J Mater Sci: Mater Med (2009) 20:1001–1007
DOI 10.1007/s10856-008-3657-1

Effects of different preparation procedures during tooth whitening on enamel bonding

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Received: 11 April 2008 / Accepted: 1 December 2008 / Published online: 13 December 2008

Abstract The objective of this study was to assess effects of some clinically related preparation procedures during tooth whitening on enamel bonding properties. Sixty-two extracted human teeth were cleaned and divided into four groups. Forty-two of the teeth were left with their natural surface intact while 20 teeth were polished to form a flat surface. Half of the tooth served as the experimental side and received one of the two whitening products: Opalescence (10% carbamide peroxide) and Crest Whitestrips (6.5% hydrogen peroxide), for 2 weeks. Post-bleaching intervals included: 1 day, 1 week, and 2 weeks. On these days, tooth (10 mm × 1.5 mm × 1.5 mm) sections were evaluated using Raman spectroscopy, scanning electron microscopy and tensile bond strength tests. *T*-test, ANOVA test, and mixed model regression analysis were used to assess the differences. No significant difference existed between natural surface and polished surface teeth for all groups at both Day One and Week Two ($P > 0.05$). On Day One, both treated groups had significant lower bond strength than the control group ($P = 0.002$). After 2 weeks, no significant difference existed between any group ($P = 0.381$). SEM indicated that resin–enamel interfaces in bleached enamel exhibited more defects in granular formations when compared to the control. Raman results indicated a lower degree of polymerization (DP) of adhesive at the interface for treated teeth surfaces. In summary, pre-bleaching surface treatments such as polish or non-polish, had no effect on bond strength. Bleaching significantly decreased bond strength initially, but after 2 weeks, bleaching had no significant effect on bond strength. Storage time had significant effect on Opalescence treated enamel, but not on control and Whitestrip treated enamel. The decrease of bond strength may be related to interfacial defects and low DP due to oxygen release after bleaching.

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1 Introduction

At-home teeth whitening systems have become popular and effective way to remove both intrinsic and extrinsic stains from teeth. Many whitening products are available on the market or through a dental professional for at-home use. There are two forms of whitening products available to the public, at-home bleaching products and in-office bleaching products. To enhance the effects of the bleaching process carbopol is added to most in office bleaching agents. Carbopol is an additive in carbamide peroxide that enhances the materials adhesion to enamel and extends the release of oxygen from peroxide [1].

The whitening agent in most whitening systems is hydrogen peroxide or carbamide peroxide which eventually converts to hydrogen peroxide and urea. Tooth discoloration is thought to be removed by hydrogen peroxide by means of oxidation [2]. Whitening materials cause changes in the morphology of enamel similar to etching that include a loss of prismatic form [3]. These changes to the surface of enamel are important to understanding what occurs during the bonding of composite to enamel. Studies have shown that for a period of up to 2 weeks after bleaching has been completed, the bond strength of the enamel surface to composite resin has a slight decrease [4–6]. However, it is argued that all the teeth were polished

to obtained flat enamel surface for bond strength studies, which might not simulate the clinical situation where natural surfaced teeth are used. In addition, the reason for reduction in bond strength of the composite resin for 2 weeks after whitening is not known yet. During the bonding process of the adhesive and composite resin to the enamel the residue from the whitening agent or the change in surface morphology could be the cause of this bond strength reduction. Studies have shown that if the surface layer of enamel is removed prior to bonding then there is no reduction in bond strength [5]. However dentists try to conserve as much healthy tooth structure as possible when performing restorations, so this is not a practical procedure for clinicians to perform during restorations. It is important to understand the change that occurs to enamel chemically and/or morphologically to cause this reduction so that a procedure can be developed for clinicians to eliminate or shorten the period of waiting 2 weeks to perform enamel surface bonding after teeth whitening. The goal of this study was to understand why there is a reduction in bond strength for 2 weeks after whitening has been completed by analyzing the enamel surface chemically and morphologically and to assess if there is a difference between polished surfaced and natural surfaced teeth for tensile bond strength tests.

2 Materials and methods

2.1 Specimen preparation

Sixty-two extracted human teeth with no surface caries or enamel defects were stored at 4°C in sterile Delbecco's phosphate saline (cellgro DPBS, Mediatech, Inc., Herndon, VA,USA) with 0.002% sodium azide were used for this study. The teeth were collected after informed consent was obtained under a protocol approved by the UMKC adult health sciences institutional review board. At the start of the study the teeth were cleaned with pumice, water, and a soft rubber prophy cup on a slow speed hand-piece. The teeth were then stored in an artificial saliva solution for the remainder of the study at 37°C. To prepare 1 L of artificial saliva 0.22 g $CaCl_2$ $2H_2O$, 0.12 g KH_2PO_4, 11.16 g KCL and distilled water were used. The pH value of the solution ranged from 7.04 to 7.07 and the solution was changed every 3 days. The pH of the solution was measured using the Accumet pH meter (Fisher Scientific, Pittsburgh, PA, USA).

2.2 Specimen selection and time intervals

Forty-two of the teeth with the natural surface left intact were randomly divided into two groups. The remaining 20 teeth were polished to create a flat enamel surface using 320, 600, and 1200 wet grit CarbiMet Discs sandpaper (Buehler Ltd, Lake Bluff, IL, USA) and were randomly divided into two groups. All the teeth were sectioned in half longitudinally. Half of the tooth served as the experimental side and received one of the two whitening products according to manufacturer's directions for 2 weeks. Twenty-one of the 42 teeth were randomly selected to receive Crest Whitestrips (6.5% hydrogen peroxide) (Procter& Gamble Company, Cincinnati, OH, USA) and the other 21 teeth received Opalescence Gel (10% carbamide peroxide) (Ultradent Products, Inc., South Jordan, Utah, USA). Ten of the 20 polished surfaced teeth were randomly selected and received Crest Whitestrips (6.5% hydrogen peroxide) and the other 10 polished teeth received Opalescence Gel (10% carbamide peroxide). The Opalescence Gel was applied for 6 h per day and the Crest Whitestrips were applied for 30 min twice a day for 2 weeks to the experimental side of the tooth. The other side of the tooth did not receive any whitening product and served as the control side. During the whitening process, the teeth were stored in an environmentally controlled chamber simulating oral cavity conditions (PLASLABS, Lansing, Mich, USA). After whitening each day the teeth were cleaned with distilled water and a tooth brush. The teeth were then placed into artificial saliva solution at 37°C.

Post-bleaching time intervals included: 1 day, 1 week, and 2 weeks for the natural surface teeth and for the polished teeth the time intervals included: 1 day and 2 weeks. For the teeth with their natural surface left intact, seven of the teeth whitened with Crest Whitestrips and seven of the teeth whitened with Opalescence where randomly selected on 1 day, 1 week, and 2 weeks. For the polished surface teeth, five teeth whitened with Crest Whitestrips and five of the teeth whitened with Opalescence where randomly selected on 1 day and 2 weeks. On these days, the teeth were sectioned in half and the roots were removed with a water-cooled diamond saw (ISOMET 1000, Buehler Ltd, Lake Bluff, IL, USA).

2.3 Application of the bonding agent and composite

The bonding was completed using the adhesive, Single Bond Plus (3 M ESPE, St. Paul, MN, USA), and composite resin, Filtek Z250 (3 M ESPE, St. Paul, MN, USA). The application of the adhesive started with etching the tooth for 15 s then rinsing the tooth with distilled water for 10 s. Next the specimen was dried with cotton and air dried for 10 s. The adhesive was then applied in two coats, the first coat was air dried for 5 s and the second coat was air dried for 10 s. Next the adhesive was cured for 10 s with visible-light source (Dentsply Spectrum 800, Milford, DE, USA) at 800 mW/cm^2. The composite resin was then applied in layers of no more than 2 mm height to a height of 5 mm

and then cured for 20 s. The specimens were then stored in distilled water at room temperature for 24 h.

2.4 Mechanical testing regimen

Rectangle bar specimens (10 mm × 1.5 mm × 1.5 mm) sectioned using a water-cooled diamond saw were used to test mechanical properties. 5 mm of the length of each specimen consisted of composite and the other 5 mm consisted of tooth. Due to the difficulty of cutting beams exactly 1.5 mm by 1.5 mm, the cross-sectional area of the beam was calculated based on the exact dimensions measured by an electronic digital caliper (Marathon, Ontario, Canada) before testing. The tensile properties were determined for all specimens from 24 to 48 h of bonding after being stored in distilled water at room temperature. Specimens were tightly and fully attached to the upper and lower grips using cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA) with the composite-enamel interface located in the middle of the 3.5 mm gap between the grips and were load at a cross-head speed of 0.5 mm/min using SSTM-500 mechanical tester (United Calibration Corporation, Huntington Beach, CA) with a 250 lb load cell. The ultimate tensile strength (UTS, MPa) of each specimen was calculated as the maximum force at the point of failure divided by the specimen cross-sectional area. Prior to mechanical testing, the specimens were carefully examined for defects. Specimens with defects were not used. ANOVA, *t*-tests, and Duncan tests were used to assess the differences between the natural surface and polished surface groups, non-treated versus treated groups, and time intervals.

2.5 Scanning electron microscopy (SEM)

The specimens were first viewed using SEM under their natural surface without coating to preserve the interface for micro-Raman spectroscopy. The dry specimens were cut into 2–3 mm long bars with a diamond saw and mounted to aluminum stubs using cyanoacrylate glue**.** The specimens' natural surface interface was analyzed with an XL30 ESEM-FEG SEM Microscope (FEI Company, Hillsboro, OR, USA) at 500 V accelerating voltage to evaluate the presence of the interface and resin tags. The specimens were then examined using micro-Raman spectroscopy, since this is a non-destructive technique, these specimens were still available to be coated and analyzed using SEM. Following micro-Raman analysis, the specimens were coated with gold–palladium to prepare for SEM. Specimens were examined at a variety of magnifications with an XL30 ESEM-FEG Microscope at 15 Kv accelerating voltage**.** Analysis was performed on the adhesive interfaces along with composite and enamel surfaces.

2.6 Micro-Raman spectroscopy

The micro-Raman spectrometer (Lab RAMHR800, Horiba Jobin Yvon, France) consisted of a laser beam (632 nm) focused through both 50× and 100× Olympus MPLAN objectives to a 1–2 μ beam diameter. Raman back-scattered light was collected through the objective and resolved with a monochromator. The spectra were recorded. The slit width of the spectrograph was set at 100 μm and the hole diameter was set at 400 μm, providing spectral resolution of 8 cm^{-1}. Two scans of spectra (with a 60 s accumulation time each) were obtained from each site. The laser power was approximately 7 mW; no thermal damage of the specimen was observed during measurement. An imaging system and high-resolution monitor were used to allow for visual identification of the position at which the Raman spectrum was obtained. Each adhesive/enamel/composite interface slab was mounted at the focus of the objective. To investigate difference of the adhesive interface for specimens in all groups from Day One, Raman spectrum from the adhesive the interface were collected The peak located at 1,637 cm^{-1} indicates un-reacted C=C double bond of the adhesive, and the peak in 1,608 cm^{-1} represents the carbon to carbon bonds in aromatic rings in the Bis-GMA molecules of the composite [7]. The ratios of the area at peak 1,637 and 1,608 cm^{-1} for both the adhesive monomer and polymer using the following equations: $R_{POLYMER}$ = Area 1,637 cm^{-1}/Area 1,608 cm^{-1} in polymerized adhesive, and $R_{MONOMER}$ = Area 1,637 cm^{-1}/Area 1,608 cm^{-1} in adhesive monomer before light curing. The degree of polymerization, (PD), of the adhesive could then be calculated using the following equation: $PD = 1 - ((R_{POLYMER})/(R_{MONOMER}))$.

The data were entered into and analyzed with SPSS 15.0 Statistical program (SPSS Inc., Chicago, IL 60606). All individual pair-wise comparison was conducted either by *t*-test or One-Way ANOVA for match design. A mixed model for multivariable regression analysis was developed with time (1 day, 7 days, and 14 days) and treatment (none, Opalescence, and Crest Whitestrips) as fixed effects and tooth surface type (natural or polished) as a random effect. The outcome variable was bond strength. Statistical significance level was set at $P = 0.05$.

3 Results

3.1 Mechanical testing

The analysis of natural surface versus polished surface tensile bond strengths for Day One and Week Two is presented in Table 1. ANOVA test showed no significant difference existed between natural surface and polished

surface teeth for all groups at both Day One and Week Two ($P > 0.05$).

With no significant difference revealed, natural surface groups and polished surface groups in the same time interval and surface treatment were combined for analysis of non-treated surfaces and treated surfaces. Also, natural surface groups and polished surface groups within the same surface treatment were combined for analysis of time interval on tensile bond strength. Table 2 shows the results for each time interval based comparing non-treated and treated teeth surfaces. On Day One, ANOVA test revealed a significant difference existed between the control group and the treated surface groups for both Crest Whitestrip and Opalescence ($P = 0.002$). For Week One time interval, a significant difference existed between the control group and the Opalescence group but no significant difference existed between the control group and the Crest Whitestrip group. For Week Two time interval, no significant difference existed between the control group and both treated groups ($P = 0.381$).

Table 3 shows the results of each treatment group compared to the three time intervals. No significant difference existed for the Crest Whitestrip group at any time interval. According to Duncan Grouping, significant differences existed for the Control group and the Opalescence group. The significant difference for the control group occurred between Day One compared to both Week One and Week Two time intervals. The significant difference for the Opalescence group occurred between Week One and Week Two time intervals.

Table 4 summarized locus of failure. Overall, composite was the most common site for facture, followed by cohesive layer and interface. These three sites accounted for the vast majority of fracture locus (81%).

Using a mixed model with time and treatment as fixed effects and tooth surface type as a random effect, multivariable regression analysis showed that treatment ($P = 0.25$) and time ($P = 0.29$) were not a significant factor for bond strength after controlling for other factors (type III test).

3.2 SEM

All the representative SEM images shown are coated with gold–palladium from Day One and the accelerating voltage was 15 Kv. Figure 1a shows a representative SEM image (magnification of 50×) from the fractured surface of a control group specimen. The fracture occurred mostly in

Table 1 Day One and Week Two results: natural surface versus polished surface

Group		*N*	Surface	Bond strength (SD)	*t*-test	*P* value
1-Control	Day One	11	Natural	28.4 (10.9)	0.855	0.401
		7	Polish	25.2 (6.1)		
	Week Two	14	Natural	18.6 (8.4)	1.362	0.180
		10	Polish	21.8 (7.2)		
2-Crest	Day One	6	Natural	21.7 (7.9)	0.825	0.419
		4	Polish	19.3 (4.0)		
	Week Two	7	Natural	20.5 (4.6)	0.372	0.714
		5	Polish	21.2 (4.4)		
3-Opalescence	Day One	6	Natural	20.0 (5.8)	0.288	0.777
		5	Polish	19.3 (4.7)		
	Week Two	7	Natural	25.2 (4.1)	2.070	0.053
		5	Polish	20.0 (6.9)		

Table 2 Results for all time intervals based on surface treatment

Time	Group	*N*	Bond strength (SD)	ANOVA test	Duncan grouping
Day 1	1-Control	18	27.0 (9.1)	$F = 6.897$	A
	2-Crest	10	20.7 (6.6)	$P = 0.002$	B
	3-Opalescence	11	19.7 (5.3)		B
Week 1	1-Control	14	22.6 (7.7)	$F = 2.705$	A
	2-Crest	7	21.3 (7.5)	$P = 0.077$	AB
	3-Opalescence	7	17.3 (4.7)		B
Week 2	1-Control	24	22.9 (6.5)	$F = 0.978$	A
	2-Crest	12	20.8 (4.4)	$P = 0.381$	A
	3-Opalescence	12	22.6 (6.2)		A

Table 3 Results based on time comparison within the same surface treatment group

Group	Time	Bond strength (SD)	ANOVA	Duncan grouping
1-Control	Day 1	27.0 (9.1)	$F = 2.682$	A
	Week 1	22.6 (7.7)	$P = 0.074$	B
	Week 2	22.9 (6.5)		B
2-Crest	Day 1	20.7 (6.6)	$F = 0.044$	A
	Week 1	21.3 (7.5)	$P = 0.957$	A
	Week 2	20.8 (4.4)		A
3-Opalescence	Day 1	19.7 (5.3)	$F = 4.017$	AB
	Week 1	17.3 (4.7)	$P = 0.024$	A
	Week 2	22.6 (6.2)		B

Table 4 Summary of locus of failure

Break point	1-Control (%)	2-Crest (%)	3-Opalescence (%)	Total (%)
Enamel	8	11	14	10
Interface	11	32	31	22
Cohesive	37	21	19	28
Composite	34	26	31	31
Damage in removal	10	11	5	9
Total	100	100	100	100

the composite and only slightly through the adhesive interface, revealing a strong adhesive layer. Figure 1b shows a magnified view (magnification of 500×) of the same control specimen as Fig. 1a at the adhesive interface showing a high degree of consistency. Figure 2a, is an image of a Crest Whitestrip specimen with magnification of 50×, a magnified view (magnification of 500×) of the fractured surface of the same Crest Whitestrip specimen at the adhesive interface is shown in Fig. 2b. The bubbling effect of the adhesive layer in this image shows a lesser regularity of the adhesive layer when compared to the control specimen. Similarly, Fig. 3a shows a representative image of fracture surface from the Opalescence group, a magnified view of the same Opalescence specimen as Fig. 3a at the adhesive interface is shown in Fig. 3b. As with the Crest Whitestrip specimen, the Opalescence specimen shows bubbling and a lesser degree of regularity of the adhesive layer.

3.3 Micro-Raman spectroscopy

Representative Raman spectra obtained from bleached groups and controls are shown in Fig. 4. The ratio of the polymer, $R_{POLYMER}$ = (Area 1,637 cm^{-1}/Area 1,608 cm^{-1}) for the control group equaled 0.205 and for the treated surface group equaled 0.302. The higher the ratio of the polymer is, the higher the amount of carbon–carbon double bonds that have not been polymerized and therefore, a lower degree of polymerization of the adhesive. The degree of polymerization, PD = $1 - ((R_{POLYMER})/(R_{MONOMER}))$, calculated for the control group equaled 0.947 and for the bleach-treated specimen equaled 0.917. These results show a higher degree of polymerization for the control group compared to the treated surface group of the adhesive layer.

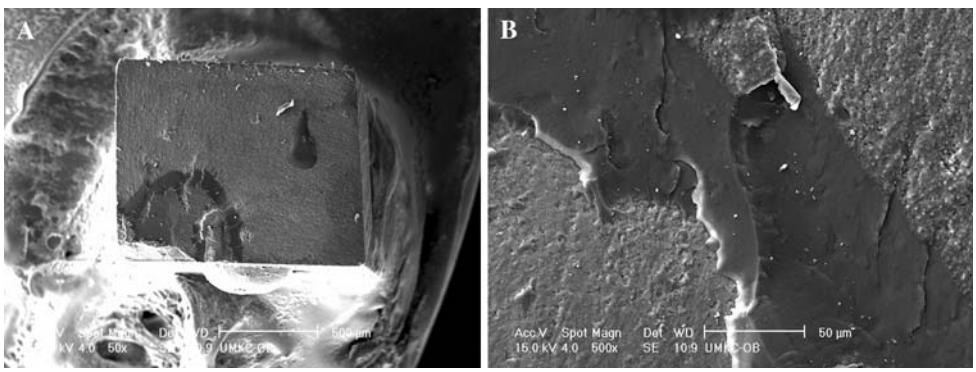

Fig. 1 Representative SEM images of the fractured surface from control group at Day One, **a** magnification = 50×; **b** magnification = 500×

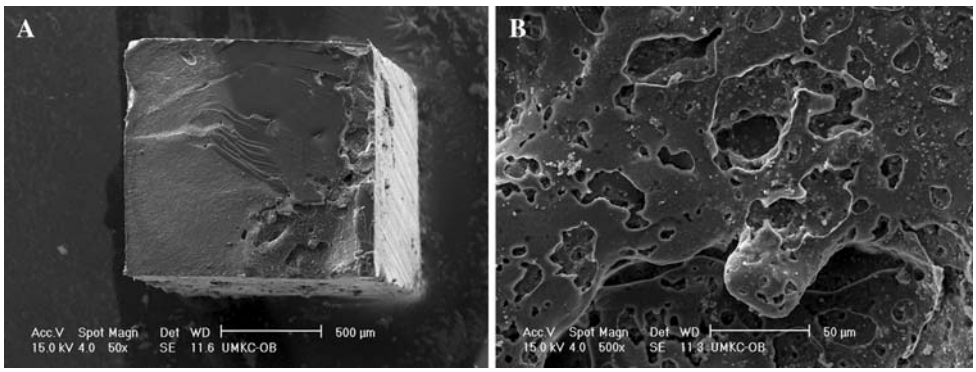

Fig. 2 Representative SEM images of the fractured surface from Crest Whitestrip group at Day One, **a** magnification = 50×; **b** magnification = 500×

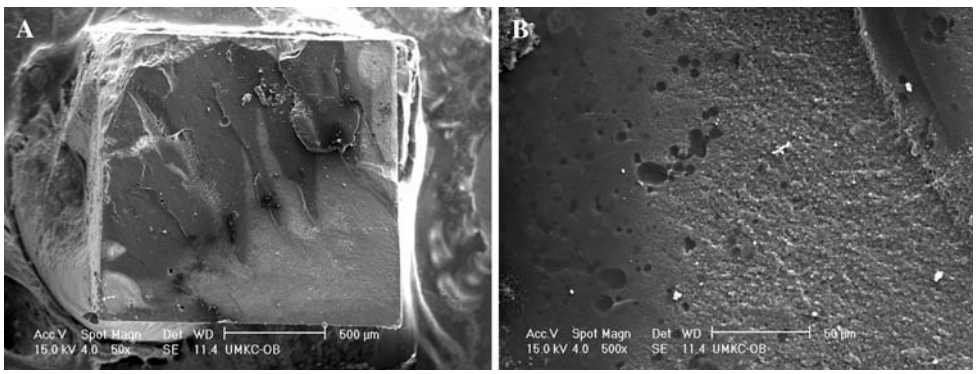

Fig. 3 Representative SEM images of the fractured surface from Opalescence group at Day One, **a** magnification = 50×; **b** magnification = 500×

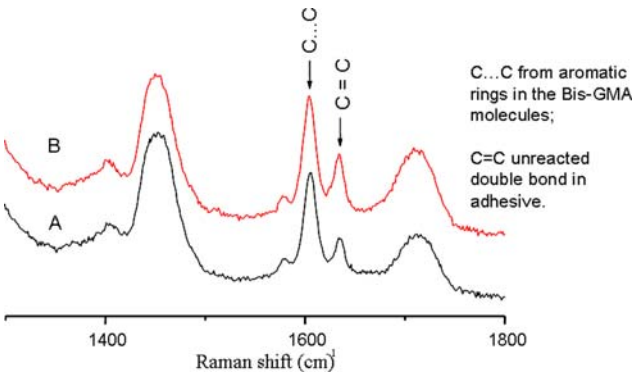

Fig. 4 Representative Raman spectra of adhesive resins in interfaces between enamel and composite. **a** from control group; **b** from Opalescence treated surface group

4 Discussion

No significant difference was found between natural surface teeth and polished surface teeth within the same group and time interval. Previous studies have only used either polished surface teeth or natural surface teeth and the reliability of comparing these studies was unknown. These results show that results from polished surface teeth studies can be compared with studies with the natural surface left intact for enamel bonding. This will be valuable to investigate studies using different concentrations of carbamide peroxide and hydrogen peroxide along with different time intervals of testing bond strength.

It was reported that bonding to enamel after application of a whitening product had a lower bond strength than bonding to a non-treated enamel surface, which is consistent with the data presented [4, 5]. Both of the treated surface groups on Day One have significantly lower UTS than the non-treated surface group. At the 2 week time interval, there was no significant difference between the control group and both the treated surface groups. It is therefore important to understand the changes to the enamel surface over this time period to understand this reduction in bond strength immediately after whitening has been completed.

The slight reduction in bond strength of the control group from Day One compared to both Week One and Week Two (Table 2) was noticed. Even though it is not considered significant through the ANOVA test, it was found to be relevant through the Duncan Grouping, and should be noted. The slight difference between the two time intervals shows that there is some factor acting on the specimens during the storage intervals. Before this study, data were lacking on the time effect on each individual group. Possibly phosphate buffered saline (PBS) and/or the artificial saliva have some effect on the enamel surface that is exhibited over the storage interval. More research is needed to understand what is causing this reduction in bond strength for the control group.

The SEM analysis of the specimens from Day One revealed that most of the fractures for the control group

occurred outside of the adhesive interface and only a small portion of the fracture in the adhesive layer. These fractures either occurred in the composite, enamel, or a combination of the both. Fractures from the treated specimens showed a greater proportion of the break occurring in the adhesive layer rather through the composite or enamel layers. These results reveal a stronger adhesive interface for the control group when compared to treated specimens on Day One which correlates well with the UTS data from Day One.

The SEM analysis of specimens in which the fracture occurred through some part of the adhesive layer revealed structural changes of the adhesive in treated surface specimens as compared to those of control surface specimens. A granular or bubbling effect was seen in the adhesive layer of the treated surface specimens. This bubbling did not occur in the control specimens, which indicates a change has occurred in the enamel surface during whitening for the treated surface specimens. Chemical analysis through Raman spectroscopy showed a lower degree of polymerization of the adhesive layer for the treated surface specimens compared to the control specimens. As known, the mechanical properties of polymer materials depend on their degree of polymerization. The lower degree of polymerization contributes to the lower bond strength for the treated specimens. To explain the lower degree of polymerization it is important to note that oxygen inhibits polymerization of the adhesive. Oxygen is released from carbamide peroxide and hydrogen peroxide during the whitening process as follows [8]:

$$H_2NCONH_2 \cdot H_2O_2 \rightarrow H_2NCONH_2 + H_2O_2$$

$$H_2O_2 \rightarrow 2HO^{\bullet}$$

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet}$$

$$HO_2^{\bullet} \rightarrow H^+ + O_2^-$$

$$2\,H_2O_2 \leftrightarrow 2H_2O + 2\{O\} \leftrightarrow 2H_2O + O_2$$

The lower degree of polymerization can be contributed to the oxygen release from the whitening agents.

The future goal of the study is to find an alternative to delayed bonding, i.e., anti-oxidants, to reverse negative effects of bleaching on bond strength, especially when immediate bonding must be performed after bleaching. This will save both the patient and dentist time and money.

5 Conclusions

Pre-bleaching surface treatments such as polish or non-polish, had no effect on bond strength. Bleaching significantly decreased bond strength at day 1, but after 2 weeks, bleaching had no significant effect on bond strength. SEM results indicated that resin–enamel interfaces in bleached enamel exhibited more defects in the granular or bubble-like forms. Raman results indicated oxygen released from bleach-treated enamel inhibited resin polymerization, caused defects in interfaces and lowered bond strengths.

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