Use of grape seed extract for improving the shear bond strength of total-etching adhesive to bleached enamel

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This study was to ascertain if grape seed extract (GSE) can restore the shear bond strength (SBS) of total-etching adhesive to enamel immediately after bleaching. Immediately after bleaching with Beyond gel, different concentrations of GSE were applied to the surface of bovine enamel for 1 min before bonding of resin composite with Adper single bond 2 or All-Bond 3 adhesive. SBS values and debonding modes were recorded. Structure of the bonding interface and elements on enamel surface were analyzed by scanning electron microscopy and X-ray photoelectron spectroscopy (XPS). SBS was found to be compromised significantly in 0 and 2.5% GSE groups. GSE (≥5%) could restore the SBS to the level of control. Failure in the adhesive joint was always the major debonding mode. No significant difference was found by XPS. Thus, GSE can restore the SBS compromised after bleaching in 1 min if the concentration is ≥5%.

Keywords: Antioxidant, Grape seed extract, Tooth-bleaching, Shear bond strength

INTRODUCTION

Tooth-bleaching is become increasingly popular and is a part of esthetic dentistry[1]. Dentists should understand the interactions between tooth-bleaching and other dental treatments, such as adhesive restorations.

Hydrogen peroxide is the main chemical used in bleaching systems[2], while studies have shown that it will adversely affect the shear bond strength (SBS) of resin composite to acid-etched enamel if bonding is undertaken immediately after bleaching[3,4]. Reduction in SBS due to tooth-bleaching has been attributed to the residual oxygen released from the bleaching agent, which may interfere with infiltration of resin into etched enamel[5] or inhibit resin polymerization[3,6,7].

A typical method avoiding this problem is to delay the bonding procedure for 1–2 weeks after bleaching when peroxide ions decompose and compromised SBS is restored[8], but sometimes patients are not so patient that they wish to finish the treatment procedures as soon as possible. In recent *in vitro* research, there is evidence that bonding can be done immediately if bleaching is followed by antioxidant application, for example, use of sodium ascorbate can restore the compromised SBS after bleaching[3,4], and two applications of 35% sodium ascorbate for 1 min each can eliminate residual hydrogen peroxide[9].

Other naturally occurring antioxidants, such as grape seed extract (GSE), contain oligomeric proanthocyanidin complexes (OPCs), which can scavenge free radicals. GSE has been shown to be more potent than sodium ascorbate[10,11], and safe to be used as an antioxidant in various clinical applications and dietary supplements[10]. Vidhya *et al.*[12] and Abraham *et al.*[13] found that if 5% GSE was applied for 10 min, the reduced SBS was restored. However, whether different concentrations of GSE in shorter application time can also restore the compromised SBS is still unknown.

We wished to compare the effects of application of 2.5–15% GSE in 1 min on improving the SBS of bleached enamel. The reason we set the application time to 1 min is that two consecutive applications of 35% sodium ascorbate for 1 min each is the minimal application time that can eliminate residual hydrogen peroxide reported till now[9], for a more potent antioxidant like GSE[10,11], 1 min may be suitable, which is also a possibly acceptable application time for most dentists. The null hypothesis was that those applications had no effect on the SBS.

Besides, we also wanted to investigate the reasons behind the SBS results. Stereomicroscopy can suggest the weakest part of the bonding interface, and whether GSE can strengthen the weakest part. Scanning electron microscopy (SEM) will reveal the affections of GSE on the structure of the bonding interface. X-ray photoelectron spectroscopy (XPS) may show the change of elements, especially oxygen, on enamel surface, which may explain the mechanism of GSE's affections on the bonding interface. They were also undertaken in the test.

MATERIALS AND METHODS

The study materials

1–1.5 years old extracted bovine incisors were used for the research. 35% hydrogen peroxide gel (Beyond, Puyang, Nanchang, China) was selected as the bleaching agent. Adper Single Bond 2 (3M, St. Paul, MN, USA) and All-Bond 3 (Bisco, Schaumbrug, IL, USA) total-etching adhesive systems were chosen as resin-enamel

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Table 1 List of materials used in the study

Material	Manufacturer	Lot Number	Principal ingredients	Steps of application
Beyond	Puyang, Nanchang, China	CB9801	35% hydrogen peroxide	Applied to the enamel surface (10 min per time) thrice consecutively, rinsed, dried
MegaNatural Gold	Polyphenolics, Madera, CA, USA	B214547	GSE, rice flour, gelatin, magnesium stearate, silica	Applied by covering enamel with saturated cotton for 1 min, rinsed, dried
GLUMA Etch 35 Gel	Heraeus Kulzer, Hanau, Germany	395074	35% o-phosphoric acid	Applied and left untouched for 15 s, rinsed, dried
Adper Single Bond 2	3M, St. Paul, MN, USA	N353081	Bis-GMA, HEMA, dimethacrylates, silica nanofiller, polyalquenoic acid copolymer, initiators, water, ethanol	Applied and gently air-blown to a film, light-cured for 20 s
All-Bond 3	Bisco, Schaumbrug, IL, USA	0700005251 (Part A) 0700005255 (Part B)	MgNTG-GMA, ethanol (Part A) Bis-GMA, BPDM, HEMA, photo initiator, stabilizer (Part B)	Applied and gently air-blown to a film, light-cured for 20 s
Z350	3M	N162941	Bis-GMA, Bis-EMA, UDMA, TEGDMA, 5–20 nm nonagglomerated silica, 5–20 nm zirconium/silica nanoagglomerate, 0.6–1.4 μm agglomerated particles	Placed incrementally and cured, formed cylindrical posts perpendicular to the enamel surface

GSE: grape seed extract; Bis-GMA: bisphenol-A-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MgNTG-GMA: magnesium nitro-tri-glycyl glycidyl methacrylate; BPDM: bisphenol dimethacrylate; Bis-EMA: bisphenol-A-glycol dimethacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethyleneglycol dimethacrylate.

adhesives. 35% o-phosphoric acid (GLUMA Etch 35 Gel, Heraeus Kulzer, Hanau, Germany), and resin composite (Z350, 3M) were used in this study.

Four different concentrations of GSE solutions were prepared by dissolving GSE powder (MegaNatural Gold, Polyphenolics, Madera, CA, USA) in distilled water. For example, 2.5% GSE solution was prepared by dissolving 2.5 g GSE powder in 97.5 mL distilled water. 5, 10, and 15% GSE solutions were prepared in the same way.

The materials used in the study are summarized in Table 1.

Specimen preparation for SBS testing

Immediately after extraction, 360 teeth were scraped clean of residual tissue, pumiced, and washed under running tap water, then stored in distilled water at 4°C until required (≤1 week). Roots at the cement-enamel junction were removed using a slow-speed diamond saw under copious water spraying. Pulp was removed with a curette. Pulp chamber was sealed with temporary crown and bridge material (Protemp 4, 3M). Crown segments were fixed (with the exposed labial enamel downwards) on a double-sided adhesive tape on a glass slide. Then, they were embedded in self-curing resin (ATR, New Century Dental, Shanghai, China) by placement of a specially made mold (Bovine Mold, Tianhejingji Technology, Beijing, China) open at both ends on the glass slide and filling the mold with resin. Labial surfaces were ground flat on a wet 600-grit sandpaper by hand in a direction like writing the Arabic number "8" for 30 s under running tap water, and rinsed thoroughly with an air/water spray for 30 s and air-dried.

The specimens were assigned randomly to 6 groups with 60 specimens per group for the following bleaching and application of GSE step: control (non-bleached); no antioxidant treatment after bleaching; 2.5% GSE solution after bleaching; 5% GSE solution after bleaching; 10% GSE solution after bleaching; 15% GSE solution after bleaching.

Bleaching and application of GSE

Bleaching agent was applied to the enamel surface (10 min per time) thrice consecutively according to manufacturer instruction. At the end of bleaching, specimens were rinsed thoroughly with an air/water spray for 30 s and air-dried. GSE solutions were applied by covering bovine enamel with saturated cotton for 1 min. After antioxidant treatment, enamel surfaces were rinsed thoroughly with an air/water spray for 30 s and air-dried.

Then the 6 groups were meanly divided into 12 groups with 30 specimens per group for the following

bonding with two different total-etching systems step.

Bonding with two different total-etching systems
Two different total-etching adhesive systems were applied according to manufacturer instructions respectively. After application of phosphoric acid, a layer of bonding resin, Adper Single Bond 2 or All-Bond 3, was applied and cured using a light-curing unit (SmartLite PS, Dentsply, Konstanz, Germany) at an output of 1,200 mW/cm^2. Distance between LED light and bonding surface was about 5 mm.

A split-Teflon mold with a circular hole (diameter and depth=3 mm) was positioned over the center of the flattened enamel surface and fixed into place with a light-curing gum protectant (Beyond). Z350 resin composite was placed incrementally and cured in the mold, and formed cylindrical posts perpendicular to the enamel surface.

Then the 12 groups were meanly divided into 24 groups with 15 specimens per group for the following SBS test after 24 h or 5,000 cycles of thermocycling.

SBS test after 24 h or 5,000 cycles of thermocycling
After 24 h storage in distilled water at 37°C, a SBS test was done using a universal testing machine (EZ-L-1kN, Shimadzu, Kyoto, Japan). Each specimen was fixed to a custom-made testing jig. A knife-edge shearing rod (crosshead speed, 1 mm/min) was used. SBS of specimens was calculated and expressed in MPa.

The other half samples underwent 5,000 cycles of thermocycling (TC-501F Thermocycling Device, Weier, Suzhou, China) from 5 to 55°C, with a dwell time of 30 s and a transfer time of 10 s, then a SBS test was done.

Assessing failure modes
Failure modes of debonded specimens were assessed under a stereomicroscope (220670, Olympus, Tokyo, Japan) at ×40 magnification and classified as: cohesive failures in the resin composite; failures in the adhesive joint; cohesive failures in enamel; mixed failures.

SEM
The labial enamel surfaces of nine other intact bovine incisors were divided into three groups: control (non-bleached); no antioxidant treatment after bleaching; 15% GSE solution after bleaching. Bonding of resin composite to enamel was done by Adper Single Bond 2 system. Samples were cut vertically to the bonding interface using a slow-speed water-cooled saw equipped with a diamond-impregnated disk (Isomet 1000, Buehler, Lake Bluff, IL, USA) and sputter coated with gold, then examined using a SEM (SUPRA 55, Carl Zeiss, Oberkochen, Germany) operating at 15 kV.

XPS
The labial enamel surface of another intact bovine incisor was divided into three parts. XPS of chemical elements was done on the enamel surface exposed to three treatments: control (non-bleached); no antioxidant treatment after bleaching; 15% GSE solution after bleaching. Samples were dried with a series of alcohol solutions and prepared at high vacuum for XPS. XPS data were obtained on an AXIS-Ultra instrument (Kratos Analytical, Manchester, UK) using monochromatic Al Kα radiation (225 W, 15 mA, 15 kV) and low-energy electron flooding for charge compensation. To compensate for surface-charge effects, binding energies were calibrated using the carbon-1s hydrocarbon peak at 284.80 eV. Data were converted into VAMAS file format and imported into a Casa XPS software package for manipulation and curve fitting. To ensure accuracy of test results, XPS was repeated in an additional two incisors.

Statistical analyses
SPSS v19 (IBM, Armonk, NY, USA) was used to analyze the results of SBS and the failure modes. Three-way ANOVA test and *t*-test were performed on the SBS values in order to compare the effects of the adhesives and the surface treatments, as well as the storage conditions. Tukey compromise *post hoc* test was performed at a significance level of α=0.05. Fisher's exact test was performed on the failure modes at a significance level of α=0.05.

RESULTS

SBS
SBS values (mean±standard deviation) are summarized in Table 2. No matter what kind of adhesive used or storage condition, SBS values decreased significantly after bleaching, and could not be restored by application of 2.5% GSE for 1 min, while, when GSE concentrations were ≥5%, compromised SBS values were restored to the same level to the control (F=27.415, P=0.000). No matter what kind of adhesive used or surface treatment, SBS values decreased significantly after thermocycling (F=186.027, P=0.000). No matter what kind of surface treatment or storage condition, there was no significant difference in SBS values between the two adhesive groups (F=0.009, P=0.925).

Distribution of failure modes
There was no significant difference in distribution of failure modes among the testing groups (P=1.000). In the 360 tested samples, 350 samples were adhesive joint failures, 7 samples were cohesive failures in resin composite, and 3 samples were mixed failures (*i.e.*, cohesive failure in resin and in adhesive joint). No other types of failures were observed. Table 3 summarizes the distribution of failure modes.

SEM
SEM revealed no evidence of discontinuity at the adhesion interface when the bonding resin was applied to acid-etched enamel (Fig. 1). In single-bleached samples, evidence of marginal gap was seen (Fig. 2). In bleached but GSE-treated samples, no evidence of discontinuity was seen (Fig. 3).

Table 2 Shear bond strength *in vitro* (MPa)

Type of adhesive	Surface treatment	Storage condition	
		24 h	Thermocycling
Adper Single Bond 2	Control (non-bleached)	16.72 (4.66)^{a,A}	11.14 (2.72)^{a,B}
	No antioxidant treatment after bleaching	11.97 (3.72)^{b,A}	7.78 (2.99)^{b,B}
	2.5% GSE solution after bleaching	12.04 (2.93)^{b,A}	7.65 (1.93)^{b,B}
	5% GSE solution after bleaching	17.41 (5.13)^{a,A}	11.59 (3.09)^{a,B}
	10% GSE solution after bleaching	18.26 (5.66)^{a,A}	12.18 (3.62)^{a,B}
	15% GSE solution after bleaching	18.34 (5.57)^{a,A}	12.25 (3.04)^{a,B}
All-Bond 3	Control (non-bleached)	18.45 (4.68)^{a,A}	12.05 (3.36)^{a,B}
	No antioxidant treatment after bleaching	12.18 (2.84)^{b,A}	7.85 (1.73)^{b,B}
	2.5% GSE solution after bleaching	11.87 (3.34)^{b,A}	8.12 (2.51)^{b,B}
	5% GSE solution after bleaching	16.74 (4.36)^{a,A}	11.01 (3.10)^{a,B}
	10% GSE solution after bleaching	17.41 (4.31)^{a,A}	11.61 (2.69)^{a,B}
	15% GSE solution after bleaching	18.10 (5.06)^{a,A}	12.39 (3.09)^{a,B}

Values are the mean (SD), n=15 per subgroup. The same small letters as superscript indicate no significant difference within the same column (same storage condition), and capital letters within the row (same combination of adhesive and treatment) (p>0.05).

Table 3 Distribution of failure modes

Group	Failure mode			
	Cohesive failures in composite resin	Failures in adhesive joint	Cohesive failures in enamel	Mixed failures in resin and adhesive joint
Control (non-bleached)	1/60	58/60	0/60	1/60
No antioxidant treatment after bleaching	0/60	60/60	0/60	0/60
2.5% GSE solution after bleaching	0/60	60/60	0/60	0/60
5% GSE solution after bleaching	1/60	59/60	0/60	0/60
10% GSE solution after bleaching	2/60	57/60	0/60	1/60
15% GSE solution after bleaching	3/60	56/60	0/60	1/60
Total	7/360	350/360	0/360	3/360

××/××: ××=number of specimens tested reporting the indicated failure mode; ××=total specimens tested.

Table 4 Atomic percent surface compositions of bovine enamel (%)

Group	Zn	F	O	N	Ca	C	P	Mg
Control (non-bleached)	0.22 (0.27)	0.26 (0.19)	34.87 (5.06)	6.78 (3.18)	6.16 (4.43)	45.98 (13.11)	5.42 (1.31)	0.32 (0.23)
No antioxidant treatment after bleaching	0.14 (0.29)	0.29 (0.08)	34.64 (5.16)	5.75 (2.57)	6.22 (3.22)	48.81 (4.19)	3.73 (0.71)	0.42 (0.14)
15% GSE solution after bleaching	0.23 (0.48)	0.22 (0.15)	33.54 (4.49)	4.85 (6.61)	7.18 (5.98)	49.12 (8.64)	4.47 (1.92)	0.40 (0.16)

Values are the mean (range), n=3 per subgroup.

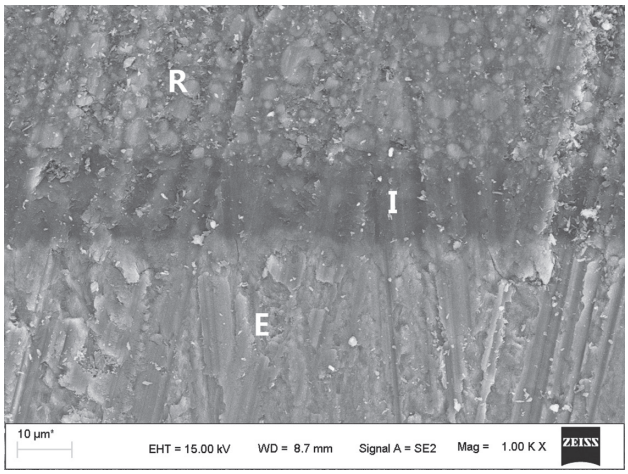

Fig. 1 SEM showing no evidence of discontinuity at the adhesion interface between bonding resin and acid-etched enamel under 1,000× magnification (control).
R, resin; E, enamel; I, adhesion interface.

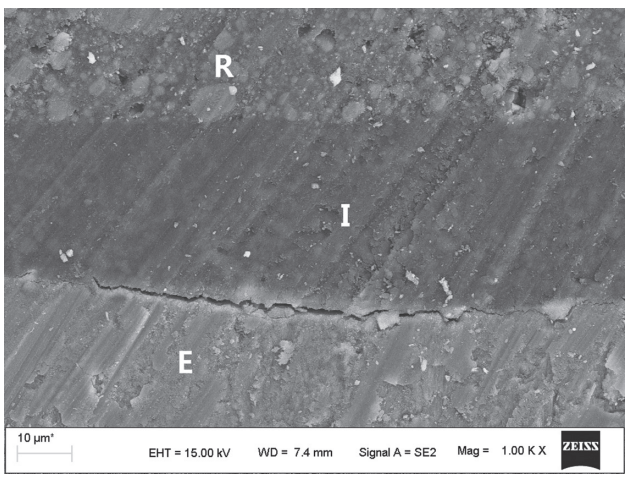

Fig. 2 SEM showing evidence of marginal gap at the adhesion interface between bonding resin and hydrogen peroxide-bleached, acid-etched enamel under 1,000× magnification.
R, resin; E, enamel; I, adhesion interface.

XPS

Semi-quantitative analyses of the elemental composition on the enamel surface are summarized in Table 4. Considering a relative error of measurement of 10–20%[14], exposure of the enamel surface to 35% hydrogen peroxide resulted in little change of oxygen concentration, and application of 15% GSE after bleaching did not elicit an obvious change in oxygen concentration. Simultaneously, the percentage of other elements did not show a change that could have a potent effect on the SBS.

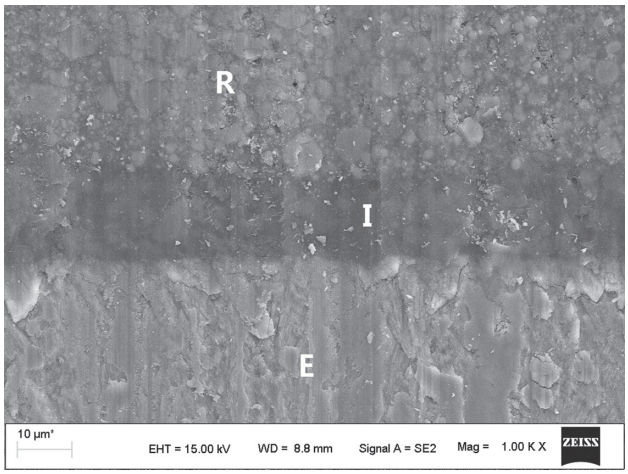

Fig. 3 SEM showing no evidence of discontinuity at the adhesion interface between bonding resin and hydrogen peroxide-bleached enamel treated with 15% GSE before acid etching.
R, resin; E, enamel; I, adhesion interface.

DISCUSSION

In laboratory settings, thermocycling is used as a model attempting to test bond degradation[15,16]. In order to get more accurate results, four experimental conditions were designed for the SBS test. Whatever adhesive used or whenever to test the samples, the SBS of total-etching adhesive to enamel decreased significantly immediately after bleaching, and the compromised SBS could be restored to the same level to that of the control by application of GSE for 1 min as long as the GSE concentration was ≥5%. Since there was no difference in SBS between "No antioxidant treatment after bleaching" and "2.5% GSE solution after bleaching" groups, the null hypothesis was partially agreed.

Bleaching agents containing hydrogen peroxide are used to treat tooth discolorations through oxidation. Hydrogen peroxide has a low molecular weight and decomposes into oxygen and hydroxyl free radicals. Free radicals released from hydrogen peroxide permeate into the enamel surface through inter-prismatic regions and attack the long-chained, dark-colored macromolecules of pigments and split them into smaller, less colored and more diffusible molecules that are removed from the structure[17].

In the present study, 35% hydrogen peroxide gel was used for three applications of 10 min each. The gel had to be applied thrice due to the rapid degradation of hydrogen peroxide (large amounts of active ingredients are available only for the first 15–20 min). Moreover, studies have shown that hydrogen peroxide used for tooth-whitening has a pH of around 7 immediately after application, while, if a single application takes too much time, the pH of the gel falls to around 5, which will increase tooth sensitivity[18].

Compromised SBS after bleaching occurs because the bleaching agent leaves a residual layer of oxygen. Studies have shown that inclusion of peroxide ions can be reversed by antioxidants. An antioxidant solution of 10% sodium ascorbate applied onto a bleached enamel surface for 10 min can restore the reduced SBS[19,20]. However, SEM has demonstrated an etched appearance on enamel surfaces after use of ascorbic acid in specimens of bleached enamel[21].

Use of plant extracts as an alternative to chemical and synthetic antioxidants has been encouraging[22]. GSE contains 90% polyphenols, and the major constitutions of polyphenols are OPCs. The latter are polymers of high molecular weight that comprise the monomers flavan-3-ol(þ) catechin and (–) epicatechin. OPCs are found at high concentrations in natural sources such as cranberries, extracts of pine bark, leaves of hazelnut trees, and bark of lemon trees. As a naturally occurring plant metabolite, GSE has been shown to be safe as an antioxidant in various clinical applications and in dietary supplements[23].

We found that SBS values were different when the resin was bonded to hydrogen peroxide-bleached, acid-etched enamel compared with those neutralized further with ≥5% GSE for 1 min and those in the control group. This finding can be explained by the mechanism of action of the antioxidant: GSE reacts with free radicals (*e.g.*, oxygen) generated by the degradation of hydrogen peroxide, thereby neutralizing them within the enamel in which they are trapped[24]. Further significant improvement of SBS did not occur as GSE concentrations increased. A possible explanation for this finding is that the reaction peaks at 1 min when the GSE concentration reaches 5%, above which the reaction decreases substantially.

Failure modes of debonded specimens were assessed to determine the weakest part of bonding in different groups. As expected, the adhesive joint was the most fragile part in all experimental groups because two completely different materials are connected at the bonding interface, which bears the most concentrated stress and is usually the most vulnerable part of bonding systems.

SBS was also examined by comparing the physical structure of the adhesion interface in samples undergoing different treatments using SEM. In control samples, the bonding resin infiltrated into acid-etched enamel and formed a tightly interlocked structure that contributed greatly to the remaining SBS. When bonding was carried out immediately after bleaching, marginal gap was very obvious, suggesting that the residual hydrogen peroxide could interfere with resin infiltration, which could explain why SBS decreased significantly immediately after bleaching. After GSE application, a continuous interface was observed again like that of the control when compromised SBS was restored.

SBS was also examined by comparing the chemical constitutions of enamel surfaces in samples undergoing different treatments using XPS. During XPS of a sample, the surface is exposed to monochromatic X-rays, which results in emission (by surface atoms) of photoelectrons having kinetic energies characteristic for each emitting atom and its respective binding state. By analyses of these photoelectrons according to their kinetic energy, semi-quantitative and structural information can be derived regarding the analyzed surface. With the exception of hydrogen, all elements are identifiable during XPS[25]. XPS allows the upper 1 to 10 atomic layers (0.5 to 5 nm, respectively) to be investigated with a detection limit of 0.1–1% and a relative error of 20%[14].

After tooth-bleaching, residual hydrogen peroxide can continue to breakdown into oxygen free radicals which are composed mainly of hydroxyl radicals[26,27]. XPS was undertaken to ascertain if the loss in adhesiveness of resin to enamel could be related to changes in elemental composition (especially oxygen percentage) on the enamel surface. We had expected the percentage of oxygen to increase significantly after bleaching, and then decrease significantly after GSE application. The outcome was not expected, but was in accordance with the work of other authors[14,25]. Interpretation of our results could be generalized into four possibilities.

First, the depth of the bonding interface is approximately several microns[28-30], which is thousands of times the depth at which XPS can detect[14]. After rinsing with water, the residual hydrogen peroxide on upper enamel (0.5–5 nm) might have been rinsed off so that a significant increase in oxygen composition could not be detected, whereas residual oxygen free radicals might have been present in deeper parts of the enamel surface, and that could also affect bonding.

Second, oxygen free radicals have unstable chemical properties. Even without external interference, the level of hydroxyl ions detected by colorimetric tests after tooth-bleaching is high only in the first 24 h[9,31]. In XPS, specimens were dried with a series of alcohol solutions and prepared at high vacuum, which took a considerable amount of time and changed the environment of the specimens. Hence, the stability of oxygen free radicals might have been affected, and possible increased levels of oxygen could not be detected.

Third, only very great change in oxygen level might be detected in every specimen. The SBS test used 360 bovine incisors, and not every specimen tested in the bleached group showed a lower SBS than that in the control. Hence, additional statistical tests might be needed to detect different levels of oxygen and more specimens might be needed.

Fourth, Ruse *et al.*[14,25] undertook similar studies to us and obtained identical results. They suggested that the reported decrease in the adhesive bond strength of resin to enamel treated with 35% hydrogen peroxide was not caused by a change in the elemental composition of the treated enamel surface.

With respect to further research, more studies are needed to: (i) provide a detailed explanation of XPS results; (ii) explore the mechanism of hydrogen peroxide in bonding of enamel; (iii) ascertain the effects of GSE in

bonding of enamel after bleaching.

CONCLUSIONS

Within the limitations of this study, the conclusion can be drawn that the compromised SBS of total-etching adhesive to enamel immediately after bleaching can be restored by GSE in 1 min as long as the GSE concentration is ≥5%.

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