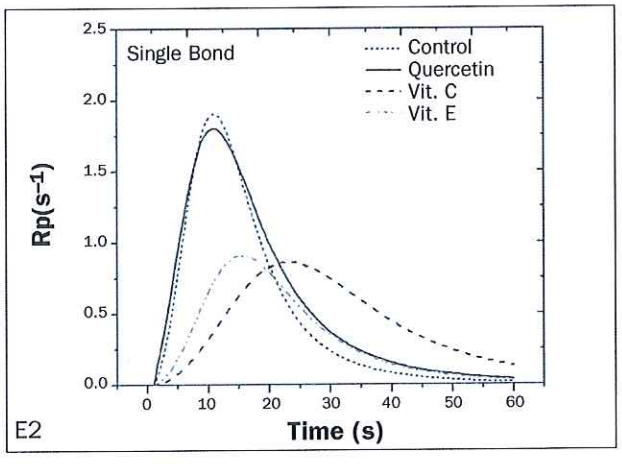
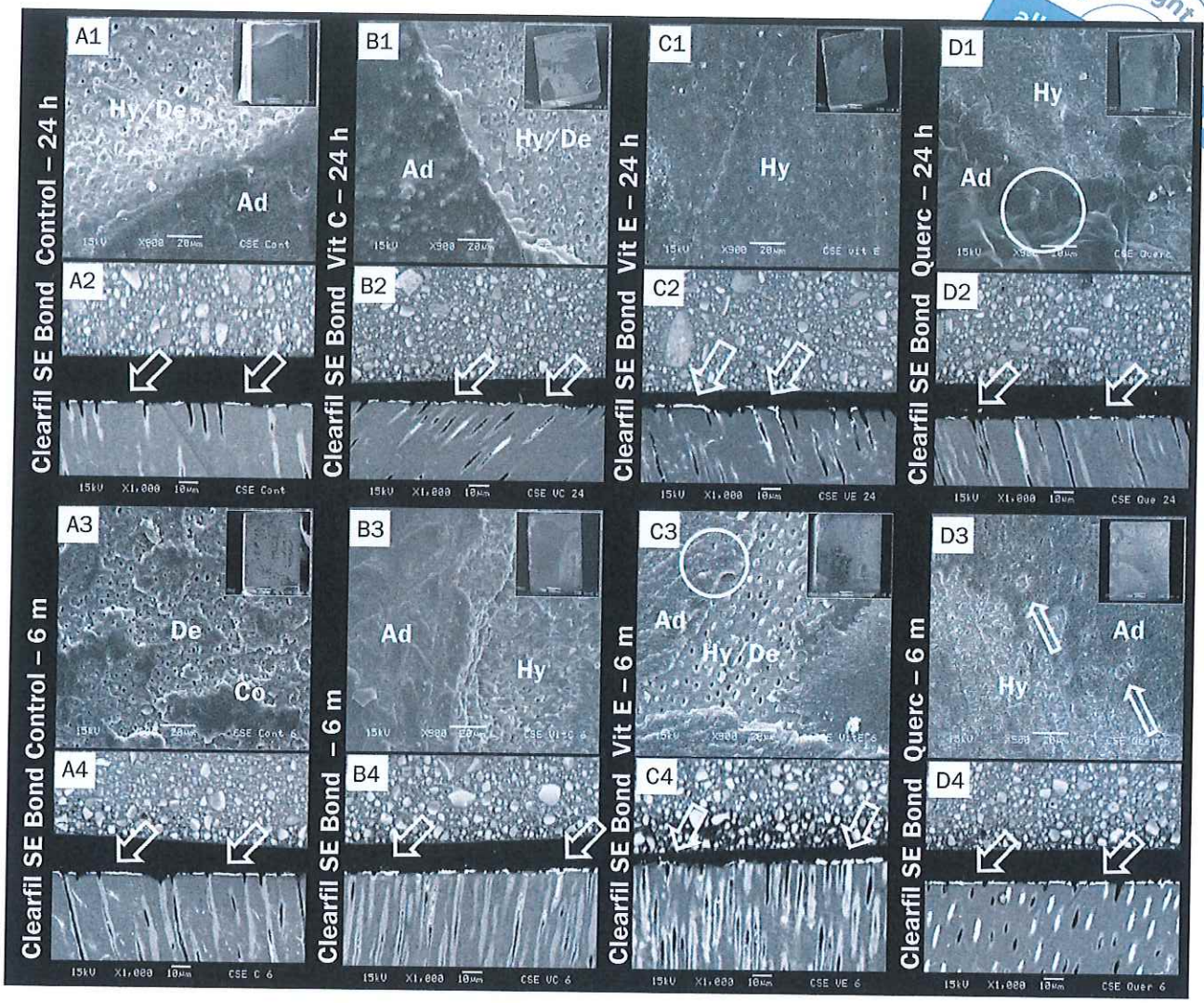
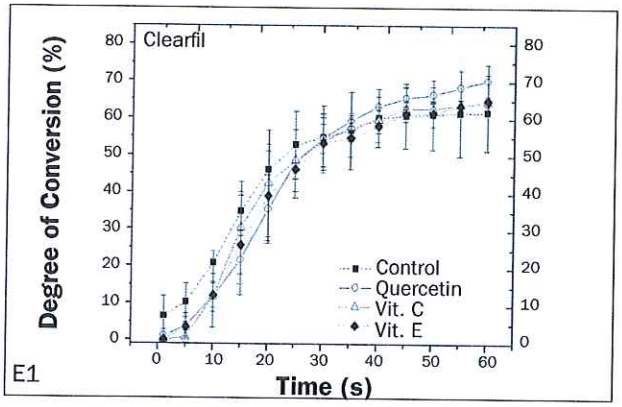


Fig 1 Scanning electron micrographs showing failure modes (A1, B1, C1, D1, A3, B3, C3, D3) and silver uptake (nanoleakage; A2, B2, C2, D2, A4, B4, C4, D4) of specimens bonded using Adper Single Bond 2. White arrows indicate the silver deposits. The graphs depict the results of degree of conversion (E1) and rate of polymerization (E2) of Adper Single Bond 2 with or without antioxidants. Figs A1 and C1: adhesive layer apparently uniform (Ad). Figs B1 (white circle) and D1: more porous and less uniform adhesive layers. Figs B3, C3, D3: uniform adhesive and hybrid layers with less permeable aspect for experimental groups after 6 months. Fig A3: evident signs of degradation, with collagen- and resin-depleted hybrid layer (Hy) (white circle). Figs E1 and E2: lower degree of conversion and rate of polymerization were obtained to all groups compared to control. Co: composite.





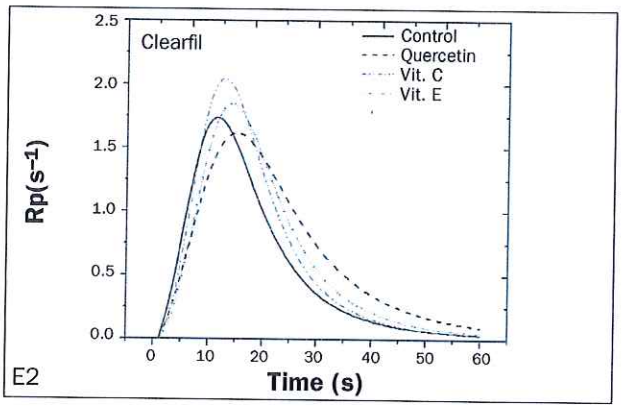
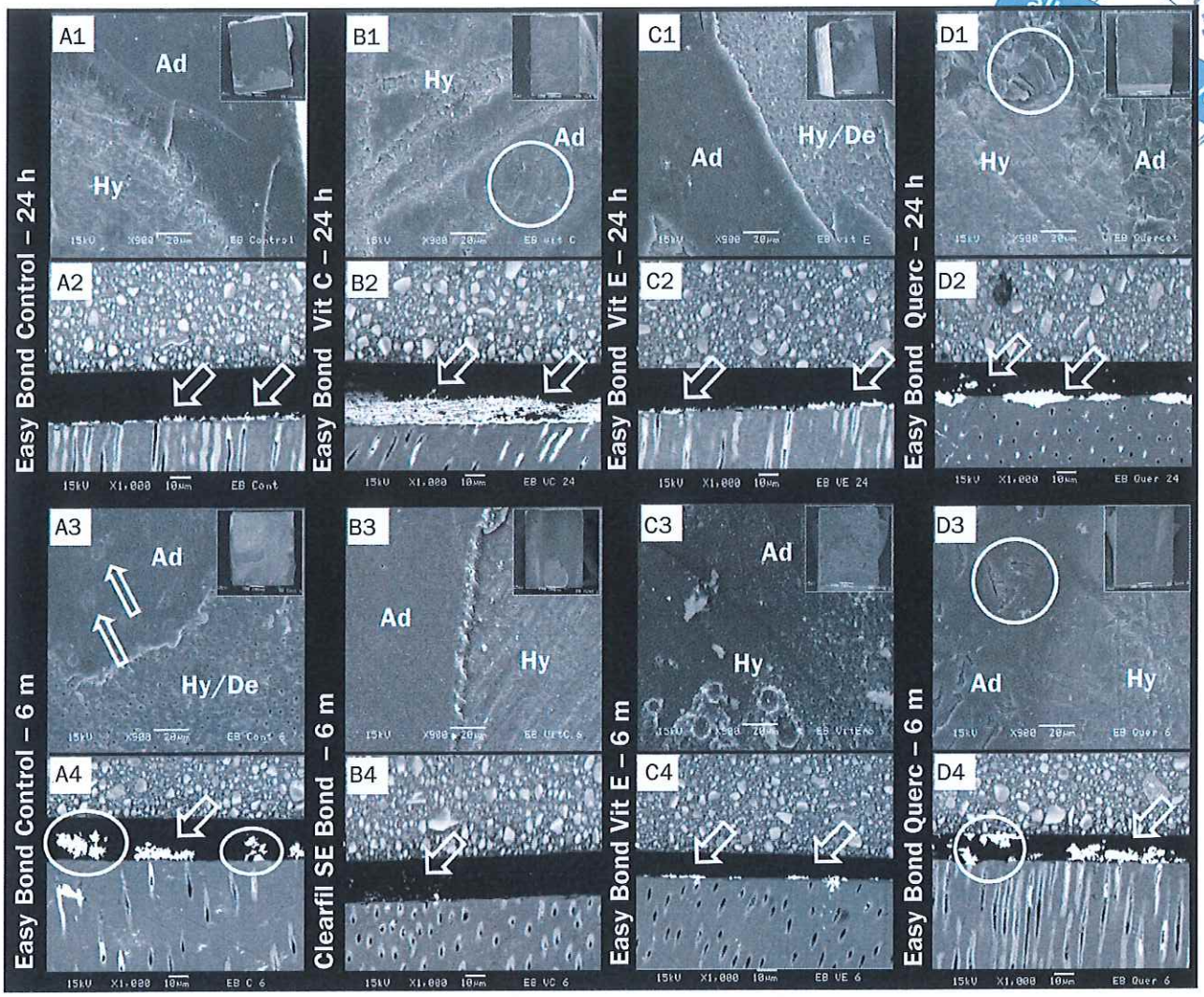
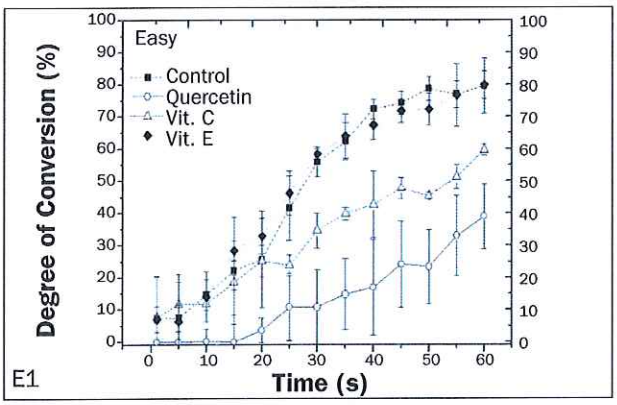


Fig 2 Scanning electron micrographs showing the failure modes (A1, B1, C1, D1, A3, B3, C3, D3) and silver uptake (nanoleakage) (A2, B2, C2, D2, A4, B4, C4, D4) of specimens bonded using Clearfil SE Bond. White arrows indicate the silver deposits. The graphs depict the results of degree of conversion (E1) and rate of polymerization (E2) with or without antioxidants incorporation. Fig C1 and Fig D1 (white circle): thinner or rough adhesive layers. Fig C3 (white circle) and Fig D3: more uniform adhesive layer despite the degradation represented as exposed dentin (De) (C3) and pores in the adhesive (Ad) layer (white arrows in D3). Fig B3: less permeable hybrid layer (Hy) after six months. Figs E1 and E2: striking changes in the degree of conversion and rate of polymerization were not observed using antioxidants. Co: composite.





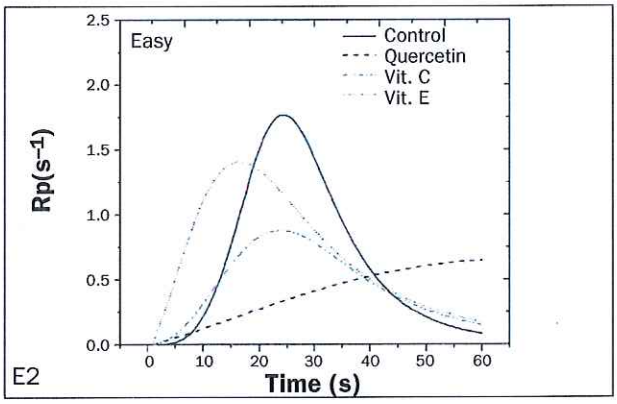


Fig 3 Scanning electron micrographs showing failure modes (A1, B1, C1, D1, A3, B3, C3, D3) and silver uptake (nanoleakage) (A2, B2, C2, D2, A4, B4, C4, D4) of specimens bonded using Adper Easy Bond. The results of degree of conversion (E1) and rate of polymerization (E2) are displayed. Fig B1 (white circle): discontinuous adhesive layer (Ad). Fig C1: permeable hybrid layer (Hy) with exposed dentin (De). Figs B3 and C3: noteworthy difference in the adhesive layer and hybrid layer was seen with uniform appearance, respectively. Fig A3 (white arrows): discontinuities in the adhesive layer, likely due to degradation. Fig D1 (white circle): adhesive layer with the crystal-like voids. White arrows indicate silver deposits. For EB control, note the increase in nanoleakage in 6 months compared to 24 h (Figs A2 and A4), with presence of water trees (white circle). The opposite behavior occurred in the Easy Bond doped with vitamin C. One can observe a notable decrease of silver deposits after 6 months compared to 24 h (Figs B2 and B4). Figs E1 and E2: lower degree of conversion and rate of polymerization were obtained using quercetin and vitamin C, but not with vitamin E.

age after 6 months (Fig 3, A2 and A4), and for EB vitamin C, which presented a remarkable decrease (Fig 3, B2 and B4).

A lower degree of conversion was obtained with all antioxidants in SB and EB (Figs 1, E1 and 3, E1), except for the EB vitamin E group, which showed similar degree of conversion to the EB control group. For CSE (Fig 2, E1), a similar degree of conversion was attained in the control and experimental groups. In general, the rate of polymerization was reduced for all antioxidant-doped adhesives compared to control groups (Figs 1, E2 and 3, E2), except for the CSE adhesive system (Fig 2, E2).

DISCUSSION

Specific antioxidants, such as hydroquinone monomethyl ether, are usually added to dental adhesives and composites at very low concentrations in order to scavenge free radicals and prevent spontaneous polymerization.[37] Recently, some studies showed that the increase of antioxidants in the composition of adhesives may retard the polymerization without impairing the final properties or stability of the resulting material.[15,24]

The quality of the polymerization process may be directly correlated with the resin-dentin microtensile bond strength and silver nitrate uptake at the bond interface.[17]

This investigation confirmed the initial adverse effects of antioxidants on the bond strength of three commercial adhesives; hence, the first null hypothesis must be rejected. In particular, vitamin C (hydrophilic antioxidant) and quercetin (amphiphilic antioxidant) reduced the bond strength of Adper Single Bond 2 in 24 h (Fig 1, E1). The relatively hydrophilic composition of this simplified adhesive[12] reflects the overall hydrophilic character, which facilitates the mixture of these antioxidants and the inhibition of polymerization free radicals. In contrast, vitamin E (hydrophobic antioxidant) led to no difference in bond strength compared to SB Control, despite the lower degree of conversion obtained (Fig 1, E1). This outcome may be attributed to an impaired mixture in different domains after phase separation. Moreover, the antioxidants have different oxidation potentials; for instance, vitamin C has a higher antioxidant capacity than does vitamin E, exerting a greater influence on the system.

For Clearfil SE Bond, all antioxidants reduced the bond strength to dentin after 24 h. The additional vitamin C in the CSE primer may have negatively affected the overall pH and its self-etching characteristics, reducing the bond strength after 24 h.[10] However, the most credible hypothesis to justify these results may be attributed to the fact that vitamin C forms relatively stable complexes with calcium.[27] Ascorbate ions may have competed with MDP functional monomer to chemically bond with calcium and hydroxyapatite.[10] Thus, with reduced chemical interaction of MDP, the bond strength was also adversely affected.[42]

In the case of more hydrophobic vitamin E and quercetin-doped CSE, inhibition of the polymerization of the adhesive system's hydrophobic di-methacrylates may occur, allowing the polymerization of mono-methacrylate monomers (ie, HEMA and MDP). These antioxidants may lead to formation of more linear polymers, which indeed compromised the bond strength.

Despite the negative results for quercetin at 24 h to SB and CSE, some antioxidants of the same type of quercetin have shown promising therapeutic outcomes. Recently, epigallocatechin-3-gallate (EGCG) was included successfully in etch-and-rinse adhesives and induced remarkable therapeutic effects, such as inhibition of the growth of Streptococcus mutans and higher bond strength than the control.[7] Proanthocyanidin from grape-seed extract also proved to be effective in improving biomechanical properties of dentin matrix, thereby enhancing preventive and reparative dental therapies.[9] Like quercetin, EGCG and proanthocyanidin are also flavonoids.

Conversely, although the presence of antioxidants decreased the bond strength of CSE after 24 h, the degree of conversion and rate of polymerization were not affected (Fig 2, E1 and E2). The composition of this adhesive – which contains photoinitiators both in the primer and the bonding resin[42] – may influence these outcomes; hence, the inhibition of polymerization in the primer might be compensated by the subsequent active photoinitiation of the bond resin.

The most hydrophilic adhesive evaluated was Adper Easy Bond. The mixture EB-vitamin C was then expected to be more detrimental (Table 4) due to similar polarity and solubility characteristics, which was confirmed. This adhesive system has initiators such as 2-(dimethylamino)-ethylmethacrylate (DMAEMA) which, compared to other co-initiators, is hydrophilic to a certain extent.[41] Vitamin C may have inhibited free radicals generated by DMAEMA in the hydrophilic domains of the adhesive blend. This reduced the degree of conversion (Fig 3, E1) and may have consequently decreased the bond strength after 24 h.

It is believed that EB is also composed of a hydrophilic photoinitiator such as trimethylbenzoyl-diphenylphosphine oxide (TPO).[16] Although camphoroquinone has been the widely used photoinitiator, it is known that more hydrophilic photoinitiators improve the polymerization of hydrophilic self-etching adhesives.[14] TPO is an alternative photoinitiator possessing an absorption peak around 380 nm and is capable of functioning independently of an amine-type coinitiator.[26] The reaction involving the TPO is characterized by a direct cleavage of the molecule and the formation of two radicals, acyl and phosphonyl.[26] In this situation, the inhibition of polymerization by vitamin C would directly act in the photosensitive molecules, inactivating the free radicals generated.

For EB quercetin, an adhesive layer with the crystal-like voids was observed, likely due to the presence of insoluble quercetin crystals (Fig 3, D1). These crystals may have been released or degraded during storage, represented by the reduced amount of voids in the adhesive layer (white circle in Fig 3, D3) after aging.

After 6 months of simulated pulpal pressure, the Adper Single Bond 2 control showed a significant decrease in bond strength, which is in agreement with a previous investigation.[12] The polymer hydrolytic degradation may be accelerated by free radicals.[35] The free radicals po-

tentially continue to act and generate more free radicals, accelerating the degradation process.[10]

Simulated pulpal pressure is considered a method of aging slower than direct water exposure of sticks.[12] However, this strategy better simulates in vivo conditions. Moreover, the aging by simulated pulpal pressure was adopted because some antioxidants could be released from the bonded interface during storage of resin-dentin sticks. This would nullify their effects over time. Possibly, the slow aging and the limited storage time were not sufficient to obtain a significant decrease in bond strength to other adhesive systems.[3]

The antioxidant incorporation maintained or increased the bond strength values obtained after 24 h after 6 months. Despite the slight changes in the pattern of nanoleakage, there was a decrease of silver deposits for EB vitamin C after 6 months; thus, the second null hypothesis must also be rejected.

Several feasible explanations may be given to support the increase of bond strength after aging, for instance, antioxidants rather than collagen or polymer matrices degraded hydrolytically, or delayed polymerization of antioxidant-doped adhesives would lead to polymers with improved resistance to degradation. Moreover, the antioxidants are available for further reaction with products of degradation even after the initial reaction with the free radicals of polymerization. This secondary reaction may release some monomers and initiators, which could lead to a late polymerization process. This continued polymerization could afford an increase in bond strength after 6 months (Tables 2 to 4). The lower degree of conversion after 24 h and the slow polymerization process seen in the rate of polymerization graphs for antioxidant-doped adhesives reinforce this hypothesis. Thus, the third null hypothesis is also rejected.

Some strategies and substances are able to overcome the degradation processes of dentin bonds, such as enzymatic inhibitors,[33] ethanol wet bonding,[25] and alternative photoinitiators.[20] Whereas they stabilize the bond strength over time, some other strategies may increase the bond strength after 24 h, eg, the usage of hydrophobic nanogel[21] or collagen cross linkers.[22] No treatment has so far increased the impaired the bond strength after aging as observed here using antioxidants.

Additional studies should emphasize other aspects, such as the analysis of the degree of conversion and rate of polymerization at the bond interface with dentin, a longer storage time of experimental adhesives with known chemical composition, and the possibility of enzyme inhibition by antioxidants.

CONCLUSION

The performance of antioxidants changed according to the adhesive system to which they were added, and antioxidant-doped adhesives appear to have a positive effect on the adhesive interface durability, since their bond strength after 24-h water storage was maintained or increased over time.

ACKNOWLEDGMENTS

We are grateful for financial support from Capes Brazil (grant 3110/2010).

REFERENCES

1. Asmussen E, Peutzfeldt A. Influence of composition on rate of polymerization contraction of light-curing resin composites. Acta Odontol Scand 2002;60:146-150.
2. Bedran-Russo AK, Pashley DH, Agee K, Drummond JL, Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res B Appl Biomater 2008;86B:330-334.
3. Belli R, Sartori N, Peruchi LD, Guimarães JC, Araújo E, Monteiro S Jr, Baratieri LN, Lohbauer U. Slow progression of dentin bond degradation during one-year water storage under simulated pulpal pressure. J Dent 2010;38:802-810.
4. 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.
5. Carrilho MR, Carvalho RM, de Goes MF, di Hipólito V, Geraldeli S, Tay FR, Pashley DH, Tjäderhane L. Chlorhexidine preserves dentin bond in vitro. J Dent Res 2007;86:90-94.
6. Deng D, Yang H, Guo J, Chen X, Zhang W, Huang C. Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces. J Dent 2014;42:1577-1585.
7. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent 2012;40:485-492.
8. Ely C, Schneider LF, Ogliari FA, Schmitt CC, Corrêa IC, Lima Gda S, Samuel SM, Piva E. Polymerization kinetics and reactivity of alternative initiators systems for use in light-activated dental resins. Dent Mater 2012;28:1199-1206.
9. Epasinghe DJ, Yiu CK, Burrow MF, Tsoi JK, Tay FR. Effect of flavonoids on the mechanical properties of demineralised dentine. J Dent 2014;42:1178-1184.
10. Erhardt MC, Osorio R, Viseras C, Toledano M. Adjunctive use of an antioxidant agent to improve resistance of hybrid layers to degradation. J Dent 2011;39:80-87.
11. Feitosa VP, Sauro S, Watson TF, Correr AB, Osorio R, Toledano M, Correr-Sobrinho L, Sinhoreti MA. Evaluation of the micro-mechanical strength of resin bonded-dentin interfaces submitted to short-term degradation strategies. J Mech Behav Biomed Mater 2012a;15:112-120.
12. Feitosa VP, Leme AA, Sauro S, Correr-Sobrinho L, Watson TF, Sinhoreti MA, Correr AB. Hydrolytic degradation of the resin-dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing. J Dent 2012b;40:1134-1143.
13. Göpferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103-114.
14. Guo X, Peng Z, Spencer P, Wang Y. Effect of initiator on photopolymerization of acidic, aqueous dental model adhesives. J Biomed Mater Res A 2009;90:1120-1127.
15. Hadis MA, Shortall AC, Palin WM. Competitive light absorbers in photoactive dental resin-based materials. Dent Mater 2012;28:831-841.
16. Hass V, Luque-Martinez I, Sabino NB, Loguercio AD, Reis A. Prolonged exposure times of one-step self-etch adhesives on adhesive properties and durability of dentine bonds. J Dent 2012;40:1090-1102.
17. Hass V, Dobrovolski M, Zander-Grande C, Martins GC, Gordillo LA, Rodrigues Accorinte Mde L, Gomes OM, Loguercio AD, Reis A. Correlation between degree of conversion, resin-dentin bond strength and nanoleakage of simplified etch-and-rinse adhesives. Dent Mater 2013;29:921-928.
18. Hilton TJ, Ferracane JL. As per manufacturer's instructions. J Esthet Restor Dent 2006;18:1-2.
19. Lamblin G, Leprince J, Devaux J, Mestdagh M, Gallez B, Leloup G. Hydroxyl radical release from dental resins: electron paramagnetic resonance evidence. Acta Biomater 2010;6:3193-3198.
20. Liu Y, Tjäderhane 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.
21. Morães RR, Garcia JW, Wilson ND, Lewis SH, Barros MD, Yang B. Improved dental adhesive formulations based on reactive nanogel additives. J Dent Res 2012;91:179-184.
22. Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical crosslinkers on caries-affected dentin bonding. J Dent Res 2009;88:1096-1100.

23. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1-16.
24. Rosentritt M, Shortall AC, Palin WM. Dynamic monitoring of curing photoactive resins: a methods comparison. Dent Mater 2010;26:565-570.
25. Sauro S, Osorio R, Watson TF, Toledano M. Assessment of the quality of resin-dentin bonded interfaces: an AFM nano-indentation, μTBS and confocal ultramorphology study. Dent Mater 2012;28:622-631.
26. Schneider LF, Cavalcante LM, Prahl SA, Pfeifer CS, Ferracane JL. Curing efficiency of dental resin composites formulated with camphorquinone or trimethylbenzoyl-diphenyl-phosphine oxide. Dent Mater 2012;28: 392-397.
27. Schubert J, Lindenbaum A. Complexes of calcium with citric acid and tricarballylic acids measured by ion exchange. Nature 1950;166:913-914.
28. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res 2002;62:447-456.
29. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, Katz JL. Adhesive/dentin interface: the weak link in the composite restoration. Ann Biomed Eng 2010;38:1989-2003.
30. Takahashi M, Nakajima M, Hosaka K, Ikeda M, Foxton RM, Tagami J. Long-term evaluation of water sorption and ultimate tensile strength of HEMA-containing/-free one-step self-etch adhesives. J Dent 2011;39:506-512.
31. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472-476.
32. Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, Breschi L, Vallittu P, Tay FR, Pashley DH. Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro. J Dent Res 2012;91:192-196.
33. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-135.
34. Toledano M, Cabello I, Yamauti M, Giannini M, Aguilera FS, Osorio E, Osorio R. Resistance to degradation of resin-dentin bonds produced by one-step self-etch adhesives. Microsc Microanal 2012;18:1480-1493.
35. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266:37-56.
36. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005;84:183-188.
37. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757-3785.
38. Van Landuyt KL, De Munck J, Mine A, Cardoso MV, Peumans M, Van Meerbeek B. Filler debonding & subhybrid-layer failures in self-etch adhesives. J Dent Res 2010;89:1045-1050.
39. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28:215-235.
40. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010;26:100-121.
41. Ye Q, Park J, Topp E, Spencer P. Effect of photoinitiators on the in vitro performance of a dentin adhesive exposed to simulated oral environment. Dent Mater 2009;25:452-458.
42. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, Van Landuyt KL, Osaka A, Suzuki K, Minagi S, Van Meerbeek B. Nanolayering of phosphoric acid ester monomer on enamel and dentin. Acta Biomater 2011;7:3187-3195.

Clinical relevance: Certain combinations of antioxidant and adhesive may inhibit the adverse effects of long-term hydrolysis thereby improving dentin bond durability.