Disinfection by methylthionine chloride and chitosan in combination with Photo radiation therapy on caries affected dentin nano hardness, adhesive integrity, and bond failure

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Abstract. – OBJECTIVE: The aim of the study was to assess the disinfection efficacy, bond integrity, and nano hardness of caries-affected dentin (CAD) surface bonded to resin cement when disinfected with chlorhexidine (CHX), Methylene blue activated by Photodynamic therapy (MB-PDT), chitosan, silver diamine fluoride (SDF), chitosan activated by PDT, and SDF-diode laser against *S. mutans*.

MATERIALS AND METHODS: A total of 60 human mandibular molars were extracted non-traumatically and gathered using ICDAS criteria. The dentin surface was prepared, leaving CAD to receive a disinfection procedure. After inoculation with *S. mutans*, the CAD samples were divided into six groups and disinfected with various disinfectants (n = 10) CHX, MB-PDT, chitosan, chitosan-PDT, SDF, and SDF+ diode laser. Survival rates of *S. mutans* were analyzed following the restoration of samples with resin cement *via* the etch and rinse method to assess SBS. Also, nano hardness was analyzed. Statistical analysis was performed by using the ANOVA and the Tukey multiple test ($p<0.05$). The Kruskal-Wallis test was used to evaluate the change in survival rate.

RESULTS: Related to the survival rates, the SDF+ diode laser displayed the highest reduction in *S. mutans* levels and chitosan presented the lowest level of disinfection. The intergroup comparison revealed that CHX and chitosan-PDT displayed comparable outcomes of *S. mutans* survival rate to that of SDF+ diode laser ($p>0.05$). Likewise, MB-PDT and SDF displayed a comparable survival rate of *S. mutans* to Chitosan disinfection ($p>0.05$). Considering SBS and nano hardness, the highest SBS and NH were exhibited by the SDF+ diode laser, and the lowest SBS and NH values were exhibited by MB-PDT. The intragroup comparison revealed that CAD specimens disinfected with Chitosan-PDT showed comparable SBS and NH values to the SDF+ diode laser ($p>0.05$). CHX, chitosan, and SDF exhibited bond values and NH comparable to MB-PDT ($p<0.05$).

CONCLUSIONS: Synergistic use of Silver diamine fluoride with diode laser and chitosan activated by PDT can be used as an alternative to CHX for controlling *S. mutans* growth, promoting enhanced bond efficacy and nano hardness for bonding resin cement to the caries-affected dentin.

Key Words:
Chlorhexidine, Silver di amine fluoride, Chitosan, Diode Laser, Methylene blue, Caries affected dentine.

Introduction

Dental caries, commonly known as tooth decay or cavities, are indeed the most prevalent chronic polymicrobial dental disorder. It is a multifactorial disease, meaning that it is caused by a combination of various factors. The etiology of dental caries can be attributed to genetic, be-

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havioral, and microbial factors[1,2]. The disruption of the ecological balance in the oral cavity due to plaque accumulation on the tooth surface is a critical contributing factor to the development of dental caries. Dental plaque is a biofilm formed by a complex community of microorganisms, primarily bacteria, which adhere to the tooth surfaces and oral tissues[3]. The cariogenic bacteria *Streptococcus mutans* (*S. mutans*), which resides in dental plaque, plays a significant role in initiating and progressing dental caries. This is primarily due to its ability to secrete an extracellular polysaccharide matrix through the action of glucosyltransferases[4]. This matrix facilitates the adhesion of *S. mutans* to the tooth surface and provides a favorable environment for other cariogenic microorganisms. Moreover, *S. mutans* can produce acids as a metabolic byproduct, which reduces oral pH, contributing to the demineralization of tooth enamel and the initiation of dental lesions[5,6].

Nevertheless, enamel demineralization is a reversible process and can be regressed by using the minimal invasive dental approach that constitutes the eradication of microbial-laden infected dentin without exterminating the caries-affected dentin (CAD) from the tooth surface to protect the pulp aiding in remineralization to secure the functionality of a tooth[7,8]. Significantly, the CAD surface is more delicate than adhesion to enamel as it is a more heterogeneous and hydrophilic tissue by nature, which might result in rehabilitation loss[9]. Additionally, the persistence of microbial colonies within the cavity can result in the fracture of the restorative-tooth interface, causing secondary caries and necessitating effective disinfection techniques to reinforce the tooth-cement bond for a better prognosis[10-12].

Among various disinfectants, chlorhexidine (CHX) has been regarded as a gold-standard broad-spectrum antimicrobial biguanide CAD surface cleanser that exterminates microbial colonies by disrupting their cell membrane[13]. It can be used as a viable antiseptic and antibacterial agent against *S. mutans* and other resistant microbial strains to control dental lesions and validate better shear bond strength (SBS)[14,15]. This is in line with the study conducted by Catalbas et al[16]. Another CAD decontaminating strategy includes the fluoride-containing prophylactic agent – silver diamine fluoride (SDF) – which is a colorless liquid that, when applied on a dentin surface, can halt carious lesions and alleviate dentin hypersensitivity due to the presence of silver and fluoride[17]. Silver provides the antibacterial effect by impeding DNA replication, blocking enzymatic actions, denaturation of proteins, and disrupting cell membranes by forming organometallic complexes within the microbial structure, while fluoride aids in promoting dentinal remineralization and averting the carious attack[18,19].

Moreover, a polysaccharide-based CAD cleanser ‘Chitosan’ that is composed of copolymers of glucosamine and N-acetylglucosamine and formed by partial deacetylation of chitin, serves as a compelling chelating agent and has been used persuasively to eradicate carious lesion by remineralizing it through the application of calcium and phosphate and may also nucleate the dentin surface *via* crystal formation[20,21]. It provides antibacterial effectiveness on adherence to the bacterial DNA and mRNA, causing cellular disruption and protein synthesis obstruction, thereby impeding the biofilm formation[22].

The introduction of Photodynamic therapy (PDT) in dentistry can be perceived as an expedient CAD disinfection protocol that relies on the activation of various photosensitizers (PS) by photosensitization under aerobic conditions and inevitably emitting reactive oxygen species (ROS) and singlet oxygen, ultimately resulting in cell death[23,24]. Among PS, methylene blue (MB), a hydrophilic phenothiazine derivative, has been considered an effective, reasonable, and widely utilized PS in numerous dental domains due to its prevalent antimicrobial potency[25,26]. Past literature[27,28] has shown that MB activated by PDT can diminish the growth of acidogenic bacteria (*S. mutans*) in the oral cavity due to ROS release. However, PDT’s role in actuating SDF and chitosan has yet to be studied and explored in eradicating *S. mutans* strains for dissuading caries advancement. Diode lasers (445 nm) have been considered effective and safe in several dental applications by eliminating bacteria and resisting carious attacks in turn securing tooth-restoration bond[29]. Significantly, no rise in temperature in biological tissue is observed while applying diode laser hence considered as an harmless antimicrobial approach[30].

Within the study’s limitations, a detailed exploration of the interaction of different disinfectants with PDT stimulation and their effects on the binding efficacy to protect CAD remains unexplored and unparalleled, requiring substantial *in-vitro* probing. Therefore, it has been hypothesized that CHX will demonstrate better antimicrobial effectiveness against *S. mutans* with enhanced SBS and nano hardness compared to other disinfection methods. Hence, the present study anticipated to assess the disinfection efficacy, bond integrity, and nano hardness of CAD surface bonded to resin cement when disinfected with CHX, MB-PDT, chitosan, SDF, chitosan-PDT, and SDF-diode laser against *S. mutans*.

Materials and Methods

Sample Preparation

Over four months, sixty human mandibular molars (n = 60) were extracted non-traumatically and gathered using ICDAS (International Caries Detection and Assessment System) standards, and all of the specimens were visually inspected for check caries severity index by employing caries detecting dye and dental explorer. After that, a bitewing x-ray was performed and caries code 4 (extensive cavity; demineralization involving the middle third of the dentin) was classified for the specimens, while stained pink dentin was determined as CAD surface. Afterward, all specimens were submerged in a disinfectant solution of 0.5% Chloramine T solution (Sparchem, Mumbai, India) for around 48 hours at 4°C. All the attached periodontal fibers, debris, plaque, and calculus were disengaged from the specimens by using an ultrasonic scaler (ESCO MEDCO, Jiangsu, China) and kept in distilled water until experimental use. Then, samples were dried and positioned vertically in a self-curing acrylic resin (Radiant Surgident, Indore, India) to the cementoenamel junction (CEJ). The study followed the technique defined by Nakajima et al[31]. Later, the CAD surface was ground with 1200-grit silicon carbide grinding discs (Buehler, UK) under flowing water and only caries-infected dentin was removed leaving the caries-affected dentin for inoculation and analyzation of diverse disinfection modes. The average cavity preparation of all specimens had a depth of 2 mm and a breadth of 3 mm to establish uniformity.

Biofilm Formation on the Specimens

The American Type Culture Collection provided gram-positive *S. mutans* (S mutans; ATCC 25175) to inoculate the CAD surface. The specimens were placed in *S. mutans* broth overnight in an orbital incubator (150 rpm) at 37°C. The bacterial cells were harvested and washed with MRS agar media using a centrifuge set at 2,700 g for 15 min. Then, bacterial suspensions were tested using the spectrophotometer with an optical density of 0.6 and wavelength of 600 nm and showed the suspension contained a concentration of 1 × 108 CFU/ml of *S. mutans*. Then, the bacterial suspension (1 × 10^8 CFU/ml) was poured on the well plates containing the specimens and were inoculated and harvested anaerobically for three weeks at 37°C to create a biofilm. To enhance the growth of the bacteria and eradication of non-adherent bacterial cells, the MRS media was changed every alternate day[32].

Experimental Groups

After microbial inoculation, a total of 60 samples (n = 60) were apportioned randomly into six groups, each containing 10 specimens (n = 10), based on the disinfection methodology being used.

Group 1 (n = 10): Treatment with CHX

CAD samples in this group were treated with a 2% CHX solution (Varni Corporation, Gujrat, India) for 60 seconds. Later, distilled water was used to clean the disinfected surface and air-dried for 5 seconds[29].

Group 2 (n = 10): Treatment with MB Activated by PDT

In this group, the CAD surface was treated with 1 ml of MB solution which was activated by PDT. The PS was illuminated and activated by a diode laser (Original Brand, China) for 60 seconds keeping at a 2 mm distance at a wavelength of 660 nm, output power of 40 mW, and energy density of 60 J/cm^2. Later, specimens were cleansed with distilled water and air-dried for 5 seconds.

Group 3 (n = 10): Treatment with Chitosan

100 µL of chitosan at a concentration of 3 mg/ml was used to treat the specimen for 60 seconds as per the study conducted by Camacho-Alonso et al[33]. The CAD surface was then washed with distilled water and air dried for 5 sec.

Group 4 (n = 10): Treatment with Chitosan Activated by PDT

100 µL of chitosan at a concentration of 3 mg/ml was used to treat the specimen along with the irradiation by PDT at a power of 50 mW for 60 seconds and later disinfected and dried for 5 seconds.

Group 5 (n = 10): CAD Surface Treatment with SDF

The CAD surface was treated with 0.04 ml of 38% SDF for 60 seconds and then cleansed with distilled water and dried for 5 seconds.

Group 6 (n = 10): CAD Surface Treatment with SDF and Diode Laser

The CAD surface was treated with 0.04 ml of 38% SDF for 30 seconds and then diode laser was used for further decontamination for a further 30 seconds at a wavelength of 445 nm. The samples were then washed and dried for 5 seconds.

Survival Rates Analyzation

All specimens were preserved in an incubator at 37ºC for 24 hours after covering them with a polyethylene sheet. After disinfection and incubation at 37°C, the survival rate of *S. mutans* was estimated by dividing the colony forming units (CFUs) of each experimental group by the CFU count of the positive control.

Survival rates = CFUs (each group) / CFU (control group)

Bonding Procedure

After disinfecting the cavity, a self-etch adhesive (Adper Prompt, 3 M ESPE AG Dental Products, Seefeld, Germany) was applied by a sterile brush over the surface for 15 seconds and air-dried gently to obtain a thin layer of adhesive all over the cavity area. Later, after etching a surface was again smeared with the bonding agent, air-dried, and light-cured for about 10 seconds by employing an LED light (Woodpecker, China) to photopolymerize the bonding agent. After the adhesive curing, composite (Filtek Z350; 3 M ESPE) was applied in increments of 2 mm into the cavity and each increment was photo-cured for a further 10 seconds after removing the excess cement[34].

All the samples were stored for 24 hours at 37°C in a humid atmosphere in an incubator (Yooning, Zhejiang, China) following the bonding of restorative material to the CAD surface. Samples were heated for 8,000 cycles with a dwell time of 30 seconds using an automated thermocycler (LongMedtech, Jiangsu, China).

Shear Bond Strength Analysis (SBS)

The binding strength between the dentinal surface and the resin cement employed was assessed using a Shear Bond Strength (SBS) test to determine the maximal load before debonding. The dentine-resin bond surfaces served as the shearing element during the shear bond test, which was conducted using a universal testing machine (UTM, Instron, MA, USA) in compression mode at a crosshead speed of 1 mm/min until fracture occurred and debonding force was documented and expressed in Megapascal (*MPa*).

Nanohardness Analysis (NH)

With the aid of an embedded nanoindentation test, the surface nano hardness of specimens was determined. The nano hardness was measured using an atomic force microscope (Optu-Edu, Beijing, China) outfitted with a corner cube diamond indenting tip (Optu-Edu, Beijing, China) in nanoindentation mode. Each specimen received an application of 55.3 µN and three indentations values were recorded and analyzed by using NanoScope software (Bruker, Billerica, MA, USA).

Statistical Analysis

The Kruskal-Walis test was used to evaluate the variance in survival rate. The mean and standard deviation for bond values were done by utilizing analysis of variance (ANOVA) and Post-Hoc Tukey. SPSS software (SPSS version 19, Inc., Chicago, IL, USA) was employed to analyze the data, with a statistical significance threshold (*p*-value) of less than 0.05.

Results

Antimicrobial Efficiency Evaluation/Survival Rates

The normal distribution of data was assessed using the Shapiro-Wilk Test. The survival rates of *S. mutans* after disinfection of CAD surface with different disinfection regimes are demonstrated in Table I. Results revealed that samples disinfected with SDF+ diode laser displayed the highest reduction in *S. mutans* levels in comparison to the other CAD decontamination methods and the use of Chitosan for CAD surface cleansing disclosed the lowest level of disinfection, the highest *S. mutans* survival rate was observed.

The intergroup comparison revealed that CHX and Chitosan activated by PDT displayed comparable outcomes of *S. mutans* survival rate to that of SDF+ diode laser while disinfecting CAD ($p>0.05$). Likewise, MB activated by PDT and SDF displayed a comparable level of survival rate of *S. mutans* to chitosan disinfection ($p>0.05$). (Figure 1).

Table I. Survival rates of *S. mutans* after different disinfection regimes

Groups	Survival rate	Standard deviation
Group 1: CHX	0.33[a]	0.08
Group 2: Methylene blue Photosensitizer activated by PDT	0.76[b]	0.25
Group 3: Chitosan	0.77[b]	0.22
Group 4: Chitosan activated by PDT	0.31[a]	0.10
Group 5: Silver diamine fluoride	0.69[b]	0.09
Group 6: Silver diamine fluoride + diode laser	0.29[a]	0.07

CHX: Chlorhexidine, PDT: Photodynamic therapy. Dissimilar letters indicate statistical significance at $p < 0.05$.

SBS Analysis

SBS values of CAD specimens after using different strategies of disinfection are demonstrated in Table II. The highest SBS was exhibited by the CAD sample disinfected with SDF+ diode laser and the lowest SBS values in CAD specimens after cavity cleansing with MB activated by PDT. The intragroup comparison revealed that CAD specimens disinfected with Chitosan activated by PDT showed comparable SBS values to the SDF+ diode laser ($p>0.05$). Whereas, CAD disinfected with CHX, chitosan, and SDF exhibited bond values comparable to MB activated by PDT ($p<0.05$) (Figure 2).

Nanohardness Analysis

Nanohardness values of CAD specimens after using different strategies of disinfection are demonstrated in Table III. The highest nano hardness was exhibited by the CAD sample disinfected with SDF+ diode laser and the lowest nano hardness in CAD specimens after cavity cleansing with MB activated by PDT. The intragroup comparison revealed that CAD specimens disinfected with Chitosan activated by PDT showed comparable nano hardness to the SDF+ diode laser ($p>0.05$). Whereas, CAD disinfected with CHX, chitosan, and SDF exhibited nano hardness values comparable to MB activated by PDT ($p<0.05$) (Figure 3).

Discussion

The current *in-vitro* study aimed to assess the effectiveness of various disinfection methods, namely CHX, MB-PDT, chitosan, SDF, chitosan-PDT, and SDF-diode laser, against *S. mutans* when bonding resin cement to CAD sur-

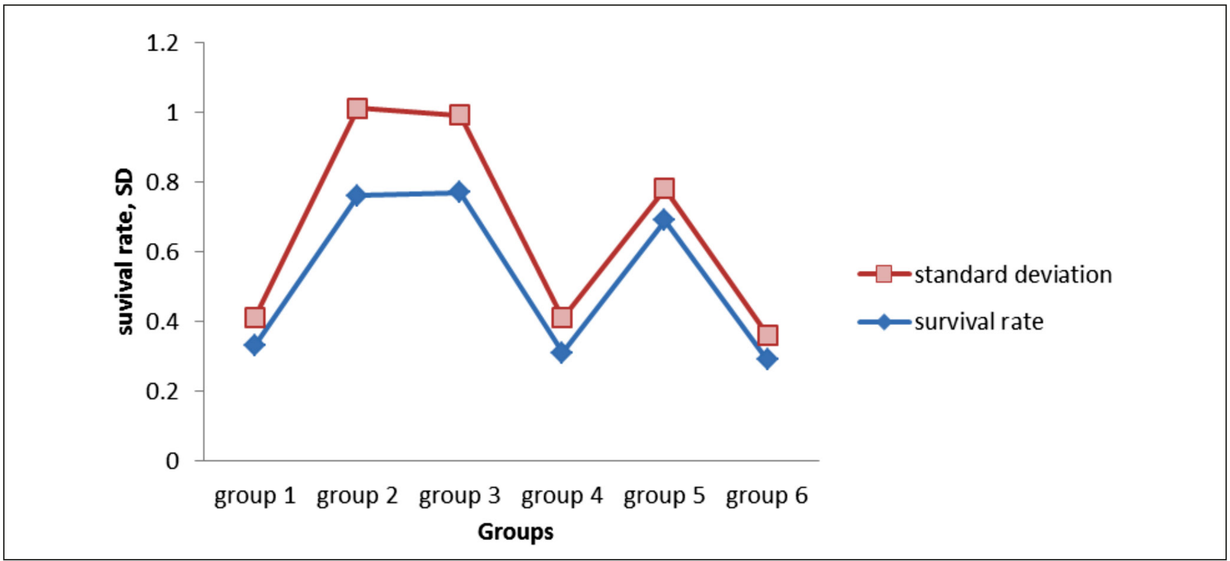

Figure 1. Survival rates of *S. mutans* after different disinfection regimes. Group 1: CHX, Group 2: Methylene blue Photosensitizer activated by PDT, Group 3: Chitosan, Group 4: Chitosan activated by PDT, Group 5: Silver diamine fluoride, Group 6: Silver diamine fluoride+ diode laser.

Table II. Shear bond strength of CAD after different strategies of disinfection.

Different methods of disinfection	Mean	SD	*p*-value
Group 1: CHX	14.21[a]	1.77	< 0.05
Group 2: Methylene blue Photosensitizer activated by PDT	13.45[a]	1.91	
Group 3: Chitosan	15.14[a]	1.13	
Group 4: Chitosan activated by PDT	18.48[b]		
Group 5: Silver diamine fluoride	15.16[a]	1.02	
Group 6: Silver diamine fluoride + diode laser	18.65[b]	1.11	

CHX: Chlorhexidine, PDT: Photodynamic therapy. The different small letter denotes statistically significant difference. !Showing significant differences among study groups (ANOVA) (Tukey multiple comparison test).

faces. The researchers hypothesized that CHX would demonstrate the highest antimicrobial efficacy against *S. mutans* while also improving the bond strength (SBS) and nano hardness compared to other disinfection methods. However, the results did not support this hypothesis, as CHX exhibited lower SBS values compared to other disinfection methods. Interestingly, the SDF-diode laser and chitosan-PDT demonstrated superior SBS, antimicrobial effectiveness, and nano hardness of the CAD surface when bonded to the resin cement, surpassing the performance of other decontamination procedures.

S. mutans possesses significant virulence factors about the pathophysiology and etiology of dental caries that can aid in microbial colonization to form biofilm on adherence to the tooth structure in turn causing weakening of the tooth-structure and ultimately affecting tooth-restorative bond leading to rehabilitation failure[35,36]. Furthermore, the tooth structure is subjected to natural demineralization and remineralization phenomenon in the oral environment but due to acid production and bacterial activity this equilibrium fails and demineralization advances causing damage to the tooth structure, reducing the SBS[37]. Hence, several disinfectants are employed in this study to inhibit carious incursion to safeguard the bond efficacy of restoration to the tooth. Also, the use of fluoride compounds, compounds with calcium and phosphate groups, lasers, and PDT can aid in securing bond efficacy.

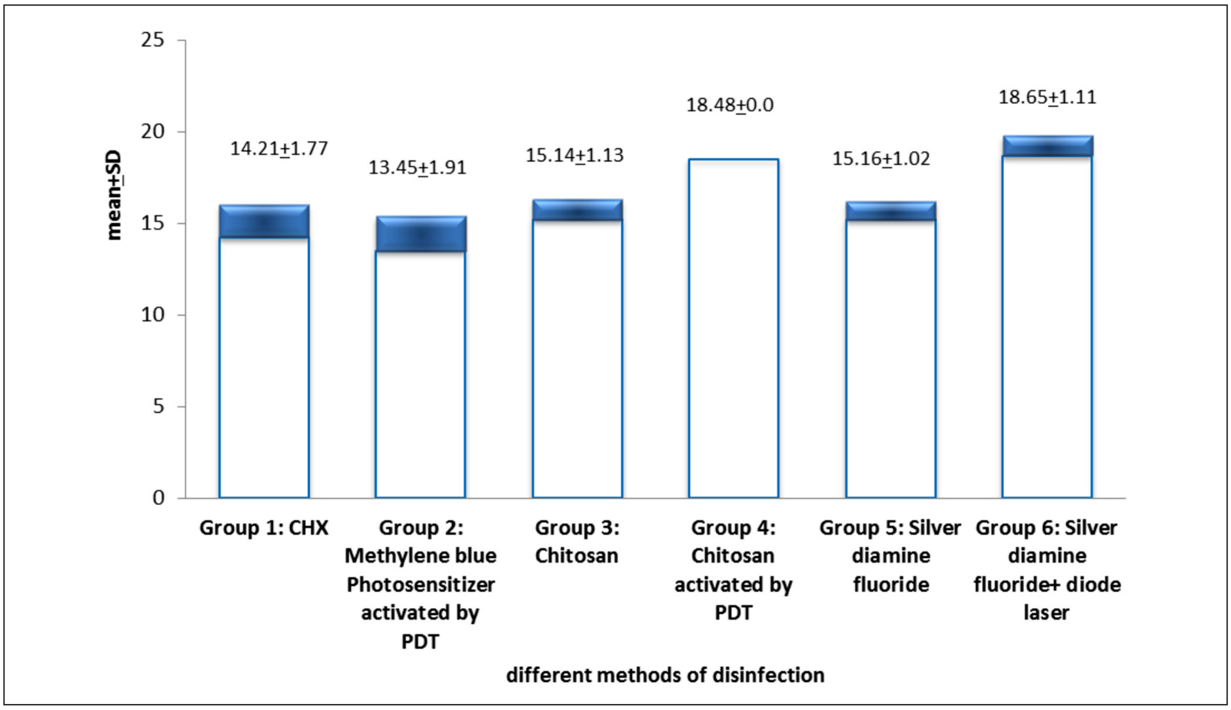

Figure 2. Shear bond strength of CAD after different strategies of disinfection.

Table III. Nanohardness after different methods of disinfection.

Methods of disinfection	Nano hardness (GPa)
Group 1: CHX	1.01 ± 0.13[a]
Group 2: Methylene blue Photosensitizer activated by PDT	0.91 ± 0.09[a]
Group 3: Chitosan	1.06 ± 0.17[a]
Group 4: Chitosan activated by PDT	1.61 ± 0.07[b]
Group 5: Silver diamine fluoride	1.00 ± 0.15[a]
Group 6: Silver diamine fluoride + diode laser	1.63 ± 0.03[b]

CHX: Chlorhexidine, PDT: Photodynamic therapy. The different small letter denotes statistically significant difference.

As per the results of survival rates of *S. mutans* after different disinfection regimes, the highest antimicrobial efficacy was displayed when the CAD surface was treated with SDF + diode laser. Its substantial elucidation is the synergistic effect of SDF's composition and diode laser effectiveness. In SDF, the presence of a higher concentration of fluoride and silver plays an essential role in arresting microbial invasion and remineralizing carious lesions[38]. A study conducted by Rosenblatt et al[17] enlightened the role of constituents in SDF. Fluoride has the affinity to bind to the microbial cell wall, and inhibits enzymatic reactions involved in carbohydrate metabolism, forming an acid-resistant surface in turn inhibiting microbial growth and biofilm formation. Silver Diamine Fluoride (SDF) has the potential to destroy cariogenic bacteria by reacting with their DNA and biological proteins. This reaction leads to the cessation of cell metabolism, thereby impeding the progression of dental caries[38]. Moreover, diode lasers after SDF treatment have shown promising effects on reducing *S. mutans* survival rates. It has been considered that after laser application, an increase in the fluoride uptake by the tooth surface has been acknowledged that also hardens and remineralizes the carious surface, augmenting SBS and restoring the tooth-cement bond[25]. Functionally, by using a diode laser to alter the polarization of an enamel component, fluoride is better retained and diffused into the enamel's inner layers, creating a fluoride reservoir[39]. Temperature can also affect the level of fluoride absorption in the dentin surface since it increases the molecules' kinetic energy, speed, and rate of impact. Hence, high disinfection efficacy, SBS, and nano hardness provided by the combined use of SDF and diode laser have made this disinfec-

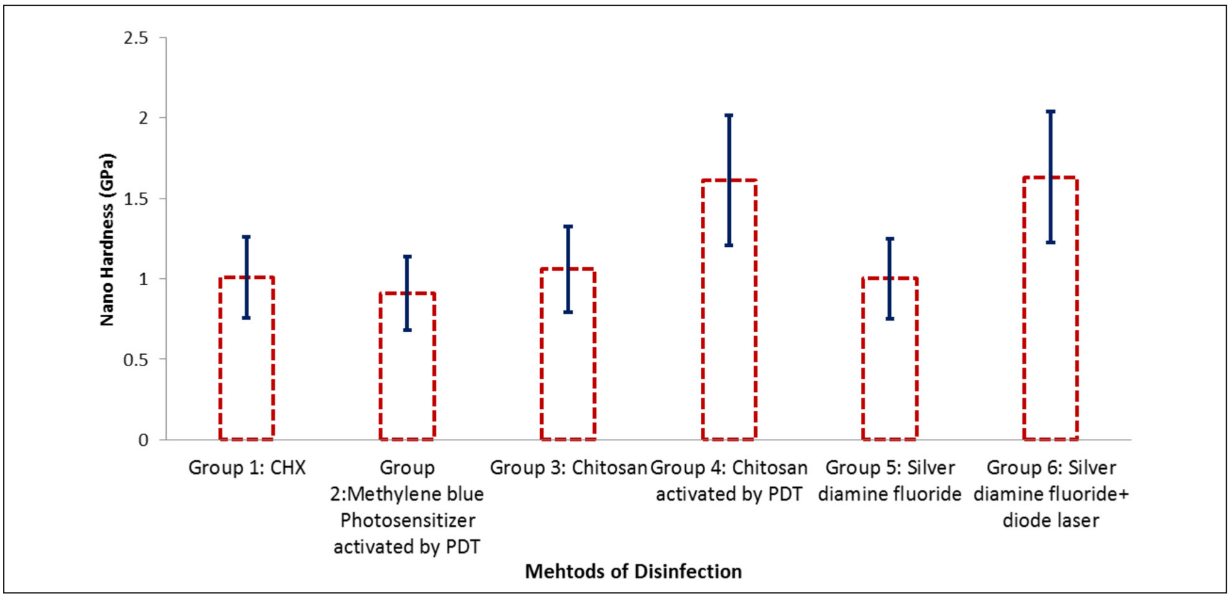

Figure 3. Nanohardness after different methods of disinfection.

tant advisable to use in dentistry. This is in harmony with the work done by Targino et al[40] and Xue et al[41] However, solo use of SDF unveiled low antimicrobial effectiveness, SBS, and NH as only fluoride and silver acted on the tooth surface for remineralization and *S. mutans* eradication.

Similarly, chitosan+PDT application also exhibited comparable disinfection, SBS, and nano hardness results to SDF+ diode laser use. The plausible interpretation of this effect was the synergistic effect of chitosan and PDT. Chitosan functions by altering the bacterial cell wall and cell membrane permeability to prevent bacterial growth as it hinders *S. mutans* from adhering to tooth surfaces and can prevent *S. mutans* from multiplying and emerging, hence inhibiting biofilm formation[42,43]. PDT yields ROS and singlet oxygen on photo illumination of PS at specific wavelengths of light that can assist in destroying *S. mutans* growth[44]. However, solo use of chitosan displayed low SBS, antimicrobial effectiveness, and NH. Still, it was observed that PDT use after chitosan application can enhance the treatment effect due to their convincing antimicrobial effect. Also, PDT has been reconnoitered as a minimally invasive approach that can affect the desired area and assassinate resistant bacterial strains, aids the tooth to remineralize with the help of chitosan as it can induce nucleation of crystals over the dentin surface by use of calcium and phosphate thereby increasing bond efficacy and NH[20,45]. Knowingly, these results are in agreement with the study steered by Azizi et al[46] and Gong et al[47].

As per the outcome of the study, CHX unveiled high antimicrobial efficacy but reduced SBS and NH when used on CAD surfaces. CHX, being a cationic biguanide, emits a positively charged cation on dissociation at physiological pH which binds to the negatively charged microbial cell wall ensuring its viable bactericidal effect causing membrane disruption and cell death, thereby reducing *S. mutans* from the CAD surface[48,49]. However, low SBS and NH are mainly due to their low depth penetration into the dentinal tubules as they fail to prevent dentin matrix metalloproteinases (MMPs) and cysteine cathepsins to degrade extracellular matrix leading to dentinal degradation[50]. A study performed by Galo et al[51] presumed similar outcomes.

Moreover, MB activated by PDT presented the lowest disinfection efficacy, SBS, and NH as per the results. It is believed that MB has an affinity for cationic compounds, it attaches to CAD through calcium (Ca^{++}) and phosphate (P^{+}) ions, forming a physical barrier between the CAD surface and filling material by precipitating calcium and phosphate over the dentinal structure[52]. Additionally, when applied to CAD surfaces, MB causes dentin to absorb water due to its hydrophilic nature, causing volumetric expansion and solubilizing the resin increment, deteriorating the bond strength and hardness values[53]. Alonaizan et al[54] deduced the akin results.

The current study should be comamended for conducting detailed investigations that explored both individual and combined applications of different disinfection techniques on *S. mutans* eradication, bond efficacy, and nano hardness. These methods involved various chemical, photoactivated, and laser-activated antimicrobial agents. It is noteworthy that the effectiveness of photodynamic therapy (PDT) can be influenced by several variables, such as the type of visible light utilized, the laser's settings, wavelength, and the interaction of the photosensitizer (PS) with the target tissue. To ensure a comprehensive evaluation of the samples, the researchers should consider employing scanning electron microscopy (SEM) and atomic force microscopy of CAD surfaces. These advanced imaging techniques will allow for a thorough assessment, providing valuable insights into the morphological and structural characteristics of the treated surfaces. By incorporating SEM and atomic force microscopy analyses, the study can better understand the effects of the disinfection techniques on the CAD surfaces at a micro and nanoscale level.

Conclusions

Synergistic use of silver diamine fluoride with diode laser and chitosan activated by PDT can be used as an alternative to CHX for controlling *S. mutans* growth, promoting enhanced bond efficacy and nano hardness for bonding resin cement to the caries-affected dentin.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Ethics Approval

The Ethical Committee of King Saud University approved the study (IRB #FC-2113, dated 2nd May 2023).

Authors' Contribution

Conceptualization.; Methodology; Software, Validation, Formal analysis, Investigation data curation, writing—original draft preparation, and writing—review and editing, visualization, supervision, and project administration, funding acquisition performed AMA, AAK, AM, HAB, MAK, EAB. All authors have read and agreed to the published version of the manuscript.

Informed Consent

Not applicable.

References

1) De Soet JJ, Van Gemert-Schriks MCM, Laine ML, Van Amerongen WE, Morré SA, Van Winkelhoff AJ. Host and microbiological factors related to dental caries development. Caries Res 2008; 42: 340-347.
2) Bordea IR, Hanna R, Chiniforush N. Evaluation of the outcome of various laser therapy applications in root canal disinfection: A systematic review. Photodiagnosis Photodyn Ther 2020; 29: 101611-10115
3) Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65: 1028-1037.
4) Kamran MA, Qasim M, Udeabor SE, Hameed MS, Mannakandath ML, Alshahrani I. Impact of riboflavin mediated photodynamic disinfection around fixed orthodontic system infected with oral bacteria. Photodiagnosis Photodyn Ther 2021; 34: 102232-102237.
5) Lemos JA, Palmer SR, Zeng L. The Biology of Streptococcus Mutans. Microbiol Spectr 2019; 7: 0051-0055.
6) Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 2008; 42: 409-418.
7) Gürgan S, Bolay Ş, Kiremitçi A. Effect of disinfectant application methods on the bond strength of composite to dentin. J Oral Rehabil 1999; 26: 836-840.
8) Shafiei F, Memarpour M. Antibacterial activity in adhesive dentistry: A literature review. Gen Dent 2012; 60: e346-e356.
9) Pinna R, Maioli M, Eramo S, Mura I, Milia E. Carious affected dentine: Its behaviour in adhesive bonding. Aust Dent J 2015; 60: 276-293.
10) AlSheikh R, Abduldaiem OY, Alkhalifa MS. Different cavity disinfectant efficacy against S.mutans and shear bond strength of caries affected dentin bonded to resin restoration. Photodiagnosis Photodyn Ther 2023; 42: 103560.
11) Shafiei F, Fekrazad R, Kiomarsi N, Shafiei E. Bond strength of two resin cements to dentin after disinfection pretreatment: Effects of Er,Cr:YSGG laser compared with chemical antibacterial agent. Photomed Laser Surg 2013; 31: 206-211.
12) Wady AF, Paleari AG, Queiroz TP. Bond strength of repaired composites with different surface. J Prosthet Dent 2003; 22: 302-308.
13) Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. J Clin Periodontol 1988; 15: 415-424.
14) McDonnell G RA. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Re 2019; 12: 147-155.
15) Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. Minerva Stomatol 2012; 61: 399-419.
16) Catalbas B, Ercan E, Dalli M, Gelgor İE, Erdemir A. Does chlorhexidine affect the shear bond strengths of orthodontic brackets? J Dent Sci 2011; 6: 76-81.
17) Rosenblatt A, Stamford TCM, Niederman R. Silver diamine fluoride: a caries “silver-fluoride bullet”. J Dent Res 2009; 88: 116-125.
18) Mei ML, Lo ECM, Chu CH. Arresting Dentine Caries with Silver Diamine Fluoride: What’s Behind It? J Dent Res 2018; 97: 751-758.
19) Gold J. Silver Diamine Fluoride Prevents Caries in Primary Teeth Superior to No Treatment, Placebo, or Fluoride Varnish. J Evid Based Dent Pract 2020; 20: 101422-101427.
20) Raafat D, Sahl H-G. Chitosan and its antimicrobial potential--a critical literature survey. Microb Biotechnol 2009; 2: 186-201.
21) Chung Y-C, Chen C-Y. Antibacterial characteristics and activity of acid-soluble chitosan. Bioresour Technol 2008; 99: 2806-2814.
22) Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. Int J Food Microbiol 2001; 71: 235-244.
23) Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. Biochem. J 2016; 473: 347-364.
24) Konopka K, Goslinski T. Photodynamic therapy in dentistry. J. Dent. Res 2007; 86: 694-707.
25) Lim Z, Cheng JL, Lim TW, Teo EG, Wong J, George S, Kishen A. Light activated disinfection: an alternative endodontic disinfection strategy. Aus Dent J 2009; 54: 108-114.
26) Catão MHC de V, Batista ALA. In vitro evaluation of the antibacterial effect of photodynamic therapy with methylene blue. Pesqui Bras Odontopediatria Clin Integr 2020; 20: 1-10.
27) Alrahlah A, Naseem M, Tanveer SA et al. Influence of disinfection of caries effected dentin with different concentration of silver diamine fluoride, curcumin and Er, Cr:YSGG on adhesive bond strength to resin composite. Photodiagnosis Photodyn Ther 2020; 32: 102065-102071.

28) Alkhudhairy FI, Alkheraif A, Bin-Shuwaish MS. Influence of different photosensitizers on push-out bond strength of fiber post to radicular dentin. Photodiagnosis Photodyn Ther 2020; 8: 1-8.
29) Alshahrani A, Abrar E, Maawadh AMI. Management of caries affected dentin (CAD) with resin modified glass ionomer cement (RMGIC) in the presence of different caries disinfectants and photosensitizers. Photodiagnosis Photodyn Ther 2020; 32: 101978-101983.
30) Pereira CA, Costa ACBP, Carreira CM, Junqueira JC, Jorge AOC. Photodynamic inactivation of Streptococcus mutans and Streptococcus sanguinis biofilms in vitro. Lasers Med Sci 2013; 28: 859-864.
31) Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J. Bonding to caries-affected dentin. Jpn Dent Sci Rev 2011; 47: 102-114.
32) Al-Saleh S, Al Rifaiy MQ, Binhasan M, Alhamdan MM, Vohra F, Abduljabbar T. Modified photoactivated methylene blue-incorporated quartz particles for dentin disinfection: A scanning electron microscope and spectroscopic analysis. Microsc Res Tech 2022; 85: 2234-2240.
33) Camacho-Alonso F, Julián-Belmonte E, Chiva-García F, Martínez-Beneyto Y. Bactericidal Efficacy of Photodynamic Therapy and Chitosan in Root Canals Experimentally Infected with Enterococcus faecalis: An In Vitro Study. Photomed Laser Surg 2017; 35: 184-189.
34) Abrar E, Naseem M, Baig QA. Antimicrobial efficacy of silver diamine fluoride in comparison to photodynamic therapy and chlorhexidine on canal disinfection and bond strength to radicular dentin. Photodiagnosis Photodyn Ther 2020; 32: 102066-102071.
35) Hamada S, Koga T, Ooshima T. Virulence factors of Streptococcus mutans and dental caries prevention. J Dent Res 1984; 63: 407-411.
36) Hossain MS, Alam S, Nibir YM. Genotypic and phenotypic characterization of Streptococcus mutans strains isolated from patients with dental caries. Iran J Microbiol 2021; 13: 449-457.
37) Banas JA. Virulence properties of Streptococcus mutans. Front Biosci 2004; 9: 1267-1277.
38) Zhao IS, Gao SS, Hiraishi N. Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int. Dent. J 2018; 68: 67-76.
39) Al-Maliky MA, Frentzen M, Meister J. Combined effects of a topical fluoride treatment and 445 nm laser irradiation of enamel against a demineralization challenge: A light and electron microscopic ex vivo study. PLoS One 2020; 15: e0237195-e0237199.
40) Targino AGR, Flores MAP, dos Santos Junior VE. An innovative approach to treating dental decay in children. A new anti-caries agent. J Mater Sci Mater Med 2014; 25: 2041-2047.
41) Xue VW, Yin IX, Niu JY, Lo ECM, Chu CH, Zhao IS. Effects of a 445 nm diode laser and silver diamine fluoride in preventing enamel demineralisation and inhibiting cariogenic bacteria. J Dent 2022; 126: 104309-104315.
42) Tarsi R, Muzzarelli RA, Guzmán CA, Pruzzo C. Inhibition of Streptococcus mutans adsorption to hydroxyapatite by low-molecular-weight chitosans. J Dent Res 1997; 76: 665-672.
43) Chen C-Y, Chung Y-C. Antibacterial effect of water-soluble chitosan on representative dental pathogens Streptococcus mutans and Lactobacilli brevis. J Appl Oral Sci 2012; 20: 620-627.
44) Terra-Garcia M, de Souza CM, Ferreira Gonçalves NM. Antimicrobial effects of photodynamic therapy with Fotoenticine on Streptococcus mutans isolated from dental caries. Photodiagnosis Photodyn Ther 2021; 34: 102303-102307.
45) Chandy T, Sharma CP. Chitosan--as a biomaterial. Biomater Artif Cells Artif Organs 1990; 18: 1-24
46) Azizi A, Shademan S, Rezai M, Rahimi A, Lawaf S. Effect of photodynamic therapy with two photosensitizers on Streptococcus mutants: In vitro study. Photodiagnosis Photodyn Ther 2016; 16: 66-71.
47) Gong J, Park H, Lee J, Seo H, Lee S. Effect of Photodynamic Therapy on Multispecies Biofilms, Including Streptococcus mutans, Lactobacillus casei, and Candida albicans. Photobiomodulation, photomedicine, laser Surg 2019; 37: 282-287.
48) Rayar S, Sadasiva K, Singh P, Thomas P, Senthilkumar K, Jayasimharaj U. Effect of 2% chlorhexidine on resin bond strength and mode of failure using two different adhesives on dentin: An in vitro study. J Pharm Bioallied Sci 2019; 11: S325-S330.
49) Rossi-Fedele G, Doramac EJ, Guastalli AR, Steier L, Poli De Figueiredo JA. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J. Endod 2012; 38: 426-431.
50) Arslan S, Yazici AR, Gorucu J. Effects of different cavity disinfectants on shear bond strength of a silorane-based resin composite. J Contemp Dent Pract 2011; 12: 279-286.
51) Galo R, Marinho MT, Telles PD da S, Borsatto MC. Shear bond strength of the adhesive/dentin interface after different etching protocols. J Conserv Dent 2021; 24: 393-398.
52) Al Jeaidi ZA. Influence of resin cements and root canal disinfection techniques on the adhesive bond strength of fibre reinforced composite post to radicular dentin. Photodiagnosis Photodyn Ther 2021; 33: 102108-102114.
53) Al Ahdal K, Al Deeb L, Al-Hamdan RS. Influence of different photosensitizers on push-out bond strength of fiber post to radicular dentin. Photodiagnosis Photodyn Ther 2020; 31: 101805-101810.
54) Alonaizan FA, Alofi RS, Alfawaz YF. Effect of Photodynamic Therapy, Er,Cr:YSGG, and Nd:YAG Laser on the Push-Out Bond Strength of Fiber Post to Root Dentin. Photobiomodulation Photomedicine Laser Surg 2020; 38: 24-29.