



The Impact of Antimicrobial Photodynamic Therapy (aPDT) with Various Photosensitizers on the Bond Strength of Orthodontic Brackets

Sepideh Seyfnia¹, Mahdjoube Goldani Moghadam², Farzaneh Ahrari^{3*}

¹School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

²Department of Orthodontics, School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

³Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

*Correspondence to

Farzaneh Ahrari,
Emails: Farzaneh.Ahrari@gmail.com;
Ahrarif@mums.ac.ir

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Abstract

Introduction: Antimicrobial photodynamic therapy (aPDT) has demonstrated effectiveness in reducing gingival hyperplasia and preventing dental caries formation. However, concerns exist about its potential impact on the bond strength of resin composites. This study aimed to evaluate the effect of aPDT with various photosensitizers on the shear bond strength (SBS) of orthodontic brackets to enamel.

Methods: A total of 102 healthy bovine central incisors were divided into six groups as follows: Group 1: control, Group 2: chlorhexidine (CHX), Group 3: aPDT with methylene blue and a 660-nm laser, Group 4: aPDT with indocyanine green and an 810-nm laser, Group 5: aPDT with curcumin and an LED, and Group 6: aPDT with riboflavin and an LED. Following the aPDT procedure, orthodontic brackets were bonded to the enamel surfaces. Shear bond strength was assessed using a universal testing machine, and the adhesive remnant index (ARI) scores were recorded. Data were analyzed using one-way ANOVA and Fisher's exact test, with a significance level set at $P < 0.05$.

Results: The highest SBS value was found in the indocyanine green group (10.31 ± 3.46) and the lowest in the control group (7.82 ± 1.86). ANOVA revealed no significant difference in shear bond strength between the groups ($P = 0.337$). Additionally, the ARI scores showed no significant variation across the study groups ($P = 0.882$).

Conclusion: Photodynamic therapy does not adversely affect the bond strength of orthodontic brackets to enamel, and thus it can be applied safely in orthodontic patients due to its proven antibacterial benefits.

Keywords: Antimicrobial, Bond strength, Dental caries, Orthodontic brackets, Photodynamic therapy, Photosensitizers, Resin composite



Introduction

Today, an increasing number of patients are seeking orthodontic treatment to enhance their facial and smile aesthetics and quality of life. Fixed orthodontic appliances enable precise control over tooth movement, essential for addressing dental problems such as crowding, spacing, and bite abnormalities. A key concern during orthodontic treatment is the development of enamel demineralization areas, called white spot lesions (WSLs), which cause unsightly white marks on the teeth.¹⁻³ The prevalence of WSLs may vary from 2% to 97% in orthodontic patients, based on previous studies.^{4,5} Poor oral hygiene and plaque retention around orthodontic attachments are primary risk factors for WSL formation in patients undergoing fixed orthodontic treatment.⁶⁻⁸

Several strategies have been suggested to prevent enamel caries during orthodontic therapy, such as precise brushing with high-fluoridated toothpaste combined with dental flossing. Additionally, remineralizing agents such

as fluoride mouthwashes and CPP-ACP paste can help prevent WSL formation and restore demineralized enamel areas.^{9,10} However, the efficacy of most preventive and remineralizing strategies depends on patient compliance. A more promising approach that does not rely on patient cooperation is the photodynamic inactivation of microorganisms contributing to oral diseases.¹¹

Photodynamic therapy (PDT) is a non-invasive, emerging technique that combines chemical agents called photosensitizers (PSs) with specific light wavelengths in the presence of oxygen molecules. This combination produces reactive oxygen species (ROS), such as singlet oxygen, which damage tumoral or harmful microbial cells.^{12,13} Antimicrobial photodynamic therapy (aPDT) is extensively applied in medicine and dentistry to manage bacterial and fungal infections. This technique offers advantages such as reproducibility and the ability to reduce bacterial biofilms without causing photo-resistant species.¹⁴ aPDT effects can be beneficial in

lowering microbial levels and decreasing the occurrence of gingivitis and enamel demineralization around orthodontic appliances.

Various photosensitizers (PSs) may be used in aPDT, each with a specific light absorption wavelength. The PSs are classified into three groups based on their chemical structure. Phenothiazine dyes, including methylene blue (MB) and toluidine blue O (TBO), are widely used in dentistry. They possess strong absorption around 600–660 nm wavelengths and are effective against a broad spectrum of microorganisms.¹⁵ Tetrapyrrole-based photosensitizers include porphyrins, chlorins, and phthalocyanines. These PSs can be activated by a broad range of wavelengths, allowing deeper tissue penetration during PDT.¹⁶ Cyanine dyes are characterized by strong absorption in the visible and near-infrared (NIR) regions. Their ability to absorb NIR light allows deeper tissue penetration, making them promising agents for photodynamic therapy and biomedical imaging. Indocyanine green (ICG), a tricarboxyanine dye, is the most widely used member of this group due to its favorable biocompatibility and low toxicity. Natural PSs, such as curcumin and riboflavin (Vitamin B2), are another group of photosensitizers that often have inherent antimicrobial and anti-inflammatory properties, complementing their photodynamic action.¹⁷

Several studies demonstrated the efficacy of aPDT in lowering microbial levels and decreasing the risk of gingival inflammation and white spot lesions during orthodontic treatment.^{18–20} However, a potential concern is the effect of aPDT on the bond strength of adhesive resins. Studies in restorative dentistry exhibited reduced bond strength of restorations bonded immediately after the bleaching process.^{21,22} This reduction has been attributed to residual oxygen radicals within the dental tissues, which may interfere with the resin infiltration and polymerization at the bonded interface.^{21–23} Following the aPDT procedure, the oxygen species may also remain on the tooth surface and alter the surface properties of enamel, potentially weakening the bond strength of orthodontic attachments. In orthodontic therapy, the integrity of bracket bonding is an essential factor to achieve precise tooth movement,²⁴ and thus any impact of aPDT on bond strength should be carefully evaluated. The reduction in bond strength would increase the risk of bracket failure, requiring re-bonding and prolonging treatment time.

There is limited evidence regarding the impact of aPDT on the bond strength of orthodontic attachments. Therefore, the present study aimed to investigate the influence of aPDT with various photosensitizers on the shear bond strength (SBS) of orthodontic brackets to enamel compared to chlorhexidine (CHX) and a control group.

Materials and Methods

Study Design and Sample Size Calculation

The sample size was calculated to be 17 teeth per group based on the data of a previous study (30), and considering $\alpha = 0.05$ and $\beta = 0.2$. The calculation was performed using

NCSS software (NCSS, Kaysville, Utah, USA).

Sample Preparation

A total of 102 sound bovine central teeth were used in this study. The bovine teeth were chosen as they have large and flat surfaces and show structural similarity to human teeth.²³ The specimens were examined under a stereomicroscope at 10× magnification to discard those with caries, cracks, or enamel chipping. Each tooth was cleaned for 15 seconds using a rubber cup and a water slurry of pumice. The specimens were then disinfected in a 0.1% (w/v) thymol solution for 24 hours, followed by storage in a saline solution at room temperature until the time of the experiment.

Grouping

A 5 × 5 mm window was marked at the middle of the buccal surface to represent the treatment area. The samples were randomly divided into six groups (n = 17) based on the assigned pretreatment procedure before bracket bonding. The study groups were as follows:

Group 1 (Control)

The teeth in the control group did not receive any pretreatment procedure.

Group 2 (CHX)

The teeth in this group were immersed in a 0.2% chlorhexidine gluconate solution (Irsha; Shafa Pharmaceutical and Health Company, Alborz, Iran) for one minute.

Group 3 (Methylene Blue-Mediated aPDT)

A 100 µg/ml solution was prepared by dissolving methylene blue (Mehrshimi, Tehran, Iran) in distilled water. The solution was applied to the buccal surface of each tooth for 5 minutes. Then, an indium-gallium-aluminum-phosphide (InGaAlP) low-level laser (wavelength 660 nm; Thor DD2 Control Unit, Thor, London, UK) was irradiated on the tooth surface for one minute in the presence of the methylene blue solution. The laser operated at a power of 200 mW and in continuous wave mode, and the probe was held manually at a distance of 1 mm from the tooth surface. The irradiation was performed in a scanning mode, delivering an energy of 12 J with an energy density of 61.1 J/cm² over the treatment area.

Group 4 (Indocyanine Green-Mediated aPDT)

A 1 mg/ml solution was prepared by dissolving indocyanine green (Chemodex, Gallen, Switzerland) in distilled water. The solution was then applied to the buccal surface of the tooth for 5 minutes. A gallium aluminum arsenide (GaAlAs) low-level laser (wavelength 810 nm; Thor DD2 Control Unit) was applied over the tooth surface for one minute. The laser operated at a power of 200 mW and in continuous wave mode (CW) at an approximate distance of 1 mm from the tooth surface. The laser beam was directed with a scanning motion to ensure covering the whole sample surface, giving 12 J energy with an energy

density of 61.1 J/cm² over the treatment window.

Group 5 (Curcumin-Mediated aPDT)

Curcumin (Merck, Darmstadt, Germany) was dissolved in distilled water containing 4% dimethyl sulfoxide (DMSO) to achieve a solution at a concentration of 40 µM. Since curcumin has low water solubility, it is traditionally dissolved in dimethylsulfoxide (DMSO).²⁵ The solution was then applied to the tooth surface for 5 minutes. A light-emitting diode (LED) with an intensity of 1200 mW/cm² (X-cure; Woodpecker, Guilin, China) was irradiated on the tooth surface for one minute. The device emitted light at a spectrum of 385–515 nm, with a central wavelength of 450 nm (blue light). The tip of the LED was held constant over the treatment area at an approximate distance of 1 mm.

Group 6 (Riboflavin-Mediated aPDT)

Riboflavin (Central Drug House Ltd., New Delhi, India) was dissolved in distilled water to produce a solution with a 100 µM concentration. The solution was then applied to the buccal surface of the tooth for 5 minutes. Afterward, the LED light (X-cure, Woodpecker) was irradiated over the tooth surface for one minute, as explained in group 5.

Bracket Bonding

Following the aPDT treatment, the teeth were rinsed and dried. The buccal surfaces were then etched with 37% phosphoric acid gel (3M) for 15 seconds, rinsed with water for 15 seconds, and dried with an oil-free air spray until a chalky, white appearance was achieved. The Transbond XT primer (3 M Unitek, Monrovia, CA, USA) was applied on the tooth surface, thinned with air spray, and light-cured for 10 seconds. Subsequently, a sufficient amount of Transbond XT adhesive was applied on the base of a central incisor bracket (Dentaurum, Ispringen, Germany), and the bracket was pressed in the middle of the buccal surface. After removing the excess material, the adhesive was cured for 20 seconds from mesial and distal directions using the LED device (X-cure, Woodpecker).

After the bonding procedure, the teeth were mounted in molds containing self-curing acrylic resin (Acropars, Tehran, Iran), using a mounting guide to ensure that the bracket base was parallel to the direction of the debonding force.

Thermocycling and Shear Bond Strength (SBS) Assessment

The teeth were stored in distilled water at 37 °C for 24 hours and then placed in a thermocycling machine. The thermal cycles were conducted in a water bath between 5°C and 55°C for 5000 cycles, with a dwell time of 30 seconds per bath.

Subsequently, the specimens were positioned in a universal testing machine (SANTAM model STM-20, Tehran, Iran) to measure the SBS of orthodontic brackets. The force was applied with a metal loop to the bracket-tooth interface using a cross-head speed of 0.5 mm/min (Figure 1). The maximum load at debonding was recorded

in Newtons (N) and converted to megapascals (MPa) by dividing the force by the bracket cross-sectional area.

Adhesive Remnant Index (ARI) Assessment

After debonding, the enamel surfaces were inspected by a single observer at 10 x magnification using a stereomicroscope (SMZ168, Motic, China). The adhesive remnant index (ARI) was used to score bond failure mode as follows:²⁶

- **Score 0:** No adhesive remained on the tooth
- **Score 1:** Less than 50% of the adhesive remained on the tooth.
- **Score 2:** More than 50% of the adhesive remained on the tooth.
- **Score 3:** All adhesive remained on the tooth, with a distinct impression of the bracket base.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test confirmed the normality of the data distribution ($P > 0.05$). A one-way analysis of variance (ANOVA) was run to compare the shear bond strength of the study groups. Fisher's exact test was used to compare the distribution of ARI scores among the groups. The significance level was set at $P < 0.05$.

Results

Table 1 presents the descriptive statistics for SBS values

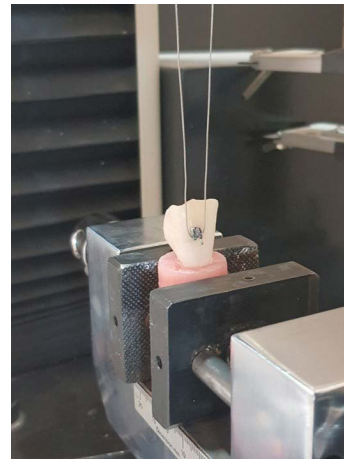


Figure 1. A sample mounted in the testing machine to assess the shear bond strength (SBS) of the bracket to enamel

Table 1. The mean and standard deviation (SD) of shear bond strength (MPa) in the study groups

Group	Definition	Mean ± SD
1	Control	7.82 ± 1.86
2	Chlorhexidine	9.53 ± 4.54
3	Methylene blue + 660 nm laser	10.12 ± 4.57
4	Indocyanine green + 810 nm laser	10.31 ± 3.46
5	Curcumin + LED	9.12 ± 3.74
6	Riboflavin + LED	9.01 ± 3.55
P-value		0.337

in the study groups. The highest shear bond strength was observed in the indocyanine green group (10.31 ± 3.46), whereas the lowest was found in the control group (7.82 ± 1.86). ANOVA revealed no significant difference in bond strength values between the study groups ($P=0.337$).

Table 2 provides the frequency and percentage (%) of ARI scores in the study groups. In all groups, the frequency of score 2 was higher, indicating that more than 50% of the adhesive was left on the tooth surface. According to Fisher's exact test, ARI scores did not significantly differ between the groups ($P=0.882$).

Discussion

This study evaluated the effect of antimicrobial photodynamic therapy (aPDT) with various photosensitizers on the SBS of orthodontic brackets, comparing the results with chlorhexidine (CHX) and a negative control group. The teeth underwent thermocycling between 5°C and 55°C to simulate the thermal variations experienced in the oral environment.

The outcomes of this study revealed no significant difference in SBS and ARI index between the groups. The highest SBS was observed in the indocyanine green (ICG) group and the lowest in the control group. The bond strength values in all groups were greater than the threshold of 7.8 MPa required in orthodontic clinical practice.²⁷ Therefore, neither aPDT nor chlorhexidine would adversely affect the bond strength of orthodontic brackets. These treatments can be used safely in orthodontic patients to lower microbial levels and decrease the risk of gingivitis and enamel demineralization around fixed appliances.

Chlorhexidine (CHX) is an antimicrobial agent widely used in orthodontic patients to control microbial plaque and reduce the risk of oral infections and periodontal problems during fixed orthodontic therapy. CHX is recognized as the gold standard for chemical plaque control and is usually used as a benchmark to allow a reliable comparison of aPDT's efficacy in reducing the bacterial load.^{28,29} Therefore, CHX was included in this study to compare its effect on the bond strength of orthodontic brackets. The outcomes of this study agree with several studies that found the application of CHX before the bonding process did not have a significant effect on the shear bond strength of brackets.³⁰⁻³² In contrast, some studies found that varnish, gel, and solution forms of CHX exhibited detrimental effects on the bond strength of orthodontic attachments,^{31,33} possibly due to interference

with the surface conditioning of enamel and prevention of proper resin penetration into the etched surface.

In the present study, four photosensitizers were used for aPDT, including methylene blue, indocyanine green, curcumin, and riboflavin. Methylene blue, a cationic phenothiazine dye, is widely used in aPDT. Upon light activation at an appropriate wavelength (typically in the red spectrum, around 660 nm), methylene blue generates reactive oxygen species (ROS), including singlet oxygen, which penetrate the bacterial cell membrane and induce oxidative damage to biological tissues. This property has been widely employed in dentistry to disrupt bacterial biofilms and oral pathogens.³⁴

Indocyanine green (ICG) is a fluorescent dye, notable for its high absorption in the near-infrared spectrum (within the wavelength range of 800–810 nm). ICG allows deep tissue penetration and efficient energy transfer during aPDT. This characteristic is particularly beneficial in treating infections in deep oral tissues or affecting microbial biofilms, which are less accessible to other photosensitizers. ICG's safety profile and effectiveness against various pathogens make it a valuable choice for enhancing the outcomes of aPDT.¹⁴ Upon activation, ICG produces reactive oxygen species and heat. Therefore, the antibacterial mechanism of ICG consists of photochemical and photothermal effects,³⁵ making it somewhat different from other PSs, which mainly rely on photochemical reactions. However, the difference in the mechanism of action of ICG did not affect the bond strength of orthotic brackets in this study, as compared to other groups.

Both curcumin and riboflavin are natural photosensitizers. Curcumin (CUR) is a polyphenolic compound derived from the rhizomes of *Curcuma longa*. It is recognized as a promising photosensitizer in photodynamic therapy (PDT) due to its safety profile and multifunctional therapeutic properties. Curcumin is activated by blue light (the wavelength range of 400–500 nm with a peak at 430 nm),³⁶ generating reactive oxygen species that exhibit potent antimicrobial and antimitotic effects.²⁵ Curcumin-mediated aPDT has shown promise in reducing microbial viability in both planktonic cultures and biofilms, implying the potential of this modality as an adjunct in the management of oral diseases.^{25,37,38} A potential limitation of curcumin is discoloration of teeth and esthetic composite restorations at high concentrations.²⁵

Riboflavin (RIB), or vitamin B2, is a water-soluble compound with intrinsic photodynamic properties.

Table 2. Distribution of ARI scores in the study groups.

Groups		0	1	2	3
Control	Control	2 (11.8%)	5 (29.4%)	9 (52.9%)	1 (5.9%)
CHX	Chlorhexidine	1 (5.9%)	4 (23.5%)	12 (70.6%)	0
MB	Methylene blue + 660 nm laser	2 (11.8%)	5 (29.4%)	10 (58.8%)	0
ICG	Indocyanine green + 810 nm laser	2 (11.8%)	6 (35.2%)	7 (41.2%)	2 (11.8%)
CUR	Curcumin + LED	1 (5.9%)	5 (29.4%)	11 (64.7%)	0
RIB	Riboflavin + LED	0	4 (23.5%)	12 (70.6%)	1 (5.9%)

Activated by blue or ultraviolet light (maximum absorption range at 445, 336, and 270 nm), riboflavin generates reactive oxygen species that exert antimicrobial effects by disrupting microbial membranes and cellular structures. Its biocompatibility and safety profile have made it a suitable choice in aPDT regimens for managing dental microbial infections. Cova et al.³⁹ demonstrated that RIB activated by ultraviolet-A (UVA) light is adequate in inhibiting matrix metalloproteinase (MMP) enzymes and enhancing collagen cross-linking in dentin. This feature may be potentially advantageous when using aPDT to disinfect deep carious lesions, as preventing collagen matrix degradation would enhance the bond strength of adhesive resins to dentin.

The outcomes of this study indicate that the reactive oxygen species created during aPDT procedure would not affect the bonding integrity of adhesive restorations, as no statistically significant difference in SBS was observed between the groups. Therefore, aPDT with various photosensitizers can be employed safely in orthodontic patients without negatively impacting the performance of orthodontic adhesives.

In the present study, all groups showed a higher distribution of ARI scores 2 and 1, implying that orthodontic adhesive remained on the tooth surface post-debonding. A specific and universally accepted ARI index from a clinical perspective has not been established.^{40,41} The distribution of ARI scores was comparable among the study groups, indicating that aPDT does not influence the retention of adhesive resins on the enamel surface.

The findings of this study are consistent with previous research demonstrating that the application of aPDT does not compromise the bond strength of orthodontic brackets. Mirhashemi et al.⁴² indicated that aPDT with both riboflavin and curcumin leads to an acceptable bond strength of orthodontic brackets with no significant difference in SBS and ARI scores between groups. In their study, the mode of failure was mainly scores 1 and 2, similar to the present findings. Hashemikamangar et al.⁴³ reported that aPDT with phycocyanin or toluidine blue did not influence the bond strength of resin composite to sound dentin. In contrast to the findings of this study, Mirhashemi et al.³⁵ reported that aPDT with either methylene blue or indocyanine green caused a significant reduction in the SBS of orthodontic brackets. In SEM images, they observed porosity and irregularity post-etching in the aPDT groups, but the typical honeycomb pattern was not evident. In a systematic review, Mendes et al.⁴⁴ concluded that the effects of aPDT on the bond strength of restorative materials vary depending on the substrate and photosensitizer used, with methylene blue specifically showing adverse effects on bond strength.⁴⁴ The discrepancy between the results of this study and previous studies may be attributed to the differences in methodology, such as the use of bovine versus human teeth, the bonding procedures, variations in thermal cycles, and the difference in the concentration of photosensitizers and light exposure regimens applied in

aPDT.

A limitation of this study is its in vitro design, which cannot fully replicate the complex conditions and dynamics of the oral environment. The shear bond strength (SBS) values achieved in laboratory settings are typically different from in vivo results due to additional factors, such as pH fluctuations, enzymatic activity, changes in temperature, and fatigue from occlusal forces in the oral environment. It should also be noted that the effects of aPDT on the bond strength of orthodontic brackets may vary based on factors such as the type and concentration of the photosensitizer, the tooth substrate, and the aPDT protocol, and thus, further research is needed to understand these variables and their impact on clinical outcomes. Future research is also suggested to assess the cumulative effects of repeated aPDT applications using different photosensitizers on SBS, as well as their clinical efficacy in preventing enamel caries and reducing periodontal complications.

Conclusion

Under the conditions used in this in vitro study, photodynamic therapy (PDT) did not adversely affect the shear bond strength of orthodontic brackets bonded to enamel. This finding suggests that aPDT can be safely integrated into orthodontic procedures without compromising bonding integrity.

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Authors' Contribution

Conceptualization: Mahdjoube Goldani Moghadam.
Data Curation: Sepideh Seyfnia.
Formal Analysis: Farzaneh Ahrari.
Funding Acquisition: Mahdjoube Goldani Moghadam.
Investigation: Sepideh Seyfnia.
Methodology: Farzaneh Ahrari.
Project Administration: Mahdjoube Goldani Moghadam.
Resources: Mahdjoube Goldani Moghadam.
Software: Farzaneh Ahrari.
Supervision: Farzaneh Ahrari.
Validation: Farzaneh Ahrari.
Writing–Original Draft: Farzaneh Ahrari.
Writing–Review & Editing: Sepideh Seyfnia, Mahdjoube Goldani Moghadam.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

No consent for publication applies to this study.

Data Availability Statement

Data will be made available on request.

Ethical Approval

Not applicable.

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