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Bonding performance of self-etch adhesives to enamel bleached with different peroxide concentrations

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The aim of this study was to evaluate the micro-shear bond strength (µSBS) of one and two steps self-etch adhesive systems after enamel bleaching with photo-activated bleaching systems of different hydrogen peroxide (HP) concentration. Occlusal enamel of forty intact human molars were flattened and assigned into four groups. GI Unbleached, GII, GIII, and GIV were bleached with Pyrenees (3.5% HP), GC TiON (20% HP), and Hi-Lite (35% HP) respectively. Enamel treatment with one and two steps self-etch adhesives (Clearfil S3 Bond- S3, and Clearfil SE Bond-SE) then micro-tubes were fixed on enamel and filled with AP-X composite (n=5). Bond was tested with the universal testing machine. Data were analyzed using two-way ANOVA and Tukey's tests at 5 % level of significance. The µSBS was significantly different between adhesives (F=154.46; $p<0.05$) and bleaching systems (F=77.33; $p<0.05$) with significant interaction. Specimens bonded with S3 shows a significantly lower µSBS than those bonded with SE ($p<0.05$) in all groups. For both adhesives the bleached groups demonstrate lower µSBS than unbleached except specimens bleached with Pyrenees and bonded with SE ($p>0.05$). A significant difference was observed between groups of the bleaching systems ($p<0.05$). Different peroxide concentrations photo-activated bleaching systems adversely affect µSBS of one and two steps self-etch adhesives. Low concentration system (Pyrenees) does not influence the bond strength of two steps adhesive.

Key Words: Micro-shear bond strength, Bleaching, Self-etch adhesives

Introduction

Teeth's bleaching is an economical and conservative method to reverse unpleasant dental discolorations. In-office or power bleaching is one of the commonly used techniques because of their efficacy, short time consuming, and immediate result. The success of in-office bleaching relies on the high peroxide concentration of 25-35% formulation to induce high oxidative power. Currently, the photo- activated in-office bleaching systems gains high popularity due to their efficiency even with low peroxide releasing capability in a range of concentrations (3.5% to 35%). Its action powered by energizing the bleach material through adding an activator or colorant to improve light absorption from various sources like halogen, LED or laser (1,2).

Depending on the bleaching outcome, a case may require replacement of the old restorations or use composite or porcelain veneers. Several reports indicated a reduction in the enamel restoration bond when performed shortly after bleaching. One of the strongly suggested reasons for lowered bond is the presence of residual peroxide/oxygen within enamel pores that will interfered with resin polymerization mechanism compromising resin tags quality and attachment (3). Based on this assumption, successful attempts were carried out to reverse weak bond via minimizing the retained peroxide and /or other oxygen radicals by application of a reducing agent, delay bonding, treating enamel with alcohol or acetone based adhesive (4-6). With all measures the question is raised as whether adopting an agent of low peroxide level can overcome or at least inhibit the detrimental effect on enamel bonding since the diffusion within dental tissue is largely governed by the concentration gradient. In addition, the procedure will eliminate concern about safety as the cytotoxicity was also influenced by the concentration (7).

The self-etch adhesive systems form a reliable bond with a fewer steps, and operative time and can be a good clinical alternative to older system uses a separate technique of etching then bonding (8). Based on application steps such systems may employ a two-steps of hydrophilic primer followed by solvent-free hydrophobic adhesive or more developed of one step which blends hydrophilic and hydrophobic monomers with water and organic solvent into one bottle solution (all-in-one). Although the all in one system is easy to use than two steps but it exhibited lower performance due to the water content needed to maintain their acidity (9). A remarkable polymerization inhibitory effect was reported in water contains adhesive a factor may control the bonding behavior variation between one step and two-steps in response to the detrimental effect of bleaching (6). A recent report demonstrates that bond strength stability of self-etch adhesive systems is more sensitive to bleaching process in comparison to other adhesives (10). Adopting photo-catalytic molecules like visible light TiO2 could speed oxidation and enhancing efficiency, enabling the reduction of peroxide. The superiority of the two-steps self-etch adhesive over one-step was reported. However, the concern about the benefit of using two-step and low peroxide photo-activated regimes to overcome the undesirable effect remains. According to our knowledge, there is limited information that compares the adhesive ability of one- and two-step self-etch adhesive systems after bleaching with low level peroxided photo activated agents (11). Therefore, the purpose of this study was to evaluate and compare the µSBS of one and two-steps self-etch adhesive systems without selective etching to enamel bleached with different in-office photo-activated bleaching systems releasing various H_2O_2 concentrations. The null hypothesis to be tested was that the differences between the one and two step adhesive systems and bleaching systems will not affect the µSBS of the resin composite.

Materials and methods

Specimen preparation:

After approval by ethical committee (no.341/11-06-2019), forty intact human molars were cleaned with pumice and roots were resected with diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under copious water. The enamel of the occlusal surfaces was wet ground with 600-grit SiC paper to create a standardized smear layer and flat enamel surfaces. Surfaces were washed and dried with oil free triple syringe.

Experimental groups and bleaching procedure:

Specimens were randomly assigned into following groups : GI without bleaching while GII, GIII, and GIV were bleached with three in-office photo activated bleaching systems of various H_2O_2 releasing ability: (Pyrenees, Mitsubishi Gas Chemical Co. Inc., Japan.) 3.5% H_2O_2; (GC TiON in-office, GC America, USA) 20% H_2O_2; and (Shofu Hi-Lite office, Kyoto, Japan) 35% H_2O_2 Table 1. Bleaching systems were prepared and applied over enamel surfaces as manufacturer's instruction. Systems were photo-activated with a halogen light unit of 700 mW/cm^2 irradiance (Optilux 501, Demetron; Danbury, CT, USA). Unit irradiation was checked with radiometer (Jetlite light tester, J. Morita, Mason Irvine, CA, USA), then the treated enamel was washed and dried with triple syringe.

Table 1: Bleaching systems used in this study.

Agent	Main Component	H_2O_2% (pH)	Application
Pyrenees			Solution 1 & 2 were mixed for 1 min, Applied in 2mm thick, irradiated for 5 min total, washed and dried. Procedure repeated three times.
Solution 1	H_2O_2 <6%, stabilizer pH adjuster < 6%; Pure water	3.5% (6.0)	
Solution 2	TiO_2 <1%; stabilizer <5%; pure water, other		
GC TiON			Reactor applied and air blown. Syringes A&B were mixed, Applied, irradiated for 1 min, left for 5 min, washed and dried. Procedure repeated three times.
Reactor	Ethanol, TiO_2, thickener, pH conditioner, water		
Syringe A	35% H_2O_2	20% (neutral)	
Syringe B	30% carbamide peroxide, glycol solvente, thickener, Ph conditioner		
Hi-Lite			Powder and liquid mixed, small a applied for 5min and irradiation for 3min. Mixture was kept for another 2min then removed, washed and dried. The procedure was repeated for three times.
Powder	Potassium persulphinate, manganese sulfate, monohydrate, hydrated amorphous sílica, mixed sodium and calcium salt	35% (4)	
Liquid	35% H_2O_2, water		

Bonding procedure:

Groups were divided into two sub-groups and bonded with one and two steps self-etch adhesives (Clearfil S3 Bond- S3 & Clearfil SE Bond-SE, Kuraray Medical Inc., Tokyo, Japan.) (n=5). For SE bond the primer was applied over enamel for 20 seconds, spread and dried with a mild air blast followed by adhesive. For the S3 bond the adhesive was applied for 20 seconds and dried. Specimen received five translucent micro-tubes of 0.75 mm internal diameter and 0.5 mm high then adhesives were photo irradiated with halogen light unit for 10 seconds. Tubes were filled with a hybrid resin composite (Clearfil AP-X, Kuraray Medical Inc., Tokyo, Japan) and photo irradiated for 40 seconds. The composition of adhesives and restorative materials are shown in Table 2. The integrity of the bonded resin composite cylinders was examined under a light microscope and any defective bond was excluded. After 24 hours of storage at 37 °C, each specimen was fixed into the testing device with a cyanoacrylate adhesive of the tabletop universal testing machine (EZ Test, Shimadzu, Kyoto, Japan). A thin stainless steel wire (0.2 mm in diameter) was fitted at the enamel composite interface and loaded at 1.0 mm/min until bond failure. The µSBS was calculated by dividing the fracture load by the surface area and result expressed in MPa.

Table 2: Self-etch adhesive systems and resin composite used in the study.

Material	Composition
Clearfil S3 bond (One-step)	MDP, HEMA, bis-GMA, dl-Camphorquinone, water, ethanol, silanated colloidal silica.
Clearfil SE bond (two-step)	**Primer:** MDP, HEMA, dimethacrylate hydrophilic, Camphorquinone, N, N-diethanol-p-toludine, water. **Adhesive**: MDP, bis-GMA, HEMA, dimethacrylate hydrophobic, Camphorquinone, N,N-diethanol-p-toludine, silanated colloidal silica.
Clearfil AP-X	Barium glass, Silica, Colloidal silica, Silicon dioxide, (71vol.%, 0.1-15 µm), Bis-GMA, TEGDMA, photoinitiator

Statistical analysis:

The data of µSBS were analyzed using two-way ANOVA to assess the influence of both parameters bleaching and adhesive systems and their interaction using the statistical package for social science (SPSS 20, SPSS Inc., Chicago, IL, USA). Tukey's HSD was used as post-hoc test. All tests were performed at a 5 % level of significance.

Fracture mode analysis:

Interfaces of all groups were evaluated for fracture mode and ten debonded regions were randomly selected from each group and photographed under scanning electron microscope (JSM-5310, JEOL, Tokyo, Japan) at x150 and x1500 magnification. Mode of failure was classified into three types: adhesive failure (when enamel exposed); cohesive failure in resin materials (adhesive or resin composite remain); and mixed failure (combination of adhesive and cohesive). The surface area ratios of each failure type were calculated. The distributions of failure modes were subjected to a non-parametric test to compare group's difference.

SEM observation:

For observation of the changes within bleached enamel surfaces an additional two specimens were prepared from each group and treated as those used for µSBS test groups. After the rinsing and dehydration procedures, the enamel surfaces were coated with gold and observed under SEM to evaluate alteration in surface morphology. Photographs were recorded at magnification x150 and x1500.

Results

Means and standard deviations of the µSBS are summarized in Table 3. Two-way ANOVA demonstrated that the µSBS was significantly different according to the adhesive types (F= 77.33; $p < 0.05$) and bleaching systems (F= 154.46; $p < 0.05$) with a significant interaction in between (F= 16.59; $p < 0.05$). Tukey's test indicated that µSBS of the resin composite bonded with both adhesives to the enamel bleached with all systems were significantly lower than those bonded to unbleached enamel (control) except specimens bonded with two-step Clearfil SE bond after bleaching with Pyrenees system ($p > 0.05$). Both unbleached and bleached specimens bonded with Clearfil S3 have demonstrated significantly lower µSBS values in comparison to those bonded with the SE bond ($p < 0.05$). A significant difference was recorded between three bleaching systems regardless of adhesive system (one or two-step) ($p < 0.05$). Failure mode percentages displayed significant variations between adhesive types and bleaching conditions ($p<0.05$) (Figure 1). Bleached groups demonstrated predominantly adhesive failures. The failure was more predominant in Hi-Lite than other agents and in groups bonded with one step than two-steps that revealed higher cohesive (Figure 2). Pyrenees associated SE failure was lower than TiON and Hi-Lite but similar to unbleached. While Pyrenees associated S3 show a higher adhesive percentage than unbleached but lower than Hi-Lite.

Table 3: Mean and (SD) of µSBS for both adhesive systems in unbleached and bleached enamel groups (MPa).

Adhesive	Unbleached	Pyrenees	GC TiON	Hi-Lite
Clearfil SE	38.0(5.6) ^{A,a}	37.3(4.3)^{A,a}	28.2(5.1) ^{B,a}	22.9(4.6) ^{C,a}
Clearfil S^3	33.2(3.3) ^{A,b}	18.3(4.4) ^{B,C,b}	20.7(6.6) ^{B,b}	15.6(4.3) ^{C,b}

Means with different upper case letters within each adhesive system and lower case letters for each bleaching condition were significantly diferente

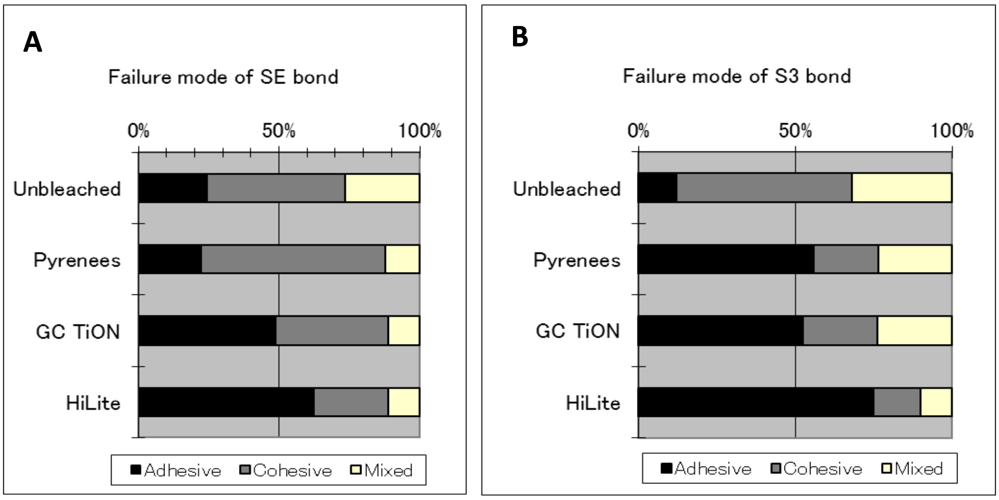

Figure 1. The percentage distribution of resin composite failure mode bonded with two (A) and one-step (B) self-etch adhesives for unbleached (control) and bleached enamel groups.

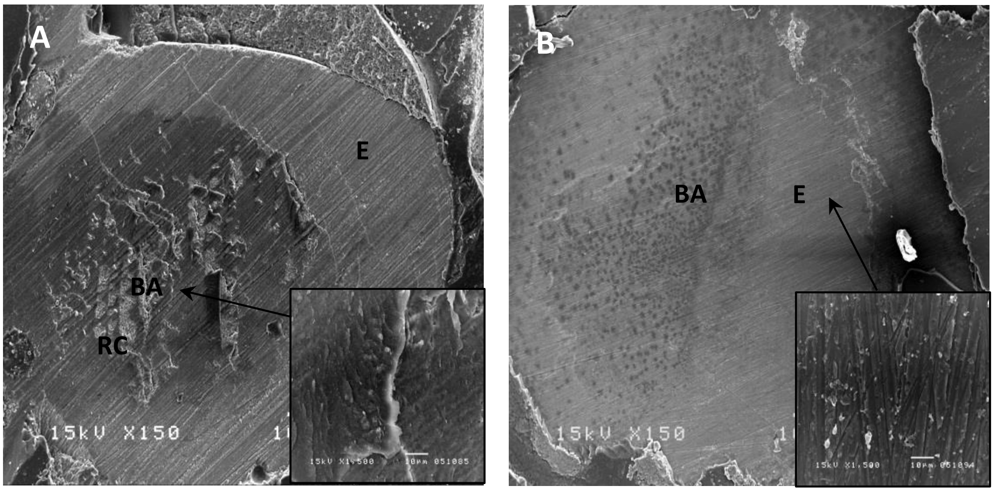

Figure 2. Representative micrograph of debonded interfaces show (A) cohesive failure predominance seen at x 150 within interface of specimen bleached with Pyrenees and bonded with two-steps Clearfil SE bond. Area of exposed enamel E, Resin composite remaining RC, and thick layer of bond agent BA magnified at x1500, and (B) adhesive failure predominance at x 150 from specimen bleached with Hi-Lite and bonded with one step Clearfil S3. Numerous dark spots spread over wide area of interface may indicate poorly polymerized bonding agent BA. The exposed enamel can be seen over whole interface. A higher magnification at x 1500 confirms the exposed enamel E.

SEM observation:

Pyrenees showed no obvious surface changes in comparison to unbleached enamel specimens (Figure 3 A&B) while enamel bleached with GC TiON and Hi-Lite systems displayed definitive changes in surface texture with amorphous chucky enamel appearance and surface porosities (Figure 3 C&D).

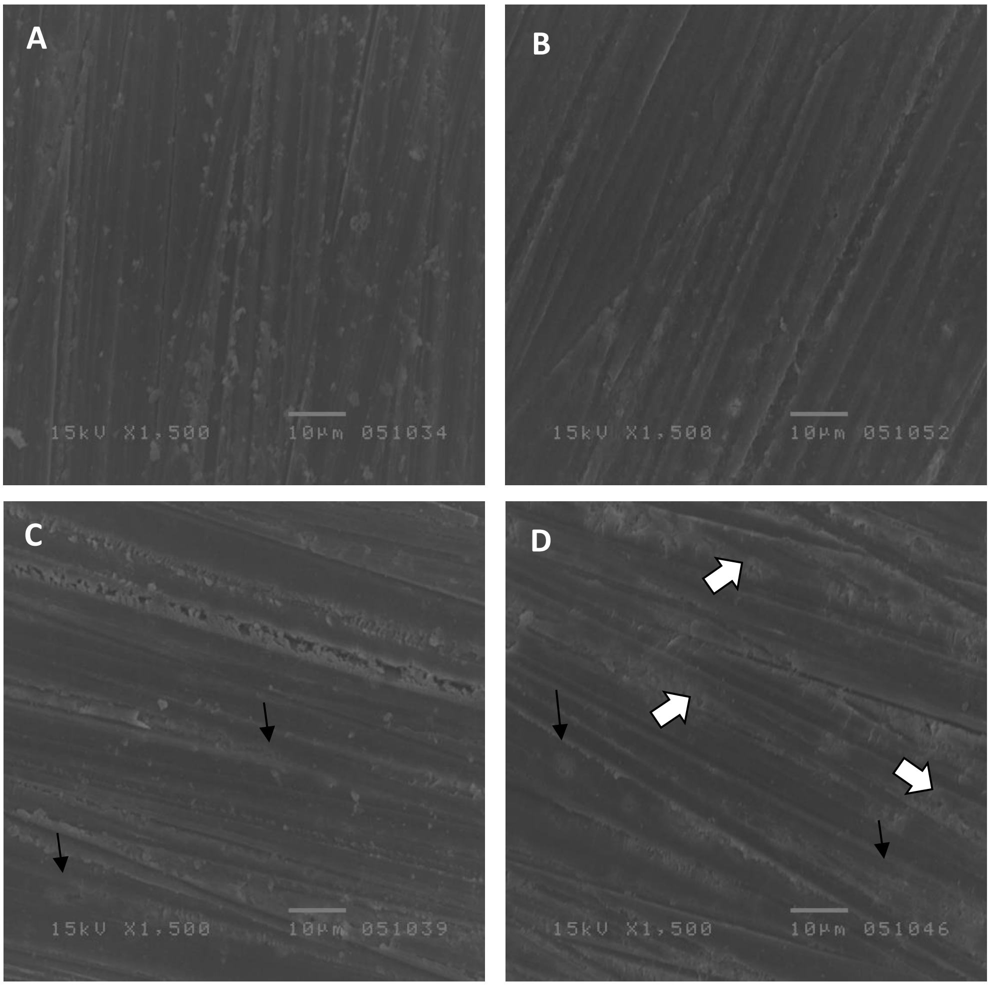

Figure 3. Representative photomicrograph of surface texture for (A) unbleached enamel reveal scratches, (B) enamel specimen bleached with Pyrenees show similar pattern to control with no obvious changes in texture, (C) GC TiON show slight changes with few number of white patches (line arrows) and (D) specimen bleached with Hi-Lite system revealed clear white patches with a chucky appearance (line arrows) and porosities (block arrows).

Discussion

The majority of teeth bleaching systems depend on H_2O_2 as a predominant and essential component. Due to simplicity and efficiency of self-etching adhesive many clinicians may encourage using for teeth restoration shortly following bleaching. This study was designed to evaluate effect of three in-office photo-activated bleaching systems elaborating various levels of H_2O_2 concentration on the enamel µSBS of two self-etch adhesive systems (One step and two-steps). Clinically in in-office bleaching the agent is applied on intact enamel surface. A procedure was not followed in this study. Flat enamel surface was prepared for the purpose of uniform bonding and standardization of bond test. Originally, the procedures of µSBS propose application of adhesive followed by air blasting for better distribution and solvent dryness. Similar steps where followed in our study without delimitation technique a possible reason for the high incidence of cohesive failures. Shimaoka et al. (12) where found that with delimitation there is a lack of cohesive failure with a higher incidence of adhesive. Similar to the other studies the bond strengths of both adhesives presented in this study were substantially reduced following enamel bleaching with all systems (9,11,13) except group bleached with Pyrenees and bonded with SE bond. The precluding of selective acid etching of enamel from the adhesion strategy should be considered as one limitation in this study and comparable research may be necessary to verify the significance of this step. Bleached groups produced adhesive failure more frequently than unbleached unlike interface of group bonded with SE bond after bleaching with Pyrenees show similar pattern to control. The reserved H_2O_2 within enamel pores will breakdown to release oxygen and water that diffuse and probably trapped within the hybrid and adhesive layer results in incomplete polymerization or interfering with resin impregnation (14,15). Incomplete polymerization was confirmed by differential calorimeter over bleached enamel (3). Optimal adhesives polymerization is an important parameter for bond durability since lower mechanical properties accompanied a low percentage of monomer to polymer conversion (16). Within overall groups the two-steps Clearfil SE adhesive produced consistently and significantly stronger µSBS than one step Clearfil S3 bond. Screening the data has demonstrated that the highest µSBS value recorded in one step adhesive failed to approximate even the lowest value in two-steps. A recent study has confirmed the advantage of Clearfil SE bond over one step after bleaching with photo-activated system (13).Compositional variation between the two adhesives may influence the result as Clearfil S3 combine the water and ethanol solvent with monomer resin blend thus called all-in-one adhesive a simplification necessitates increasing in the hydrophilicity of the system. Water rich adhesive can be detrimental to bond efficiency and stability (17,18). Presence of ethanol in one step was unable to counteract the adverse effect of residual peroxide as a proposed by some researches. Furthermore one step adhesives in nature are more permeable and more prone to nanoleakage thus high oxygen emanated from bleaching agents may easily trapped and compromising interface by polymerization rate inhibitory effect. SEM images displayed dark spot areas and porous aspect that might represent gaseous bubbling action suggesting the residual water and oxygen content affecting resin polymerization (Figure 2). A direct inverse correlation has been declared between the extent of polymerization of the adhesive films and permeability (16,19). Although two steps adhesive composed of primer contains water to help monomer ionization air dryness can easily remove excess water from applied primer on enamel. The second bonding resin layer plays an important role in substantial reduction of water sorption and penetration within adhesive interfaces by creating a hydrophobic barrier. High rate of adhesive polymerization in two-steps adhesives has been approved (20). Previous nanoleakage study cleared up this suggestion when they found the hydrophobic monomer may not mixed with excessive amount of water (21).

Pyrenees is innovative low 3.5 % H_2O_2 in-office photo activated bleaching agent includes visible-light titanium dioxide photo-catalytic molecules. Whitening efficiency of this agent has been investigated extensively under different light sources and in comparison with other in-office agents of highly concentrated H_2O_2. A promising result has associate activation with halogen light and when compared to agents of higher concentration (2,22). The important finding of this study is the absence of the reduction in µSBS of the specimens bleached with Pyrenees and bonded with two-steps self-etch adhesive. Failure mode of these specimens was almost similar to control. This was not the case in one-step adhesive as the value of µSBS was dramatically reduced in spite of using low peroxide bleaching agent. The reason probably related to the compositional variation and number of application step that strongly influence their polymerization rate later on bonding ability. Findings imply that the deleterious effect of low peroxide agent on the bond was dependent on the type of adhesive system an inference parallel to the recent report (13). A correlated result was also found with other study that evaluated microtensile strength of same adhesive systems used in this study after Pyrenees internal bleaching (23). The similarity in the result may indicate that bonding performance of the two-step adhesive will not affected by bleaching technique whenever low peroxide agent used. Hi-Lite is acidic powerful in-office bleaching system of 35% H_2O_2 while GC TiON in-office is a neutral system liberates approximately 20% of H_2O_2 in the final mixture therefore it may be considered as an intermediate concentrated agent between Pyrenees and Hi-Lite. For better efficacy activity of GC TiON was compensated by initial application of reactor contains visible light TiO2 photocatalyst molecules to accelerate bleaching of the next mixture. According to the result of this study a sever decrease in µSBS was associated both agents (Hi-Lite and GC TiON) irrespective of adhesive when compared to control group. A significant difference was also recorded between two agents where the bond value in group bleached with TiON was higher than Hi-Lite. These results were irrespective to the bonding system. The variation in concentration of H_2O_2 may be one of the major reasons responsible on bond strength diversity (24). System of higher level may give rise to a more compromised resin enamel interface. A suggestion indirectly supported by bond reversibility when agent of high peroxide concentration needs a longer post bleaching waiting period than low agent. Unlu et al. (5) find that one day period was sufficient to restore bond for 10% carbamide to bleached group meanwhile one-week interval was required for 35% H_2O_2.

In consistency with bond strength outcomes related both systems comma the morphological alteration observed over enamel bleached with Hi-Lite agent was more pronounced than what was seen on surfaces bleached with GC TiON has another factor may partially affect bond strength result. Increased porosity manifested by an "over-etched" appearance on enamel bleached by Hi-Lite was evident. The clear etching pattern and white patches on the enamel of Hi-Lite group suggesting acid demineralization. The high concentration of H_2O_2 and low pH=4 of Hi-Lite solution could participate in this enamel alteration. In contrast the GC TiON has lower concentration and neutral pH which is above the critical value of enamel demineralization (25,26). Based on the result of the study the null hypothesis was rejected as different systems of bleaching agent and adhesive affected the composite enamel µSBS value. One factor to be acknowledged is overlooking of non-light bleaching of the implemented systems a deprivation may need evaluation. It might be concluded that all photo-activated bleaching systems tested in this study adversely affects µSBS of one and two steps self-etch adhesives. System of lowest H_2O_2 concentration (Pyrenees) doesn't exert harmful effect on the µSBS of composite when bonded with two-steps adhesive. Although this study addressed a promising result of Pyrenees bleaching effect in relation to adhesive dentistry but the eventual result must be interpreted with caution because the whitening efficiency of this agent in comparison to other of higher concentration was not sufficiently reported clinically and further investigation into this area is necessary.

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Resumo

O objetivo deste estudo foi avaliar a resistência ao cisalhamento (µSBS) de sistemas auto-adesivos de um e dois passos após clareamento do esmalte com sistemas de clareamento foto-ativados de diferentes concentrações de peróxido de hidrogênio (HP). O esmalte oclusal de quarenta molares humanos intactos foi aplainado e atribuído em quatro grupos. GI Unbleached, GII, GIII, e GIV foram branqueados com Pyrenees (3,5% HP), GC TiON (20% HP), e Hi-Lite (35% HP) respectivamente. Tratamento de esmalte com adesivos de um e dois passos auto-adesivos (Clearfil S3 Bond- S3, e Clearfil SE Bond-SE), depois os microtubos foram fixados no esmalte e preenchidos com composto AP-X (n=5). A resistência ao cisalhamento foi testada com a máquina universal de testes. Os dados foram analisados usando os testes de ANOVA e Tukey de duas vias a um nível de 5% de significância. O µSBS foi significativamente diferente entre adesivos (F=154,46; p<0,05) e sistemas de clareadores (F=77,33; p<0,05) com interação significativa. Os espécimes colados com S3 mostram um µSBS significativamente inferior aos colados com SE (p<0,05) em todos os grupos. Para ambos os adesivos, os grupos clareados demonstram µSBS inferiores aos não clareados exceto os espécimes clareados com Pyrenees e colados com SE (p>0,05). Foi observada uma diferença significativa entre os grupos dos sistemas de clareamento (p<0,05). Diferentes concentrações de peróxidos foto-ativados nos sistemas de clareamento afetam negativamente µSBS dos adesivos de uma e duas etapas de auto-colagem. O sistema de baixa concentração (Pyrenees) não influenciou a resistência ao cisalhamento do adesivo de duas etapas.

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