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Original Article

Potential of Erythrosine-Mediated Photodynamic Therapy as a Cavity Disinfectant: Antibacterial Efficacy and Bonding Ability

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Abstract

This study aimed to evaluate the antibacterial efficacy of erythrosine-mediated photodynamic therapy (PDT) against *Streptococcus mutans* (*S. mutans*) in dentin and its effect on the shear bond strength (SBS) of composite resin to dentin. Eighty extracted human noncarious premolars were used in this study. Forty teeth were used for the antibacterial activity test, while the remaining 40 were used for the SBS test. Both experiments were conducted with 4 experimental groups (n = 10): control (distilled water), sodium hypochlorite (NaOCl, 6%), chlorhexidine (CHX, 0.12%), and erythrosine-mediated PDT. Antibacterial effects were evaluated by counting *S. mutans* colony-forming units (CFUs). The SBS of composite resins to dentin was measured using a universal testing machine. All treatments (NaOCl, CHX, and PDT) demonstrated statistically significant differences in antibacterial activity compared with the control group ($p < 0.05$). The antibacterial effects were ranked from strongest to weakest as follows: NaOCl, PDT, and CHX. In the SBS test, the NaOCl group exhibited a statistically significant difference compared with the CHX, PDT, and control groups ($p < 0.05$), with the lowest bond strength. No statistically significant differences were found among the CHX, PDT, and control groups ($p > 0.05$). Erythrosine-mediated PDT exhibited significant antibacterial effects against *S. mutans*, with higher antibacterial activity than CHX but lower than NaOCl. Only NaOCl negatively affected the bond strength of composite resin to dentin. In conclusion, erythrosine-mediated PDT shows potential as a cavity disinfectant due to its significant antibacterial effects against *S. mutans* and lack of adverse effects on bond strength. [J Korean Acad Pediatr Dent 2024;51(3):290-298]

Keywords

Streptococcus mutans, Shear bond strength, Cavity disinfectant, Erythrosine, Photodynamic therapy (PDT)

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Introduction

Dental caries is a critical global public health problem and the most widespread non-communicable disease. According to the 2016 Global Burden of Disease Study, dental caries affected 3.58 billion people in their permanent teeth and 560 million children in their deciduous teeth[1]. The gold standard for cavity preparation involves using mechanical instruments to remove caries and shape the cavity, followed by filling the cavity with restorative materials to restore the tooth's function and aesthetics.

Cavity preparation is a procedure that attempts to remove all infected dentin before placing restorative materials; however, bacterial remnants can remain during and after cavity preparation[2]. Over time, they can increase pulpal sensitivity or cause pulpal inflammation and secondary caries, which may require replacement of the restoration[3,4]. Various antimicrobial agents have been used to achieve higher levels of bacterial eradication against pathogens responsible for recurrence and postoperative sensitivity. Various antibacterial treatment modalities, including chlorhexidine (CHX), sodium hypochlorite (NaOCl), citric acid, have been investigated for their antimicrobial treatment effectiveness[5]. However, previous studies have demonstrated that residual bacteria may persist following the application of disinfectants. Furthermore, the concentrations of these disinfectants required for effective antimicrobial action may potentially induce cytotoxic effects on adjacent tissues[6].

In light of this, photodynamic therapy (PDT) has surfaced as a promising alternative to chemical disinfectants, capturing the interest of researchers in the field. PDT is a technique that combines a dye (also referred to as a photosensitizer), a low-energy light source with an appropriate wavelength, and molecular oxygen ($^3O_2$) to generate reactive oxygen species (ROS). ROS promote the inactivation of microorganisms by damaging bacterial cell components through mechanisms such as compromising the integrity of the cytoplasmic membrane, leading to increased cellular permeability[7], and oxidizing double bonds in biological molecules and macromolecules[8]. PDT has applications in treating bacterial infections, cancer, and viral diseases, and has also become a significant area of research in dental treatment.

In previous in vitro studies, various photosensitizers employed in PDT have been investigated. There are many photosensitizer options available, including phenothiazine dyes, phthalocyanines, and porphyrins. Among the range of photosensitizers applied in PDT, erythrosine has proven to be a promising agent in the inhibition of cariogenic bacterial strains[9,10]. Wood et al.[11] reported that erythrosine exhibits the highest efficacy as a photosensitizer in PDT against *Streptococcus mutans* (*S. mutans*) biofilms, which are the primary cariogenic bacteria. The main advantage of erythrosine over other photosensitizers lies in its non-toxic nature and FDA approval for use in dentistry and food, which means that it has a low clinical risk[11].

Achieving a strong bond at the dentin-restorative interface is also crucial for the clinical effectiveness and longevity of adhesive restoration[12]. Low bond strength renders the tooth-restoration interface more susceptible to microleakage and recurrent caries[13]. Whether the application of caries disinfectants affects the sealing ability of adhesive bonding resins has been controversial in previous studies[14-17]. However, existing literature has not yet examined the influence of erythrosine-mediated PDT on bond strength when utilized for dentin disinfection. The photosensitizer dyes used in PDT may affect the adhesion of the composites to dentin because residual structural molecules may interfere with the adhesion process.

This in vitro study aimed to determine the potential of erythrosine-mediated PDT as a cavity disinfectant by investigating its efficacy against *S. mutans* in dentin and its influence on the composite-dentin shear bond strength. The disinfection protocols used in this study included NaOCl, CHX, and erythrosine-mediated PDT.

Materials and Methods

This study was approved by the Institutional Review Board of Gangneung-Wonju National University Dental

Hospital (IRB No: GWNUDH-2023-A015). Eighty extracted human noncarious premolars were used in this study. After removing calculus and soft tissue, the teeth were stored in distilled water at 4°C until use. After all the teeth were randomly assigned, 40 were used for the antibacterial activity test and 40 for the shear bond strength (SBS) test.

1. Antibacterial activity test

1) Specimen preparation

Forty extracted human noncarious premolars were used. After scaling and cleansing, using a low-speed diamond disk under water, dentin specimens with (3.0 × 3.0 × 2.0 mm) were prepared. The specimens were polished using 600-grit silicon carbide paper disks (CC-357, PACO Co., Ltd., Yeoju, Korea). Only one 3.0 × 3.0 mm surface was left exposed, and the remaining areas were coated with a silicone impression material (Examixfine injection type, GC Corporation, Tokyo, Japan). The specimens were sterilized with ethylene oxide gas.

2) Biofilm formation on the specimens

For biofilm formation, *S. mutans* ATCC 25175 was provided by the Department of Oral Microbiology, College of Dentistry, Gangneung-Wonju National University. *S. mutans* was cultured in Brain-Heart Infusion (BHI) broth (Becton, Dickinson and Company, Sparks, MD, USA) at 37°C under 5% CO_2 for 18 h. Bacterial turbidity was subsequently assessed using a spectrophotometer (Smart Plus 2700; Young-woo Instrument, Seoul, Korea), and the *S. mutans* suspension was prepared at a concentration of 1.0×10^9 colony-forming units (CFU)/mL. The specimens were placed in a 12-well plate with 1800 μL of BHI broth containing 1% sucrose and 200 μL of *S. mutans* suspension, and cultured for 24 h under conditions of 5% CO_2 at 37°C.

3) Dentin surface treatments

The specimens were randomly allocated into 4 treatment groups: a control group treated with distilled water and 3 experimental groups treated with either 6% NaOCl, 0.12% CHX, or PDT (n = 10 each). In the control group, after being rinsed with distilled water for 10 sec and dried in oil-free air without disinfection, the specimens were prepared for further testing. In the NaOCl and CHX groups, 40 μl of disinfectant (NaOCl, CHX) was applied to the specimen and rubbed for 20 sec using a sterile microbrush tip. After disinfection, the surfaces were rinsed with distilled water for 10 sec and air-dried. In the PDT group, the specimens underwent erythrosine-mediated PDT. A 40 μM erythrosine solution was prepared by dissolving erythrosine (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (PBS). The prepared erythrosine solution was then stored at room temperature in 50 mL tubes that were protected from light exposure. A light-emitting diode (LED; VALO™, Ultradent Products Inc., South Jordan, UT, USA) with a wavelength range of 395 - 515 nm was employed as the light source in this study. At an irradiation distance of 10 mm, the power intensity was recorded as 1800 mW/cm^2, with the output being measured using a photometer (DigiRate radiometer; Monitex, Taiwan). The specimens were treated with 40 μL of 40 μM erythrosine and then incubated in a dark room for 3 minutes. Subsequently, light irradiation was applied for 18 sec from a 10 mm distance.

4) Colony-forming units (CFUs) count

Subsequent to the surface treatment, the specimens underwent 2 PBS wash cycles to remove loosely adhered bacteria and were then subjected to sonication (VC 100; Sonics & Materials Inc., Danbury, CT, USA) for 10 sec to procure a bacterial suspension. The bacterial suspension was diluted 1:1000 with PBS, and a 50 μL aliquot was obtained. The aliquot was plated on a blood agar plate (Hanil-KOMED, Seongnam, Korea) and incubated at 37°C in 5% CO_2 for 72 h. Bacterial CFU counts were analyzed after conversion to log values.

2. Shear bond strength (SBS) test

1) Sample preparation

The roots of 40 teeth were embedded in self-cured acrylic resin in a square plastic mold. After the self-cured

acrylic resin was polymerized, the teeth were removed from the mold. A flat surface of the superficial dentin was exposed in the middle of the occlusal surface of each tooth using a low-speed diamond disk under water and polished with 600-grit silicon carbide paper disks.

2) Dentin surface treatments

The specimens were randomly allocated into 4 treatment groups (n = 10 each). Each specimen was treated according to the corresponding treatment group. The surface treatment methods for each group were identical to those used for the antibacterial activity tests, and the specimens were air-dried.

3) Restorative procedures

After the treatment, the specimens underwent a 15 sec etching process using 32% phosphoric acid gel (Scotchbond Universal Etchant; 3M ESPE, St. Paul, MN, USA). Subsequently, they were rinsed thoroughly with water spray for 10 sec and then gently air-dried for an additional 10 sec. Scotchbond Universal Adhesive (3M ESPE) was applied as the dentin adhesive, gently agitated with a microbrush tip for 20 sec, and then carefully air-dried for 5 sec to facilitate solvent evaporation. Following this, the specimens were light-cured for 10 sec using the LED light-curing unit. The nanohybrid composite resin (Filtek Z-350 XT, Shade: A2, 3M ESPE) was placed on the bonded area using a Teflon mold (3 mm diameter and 3 mm height) in 2 layers. Each layer was polymerized for 20 sec. The specimens were maintained in distilled water at 37°C for 24 h.

4) Testing of shear bond strength (SBS)

A universal testing machine (RB-306, R&B Inc., Daejeon, Korea) was used to measure the SBS of each specimen to composite resin. The measurement was performed by applying a shear force of 500 kgf at a crosshead speed of 1 mm/min. Shear bond strength values of the composite resin to dentin were obtained in Newtons (N) and then expressed in MPa by considering the composite build-up's cross-sectional area of 7.06 mm^2.

3. Statistical analysis

Data were analyzed using SPSS (version 29.0; IBM, Armonk, NY, USA). The Shapiro-Wilk test revealed that log_{10}CFU/mL and SBS values followed a normal distribution. One-way analysis of variance (ANOVA) was used to compare the differences in both experimental values. Subsequently, Tukey's honestly significant difference post hoc test was applied to ascertain the statistical significance of multiple comparisons ($\alpha = 0.05$).

Results

1. Antibacterial activity

The mean and standard deviation of log_{10}CFU/mL for all groups are presented in Fig. 1. Bacterial colonies grown on the blood agar plates for each group are shown in Fig. 2. All treatment modality groups exhibited statistically significant superior antimicrobial efficacies compared to the control group ($p < 0.05$). The NaOCl group showed complete bacterial elimination, followed by the PDT group and the CHX group. Statistically significant differences were observed among all groups: NaOCl, PDT, and CHX ($p < 0.05$ for all intergroup comparisons).

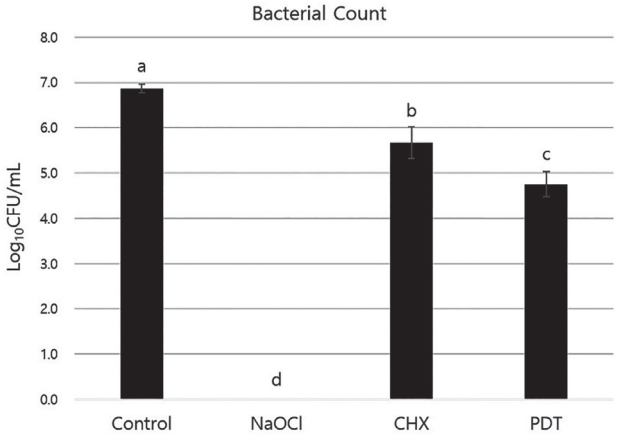

Fig. 1. Bacterial counts for each group.
Different superscript letters indicate significant differences by Tukey's HSD test for post hoc analysis ($p < 0.05$).
NaOCl: sodium hypochlorite; CHX: chlorhexidine; PDT: photodynamic therapy.

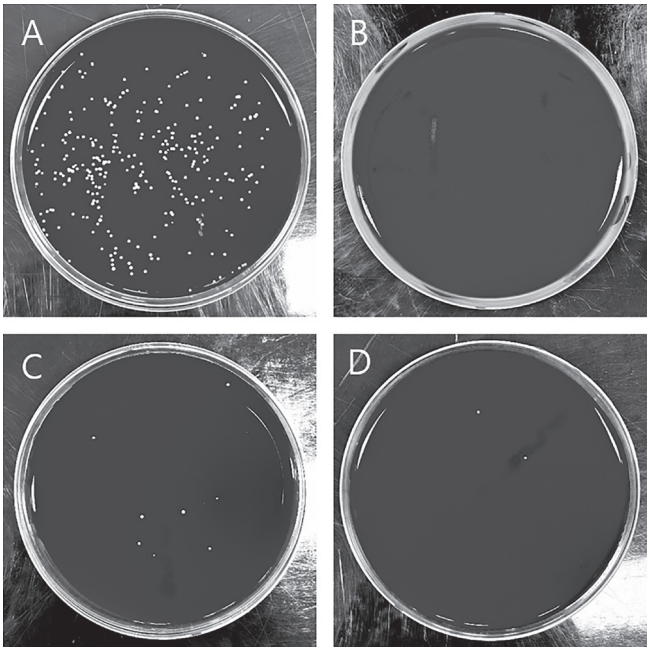

Fig. 2. Bacterial colonies grown on the blood agar plates for each group. (A) Control group, (B) NaOCl group, (C) CHX group, (D) PDT group.
NaOCl: sodium hypochlorite; CHX: chlorhexidine; PDT: photodynamic therapy.

2. Shear bond strength (SBS)

The mean and standard deviation of shear bond strength (MPa) for all groups are presented in Table 1. The NaOCl group, which had a weaker bond strength, exhibited a statistically significant difference compared to the CHX, PDT, and control groups ($p < 0.05$). Intergroup comparisons revealed no statistically significant differences in shear bond strength among the CHX, PDT, and control groups ($p > 0.05$).

Table 1. The mean and standard deviation of shear bond strength (MPa) for each group

Group (n = 40)	Shear bond strength (Mean ± Standard deviation, MPa)	*p* value
Control (n = 10)	26.50 ± 4.80[a]	
NaOCl (n = 10)	19.17 ± 3.52[b]	< 0.0001
CHX (n = 10)	24.34 ± 2.88[a]	0.529
PDT (n = 10)	26.86 ± 3.32[a]	0.996

p value from the 1-way ANOVA.
Different superscript letters indicate significant differences by Tukey's HSD test for post hoc analysis ($p < 0.05$).
NaOCl: sodium hypochlorite; CHX: chlorhexidine; PDT: Photodynamic therapy.

Discussion

The use of antibacterial disinfectants after cavity preparation has been suggested to effectively inhibit bacterial activity[15]. NaOCl and CHX have been shown to be effective antibacterial agents in previous studies[18]. NaOCl exhibits antibacterial effects through its organic tissue dissolving properties, which prevent bacterial adhesion to both other bacteria and dentinal walls. CHX is both bactericidal and bacteriostatic, primarily acting on the phospholipids of bacterial cell walls. It exerts the effect by altering the cell membrane permeability, leading to the leakage of intracellular components and the disruption of bacterial metabolism[19].

Similarly to previous findings, in this study, the *S. mutans* cell count was significantly lower in the CHX and NaOCl treatment groups compared to the control group ($p < 0.05$). According to our results, 6% NaOCl showed a significantly higher antibacterial effect than 0.12% CHX ($p < 0.05$), with no growth of *S. mutans* observed in any of the samples. This is similar to a study by Arias-Moliz et al.[20], who found that 5.25% NaOCl was more effective than 2% CHX. In this study, the *S. mutans* cell count was significantly lower in the PDT treatment group compared to the control group ($p < 0.05$), demonstrating that erythrosine-mediated PDT exhibited significant antimicrobial effects. The antimicrobial activity of activated erythrosine was lower than that of NaOCl ($p < 0.05$), but higher than that of CHX ($p < 0.05$). PDT is a proven technology for eliminating unhealthy cells and microorganisms. When a photosensitizer is irradiated using a light source of an appropriate wavelength, the cell wall is destroyed by the oxygen radicals generated when light is absorbed by the photosensitizer[21,22]. PDT is a relatively conservative treatment strategy that offers several advantages. It is a non-invasive, repeatable, and cost-effective treatment method that has no unwarranted systemic effects, making it comfortable for patients[11,23,24]. Compared to chemical disinfectant agents, the advantage of PDT is

that it provides rapid bacterial death while maintaining normal microbial flora, eliminating the need for high concentrations of chemicals, thereby reducing the development of microbial resistance[25].

Erythrosine, a commonly used dental biofilm staining agent, was used as a photosensitizer in this study[26]. Studies have revealed that it can be activated by specific visible light irradiation (500 - 550 nm) to generate ROS, and the blue LED light used in dental offices has a suitable wavelength for this activation[27]. According to the results of this study, 40 μM erythrosine activated by blue LED light exhibits strong antibacterial activity against *S. mutans*. The erythrosine concentration of 40 μM in this study was set based on the study by Choi et al.[28], which also claimed a significant *S. mutans* reduction in colony count after PDT with 20 - 40 μM erythrosine. In this study, a markedly lower concentration than the 9 - 25 mM range typically employed in clinical dental applications as plaque disclosing agents for revealing changes in dental biofilms was used. According to previous studies, there is a tendency for the antimicrobial effect of PDT to be strengthened by raising the concentration of erythrosine[28]. Even at high concentrations, erythrosine exhibits antimicrobial properties against certain microbes without light exposure[29].

Due to their reported antibacterial properties, cavity disinfectants can be used to remove cariogenic bacteria and toxins that remain after tooth preparation, resulting in a reduction of pulpal damage[3]. Due to its well-known disinfecting properties, NaOCl has been widely used in clinical settings as one of the most common cavity disinfectants[30,31]. Although it has been suggested that NaOCl treatment on the dentin surface may improve bond strength by deproteinizing the substrate, resulting in a porous structure with numerous irregularities, conflicting research findings continue to be reported in the literature[32-34]. According to previous in vitro studies, NaOCl treatment on the dentin surface may either have no effect or decrease the bond strength, depending on the application method and the adhesive system employed[32-36].

In this study, dentin surface treatment with 6% NaOCl resulted in a significant decrease in bond strength and exhibited the lowest bond strength among the groups evaluated. The results of this study are consistent with the findings reported by Ozturk et al.[37], which reported that dentin surface treatment with 5% NaOCl, a concentration comparable to that employed in the present study, results in a decrease in bond strength. Previous studies have proposed several mechanisms by which NaOCl can negatively affect the dentin bond strength. First, the removal of dentin collagen fibers disrupts the formation of a consistent hybrid layer[38]. Another possible explanation is the presence of reactive residual free radicals in NaOCl-treated dentin, which can compete with vinyl free radicals during polymerization, leading to incomplete polymerization of the adhesive resin[39].

CHX is considered the gold standard for cavity disinfectants owing to its reported antibacterial properties. However, the effects of CHX on the bond strength between resin and dentin remain a subject of debate. Several studies have reported that CHX exerted an adverse impact on bond strength[40,41]. Conversely, other studies have reported that the application of CHX on dentin did not adversely affect the bond strength between composite resin and dentin[42-45].

In the present study, the application of CHX on the dentin surface demonstrated stability, exhibiting bond strength values similar to those of the control group ($p > 0.05$). This study utilized a concentration of 0.12%, as it is the most frequently prescribed strength. The findings of the current investigation align with multiple prior studies that have shown the application of CHX to dentin before acid etching does not reduce the bond strength of the composite resin to dentin[42-45]. Experimental evidence suggests that CHX, acting as a matrix metalloproteinase (MMP) inhibitor, has been shown to mitigate the auto-degradation of exposed collagen fibrils within inadequately polymerized hybrid layers, thereby enhancing the long-term durability of the bond strength[46].

The study results showed that erythrosine-mediated PDT resulted in favorable shear bond strength, similar to that of the control group ($p > 0.05$). As previously reported, because resin materials are sensitive to prior

dentin contamination, the adhesive strength of the restoration may be compromised as dentin is affected by the residual dye solution[47]. Conversely, the findings of the current investigation demonstrated that erythrosine-mediated PDT prior to the restorative protocol did not interfere with composite bonding to dentin. However, it is important to note that the bond strength to dentin may vary depending on the conditioning technique, adhesive, energy and irradiation time of the light source used in PDT, and the type and concentration of the photosensitizer used. Future research should explore the effects of different PDT parameters on composite bonding to dentin.

Based on our literature review, this study appears to be one of the few to investigate both the antibacterial efficacy of erythrosine-mediated PDT against *S. mutans* on human dentin and its impact on the bond strength of composite resin. Nevertheless, the first limitation of this study is that it was performed only on initial bacterial biofilm, which might not precisely represent the conditions found in actual clinical situations. Another limitation of this study is that it was performed on a single cariogenic bacterial species. However, dental caries can be caused by various microorganisms. Further laboratory studies should be conducted to investigate the impact of erythrosine-mediated PDT on further compromised tooth structures, such as those observed in caries-affected dentin or hypomineralized teeth. Additionally, this study did not include an analysis of failure modes. Future research should incorporate a detailed analysis of failure modes using microscopic techniques.

Conclusion

Despite the limitations of this study, erythrosine-mediated PDT exhibited significant antibacterial effects against *S. mutans*, with higher antibacterial activity than CHX but lower antibacterial activity than NaOCl. Among the methods evaluated, NaOCl was the only treatment that exerted a negative effect on the bond strength between the composite resin and the dentin substrate. Erythrosine-mediated PDT shows potential as a cavity disinfectant for clinical use, demonstrating significant antibacterial efficacy and favorable bonding ability.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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