

Original Article

Shear Bond Strength of Resin Bonded to Bleached Enamel Using Different Modified 35% Hydrogen Peroxides

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Abstract

Statement of Problem: Bleaching systems with different concentrations and applications are widely used to improve the visual appearance of the teeth, but one of the complications of these materials is reduction of bond strength for immediately bonding to the bleached enamel.

Objectives: The aim of this study was to evaluate the influence of using different modified hydrogen peroxide bleaching agents on the shear bond strength of composite resin bonded to the bleached enamel.

Materials and Methods: Forty-eight sound extracted premolar teeth were collected, sectioned 1 mm below the CEJ to detach the root. The proximal surfaces of the teeth were flattened using diamond disks and silicon carbide papers to achieve flat homogeneous enamel surfaces without exposure to the dentin. The teeth were randomly divided into four groups as follows (n = 12): group 1: bleaching with 35% hydrogen peroxide gel; group 2: bleaching with 35% hydrogen peroxide gel contained (casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)); group 3: bleaching with 35% hydrogen peroxide gel combined with fluoride; and group 4: bleaching with 35% hydrogen peroxide applying one week before resin restoration placement. Composite resin, Clearfil AP-X (Kuraray, Tokyo, Japan), was bonded on each tooth in the mould (4 mm diameter × 3 mm height) using Clearfil SE Bond (Kuraray, Tokyo, Japan). After 24 hours of storage and 1000 cycles of thermocycling, the shear bond strength of the specimens at a cross-head speed of 0.5 mm/min was measured in MPa. Data were analyzed using ANOVA and Tukey's post-hoc test.

Results: The minimum and maximum mean shear bond strength values were observed in groups 2 (15.82 ± 4.41) and 4 (21.00 ± 3.90), respectively. Multiple comparisons of groups revealed no significant differences among the groups except between group 4 and all the other groups. The most common type of failure was adhesive.

Conclusions: Using modified bleaching agents decreased the bond strength of the composite resin to the enamel when it was used immediately after bleaching.

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Introduction

Vital tooth bleaching which is a routine treatment in modern dental practice is accomplished by either an at-home technique or in-office procedures with high-concentration bleaching agents [1,2]. Although the clinical effectiveness of tooth bleaching has been demonstrated extensively [3], there are some concerns about potential complications. Whitening agents have adverse effects on the dental pulp [4,5] may decrease micro-hardness of the bleached substrate [6], and have deleterious effect on bond strength of the resin materials [7,8]. One of the theories regarding the deleterious effect of bleaching on the bond strength of resin materials is related to the decrease in bond strength with the free radicals from oxygen that remain in the dental tissues released by the bleaching agents. After an adhesive system application, the oxygen responds to the closures of the forming polymeric chains, finishing the polymeric extension, lessening the level of transformation of the adhesive system and resin composites and declining the bond quality [9]. The use of bleaching techniques modified by a remineralizing agent called CPP-ACP (casein phosphopeptide amorphous calcium phosphate) has been suggested in an attempt to recover minerals that are lost during bleaching [10]. A recent study revealed that the associative utilization of CPP-ACP and high concentration hydrogen peroxide may be a fruitful strategy for diminishing tooth sensitivity and restricting changes in the enamel morphology during in-office bleaching [11]. Another modification is using fluoride with bleaching agents to prevent either hypersensitivity or demineralization accompanying tooth-whitening therapy. The addition of sodium fluoride to the bleaching agent was found to generate fluoridated hydroxyapatite and calcium fluoride crystals on the enamel surfaces, which potentially accelerated the remineralization of the bleached enamel [12]. But there is inadequate evidence on the influence of hydrogen peroxide and CPP-ACP on composite-enamel bonding. Additionally, despite profound scientific suggestions which support the remineralization capability of fluoride [13], the impact of fluoride on resin-enamel holding is dubious. Decreased resin bond strength has been reported for fluoride-treated enamel, particularly

when acidulated sodium fluoride was utilized [14]. Hence, mixing fluoride and oxidizing agents might furthermore hinder the resin-enamel bonding or prolong the recovery period for suitable bonding. Nonetheless, the developing utilization of fluoridated and daily uses of dentifrices prompted us to re-evaluate the impact of fluoride on adhesive properties [15,16]. Whether a higher concentration of fluoride counteracts the adhesive quality is also questionable. At present, little work has been done on assessing the resin bond quality of the enamel treated with modified blanching agents.

The aim of this study was to evaluate the influence of modified hydrogen peroxide on the shear bond strength of composite resin to the bleached enamel. Therefore, the null hypothesis was that bond strength and mode of failure is similar in hydrogen peroxide and modified hydrogen peroxide bleaching agents.

Materials and Methods

Specimen preparation

In the present study, 48 non-carious, crack-free human premolars extracted for orthodontic reasons were used. The selected teeth were collected for a period of four months in private practices, under a protocol approved by the Ethics Committee of the University of Medical Sciences (N # 910709). The teeth were kept in 10% formalin (Shahid Ghazi Co., Tabriz, Iran) for one week at room temperature for disinfection and then cleaned with a periodontal curette and placed in distilled water that was changed every week. The mesial or distal surfaces of the premolars were flattened using diamond disks with a low speed handpiece under air and water spray and 600 grit silicon carbide paper to achieve flat homogeneous enamel surfaces without exposure to the dentin. Specimens were sectioned 1 mm below the CEJ and the roots of the teeth were detached. The mesial or distal surface of the premolar crown up to CEJ, were fixed in 25 (W) × 35 (L) × 15 (H) mm metal mold containing self-curing acrylic resin (Acropars TRII, Marlic medical Industries, Tehran, Iran). The longitudinal axis of each tooth was horizontal with the proximal surfaces facing up (Figure 1). The specimens were kept in cold water until the acrylic resin was completely cured to control the thermal effects generated by acrylic

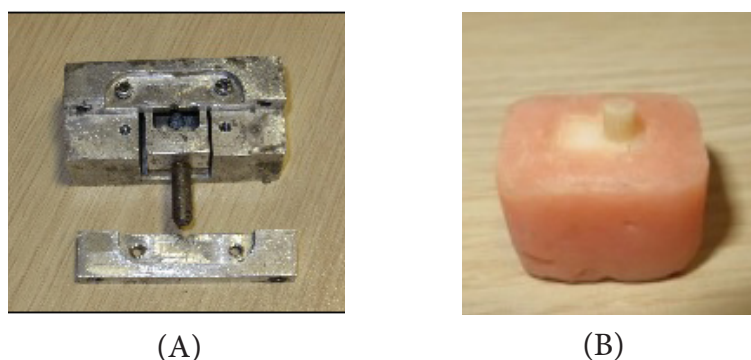


Figure 1: Specimens prepared for shear bond strength in the metal mould (A), using acrylic resin (B)

resin setting reaction. The detail information of the materials used in the present study is listed in Table 1.

Bleaching procedure

The specimens were randomly assigned into four following groups (n = 12):

Group 1: 35% hydrogen peroxide gel of 1 mm thickness was applied to the entire enamel surface. Three applications of 15 min were made for each

tooth. After each application, the gel was rinsed off from the enamel surface under running distilled water for 30s.

Group 2: bleaching with the mixture of Whiteness HP (Hydrogen peroxide 35%) and MI Paste (CPP-ACP) was applied as mentioned in group 1. The Whiteness HP/ MI mixture was prepared freshly by hand mixing with plastic pallet in a well; 0.6 mL of the hydrogen peroxide solution with 0.2

Table 1: Names, composition and manufacturers of the products used in the present study

Product	Composition	Manufacturer
Hydrogen peroxide (HP) 35% - Whiteness HP	Hydrogen peroxide, thickening agent, red dye, glycol, and water	FGM Produtos Odontológicos, Joinville, SC, Brazil
MI paste	Pure water, CPP-ACP, d-sorbitol, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, and butyl p-hydroxybenzoate	GC Corporation, Tokyo, Japan
Desensibilize KF 2%	Potassium Nitrate (5%) and Sodium Fluoride (2%)	FGM Produtos Odontológicos, Joinville, SC, Brazil
Clearfil AP-X composite resin	Bis-GMA, TEGDMA Barium glass, silica	Kuraray Medical Inc., Kurashiki, Japan
Clearfil SE Bond	Primer: MDP, HEMA, hydrophilic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine Adhesive: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, Camphorquinone, N,N-Diethanol p-toluidine, Silanized Colloidal Silica	Kuraray Medical Inc., Kurashiki, Japan

Abbreviations: Bis-GMA, bisphenol A diglycidylmethacrylate; TEGDMA, triethylene glycol dimethacrylate; 10-MDP, 10-Methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate.

Table 2: Shear bond strength of composite resin to bleached enamel with various modified bleaching gel applications (n = 12)

Groups	Description	Mean \pm (SD)
G1	Application of 35% hydrogen peroxide gel immediately before placing resin restoration	16.30 \pm (2.98) ^a
G2	Application of 35% hydrogen peroxide gel + CPP-ACP immediately before placing resin restoration	15.82 \pm (4.41) ^a
G3	Application of 35% hydrogen peroxide gel + fluoride immediately before placing resin restoration	16.58 \pm (4.17) ^a
G4	Application of 35% hydrogen peroxide one week before resin placing resin restoration	21.00 \pm (3.90) ^b

*Similar letters have no significant differences ($p > 0.05$)

mL of MI Paste, and one drop of the thickening agent until a homogeneous paste was obtained.

Group 3: bleaching with 35% hydrogen peroxide gel (2.25 g) combined with 0.75 g potassium fluoride weighed by a digital scale (KEM, Hong, China) and balanced with an accuracy of 0.005 g; the mixture was applied as mentioned in group 1.

Group 4: bleaching with 35% hydrogen peroxide applied one week before resin restoration. Bleaching procedures were performed the same as group 1 and after bleaching regime, the specimens were stored at 37 °C and 100% humidity for one week.

Bonding Procedure

For the groups 1 to 3, resin restoration was placed immediately after the bleaching agents were applied. Firstly, the self-etching primer (Clearfil SE Bond, Kuraray, Tokyo, Japan) was applied and spread over the enamel surface for 20 seconds. A light jet of air was applied to spread the primer

uniformly, and the adhesive (Clearfil SE Bond) was applied and light cured using Optilux 500 (Demetron-Kerr, USA) with a light intensity of 500 mW/cm² for 20 seconds in accordance with the manufacturer's instructions. After using the adhesive system and utilizing a special cylindrical metal mould (4 mm diameter \times 3 mm height), the composite resin, Clearfil AP-X, shade A3, was applied using incremental technique with a depth of 1.5 mm. Each increment was light cured for 20s. All specimens were incubated at 37°C and 100% humidity for 24 hours. The moulds were removed and the specimens were thermocycled (5-55°C, 1000 cycle 60-second dwelling time and 30 second transfer time) by an automatic thermocycler (Laab co. Mashhad, Iran). All procedures were performed by a single operator.

Bond Strength Test

The shear bond strength was tested in a universal testing machine (Santam, Tehran, Iran)

Table 3: Fracture modes of the bonding between composite resin and bleached enamel with various modified bleaching gel applications (n = 12)

Group	Adhesive	Cohesive	Mixed
1	9 (75.0%)	1 (8.3%)	2 (16.6%)
2	8 (66.6%)	2 (16.6%)	2 (16.6%)
3	8 (66.6%)	1 (8.3%)	3 (25.0%)
4	2 (16.6%)	5 (41.6%)	5 (41.6%)

at a cross-head speed of 0.5 mm/min. A parallel knife-edge shearing device was aligned over the bonded surface near the interface and the force was loaded to failure. Means and standard deviations were calculated in MPa by the following equation: $\text{MPa} = \text{N}/\text{mm}^2$. The mode of failure was determined by examining the specimens under a stereoscopic microscope (Blue light, Nikon, California, USA) at $\times 30$ magnification and classified as adhesive (adhesion failure), cohesive or mixed failures.

Statistical Analysis

Normal distribution of the data was confirmed using the Kolmogorov-Smirnov test, and data were analyzed using one-way ANOVA and Tukey's post-hoc test with a pre-set significance level of 0.05.

Results

The mean and standard deviations of shear bond strength values (MPa) for the experimental groups are presented in Table 2. The minimum and maximum mean shear bond strength values were observed in groups 2 and 4, respectively. One-way ANOVA showed significant differences among the study groups ($p = 0.001$). Post hoc Tukey's HSD test did not reveal any significant differences between the groups except for group 4 and all the other groups ($p < 0.05$). The mean shear bond strength in group 4 (21 ± 3.90) was significantly greater than those of other groups. Adhesive failure was the most common type of detachment among the groups with the exception of group 4 which had cohesive and mixed modes of failure. The number of different failure modes in various experimental groups is also displayed in Table 3. Various types of failure are demonstrated in Figure 2.

Discussion

To prevent either hypersensitivity or demineralization accompanying tooth-whitening therapy, some manufacturers have incorporated ingredients such as potassium nitrate, calcium phosphate, and fluoride into the bleaching agents. However, the influence of these additive ingredients on resin bonding is controversial. The present study evaluated the influence of modified hydrogen peroxides on the shear bond strength of resin materials to the bleached enamel. The adverse effects of bleaching agents on immediate resin-enamel bond strength are well documented [7-9,17-20]. A series of studies by Titley *et al.* [17,18] revealed the effects of bleaching with 25–35% hydrogen peroxide in impeding adhesion of the resin composite to the bleached enamel. Following bleaching, bond strength can be reduced to approximately 60% to 67% of that which is possible with the unbleached teeth, depending on the type and concentration of the bleaching materials [19]. Two major mechanisms have been proposed for the reduction in bond strength: the presence of residual oxygen that prevents resin infiltration and polymerization [17,18,20], or the occurrence of alterations in the morphology or organic and mineral components of the enamel and dentin [21-23]. In particular, a study by Perdigao *et al.* [22] showed that changes in the proteins and mineral content of the most superficial layers of the enamel might reduce the bond strength. Hegedus *et al.* [24] believe that changes in organic content may alter the mechanical properties, e.g. fracture toughness of the enamel and dentin. Since fracture toughness is considered to be an indicator of the resistance of the enamel against the propagation of

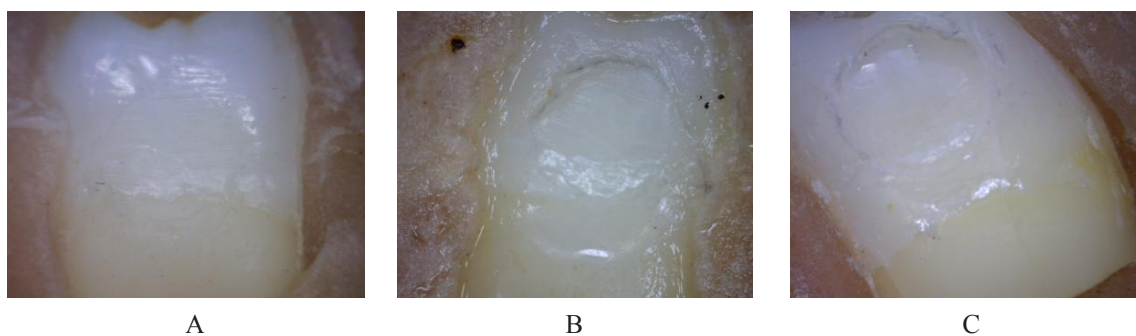


Figure 2: Different modes of failure: A) Adhesive, B) Cohesive, C) Mixed.

cracks, changes in this property can reduce the bond strength [25]. In addition, morphological alterations in the enamel following bleaching lead to a poor bonding surface due to elimination of the mineral content, increased porosity and/or alterations in the organic matrix [22,24,25].

Various methods have been suggested to improve the compromised bond to the bleached enamel and dentin, including using antioxidant materials [26-27], postponing the procedure for 24 hours to three weeks [20,28-30], and applying whitening agents with fluoride [31]. CPP-ACP has been demonstrated in animal and human studies to significantly reduce caries activity and promote the enamel subsurface remineralization. CPP-ACP advances remineralization by giving a supply of Ca²⁺ and PO₄²⁻ ions near the tooth surface and permits mobilization of these ions in regions of acid challenge [11,32]. The result of our study revealed that the concomitant use of CPP-ACP and hydrogen peroxide couldn't improve the compromised bond to the bleached enamel. Enamel treated with CPP-ACP has been found to be more resistant to a subsequent acid challenge. It seems that the reduced bond strength to the CPP-ACP treated enamel may be due to the inability of Clearfil SE Bond as a mild self-etch adhesive to effectively etch and penetrate to the hyper-mineralized enamel surface. Moreover, the remaining CPP-ACP complexes may stay on the enamel surface and be consolidated into the bonding layer or restrain the bond between the Clearfil SE Bond and enamel. Chuang *et al.* [33] revealed that treatment with 0.37% fluoridated carbamide peroxide maintained the microtensile bond strength as effectively as the unbleached enamel. The result of our study revealed that the concomitant use of fluoride and hydrogen peroxide couldn't improve the compromised bond to the bleached enamel. A study also revealed that fluoride treatment post-bleaching did not prevent the reduction in the enamel/resin shear bond strength in the time-period from immediately after treatment to 14 days after treatment [34]. The results of this study revealed that using modified bleaching agents acts as unmodified bleaching agents in decreasing the bond strength of the composite resin to the enamel if it was used immediately before composite restoration. However, if the enamel was bleached one week before restoration, the bond strength of

the composite resin to the bleached enamel was increased significantly. So our null hypothesis is rejected. In the present study with respect to the failure mode, the most frequently observed failure mode in groups 1, 2 and 3 was adhesive. It seems that the entrapped oxygen is regarded as the key cause of the inferior bond quality; the structural flaws on the enamel are also responsible for these changes. Also, the micro porosities in the enamel prisms and interprismatic regions may decline the bond strength by serving as stress promotion to cause adhesive fracture of the basic enamel during shear testing. In addition, as the bond strength increased, the frequency of adhesive failure decreased. In group 4 where the bond strength was high, we had mixed failure. In this study, both bond strength and failure modes on the bleached enamel were not related to include CPP-ACP and fluoride in HP. Further studies are suggested to be carried out on the potential benefits of bleaching with various modified whitening agents on the enamel and dentin followed by other composite resin and adhesive systems application.

Conclusions

In view of the limitations of this study, it could be concluded that application of modified hydrogen peroxide gels cannot improve the immediately compromised bond to the bleached enamel. The restorative procedure of bleached teeth using modified 35% hydrogen peroxide may have an adverse effect on directly bonding performance.

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References

1. Bernardon JK, Ferrari P, Baratieri LN, *et al.* Comparison of treatment time versus patient satisfaction in at-home and in-office tooth

- bleaching therapy. *J Prosthet Dent.* 2015.
- Perdigão J, Baratieri LN, Arcari GM. Contemporary trends and techniques in tooth whitening: a review. *Pract Proced Aesthet Dent.* 2004;16:185–192.
 - Joiner A. The bleaching of teeth: A review of the literature. *J Dent.* 2006;34:412-419.
 - Costa CA, Riehl H, Kina JF, *et al.* Human pulp responses to in-office tooth bleaching. *Oral Surg Oral Med Oral Pathol Oral Radiol and Endod.* 2010;109:59-64.
 - Kina JF, Huck C, Riehl H, *et al.* Response of human pulps after professionally applied vital tooth bleaching. *Int Endod J.* 2010;43:572-580.
 - Sasaki RT, Arcanjo AJ, Florio FM, *et al.* Micromorphology and microhardness of enamel after treatment with home-use bleaching agents containing 10% carbamide peroxide and 7.5% hydrogen peroxide. *J Appl Oral Sci.* 2009;17:611-616.
 - Barbosa CM, Sasaki RT, Florio FM, *et al.* Influence of time on bond strength after bleaching with 35% hydrogen peroxide. *J Contemp Dent Pract.* 2008;9:81-88.
 - Lima AF, Fonseca FM, Cavalcanti AN, *et al.* Effect of the diffusion of bleaching agents through enamel on dentin bonding at different depths. *Am J Dent.* 2010;23:113-115.
 - Suh BI. Oxygen-inhibited layer in adhesion dentistry. *J Esthet Restor Dent.* 2004;16:316-323.
 - Bayrak S, Tunc ES, Sonmez IS, *et al.* Effects of casein phosphopeptide -amorphous calcium phosphate (CPP-ACP) application on Enamel microhardness after bleaching. *Am J Dent.* 2009;22:393-396.
 - Borges BC, Pinheiro HM, Feitosa DA, *et al.* Preliminary study of a novel in-office bleaching therapy modified with a casein phosphopeptide-amorphous calcium phosphate. *Microsc Res Tech.* 2012;75:1571-1575.
 - Tanizawa Y. Reaction characteristics of a tooth-bleaching agent containing H₂O₂ and NaF: in vitro study of crystal structure change in treated hydroxyapatite and chemical states of incorporated fluorine. *J Cosmet Sci.* 2005;27:295-296.
 - Hellwig E, Lennon AM. Systemic versus topical fluoride. *Caries Res.* 2004;38:258–262.
 - Barcroft BD, Childers KR, Harris EF. Effects of acidulated and neutral NaF solutions on bond strengths. *Pediatr Dent.* 1990;12:180–182.
 - Garcia-Godoy F, O'Quinn JA, Donohue M. Effect of a triclosan/copolymer/fluoride dentifrice prophylaxis on the shear bond strength of a composite resin to enamel. *Am J Dent.* 1991;4:167–169.
 - El-Housseiny A, Sharaf AA. Evaluation of fissure sealant applied to topical fluoride treated teeth. *J Clin Pediatr Dent.* 2005;29:215–219.
 - Titley KC, Torneck CD, Smith DC, *et al.* Scanning electron microscopy observations on the penetration and structure of resin tags in bleached and unbleached bovine enamel. *J Endod.* 1991;17:72–75.
 - Titley KC, Torneck CD, Ruse ND. The effect of carbamide-peroxide gel on the shear bond strength of a microfil resin to bovine enamel. *J Dent Res.* 1992;71:20-24.
 - Unlu N, Cobankara FK, Ozer F. Effect of elapsed time following bleaching on the shear bond strength of composite resin to enamel. *J Biomed Mater Res B Appl Biomater.* 2008;84:363-368.
 - Torneck CD, Titley KC, Smith DC, *et al.* Effect of water leaching the adhesion of composite resin to bleached and unbleached bovine enamel. *J Endod.* 1991;17:156-160.
 - Barkhordar RA, Kempler D, Plesh O. Effect of nonvital tooth bleaching on microleakage of resin composite restorations. *Quintessence Int.* 1997;28:341-344.
 - Perdigão J, Francci C, Swift EJ Jr., *et al.* Ultra-morphological study of the interaction of dental adhesives with carbamide peroxide-bleached enamel. *Am J Dent.* 1998;11:291-301.
 - Toko T, Hisamitsu H. Shear bond strength of composite resin to unbleached and bleached human dentine. *Asian J Aesthet Dent.* 1993;1:33-36.
 - Hegedus C, Bistey T, Flora-Nagy E, *et al.* An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent.* 1999;27:509-515.
 - Berger SB, Pazenhausen R, Martinelli N, *et al.* Effect of bleaching agents on the flexural strength of bovine dentin. *J Contemp Dent Pract.* 2014;15:24-27.

26. Comlekoglu ME, Gokce B, Kaya AD, *et al.* Reversal of reduced bond strength after bleaching. *Gen Dent.* 2010;58:264-265.
27. Gurgan S, Alpaslan T, Kiremitci A, *et al.* Effect of different adhesive systems and laser treatment on the shear bond strength of bleached enamel. *J Dent.* 2009;37:527-534.
28. Bittencourt ME, Trentin MS, Linden MS, *et al.* Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations. *J Am Dent Assoc.* 2010;141:300-306.
29. May LG, Salvia AC, Souza RO, *et al.* Effect of sodium ascorbate and the time lapse before cementation after internal bleaching on bond strength between dentin and ceramic. *J Prosthodont.* 2010;19:374-380.
30. McGuckin RS, Thurmond BA, Osovitz S. Enamel shear bond strengths after vital bleaching. *Am J Dent.* 1992;5:216-222.
31. Swift EJ, Jr. Effects of bleaching on tooth structure and restorations, part II: enamel bonding. *J Esthet Restor Dent.* 2008;20:68-73.
32. Borges BC, de Vasconcelos AA, Cunha AG, *et al.* Preliminary clinical reports of a novel night-guard tooth bleaching technique modified by casein phosphopeptide-amorphous calcium phosphate (CCP-ACP). *Eur J Esthet Dent.* 2011;6:446-453.
33. Chuang S-F, Chen H-P, Chang C-H, *et al.* Effect of fluoridated carbamide peroxide gels on enamel microtensile bond strength. *Eur J Oral Sci.* 2009;117:435-441.
34. Metz MJ, Cochran MA, Matis BA, *et al.* Clinical evaluation of 15% carbamide peroxide on the surface microhardness and shear bond strength of human enamel. *Oper Dent.* 2007;32:427-436.