



Comparative Efficacy of Photodynamic Therapy and Conventional Methods for the Disinfection of Clear Thermoplastic Sheets: An In Vitro Study

Leila Pourmoussa¹, Amirhossein Mirhashemi^{1,2*}, Mohammad Ali Keshvad², Sogol Saberi¹, Yasaman Sanaee², Maryam Pourhajibagher^{3**}

1. Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

2. Department of Orthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

3. Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Article Info

Article type:

Original Article

Article History:

Received: 06 Jul 2025

Accepted: 15 Jan 2026

Published: 10 Jul 2026

Corresponding authors:

*Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Email: mirhashemi@sina.tums.ac.ir

**Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Email: m-pourhajibagher@sina.tums.ac.ir

ABSTRACT

Objectives: This study compared the efficacy of photodynamic therapy (PDT) with that of conventional methods for disinfection of clear thermoplastic sheets used for the fabrication of aligners.

Materials and Methods: In this study, 70 pieces of clear dental aligners measuring 15×15mm were gamma-sterilized, and randomly assigned to two groups (n=35) for inoculation with *Streptococcus mutans* and *Lactobacillus acidophilus*. After biofilm formation, the specimens were randomly assigned to 7 subgroups for disinfection with 0.2% chlorhexidine (CHX), 2.5% acetic acid, 3% hydrogen peroxide (HP), 100 µL/mL methylene blue (MB), PDT with MB and diode laser, diode laser alone, and distilled water (control). After sonication and culture, the colonies were counted and compared using ANOVA, Tamhane, and one sample t-test (alpha=0.05).

Results: HP showed the highest efficacy in both bacterial groups (P<0.05). In *S. mutans* group, CHX had a significantly higher efficacy than the other modalities (P<0.05). Distilled water showed no significant difference compared with MB or laser (P>0.05), and acetic acid showed no significant difference compared with PDT (P>0.05). In the *L. acidophilus* group, CHX demonstrated significantly higher efficacy than the other modalities (P<0.05). Distilled water showed no significant difference compared with laser, and acetic acid showed no significant difference compared with PDT (P>0.05).

Conclusion: Within the limitations of this in vitro study, PDT demonstrated moderate antibacterial activity against *S. mutans* and *L. acidophilus* biofilms. However, HP followed by CHX showed significantly greater antibacterial efficacy and remained the most effective disinfection approach among those evaluated.

Keywords: *Lactobacillus acidophilus*; Lasers; Methylene Blue; Orthodontic Appliances; Photochemotherapy; *Streptococcus mutans*

- **Cite this article as:** Pourmoussa L, Mirhashemi A, Keshvad MA, Saberi S, Sanaee Y, Pourhajibagher M. Comparative Efficacy of Photodynamic Therapy and Conventional Methods for the Disinfection of Clear Thermoplastic Sheets: An In Vitro Study. *Front Dent.* 2026;23:29. <http://doi.org/10.18502/fid.v23i29.22719>

INTRODUCTION

The demand for orthodontic clear aligners is on the rise. They have gained increasing popularity due to their esthetic appearance

and ease of use [1,2]. Nonetheless, plaque accumulation and proliferation of microorganisms on their surface are a major concern. Evidence has demonstrated the

colonization of *Streptococcus mutans* and lactobacilli, particularly *Lactobacillus acidophilus* on the surface of thermoplastic orthodontic appliances. Insufficient bacterial biofilm removal can lead to enamel demineralization and the development of white spot lesions. It can also lead to gingivitis, periodontitis, gingival hyperplasia, or gingival recession [3]. A previous study reported severe gingival inflammation and carious lesions after 4 months of clear aligner use in patients who did not clean their aligners [4]. If left untreated, such problems can also lead to systemic infections [4]. Thus, proper and effective cleaning and disinfection of clear aligners are of utmost importance.

The recommended protocol for wearing clear aligners includes wearing the aligners for 20 to 24 hours per day, removing them during meals, and brushing them before reinsertion [5]. Different protocols have been suggested for the cleaning of removable orthodontic appliances and clear aligners [6–8]. The conventional methods for disinfection of clear aligners include the use of chemical agents such as chlorhexidine (CHX), peroxide tablets, mouthwashes, or other disinfecting agents [9–13]. However, these methods have drawbacks, such as causing discoloration, corrosion, and reducing the clinical service life of aligners. Additionally, they are often not effective enough to eliminate all microorganisms. Moreover, determining and adhering to the optimal disinfection time when using chemical agents can be challenging for patients [14,15]. Systematic reviews evaluating the best protocol for the disinfection of clear aligners failed to reach a definite conclusion regarding the optimal method [16,17] and called for further studies to directly compare the efficacy of different disinfection protocols. It appears that a multi-step protocol comprising a mechanical and a chemical method may yield optimal results, warranting further investigation.

Photodynamic therapy (PDT) is a novel disinfection technique based on the activation of a photosensitizer by a specific wavelength of light and the subsequent production of reactive oxygen species (ROS) capable of eliminating microorganisms such as *S. mutans*

[18,19]. This method has advantages such as broad-spectrum antimicrobial activity against a wide range of Gram-positive and Gram-negative microorganisms, the absence of microbial resistance, and a high level of safety for host tissues [20]. Methylene blue (MB) and toluidine blue are the most commonly used photosensitizers in PDT [19]. Considering the advantages of PDT, it may be suitable for the surface disinfection of orthodontic appliances and clear aligners [21]. Considering the significance of effective disinfection of clear aligners and the advantages of PDT, this study aimed to compare the efficacy of PDT with that of conventional methods for the disinfection of clear thermoplastic sheets used for the fabrication of clear aligners.

MATERIALS AND METHODS

This in vitro, experimental study evaluated hard thermoplastic sheets of polyethylene terephthalate glycol (Bio-Art; Brazil) with a thickness of 1mm, which were cut into 15×15 mm square-shaped pieces using scissors and gamma sterilized (Gamacell GC-220; Nordion, Canada) at a dose of 25kGy for 30 minutes. The study protocol was ethically approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.BLC.1403.015).

Sample size:

The sample size was calculated to be 5 samples per subgroup in each of the 7 subgroups of each microorganism group using a one-way ANOVA power analysis performed with PASS software (version 21), assuming an α level of 0.05, a β level of 0.2, a mean standard deviation of 2.5, and an effect size of 0.75, according to a previous study [22].

Bacterial culture:

Under sterile conditions, vials of lyophilized bacteria (obtained from the Pasteur Institute of Iran) were reconstituted in 1–2mL of culture medium and cultured. A swab dipped in the microbial suspension was used for culture of *S. mutans* (ATCC35668) and *L. acidophilus* (ATCC314) on agar plates. *L. acidophilus* was cultured in MRS broth (Merck, Germany), whereas *S. mutans* was cultured in brain-heart infusion (BHI) broth

(Merck, Germany) supplemented with 1% sucrose. Both microorganisms were incubated at 37°C in a 5% CO₂ atmosphere for 24 hours. To determine the concentration of each microorganism, the optical density of the bacterial suspensions was measured spectrophotometrically at 600nm. Accordingly, a bacterial suspension equivalent to the 0.5 McFarland standard (1.5×10^8 colony-forming units/mL [CFU/mL]) was prepared. Figure 1 presents a schematic illustration of the experimental procedures performed in this study.

Biofilm formation on clear thermoplastic sheets:

Of 70 pieces of clear thermoplastic sheets, 35 were randomly assigned to the *S. mutans*, and 35 to the *L. acidophilus* group. Next, they were immersed in 5mL of the respective microbial suspension, placed in a candle jar (5% CO₂), and incubated at 37°C for 48 hours to allow biofilm formation on their surface.

Disinfection of thermoplastic sheets:

The specimens in each microorganism group were further randomly subdivided into 7 subgroups (n=5) for disinfection with

different protocols as follows:

1. 5mL of 0.2% CHX (Irsha, Shafay-Iran Pharmaceuticals, Iran). CHX served as the positive control because of its well-established antimicrobial efficacy.
2. 5mL of 2.5% acetic acid (Merck, Germany)
3. 5mL of 3% hydrogen peroxide (HP; Merck, Germany)
4. 5mL of MB at a concentration of 100µL/mL (Merck, Germany)
5. PDT with MB as the photosensitizer and irradiation using a 660nm diode laser. The specimens were first immersed in 5mL of MB in the dark for 5 minutes and then rinsed with distilled water. Subsequently, each side of the specimen was irradiated using a continuous-wave (CW) diode laser (Thor LX2; THOR Photomedicine Ltd., UK) at an output power of 200mW. The laser beam was delivered through an applicator with a spot area of 0.5cm², corresponding to a power density of 400mW/cm². Irradiation was performed from a distance of 1mm for 60s per side, delivering 12J of energy and an energy density (fluence) of 24J/cm² to each surface.

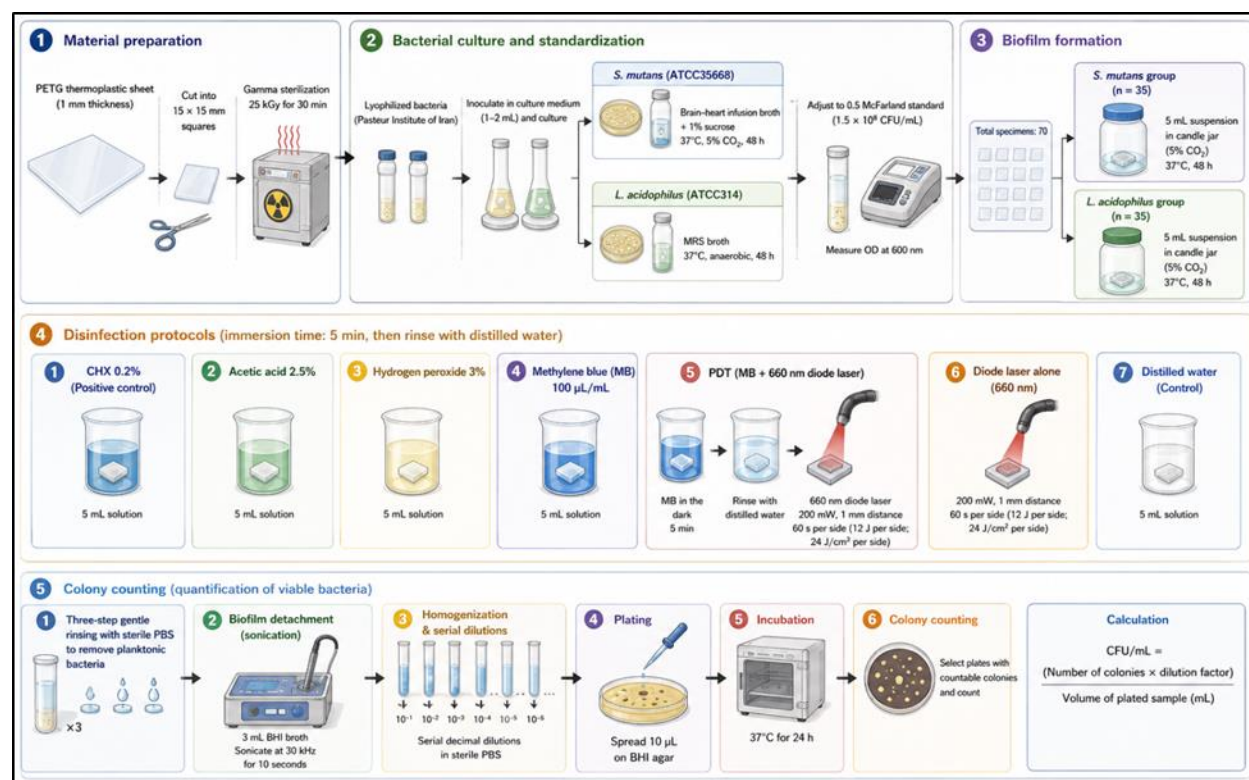


Figure 1. Schematic illustration of the experimental procedures performed in this study.

6. Diode laser irradiation alone using the same irradiation parameters

7. 5mL of distilled water as the control group
The immersion time of the specimens in each disinfecting solution was 5 minutes, and they were subsequently rinsed with distilled water

Colony counting:

The specimens underwent three gentle rinses with sterile phosphate-buffered saline (PBS) to remove planktonic bacteria loosely attached to the surface. Next, to detach the microbial biofilm, each specimen was independently placed in a sterile tube containing 3mL of brain-heart infusion broth and sonicated (Bandelin, Germany) at 30kHz for 10 seconds. The biofilm-containing suspensions were homogenized and serially diluted in phosphate-buffered saline. Next, 10µL of each diluted suspension was spread onto BHI agar (Merck, Germany) and incubated at 37°C for 24 hours. Finally, the antimicrobial efficacy of each method was determined by colony counting and expressed as CFU/mL. For quantitative bacterial enumeration, serial decimal dilutions of the collected bacterial suspensions were prepared, and appropriate dilutions were plated onto the relevant culture media. After incubation, plates containing a countable number of colonies were selected for colony enumeration. The bacterial concentration was calculated and expressed as colony-forming units per milliliter (CFU/mL) using the following formula:

$$CFU/mL = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume of plated sample (mL)}}$$

Plates showing no detectable colony growth were recorded as zero CFU at the corresponding dilution, whereas plates with an uncountable number of colonies were excluded from quantitative analysis. The dilution factor and plated volume were considered in all CFU/mL calculations to ensure accurate estimation of bacterial viability.

Statistical analysis:

The normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and the homogeneity of variances was evaluated using Levene's test. Based on the

results, overall comparisons were performed using one-way ANOVA, followed by pairwise comparisons using Tamhane's T2 post hoc test. The subgroup with zero colony counts was excluded from this analysis. Finally, a one-sample t-test was used to compare all subgroups with the HP subgroup. All statistical analyses were performed using SPSS software (version 24), and $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 presents the measures of central tendency and dispersion for *S. mutans* and *L. acidophilus* colony counts in all subgroups except for the HP subgroup (the zero-colony subgroup). One-way ANOVA showed a significant difference in both *S. mutans* ($P < 0.001$) and *L. acidophilus* ($P < 0.001$) colony counts among the different subgroups. Pairwise comparisons of the subgroups with respect to the *S. mutans* and *L. acidophilus* colony counts within each microorganism group were then performed using Tamhane's T2 post hoc test (Table 2). As shown, the *S. mutans* colony count was not significantly different among the distilled water (control), MB, and laser subgroups ($P > 0.05$). However, these three subgroups showed significantly higher *S. mutans* colony counts than the other subgroups ($P < 0.05$). The *S. mutans* colony counts in the acetic acid and PDT subgroups were not significantly different ($P > 0.05$), whereas the CHX subgroup showed significantly lower colony counts than all other subgroups ($P < 0.05$).

Regarding *L. acidophilus*, the results showed a significantly higher colony count in the control subgroup than in all other subgroups ($P < 0.05$), except for the laser subgroup ($P > 0.05$). The *L. acidophilus* colony count in the MB subgroup was not significantly different from that in the laser subgroup ($P > 0.05$); however, it was significantly higher than those observed in the other subgroups and significantly lower than that observed in the control subgroup ($P < 0.05$). The *L. acidophilus* colony counts in the acetic acid and PDT subgroups were not significantly different ($P > 0.05$), whereas the CHX subgroup showed significantly lower colony counts than all other subgroups ($P < 0.05$).

Table 1. Measures of central tendency and dispersion for *S. mutans* and *L. acidophilus* colony counts in all subgroups (n = 5), excluding the HP subgroup (zero-colony subgroup)

Group	Subgroup	Mean (CFU/mL)	SD (CFU/mL)	Min	Max
<i>S. mutans</i>	Control	8.22×10^5	1.41×10^5	6.30×10^5	9.70×10^5
	CHX	9.72×10^4	8.53×10^3	8.70×10^4	1.10×10^5
	Acetic acid	3.20×10^5	7.68×10^4	2.50×10^5	4.30×10^5
	MB	6.40×10^5	2.55×10^4	6.10×10^5	6.80×10^5
	Laser	6.74×10^5	3.97×10^4	6.20×10^5	7.20×10^5
	PDT	1.88×10^5	3.35×10^4	1.50×10^5	2.30×10^5
	Total	4.57×10^5	2.81×10^5	8.70×10^4	9.70×10^5
<i>L. acidophilus</i>	Control	8.52×10^5	5.40×10^4	7.80×10^5	9.20×10^5
	CHX	1.13×10^5	2.46×10^4	8.60×10^4	1.50×10^5
	Acetic acid	3.40×10^5	6.60×10^4	2.60×10^5	4.40×10^5
	MB	6.88×10^5	6.14×10^4	6.20×10^5	7.80×10^5
	Laser	7.28×10^5	5.76×10^4	6.60×10^5	8.10×10^5
	PDT	2.54×10^5	2.88×10^4	2.30×10^5	2.90×10^5
	Total	4.96×10^5	2.82×10^5	8.60×10^4	9.20×10^5

MB: Methylene blue; PDT: Photodynamic therapy; CHX: Chlorhexidine.

HP reduced the colony counts of both microorganisms to zero. Therefore, each subgroup was compared with the HP subgroup with respect to the *S. mutans* and *L. acidophilus* colony counts (Table 3). The results showed significantly higher colony counts of both *S. mutans* and *L. acidophilus* in all subgroups compared with the HP subgroup ($P < 0.05$).

DISCUSSION

This study compared the efficacy of PDT and the conventional methods for disinfection of clear thermoplastic sheets used for the fabrication of clear aligners. The results showed that although PDT with a diode laser was less effective than HP and CHX, it significantly decreased the *S. mutans* and *L. acidophilus* colony counts. Considering the present results, it appears that the type of photosensitizer plays an important role in the efficacy of PDT against different microorganisms. Silva Teófilo et al. [23] in their systematic review stated that toluidine blue and MB had higher antibacterial activity than other photosensitizers against cariogenic bacteria present in biofilm. Thus, MB was used in the present study.

In the current study, disinfection with acetic acid, PDT, CHX, and HP had a higher efficacy in

reducing *L. acidophilus* and *S. mutans* colony counts than distilled water, which was in line with previous findings [24–26]. Kiatwarawut et al. [24] found that application of 0.625% acetic acid for disinfection of copolyester and polyurethane retainers decreased the *S. mutans* colony count. However, they added that acetic acid was not capable of removing the biofilm, and it needed to be accompanied by mechanical plaque removal.

In the present study, HP showed the highest antibacterial efficacy compared with the other modalities. However, studies regarding its efficacy are scarce. Akgün et al. [25] compared the antimicrobial activity of peroxide-based tablets (along with tooth brushing) and acetic acid solution (along with tooth brushing) against *S. mutans* and *L. acidophilus* on the surface of orthodontic retainers with tooth brushing alone. They reported that tooth brushing alone was not sufficient to eliminate bacteria and that the use of chemical agents was necessary to reduce the bacterial load on orthodontic appliances. They also reported comparable antimicrobial efficacy of peroxide-based tablets and 5% acetic acid against *S. mutans* and *L. acidophilus*. It appears that differences in study design and methodology, as well as variations in the

Table 2. Pairwise comparisons of *S. mutans* and *L. acidophilus* colony counts among subgroups within each microorganism group using Tamhane's test

Group	Subgroups compared	Mean difference	95% CI (Lower bound)	95% CI (Upper bound)	P value
<i>S. mutans</i>	Control vs CHX	724800.00000	334923.2122	1114676.7878	0.005
	Control vs Acetic acid	502000.00000	171648.1370	832351.8630	0.006
	Control vs MB	182000.00000	-194879.0964	558879.0964	0.487
	Control vs Laser	148000.00000	-212862.0490	508862.0490	0.702
	Control vs PDT	634000.00000	265879.7855	1002120.2145	0.005
	CHX vs Acetic acid	-222800.00000	-432979.9981	-12620.0019	0.040
	CHX vs MB	-542800.00000	-606562.0238	-479037.9762	0.000
	CHX vs Laser	-576800.00000	-681635.4013	-471964.5987	0.000
	CHX vs PDT	-90800.00000	-177528.4202	-4071.5798	0.042
	Acetic acid vs MB	-320000.00000	-512312.0668	-127687.9332	0.005
	Acetic acid vs Laser	-354000.00000	-534835.4452	-173164.5548	0.001
	Acetic acid vs PDT	132000.00000	-52725.2188	316725.2188	0.197
	MB vs Laser	-34000.00000	-126680.0601	58680.0601	0.917
	MB vs PDT	452000.00000	372666.4315	531333.5685	0.000
	Laser vs PDT	486000.00000	389553.2406	582446.7594	0.000
<i>L. acidophilus</i>	Control vs CHX	739400.00000	610197.9310	868602.0690	0.000
	Control vs Acetic acid	512000.00000	353157.3891	670842.6109	0.000
	Control vs MB	164000.00000	12922.8571	315077.1429	0.031
	Control vs Laser	124000.00000	-21245.5150	269245.5150	0.114
	Control vs PDT	598000.00000	471133.6474	724866.3526	0.000
	CHX vs Acetic acid	-227400.00000	-389749.7825	-65050.2175	0.011
	CHX vs MB	-575400.00000	-724925.2616	-425874.7384	0.000
	CHX vs Laser	-615400.00000	-754411.9001	-476388.0999	0.000
	CHX vs PDT	-141400.00000	-211555.0958	-71244.9042	0.001
	Acetic acid vs MB	-348000.00000	-513749.1242	-182250.8758	0.000
	Acetic acid vs Laser	-388000.00000	-549880.3584	-226119.6416	0.000
	Acetic acid vs PDT	86000.00000	-72557.7822	244557.7822	0.461
	MB vs Laser	-40000.00000	-194817.5953	114817.5953	0.997
	MB vs PDT	434000.00000	287813.2362	580186.7638	0.000
	Laser vs PDT	474000.00000	337857.5699	610142.4301	0.000

CI: Confidence interval; CHX: Chlorhexidine; MB: Methylene blue; PDT: Photodynamic therapy.

concentration of the solutions used, may be responsible for the differences in the results among the studies.

Hakami and Alshehri [26] reported that application of PDT and 0.12% CHX decreased the *S. mutans* colony count; however, the efficacy of PDT was higher than that of CHX in their study, which was in contrast to the present findings. This difference may be attributed to differences in study design and methodology.

In the present study, the specimens were immersed in 0.2% CHX for 5 minutes, whereas they used 0.12% CHX for 3 minutes. Also, a 660nm laser with a power density of 400mW/cm² was used for PDT in the present study, whereas they used a 685nm laser with a power density of 223mW/cm² for PDT.

In the current study, 0.2% CHX ranked second after HP in terms of antimicrobial activity among the tested modalities. In contrast,

Table 3. Comparison of *S. mutans* and *L. acidophilus* colony counts between the HP subgroup and the other subgroups using the one-sample t-test

Subgroup	Group	Mean difference	95% CI (Lower bound)	95% CI (Upper bound)	P value
Control	<i>S. mutans</i>	822000.00000	646973.8167	997026.1833	0.000
	<i>L. acidophilus</i>	852000.00000	784904.1723	919095.8277	0.000
CHX	<i>S. mutans</i>	97200.00000	86613.0395	107786.9605	0.000
	<i>L. acidophilus</i>	112600.00000	82114.6833	143085.3167	0.001
Acetic acid	<i>S. mutans</i>	320000.00000	224625.9786	415374.0214	0.001
	<i>L. acidophilus</i>	340000.00000	258106.6348	421893.3652	0.000
MB	<i>S. mutans</i>	640000.00000	608343.6552	671656.3448	0.000
	<i>L. acidophilus</i>	688000.00000	611761.4261	764238.5739	0.000
Laser	<i>S. mutans</i>	674000.00000	624644.8322	723355.1678	0.000
	<i>L. acidophilus</i>	728000.00000	656456.0143	799543.9857	0.000
PDT	<i>S. mutans</i>	188000.00000	146445.9747	229554.0253	0.000
	<i>L. acidophilus</i>	254000.00000	218228.0072	289771.9928	0.000

CI: Confidence interval; CHX: Chlorhexidine; MB: Methylene blue; PDT: Photodynamic therapy.

Paramita et al. [27] reported that 0.1% CHX had no significant effect on Gram-positive bacteria. Also, Shpack et al. [28] reported that CHX had no significant effect on bacteria on paper and thermoplastic discs. They explained that, since the cationic molecules of CHX depend on the negative charge of the surface to exert their antimicrobial effect, the positive charge of thermoplastic materials prevents CHX from being absorbed and neutralizes its effect.

In the present study, laser and distilled water had equal efficacy against *L. acidophilus*. Also, MB, laser, and distilled water showed no significant difference in efficacy against *S. mutans*. Comparable efficacy of laser and MB against *S. mutans* was also reported by Azizi et al. [29]. Nonetheless, they found a significant difference in the efficacy of laser and MB compared with the control group, which may be due to the fact that they used saline as the control group, whereas distilled water was used as the control in the present study. Méndez et al. [30] reported higher antimicrobial activity of laser than MB and the control group in reducing lactobacilli colonies, which was in contrast to the present findings. The reason may be attributed to the use of different Lactobacillus strains and differences in their susceptibility to different antimicrobial agents.

A search of the literature by the authors yielded a limited number of studies comparing different disinfection protocols. Sobouti et al. [31] compared the efficacy of PDT with curcumin and a light-emitting diode (LED), CHX, sodium fluoride, and HP for disinfection of acrylic resins. They showed significant effects of the type of disinfection protocol and the type of microorganism on colony count, such that the highest *Enterococcus faecalis* colony count was recorded in the sodium fluoride group, and the highest *E. faecalis* and *Streptococcus salivarius* colony counts were recorded in the HP group. Bacterial proliferation was absent or insignificant in the PDT and CHX groups. The difference between their results and the present findings may be explained by differences in the type of retainers, photosensitizers, light source and wavelength, concentration of disinfectants, duration of exposure, type of culture medium, and sample size.

According to the present results, PDT may serve as an alternative option to CHX for possible use in clinics and dental offices during periodic patient visits (4 to 8 weeks in patients undergoing aligner therapy and every 6 months in patients with Essix retainers). Lack of complete simulation of the clinical setting, failure to account for the role of saliva, oral temperature, and masticatory forces, the

omission of mechanical plaque removal methods such as tooth brushing with different toothpastes, and the evaluation of only two microorganisms using one type of photosensitizer and one type of laser with one energy density were the main limitations of this study. Another important consideration is the potential staining effect of MB on clear thermoplastic aligners. Although the specimens were rinsed after photosensitizer application, MB is known to temporarily discolor transparent materials, which may limit the clinical acceptability of PDT, particularly for home use. Furthermore, surface characterization techniques (e.g., scanning electron microscopy, profilometry, or contact angle measurements) were not included in the present study to evaluate potential changes in surface topography, roughness, or wettability following disinfection. Although such physicochemical alterations may contribute to variations in bacterial adhesion, the observed reductions in bacterial viability are considered to primarily reflect the antimicrobial performance of the tested protocols under the evaluated conditions. Future studies incorporating comprehensive surface characterization would provide further insight into the potential contribution of surface-related effects.

Although PDT demonstrated antibacterial activity, its clinical application for routine home disinfection of clear aligners may currently be limited because it requires a photosensitizer, a dedicated light source, and additional treatment costs. Therefore, considering the superior efficacy and lower cost of HP and CHX observed in the present study, PDT may currently be more appropriate for selected clinical applications rather than routine home disinfection. Future studies should therefore evaluate the effects of PDT on the optical properties, color stability, esthetics, and surface characteristics of clear aligners, while also investigating its efficacy against other oral microbial species using different photosensitizers, laser wavelengths, and exposure settings. In addition, the complementary role of mechanical plaque removal, different retainer materials and

thicknesses, and well-designed clinical studies should be considered to verify the effectiveness and clinical applicability of the evaluated disinfection protocols.

CONCLUSION

Within the limitations of this in vitro study, PDT demonstrated moderate antibacterial activity against *S. mutans* and *L. acidophilus*. However, hydrogen peroxide, followed by chlorhexidine, exhibited significantly greater antibacterial efficacy. Therefore, PDT may be considered only as a potential alternative disinfection approach, and further in vivo and clinical studies are required before recommending its routine clinical use.

ACKNOWLEDGEMENT

This article was derived from the Doctor of Dental Surgery (DDS) thesis of Leila Pourmoussa, conducted at Tehran University of Medical Sciences.

CONFLICT OF INTEREST STATEMENT

None declared.

GENERATIVE AI IN SCIENTIFIC WRITING

ChatGPT (version 5.2) was used solely to assist with grammar correction, improvement of manuscript readability, and preparation of schematic illustrations.

REFERENCES

1. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Efficacy of clear aligners in controlling orthodontic tooth movement: a systematic review. *Angle Orthod.* 2015 Sep;85(5):881-9.
2. Hirani S, Patel U, Patel N. Invisible orthodontics-a review. *IOSR J Dent Med Sci.* 2016 Jun;15(6):56-62.
3. Ren Y, Jongsma MA, Mei L, van der Mei HC, Busscher HJ. Orthodontic treatment with fixed appliances and biofilm formation--a potential public health threat? *Clin Oral Investig.* 2014 Sep;18(7):1711-8.
4. Moshiri M, Eckhart JE, McShane P, German DS. Consequences of poor oral hygiene during aligner therapy. *J Clin Orthod.* 2013 Aug;47(8):494-8.
5. Lagravère MO, Flores-Mir C. The treatment effects of Invisalign orthodontic aligners: a systematic review. *J Am Dent Assoc.* 2005 Dec;136(12):1724-9.

6. Wible E, Agarwal M, Altun S, Ramir T, Viana G, Evans C, et al. Long-term effects of different cleaning methods on copolyester retainer properties. *Angle Orthod.* 2019 Mar;89(2):221-227.
7. Chang CS, Al-Awadi S, Ready D, Noar J. An assessment of the effectiveness of mechanical and chemical cleaning of Essix orthodontic retainer. *J Orthod.* 2014 Jun;41(2):110-7.
8. Albanna RH, Farawanah HM, Aldrees AM. Microbial evaluation of the effectiveness of different methods for cleansing clear orthodontic retainers: A randomized clinical trial. *Angle Orthod.* 2017 May;87(3):460-465.
9. Apratim A, Shah SS, Sinha M, Agrawal M, Chhaparia N, Abubakkar A. Denture hygiene habits among elderly patients wearing complete dentures. *J Contemp Dent Pract.* 2013 Nov 1;14(6):1161-4.
10. Baran I, Nalçacı R. Self-reported denture hygiene habits and oral tissue conditions of complete denture wearers. *Arch Gerontol Geriatr.* 2009 Sep-Oct;49(2):237-241.
11. Barreiro DM, Scheid PA, May LG, Unfer B, Braun KO. Evaluation of procedures employed for the maintenance of removable dentures in elderly individuals. *Oral Health Prev Dent.* 2009;7(3):243-9.
12. Axe AS, Varghese R, Bosma M, Kitson N, Bradshaw DJ. Dental health professional recommendation and consumer habits in denture cleansing. *J Prosthet Dent.* 2016 Feb;115(2):183-8.
13. Freitas LB, Vassilakos N, Arnebrant T. Interactions of chlorhexidine with salivary films adsorbed at solid/liquid and air/liquid interfaces. *J Periodontal Res.* 1993 Mar;28(2):92-7.
14. Wible E, Agarwal M, Altun S, Ramir T, Viana G, Evans C, et al. Long-term effects of various cleaning methods on polypropylene/ethylene copolymer retainer material. *Angle Orthod.* 2019 May;89(3):432-437.
15. McGowan MJ, Shimoda LM, Woolsey GD. Effects of sodium hypochlorite on denture base metals during immersion for short-term sterilization. *J Prosthet Dent.* 1988 Aug;60(2):212-8.
16. de Souza RF, de Freitas Oliveira Paranhos H, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. *Cochrane Database Syst Rev.* 2009 Oct 7;2009(4):CD007395.
17. Charavet C, Gourdain Z, Graveline L, Lupi L. Cleaning and Disinfection Protocols for Clear Orthodontic Aligners: A Systematic Review. *Healthcare (Basel).* 2022 Feb 10;10(2):340.
18. Inchingolo AD, Malcangi G, Semjonova A, Inchingolo AM, Patano A, Coloccia G, et al. Oralbiotica/Oralbiotics: The Impact of Oral Microbiota on Dental Health and Demineralization: A Systematic Review of the Literature. *Children (Basel).* 2022 Jul 8;9(7):1014.
19. Ghorbani J, Rahban D, Aghamiri S, Teymouri A, Bahador A. Photosensitizers in antibacterial photodynamic therapy: an overview. *Laser Ther.* 2018 Dec 31;27(4):293-302.
20. Ricci Donato HA, Pratavieira S, Grecco C, Brugnera-Júnior A, Bagnato VS, Kurachi C. Clinical Comparison of Two Photosensitizers for Oral Cavity Decontamination. *Photomed Laser Surg.* 2017 Feb;35(2):105-110.
21. Al-Shammery D, Michelogiannakis D, Ahmed ZU, Ahmed HB, Rossouw PE, Romanos GE, Javed F. Scope of antimicrobial photodynamic therapy in Orthodontics and related research: A review. *Photodiagnosis Photodyn Ther.* 2019 Mar;25:456-459.
22. Sayar F, Karimi MR, Boroujerdi S. Efficacy of antimicrobial photodynamic therapy with chitosan nanoparticles for decontamination of dental implants contaminated with *Aggregatibacter actinomycetemcomitans*. *Sci Rep.* 2025 Oct 1;15(1):34213.
23. Silva Teófilo MÍ, de Carvalho Russi TMAZ, de Barros Silva PG, Balhaddad AA, Melo MAS, Rolim JPML. The Impact of Photosensitizer Selection on Bactericidal Efficacy Of PDT against Cariogenic Biofilms: A Systematic Review and Meta-Analysis. *Photodiagnosis Photodyn Ther.* 2021 Mar;33:102046.
24. Kiatwarawut K, Rokaya D, Sirisoontorn I. Antimicrobial Activity of Various Disinfectants to Clean Thermoplastic Polymeric Appliances in Orthodontics. *Polymers (Basel).* 2022 May 31;14(11):2256.
25. Akgün FA, Şenışık NE, Çetin ES. Evaluation of the Efficacy of Different Cleaning Methods for Orthodontic Thermoplastic Retainers in terms of Bacterial Colonization. *Turk J Orthod.* 2019 Dec 1;32(4):219-228.
26. Hakami Z, Alshehri AH. Antimicrobial efficacy, optical properties and flexural strength following antimicrobial photodynamic therapy over vacuum-formed orthodontic retainers. *Photodiagnosis Photodyn Ther.* 2022 Sep;39:102951.
27. Paramita C, Purwanegara MK, Hoesin F. Comparison of the efficacy of two disinfectants for controlling gram-positive bacteria on thermoplastic retainers in dental plaques. *International Journal of Applied Pharmaceutics.* 2019 Apr;11:126-9.
28. Shpack N, Greenstein RB, Vardimon A. RE: response to: Efficacy of three hygienic protocols in reducing biofilm adherence to removable thermoplastic appliance. *The Angle Orthodontist* 2014(1) 161-170. *Angle Orthod.* 2014

May;84(3):574.

29. Azizi A, Shohrati P, Goudarzi M, Lawaf S, Rahimi A. Comparison of the effect of photodynamic therapy with curcumin and methylene Blue on streptococcus mutans bacterial colonies. Photodiagnosis Photodyn Ther. 2019 Sep;27:203-209.

30. Méndez DAC, Gutierrez E, Dionísio EJ, Oliveira TM, Buzalaf MAR, Rios D, Machado MAAM, Cruvinel T. Effect of methylene blue-mediated

antimicrobial photodynamic therapy on dentin caries microcosms. Lasers Med Sci. 2018 Apr;33(3):479-487.

31. Sobouti F, Dadgar S, Salehi A, Rezaei Kalantari N, Aryana M, Goli HR, et al. In Vitro Comparative Evaluation of the Efficacy of Antimicrobial Photodynamic Therapy, Chlorhexidine, Sodium Fluoride, and Hydrogen Peroxide for Acrylic Resin Disinfection. J Lasers Med Sci. 2023 Nov 4;14:e52.