Original Article

Effect of Bleaching on Shear Bond Strength of Composite Resins to Bovine Enamel Using Three Bonding Agents

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Abstract

Background and Aim: In many studies reduction in bond strength of bleached enamel has been pointed out. The decrease in bond strength raised concern in cosmetic dentistry. However, there is little information about the effects of bleaching on reduction of enamel bond strength in new bonding systems. This study aimed to investigate the effects of three dental bonding agents on shear bond strength of a composite to bleached enamel.

Materials and Methods: In this in vitro study, sixty intact bovine mandibular incisors were collected and randomly divided into six groups (n=10). In group A, the facial enamel was etched with 35% phosphoric acid gel, then a single layer of bonding agent (SingleBond, 3M, ESPE) was applied and light-cured for 40 seconds by a light-curing device (ColtoluxII colten) with 400 mw/cm² light intensity according to the manufacturer’s instructions. In group B: after etching of enamel surfaces, Clearfil SE-Bond (Kuraray Medical Inc.) was used. In group C: after etching of enamel surface, Clearfil S3 Bond (Kuraray, Medical Inc.) was used. In groups D, E, and F 35% carbamide peroxide (Opalescence, Ultradent Products Inc.) was applied on the enamel surfaces for 5 hours according to the manufacturer’s recommendations. Afterwards the specimens were rinsed thoroughly with distilled water for 60 seconds and dried with compressed air. Immediately after bleaching, bonding regimes similar to groups A, B, and C were carried out in groups D, E, and F, respectively. Then, cylinders of hybrid composite were bonded to the facial enamel surface of all specimens, using 3 dental bonding agents. After 24 hours, the specimens were thermocycled (5–55°C, 1000cycles), and then subjected to shear bond testing by a universal machine Zwick (Zo20, Germany). The data were analyzed by one- and two-way ANOVA and post hoc tests. All statistical tests were performed with SPSS version 11.

Results: The mean shear bond strength of SingleBond to bleached and unbleached enamel was 8.36±3.30 and 20.17±6.52 MPa, respectively. For Clearfil SE bond, bleached enamel exhibited bond strengths of 12.25 ±6.61MPa and unbleached enamel exhibited a bond strength value of 17.66±5.82 Mpa. The bond strength for Clearfil S3 bond was 6.66±2.57 Mpa to bleached and 10.40±3.65Mpa to unbleached enamel.

Conclusion: Shear bond strength of hybrid composite to bleached enamel in three experimental groups decreased but this reduction was not statistically significant for Clearfil SE bond.

Key Words: Bleaching shear bond strength, Bonding agent, Composite resin, Enamel
Introduction
Discoloration of teeth, especially the anterior teeth, is a serious problem in cosmetic dentistry and requires an effective treatment [1]. Reduced caries prevalence accompanied with increased living standards and improved awareness about cosmetic dentistry has provided a chance for the patients to seek more extensively for their cosmetic needs [2]. In the past, severe internal discolorations of vital and non-vital teeth were treated with crowns and veneers. This was often followed by damage to the tooth structure and biological problems [3]. With advances in dental materials, more conservative methods were proposed to solve these problems including bleaching and micro-abrasion [4]. Bleaching is used in mild discolorations such as fluorosis and in severe cases such as tetracycline pigmentation, or as a pre-treatment adjunct to laminate veneers [5]. Sometimes composite restorations are needed after bleaching and sometimes bleaching reveals defects in teeth that may require replacing previous restorations. Use of composite resins immediately after bleaching is still controversial [6]. Therefore, there are many reports on the reciprocal effects of bleaching materials and bond strength of composite resin materials to enamel. Many studies have reported a significant reduction in bond strength of composite material in bleached enamel compared to unbleached enamel [2, 3, and 7]. Several studies have shown that bond strength of composite resins decreases after bleaching. The delay period required to restore the bond strength following bleaching is still a matter of debate, but a one-week delay returns the bond strength to the unbleached tooth level [8]. As such, this study aimed to compare the effects of bleaching materials on shear bond strength of composite resin to bovine enamel using three types of bonding agents.

Materials and Methods
In this experimental study, 60 freshly extracted, caries-free, intact bovine mandibular incisor were collected and cleaned in water. Roots were amputated from the cervical 1/3, and pulp tissues were removed using a file. Then, the pulp chamber was rinsed with saline, and teeth were stored in 0.5% thymol solution at 25 °C for a week. Next, with the crown facing upward, samples were placed in a silicon matrix, and self-curing acrylic resin was poured into the matrix up to cementoenamel junction (CEJ). To prevent the thermal effects produced in acrylic resin, matrix was kept in water until the resin was completely cured. Then the teeth were removed from the matrix and their facial surfaces were polished with pumice, rubbercap, and a slow-speed hand-piece for one minute. Samples were randomly divided into 6 equal groups of 10 each.

Group A: The facial enamel surface of the samples were etched with 35% phosphoric acid gel for 15 seconds (Kimia, Iran), rinsed with water for 15 seconds, according to the manufacturer’s instructions and then dried with a mild air flow for 5 seconds. Next, a layer of bonding agent (Single Bond, 3M Dental Products, 3M, ESPE, Germany) as per manufacturer’s instructions was applied with a gentle movement of applicator, and then dried with mild air flow to dry. It was then cured using a light curing unit (Coltolux II, Coltene, Germany) at 400 mW/cm² for 40 seconds.

Group B: The self-etching Clearfil SE Bond (Kuraray, Japan) was used according to the manufacturer’s instructions. First, the primer was placed by an applicator onto the enamel surface for 20 seconds, and dispersed and thinned by a moderate air current. Then, the bonding agent was applied to the enamel surface using another applicator, and spread by a mild air spray. Finally, it was cured by the same light curing unit.

Group C: The self-etching Clearfil S3 bonding agent (Kuraray, Japan) was used according to the manufacturer’s instructions. The bonding agent was applied to the enamel surface for 20 seconds using an applicator, and dispersed by a moderate air flow for 5 seconds. Finally, it was cured for 10 seconds using the same light curing unit.

Groups D, E, F: According to the manufacturer’s instructions, 35% carbamide peroxide (Opalescence Tooth Whitening System, Ultradent Products...
Inc) was applied to the enamel surface for 5 hours. Then, samples were vigorously washed with distilled water for 60 seconds, and air dried. Immediately after bleaching, as in groups A, B, C bonding was applied to groups D, E, F in the same order. Subsequently, plastic molds with 3 mm heights and internal diameters of 3mm were used for placing the composite. Plastic molds were filled with a hybrid composite of A2 color (A2, Ivoclar Vivadent, Dinical Tetric Ceram). The excess restorative material around the contact area was carefully removed by scalpel from around and then cured in five directions (4 lateral sides and 1 top), in which each side was cured for 20 seconds (100 seconds in total). Finally, the plastic mold was gently cut with scalpel No. 11, and removed. Then, samples underwent 1000 cycle of thermo-cycling in 55-5 °C water (20 seconds in each water), and after storage for one week in normal saline at 25 °C, they were positioned in a universal testing machine (Zwick, Z0 20, Germany) at a crosshead speed of 0.5 mm/min in order to evaluate the bond strength (figure 2). Each sample was separately put in a specific place, and a maximum shearing force of 100 MPa was applied exactly at the junction between the enamel and composite (CEJ), and thus, the force at fracture was calculated in MPa.

After verifying the normal distribution of data with one-sample Kolmogorov-Smirnov test, the two-way ANOVA test was used to determine the effects of bonding and bleaching on shear bond strength. Considering the significance of reciprocal effects of the variables, the independent sample t-test was used to determine the bleaching effect on each bonding type. To compare the bonding strength of each bonding agent in bleached and unbleached groups, one-way ANOVA and post hoc (Tamhane type) tests were used (given inequality of variances in different groups). The raw data were analyzed by SPSS-11 software.

**Results**

The mean shear bond strength of single bond to bleached enamel was 8.36±3.3 MPa, and that for unbleached enamel was 20.17±6.52 MPa. With Clearfil SE bond to bleached enamel, the shear bond strength was 12.25±6.61 MPa, and that for unbleached enamel was 17.66±5.82 MPa. In the case of Clearfil S3 bond to bleached enamel, mean shear bond strength was 6.66±2.57 MPa, and that for unbleached enamel was 10.4±3.65 MPa. The results of two-way ANOVA test showed significant effects of bonding (p<0.001), bleaching (p<0.001), and interaction of two independent variables (p=0.036) on the shear bond strength. The shear bond strength distinctly decreased in all samples after bleaching. Statistically, the difference between group B (unbleached Clearfil SE bond) and group E (bleached Clearfil SE bond) was not significant (p=0.075). While, in the case of single bond (p<0.001) and Clearfil S3 bond (p=0.021), the difference between bleached and unbleached groups was significant. In bleached groups, no significant difference was observed in the three types of bonding agents (p=0.089), which is a little more than the crucial value of P (p=0.05). In the unbleached groups, the level of bond in Clearfil S3 bond was significantly lower than in the other two groups (p<0.05), but no significant difference was observed between level of bond in Clearfil SE bond and single bond (p=0.757). (Table 1)
Table 1. Mean shear bond strength, standard deviation, and standard error for all experimental groups

| Bonding   | Bleach  | Number | Mean   | Standard deviation | Standard error |
|-----------|---------|--------|--------|--------------------|               |
| Shear     | S3 bond | No     | 10/4   | 3/65994            | 1/15738        |
|           | Yes     | 9      | 6/6611 | 2/57689            | 0/85896        |
| Shear     | SE bond | No     | 17/668 | 5/82003            | 1/84046        |
|           | Yes     | 9      | 12/2567| 6/61014            | 2/20338        |
| Shear     | Single bond | No     | 20/178 | 6/52971            | 2/06488        |
|           | Yes     | 10     | 8/363  | 3/30493            | 1/04511        |

Discussion

Nowadays, bleaching is of widespread acceptance amongst dentists. Sometimes, performing bonded restorations is necessary after bleaching. There are many articles on the interference of bleaching materials with bonding systems and composite resins. Previous studies show that bleaching interferes with adhesion of composite resins to teeth. Several studies have shown that the composite resin shear strength is decreased after bleaching treatment [8]. In the present study, as in many other studies, bovine teeth were used to compare shear bond strength of restorative materials to the bleached and unbleached enamel surfaces because preparation and collection of a large number of healthy intact human teeth would be very difficult. These results were confirmed by studies conducted on human teeth [9, 10]. The present study is in line with many of the past studies, and showed that after bleaching, shear bond strength decreased in all groups, but this decrease was insignificant in Clearfil SE bond. To explain reduction in shear bond strength after bleaching, studies show that changes due to reduced mineral content, increased porosity, together with reduction in enamel prisms cause weak surface bonding [11, 12]. Dishman et al. in 1994 showed that the quality of composite bonding to enamel may be compromised by a reduction in number of present resin tags. So, they proposed that the effect of bleaching on shear bond strength is due to the bleaching mechanism, which impedes polymerization of resin bonding agent [12]. Garcia et al. in 1993 examined the bleaching effect on shear bond strength of composite to enamel, and found that shear bond strength of composite to enamel reduced 24 hours after bleaching, which is in agreement with results found in this study [13]. Perdigo et al. point out in their studies that loss of calcium, reduction in microhardness, and changes in organic component can be important factors in reducing the bond strength to enamel after bleaching [14]. Van der Vyver P.J, Titley K.C, Dishman M.V, and Ghavam in separate studies have shown that the reduction in bond strength could be due to permeation of hydrogen peroxide into enamel and formation of free-radicals, which inhibit the initiation of polymerization and formation of resin tags, which concurs with the results of this study [9, 15, 12, 16]. Zho et al. in 2000 showed that peroxide ions can replace free radicals in the apatite hydroxide network, and thus, produce apatite peroxide, causing destruction of the enamel prisms structure [17]. Several methods have been proposed for prevention from the clinical problems associated with reduced bond strength after bleaching: the most common is delaying application of bonding agent (any type) after bleaching [16]. Shimahara M.S et al. and Van Der Vyver et al. in 2004 reported that the best time for restoration of enamel and dentin is two weeks after bleaching, since the resin bond strength to enamel be improved [12-18]. Bulucu et al. also found that in the samples restored two weeks after bleaching, the difference in bond strength, compared to control group was insignificant. They also stated that the type of light cure system did not affect bond strength [19]. Other studies have shown that storing the samples in distilled water or in artificial saliva after bleaching and before bonding can improve resin bond strength by removing layer of...
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oxide from enamel surface induced by bleaching [20]. Khalili et al. stated that bleaching induced oxygen concentration onto the enamel surface inhibits curing of some resin tags, so restorative composite resins and bonding materials that contain accelerators - which facilitate polymerization and formation of long polymer chains- released monomeric free radicals that react with oxygen and inhibit polymerization [21]. Margeson et al. in 1990 stated that bond strength increases when free oxygen is reduced or destroyed by Argon [22]. Leonetti et al. found that laser Er:YAG (λ=2.94 μm, 25.56 J/cm²) had no effect on the bond tensile strength after bleaching and before restoration [23]. The results of Lai et al. and Kimyei et al. studies indicate that application of antioxidants like sodium ascorbate and sodium sulfite for 3 minute, after bleaching and before bonding, improved and even increased enamel bond strength to the level of the control group [24, 25]. Companies like Ultradent have marketed products such as Buster Bleach that can improve bond strength by reducing surface activated oxygen. Other studies consider the presence of organic solvents in bonding materials effective in reducing the effect of bleaching on enamel bond strength [26-28]. Nour-eldin A.K et al. proposed that acetone was the best solvent for resin application on conditioned and bleached tooth surface [20]. Sung et al. expressed that alcohol-based bonding can better neutralize adverse effects of bleaching (due to alcohol reaction with residual oxygen) and consequently increase bond strength. Sung et al. concluded that if bonding was applied immediately after bleaching, alcohol-based bonding might cause less reduction in shear bond strength. But if there was a few days delay in restoration of tooth, the type of bonding material would not be very effective [2]. Shinahara and Mortazavi et al. in separate studies found that solvent agent in adhesives systems - water, acetone, or ethanol- cannot diminish negative effects of bleaching [18-27]. Lopes et al. in 2006 compared shear bond strengths of 4 acetone-based bonding systems and one-bottle to dentin and enamel with ethanol-based bonding and concluded that solvent type did not significantly influence enamel bond strength, but had an important effect on bonding to the dentin, and ethanol-based bonding produced better bond strength compared to acetone-based systems [29]. Miyazaki M et al. also found that shear bond strength clearly reduced, when bonding was applied to samples immediately after bleaching [30]. Results of the present study also showed a significant reduction in shear bond strength of composite to bleached tooth (immediately restored) compared to unbleached tooth. Bonding systems used in the present study were Clearfil SE bond and Single Bond, but in samples that were washed in water and bonding applied 24 hours after bleaching, only a small reduction was observed, which was statistically insignificant. Researchers have proposed that if samples are kept in water for 24 hours after bleaching, shear bond strength to enamel in self-etch systems will be less affected by bleaching materials. In the present study also, in case of Single Bond, shear bond strength was significantly reduced after bleaching, but the level of resin polymerization did not reach zero. This can be attributable to its ethanol-based solvent which was able to reduce the effects of the superficial oxide layer. Therefore, complete polymerization of the resin was not impeded. In Clearfil SE Bond, reduction in bond strength was also evident, though not statistically significant. In this case also, the alcohol present in HEMA was able to lower the effects of oxide layer to some extent. However, the higher bond strength of Clearfil SE Bond after bleaching is thought to be associated with the presence of two alcohol layers in HEMA, both in the first layer and in the bond, which can react with more free oxygen radicals and neutralize them. Perhaps, it could also be explained in the following manner; in Clearfil SE bond, when applying the first layer, nearly 20 seconds time elapses, which allows better infusion of solvent, and since it is not washed off, the 10 MDP and HEMA present in the first layer cover the superficial oxide layer and consequently prevents its negative effects reaching the second layer of bond. While in the case of Single Bond, the phosphoric acid that is used at the be-
beginning may expose more oxygen layer to the surface by removing smear layer, and as a result monomer comes into direct contact with oxygen. Also, in Clearfil SE Bond, despite its weak acidity and mild etching, the amount of mineral ions involved in hybrid bonding layer is expected to increase, which will lead to an increase in bond strength [31]. Since Clearfil SE bond contains 10 weight percent filler it has some desirable mechanical properties, which elevates bond strength to enamel. The low viscosity filler in Clearfil SE Bond with its high tensile strength can neutralize polymerization shrinkage forces [32]. It should be noted that the 10 MDP monomer (Methacryloxydecyl Dehydrogenephosphate), developed by Kuraray Company, due to its polar property, increases the bond strength through chemical bonding with mineral ions like calcium that exist in residual hydroxyapatite [32, 33]. In the present study, as well as in a study by Jaberi-Ansari et al., despite claims of increased bond strength by manufacturer (Kuraray Medical Inc), Clearfil S3 Bond showed the lowest bond strength of all three bonding materials tested [28]. A study by Senavongse et al. also confirmed this result, and found that the lowest bond strength to intact enamel was associated with one-bottle self-etch systems [33].

The Kuraray company attempted to increase permeation of bond into enamel, thereby increase bonding strength of Clearfill S3 bond by increasing acidity of monomers, but this acidity caused destruction of the primer before increasing permeation. Also, addition of filler and 10-MDP polymer to Clearfill S3 Bond did not help increase bond strength. A major drawback in Clearfil S3 Bond is being one stage, and due to the presence of both hydrophilic and hydrophobic monomers in one bottle, it may impair their performance and lower the efficacy of each component, and consequently reduce bond strength achieved by Clearfil S3 Bond. In this regard, Jaberi-Ansari argued that being one-stage means water and solvents of the bond such as alcohol or acetone exists in bonding solution. After application of the bond and before hardening by light, the water and solvents are expected to evaporate by air current. If these materials are not fully removed from the bonding, they can cause reduction in bond strength, through their negative effects on the polymerization process [28]. By drying bonding layer of Clearfil S3 Bond, and removing solvents from the primer, the resin layer thins, and due to formation of air-inhibited layer after radiation of light, polymerization of resin may not be fully achieved. Under this condition, the levels of non-polymerized acidic monomers increase. These agents attack the resin composite polymerization initiator system and interfere with its polymerization, which is followed by reduced bond strength. Also, unlike in Clearfil SE Bond, only one bonding layer is used in Clearfil S3 Bond. Here, again the protective effect of first layer (prime) in Clearfil SE bond that was explained earlier is an issue. In Clearfil S3 Bond monomer and acid are simultaneously exposed to activated oxygen, and thus, the oxide layer produced impedes resin polymerization and bond strength is reduced. In a study by Chuang S.F et al in 2007, similar to present study 10% carbamide peroxide containing different percentages of fluoride were used and presented that enamel composite bond may be compromised due to reduced number of resin tags, which inhibits polymerization of bonding resin. A delay of one to two weeks in application of bonding improves bond strength [34]. Barcillos et al. in 2010 examined the effect of carbamide peroxide gel 10%, 15%, and 20% on shear bond strength of Z 350-tiltech composite to tooth enamel and dentin, and the results were analyzed by one-way ANOVA and Tukey post hoc tests, with a conclusion that bond strength of composite to bleached enamel and dentin was influenced by different percentages of carbamide peroxide gel, which concurs with results obtained in this study [35]. In a study by Faiz & Khoroushi et al. in 2011, the conclusion was that, using antioxidants as buffering agents after bleaching, and a delay of one week in application of composite bond, the bond strength could be significantly improved [36]. Mazaheri et al. in their 2011 study examined bond strength of Z100 resin and reinforced glass iono-
mer with Vitremer resin to bleached enamel with 9.5% hydrogen peroxide and concluded that application of 10% sodium ascorbate hydrogel immediately before bonding and delay bonding for one week eliminated the negative effects of bleaching on enamel bond strength of composite resin and reinforced glass ionomer [37].

Conclusion
Given the conditions and limitations in this study and results of the statistical analysis, the following conclusions may be drawn:

1. When bonding agents are applied immediately after bleaching, bond strength drastically decreases.

2. If it is imperative that composite restoration be carried out immediately after bleaching, then the two-stage self-etch systems such as Clearfil SE Bond should be used. The single-stage self-etch bonding systems such as Clearfil S3 Bond is not recommended for use on non-etched enamel, due to the bond strength of lower than 17 MPa (minimum strength to withstand composite polymerization shrinkage).

Acknowledgements
The present article is part of research dissertation of restorative dentistry department. This research project was approved by the Faculty of Dentistry, Tehran University of Medical Sciences Research center, project number 3775. All concerned authorities are appreciated. Also, special thanks are given to Dr. M.J. Kharazi-Fard for his supervision of stages of statistical analysis.

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