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Investigating the Effect of Photodynamic Therapy With a 660 nm Laser With Methylene Blue and Cold Atmospheric Plasma Therapy on *Streptococcus sanguinis*

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Abstract

Introduction: This study was conducted to investigate the effect of photodynamic therapy (PDT) with a 660 nm laser along with methylene blue (MB) and cold atmospheric plasma therapy (CAP) on *Streptococcus sanguinis*. Since *S. sanguinis* plays a pivotal role in biofilm formation, alongside its ability to survive in the bloodstream, it significantly heightens the risk of infective endocarditis.

Methods: In this in-vitro study, the *S. sanguinis* strain was cultured on a blood agar medium, and the samples were evenly distributed among eight experimental groups using equalization methods with nine repetitions. The experimental groups were 8 groups. Following the interventions, the samples were cultured using the pour plate method. After 48 hours, the samples were retrieved, and the number of bacterial colonies was counted. The changes in colony numbers, after normal transformation, were analyzed using the repeated measures ANOVA model, with the between-subject factor considered in the analysis.

Results: The highest colony count was observed in the control group. Particularly, in both the PLASMA group and the group treated with PLASMA+PDT using the 660 nm laser alone, there was no significant difference from the control group (PLASMA: $P>0.05$; PLASMA+PDT: $P>0.05$). However, the combination of CAP, PDT with the 660 nm laser, and MB demonstrated a significant reduction in colony counts compared to the control group (PLASMA+PDT+MB: $P=0.000$), making it the most effective intervention in this study.

Conclusion: The results demonstrated that the combination of CAP and PDT using a 660 nm laser with MB achieved the most significant reduction in *S. sanguinis* colonies.

Keywords: *Streptococcus sanguinis*; Photodynamic therapy; Cold atmospheric plasma therapy; Methylene blue

Introduction

The oral cavity hosts a wide range of microorganisms, including viruses, bacteria, and fungi, creating an environment conducive to microbial proliferation. While many of these microorganisms form part of the normal oral flora, certain conditions can lead to their pathogenic transformation.[1] One example is *Streptococcus sanguinis*, a native Gram-positive bacterium crucial for oral and dental health. However, under opportunistic conditions, this bacterium may act as a pathogen. Factors such as temperature can influence the balance of microbial communities in the oral cavity,[2] and when this balance is disrupted, *S. sanguinis* may pose a significant threat to oral health, and if it enters the bloodstream, its virulence factors allow it to persist, evade immune cells, and form vegetations on heart valves, leading to an increased risk of endocarditis and potentially contributing to atherosclerosis. Given these risks, it is crucial to promptly reduce the bacterial load of *S. sanguinis* in the oral cavity, especially when its natural balance is disrupted, to prevent potential pathogenic effects.[3-6]

One effective approach is photodynamic therapy (PDT), which involves using a light source at a specific wavelength to activate a photosensitizer (PS). This PS absorbs light, shifting from a singlet state to an excited triplet state, leading to the generation of reactive oxygen species (ROS) that can destroy bacterial cells.[7,8] The effectiveness of antibacterial PDT has been demonstrated in various microbial species, including Lactobacillus and Streptococcus mutans.[9] Notably, *S. sanguinis* has also shown a significant reduction in growth when subjected to this modern, non-invasive therapeutic method.[10]

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Another promising technology in this field is cold atmospheric plasma (CAP), a form of non-thermal plasma with applications in dentistry. Unlike thermal plasma, which is destructive due to its high energy density, CAP is safe for medical use and has shown efficacy in biofilm removal and dental implant disinfection.[11-13] Numerous studies have demonstrated CAP's antimicrobial properties, making it a potential alternative or complement to antibiotics. Given the global rise in antibiotic resistance, which poses a serious threat to human health, CAP presents a promising solution.[14,15] This therapy has been shown to be effective against *S. sanguinis*, independent of bacterial resistance to antibiotics.[16,17]

In light of these findings, combining PDT with a 660 nm laser and CAP offers a powerful approach to combating *S. sanguinis*. The goal of this research is to identify the most efficient treatment method for reducing the bacterial load of *S. sanguinis*, serving as an adjunct therapy to enhance the outcomes of the primary treatment. This approach is particularly valuable in cases where the disruption of the bacterial balance plays a critical role in the disease progression, offering a complementary solution to improve patient prognosis.

Materials and methods

Sample Size

In this in-vitro study, the sample size was determined based on the results of the Pérez-Laguna et al study, using the one-way ANOVA power analysis option in PASS 11 software, in this in-vitro study, the sample size was determined using the one-way ANOVA power analysis option in PASS 11 software, based on the results of the Pérez-Laguna et al study. The parameters were set as follows: $\beta = 0.2$, $\alpha = 0.05$, an average standard deviation of 1.1 for the logarithm of the number of colonies, and an effect size of 0.62. The minimum required sample size for each group was calculated to be nine.[10] The samples were then randomly assigned to eight groups using a simple randomization method.

Cultivation of the Microorganism

To obtain the required bacterial strain, *Streptococcus sanguinis* PTCC1449 was sourced from the National Center of Genetic and Biological Resources of Iran. For the initial culture, the bacteria were diluted in physiological saline and inoculated onto petri dishes containing 5% sheep blood. These plates were placed in an anaerobic jar for 48 hours, followed by incubation in an aerobic environment at 37 °C. After incubation, bacterial colonies were harvested using a sterile loop and transferred into a liquid trypsin medium. To standardize turbidity, several rounds of dilution were performed, and the optical absorbance of the prepared suspensions was measured at 620 nm using a spectrophotometer (Thermo Scientific, Nanodrop One, U.S.). When an optical absorbance of 0.09 was achieved, it corresponded to a standard turbidity equivalent to half of the McFarland scale.[10]

Experimental Groups

Based on the number of study groups and samples, which included eight groups with nine repetitions per group, the final bacterial suspension was divided into 72 microtubes. In the groups treated with methylene blue (MB), 125 µL of a 0.01% MB solution was administered. This solution was prepared by diluting 1 mg of MB (Merck Co., Germany) in 10 mL of sterile saline to achieve the desired concentration. The final concentration of MB was 0.1 mg/mL. This was done under light-restricted conditions. For the remaining groups, 125 µL of phosphate-buffered saline (PBS) was added to equalize the sample volumes.[18]

In the group treated with penicillin, 1 200 000 units of penicillin G benzathine were diluted to 100 000 units, and 125 µL of this solution was added to the sample. The samples were then transferred to the Laser and Plasma Department at the Islamic Azad University, Tehran Dental Faculty, under dark conditions.

The groups were structured as follows:

1. Negative control (NC) group: 125 µL of bacterial suspension with 125 µL of PBS. No intervention was done for the first group, which was the control group.
2. Positive control (PC) group: 125 µL of bacterial suspension with 125 µL of aforementioned diluted penicillin.
3. Methylene blue (MB) group: 125 µL of bacterial suspension with 125 µL of MB 0.01%. No intervention was done in the Laser department.
4. Photodynamic therapy + methylene blue (PDT + MB) group: 125 µL of bacterial suspension with 125 µL of MB 0.01% which was irradiated for 60 seconds with a 660 nm laser (Sirona, Germany) and 100 mW power with an irradiation dose of 15.6 J/cm^2 (according to 0.1 W power, 60 seconds irradiation and the radius of the tip). The applicator was positioned 1 cm from the sample, with a tip diameter of 7 mm.[18]
5. Cold atmospheric plasma therapy + methylene blue (PLASMA + MB) group: 125 µL of bacterial suspension with 125 µL of MB 0.01%. CAP (Plasma art, Naria Tech Co., Iran) was used for this group with a 3 L/min flow rate, intensity of 4, input power of 55 W, and 50 kHz frequency during 60 seconds. The applicator had a 4 mm diameter.[19]
6. Cold atmospheric plasma therapy + photodynamic therapy (PLASMA + PDT) group: 125 µL of bacterial suspension with 125 µL of PBS which was firstly irradiated for 60 seconds with a 660 nm laser (Sirona, Germany) and 100 mW power with an irradiation dose of 15.6 J/cm^2. The applicator was positioned 1 cm from the sample, with a tip diameter of 7 mm. Then, for the intervened sample, CAP (Plasma art, Naria Tech Co., Iran) was used with a 3 L/min flow

rate, intensity of 4, input power of 55 W, and 50 kHz frequency during 60 seconds. The applicator had a 4 mm diameter.

7. Cold atmospheric plasma therapy + photodynamic therapy + methylene blue (PLASMA + PDT + MB) group: 125 µL of bacterial suspension with 125 µL of MB 0.01% which was firstly irradiated for 60 s with a 660 nm laser (Sirona, Germany) and 100 mW power with an irradiation dose of 15.6 J/cm^2. The applicator was positioned 1 cm from the sample, with a tip diameter of 7 mm. Then, for the intervened sample, CAP (Plasma art, Naria Tech Co., Iran) was used with a 3 L/min flow rate, intensity of 4, input power of 55 W, and 50 kHz frequency during 60 seconds. The applicator had a 4 mm diameter.
8. Cold atmospheric plasma therapy (PLASMA) group: 125 µL of bacterial suspension with 125 µL of PBS. CAP (Plasma art, Naria Tech Co., Iran) was used for this group with a 3 L/min flow rate, intensity of 4, input power of 55 W, and 50 kHz frequency during 60 seconds. The applicator had a 4 mm diameter.

Although the laboratory environment and all materials used were sterile, a 0.45 nm syringe filter was employed to further increase experimental accuracy.

Secondary Cultivation

After the interventions, the samples were transferred to the Microbiology Research Institute of Tehran Islamic Azad University of Medical Sciences. The bacteria were cultured using the pour plate method on blood agar prepared on the day of the final intervention. The plates were incubated in an anaerobic jar at 37 °C.

Sample Counting

After 48 hours, the plates were removed from the incubator, and the bacterial colonies were counted. The number of colony forming units (CFUs) on the culture medium was recorded for each group. For plates with few colonies, all colonies were counted directly. For plates with a high colony count, the Petri dishes were divided into smaller sections, and the number of colonies in one section was multiplied by the number of divisions. A summary of the methodology can be observed in Figure 1.

Statistical Analysis

The data from the colony counts, both direct and indirect, were analyzed using the repeated measures ANOVA model, considering the between-subject factor.

Results

In Table 1, the descriptive statistics, including the mean, standard deviation, and the minimum and maximum values for each study group, are presented with a 95% confidence interval.

From Table 1 and Figure 2, it is evident that the control group had the highest average number of bacterial colonies, while the group treated with CAP therapy combined with PDT using a 660 nm laser and MB showed the lowest average colony count. Additionally, the table details the highest and lowest colony numbers in other groups.

Since the data distribution was not normal, a logarithmic transformation was applied. Table 2 displays the logarithmic values of colony numbers, showing a significant difference between the groups ($P<0.001$, Table 3). Due to unequal data dispersion ($P=0.003$, Table 4), the Tamhane post-hoc test was used to compare the groups, with the control group having the highest colony count (Table 5).

The analysis revealed that plasma therapy alone did not significantly reduce bacterial colonies ($P=0.484$, indicating $P>0.05$). Similarly, no significant difference was observed between the initial and final colony counts

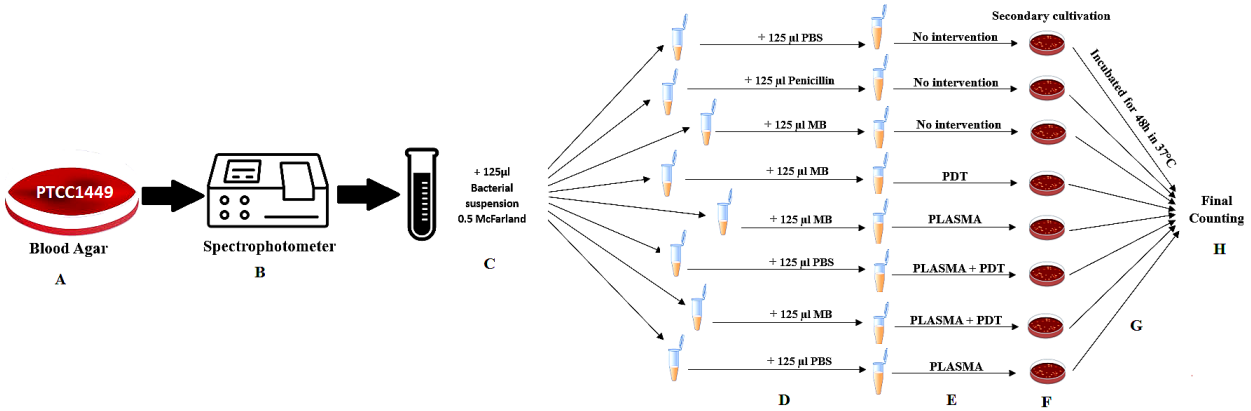

Figure 1. A Brief Introduction to Methodology. A: *Streptococcus sanguinis* was cultivated using the Blood Agar medium and incubation at 37°C for 48 hours. B: After diluting the bacteria in liquid trypsin, the desired turbidity was measured using a spectrophotometer. C: 125 µL of 0.5 McFarland bacterial suspension was added to each 72 microtubes. D: 125 µL of phosphate buffered saline was added to NC, PLASMA + PDT and PLASMA groups. 125 µL of diluted penicillin was added to the PC group. 125 µL of MB was added to MB, PDT + MB, PLASMA + MB and PLASMA + PDT + MB groups. E: The needed interventions operated. F: The samples were cultivated again in Blood Agar using the pour plate method. G: Samples were placed in an anaerobic jar and incubated at 37°C for 48 hours. H: Bacterial colonies were counted directly and indirectly at the end of the incubation

Table 1. Mean, Standard Deviation, and Minimum and Maximum Number of Bacterial Colonies (CFU)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	9	684.8889	237.60284	79.20095	502.2512	867.5266	324.00	1136.00
MB	9	42.2222	23.37080	7.79027	24.2578	60.1866	21.00	98.00
PDT + MB	9	26.0000	5.00000	1.66667	22.1567	29.8433	19.00	35.00
PLASMA + MB	9	39.6667	7.96869	2.65623	33.5414	45.7919	30.00	52.00
PLASMA + PDT	9	492.2222	351.79887	117.26629	221.8057	762.6388	32.00	1104.00
PLASMA + PDT + MB	9	23.4444	10.05126	3.35042	15.7184	31.1705	8.00	39.00
PLASMA	9	408.2222	285.18844	95.06281	189.0070	627.4375	96.00	880.00
Total	63	245.2381	317.56639	40.00960	165.2600	325.2162	8.00	1136.00

Table 2. Logarithmic Analysis of the Groups

	N	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	9	2.8113	0.15777	0.05259	2.6900	2.9325	2.51	3.06
MB	9	1.5787	0.20440	0.06813	1.4216	1.7358	1.32	1.99
PDT + MB	9	1.4080	0.08243	0.02748	1.3446	1.4713	1.28	1.54
PLASMA + MB	9	1.5907	0.08705	0.02902	1.5238	1.6576	1.48	1.72
PLASMA + PDT	9	2.5412	0.46197	0.15399	2.1861	2.8963	1.51	3.04
PLASMA + PDT + MB	9	1.3251	0.22459	0.07486	1.1525	1.4978	0.90	1.59
PLASMA	9	2.4994	0.34505	0.11502	2.2342	2.7646	1.98	2.94
Total	63	1.9649	0.63241	0.07968	1.8056	2.1242	0.90	3.06

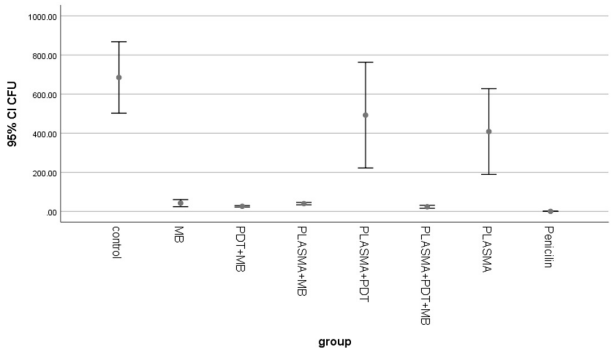

Figure 2. Error Bar Plot of CFU Counts Across Treatment Groups (95% CI)

Table 3. ANOVA Analysis of Logarithmic Differences Between Groups

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	21.085	6	3.514	53.020	0.000
Within groups	3.712	56	0.066		
Total	24.797	62			

Table 4. Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
log. CFU	Based on Mean	3.731	6	56	0.003
	Based on Median	2.957	6	56	0.014
	Based on Median and with adjusted *df*	2.957	6	22.977	0.027
	Based on trimmed mean	3.599	6	56	0.004

in the group treated with plasma therapy and PDT ($P = 0.944$, $P > 0.05$).

On the other hand, the MB group showed a significant reduction in bacterial colonies ($P < 0.05$). Furthermore, both plasma therapy combined with MB and PDT combined with MB demonstrated significant reductions in colony numbers compared to the control group ($P < 0.05$), although there were still notable differences in efficacy between these two groups (Sig. = 0.007).

Finally, the combination of plasma therapy, PDT, and MB showed the greatest antibacterial effect (Sig. = 0.000, $P < 0.05$), leading to a substantial reduction in bacterial colonies.

It should also be noted that no bacterial colonies were observed in the petri dishes treated with penicillin, owing to its strong antibacterial effects. As a result, no colony counts were performed in this group, as the number of colonies was zero.

Discussion

Based on the final results and the method used in this study, the initial bacterial culture conditions were generally similar to those in the study by Pérez-Laguna et al.[10] However, there were a few differences. For example, they also studied the effect of Rose Bengal as another photosensitizing agent. Additionally, they used distilled water as a solvent to equalize the solution volumes, rather than PBS, believing that saline contains less oxygen

Table 5. Tamhane's Post Hoc Analysis for Multiple Comparisons

(I) Group		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	MB	1.23255	0.08607	0.000	0.9198	1.5453
	PDT + MB	1.40330	0.05933	0.000	1.1769	1.6296
	PLASMA + MB	1.22061	0.06006	0.000	0.9933	1.4479
	PLASMA + PDT	0.27010	0.16272	0.944	-0.3873	0.9275
	PLASMA + PDT + MB	1.48613	0.09149	0.000	1.1505	1.8217
	PLASMA	0.31186	0.12647	0.484	-0.1800	0.8037
MB	control	-1.23255	0.08607	0.000	-1.5453	-0.9198
	PDT + MB	0.17075	0.07346	0.587	-0.1200	0.4615
	PLASMA + MB	-0.01194	0.07405	1.000	-0.3028	0.2789
	PLASMA + PDT	-.96245	0.16839	0.003	-1.6204	-0.3045
	PLASMA + PDT + MB	0.25358	0.10123	0.394	-0.1106	0.6178
	PLASMA	-.92069	0.13368	0.000	-1.4217	-0.4197
PDT + MB	control	-1.40330	0.05933	0.000	-1.6296	-1.1769
	MB	-0.17075	0.07346	0.587	-0.4615	0.1200
	PLASMA + MB	-.18269	0.03996	0.007	-0.3263	-0.0391
	PLASMA + PDT	-1.13320	0.15642	0.001	-1.7977	-0.4687
	PLASMA + PDT + MB	0.08283	0.07975	1.000	-0.2366	0.4022
	PLASMA	-1.09143	0.11825	0.000	-1.5854	-0.5975
PLASMA + MB	control	-1.22061	0.06006	0.000	-1.4479	-0.9933
	MB	0.01194	0.07405	1.000	-0.2789	0.3028
	PDT + MB	.18269	0.03996	0.007	0.0391	0.3263
	PLASMA + PDT	-.95051	0.15670	0.005	-1.6145	-0.2865
	PLASMA + PDT + MB	0.26552	0.08029	0.147	-0.0539	0.5849
	PLASMA	-.90874	0.11862	0.001	-1.4022	-0.4153
PLASMA + PDT	control	-0.27010	0.16272	0.944	-0.9275	0.3873
	MB	.96245	0.16839	0.003	0.3045	1.6204
	PDT + MB	1.13320	0.15642	0.001	0.4687	1.7977
	PLASMA + MB	.95051	0.15670	0.005	0.2865	1.6145
	PLASMA + PDT + MB	1.21603	0.17122	0.000	0.5560	1.8761
	PLASMA	0.04176	0.19220	1.000	-0.6588	0.7424
PLASMA + PDT + MB	control	-1.48613	0.09149	0.000	-1.8217	-1.1505
	MB	-0.25358	0.10123	0.394	-0.6178	0.1106
	PDT + MB	-0.08283	0.07975	1.000	-0.4022	0.2366
	PLASMA + MB	-0.26552	0.08029	0.147	-0.5849	0.0539
	PLASMA + PDT	-1.21603	0.17122	0.000	-1.8761	-0.5560
	PLASMA	-1.17426	0.13724	0.000	-1.6822	-0.6663
PLASMA	control	-0.31186	0.12647	0.484	-0.8037	0.1800
	MB	.92069	0.13368	0.000	0.4197	1.4217
	PDT + MB	1.09143	0.11825	0.000	0.5975	1.5854
	PLASMA + MB	.90874	0.11862	0.001	0.4153	1.4022
	PLASMA + PDT	-0.04176	0.19220	1.000	-0.7424	0.6588
	PLASMA + PDT + MB	1.17426	0.13724	0.000	0.6663	1.6822

compared to distilled water, which could affect the effectiveness of the treatment. However, further research is required to confirm this. One advantage of this study is the use of shorter time and distance. Previous studies employed fixed light sources, but with current technology, including portable lasers, it was possible to reduce the

distance from 17 cm to 1 cm and the exposure time from around 43 minutes to 1 minute as it has been performed in this study.[10]

According to the publications reviewed, including the study by Entezari et al, low-power lasers used in dental treatments typically range from 100 mW to 500 mW, depending on the specific application.[18] Furthermore, in antibacterial photodynamic therapy (aPDT), a power setting of 100 mW has shown promising and efficient results, as demonstrated in various studies across different applications.[20] Additionally, a previously published case report highlighted the successful use of PDT with 100 mW power in the treatment of dental caries, resulting in a significant microbial reduction.[21]

Regarding PDT intervention with MB, it was shown that if a PS such as MB is used at the exact wavelength that is the peak of its absorption point, it can eliminate pathogenic microorganisms, which is consistent with the study by Chan and Lai.[22] Additionally, Studies have shown that PDT is significantly less effective in dental treatments when performed without a PS.[23] Also, due to the antibacterial nature of the PS, compared to other materials and according to the results of previous studies that have compared it with materials such as Indocyanine green, the use of MB was associated with better results and therefore was chosen as our target material.[24] Furthermore, compared to other potent PSs like Rose Bengal (RB), MB is effective against a wider range of microbial species, including fungal pathogens.[25,26] MB also requires lower light intensities and shorter exposure times, whereas RB needs a green light for activation, which increases the risk of tissue damage. Although MB has low solubility and potential cytotoxic effects, it has been widely recognized as safe when used at optimal concentrations, having been approved by the FDA.[27] For antimicrobial PDT, safe MB concentrations reported in various studies range from 0.016–0.16 mg/mL.[23,28] In line with these recommendations, our experiments used a safe MB concentration of 0.01% (0.1 mg/mL), as supported by other studies.[18]

As mentioned in the previous section, the intervention groups utilizing CAP therapy employed a flow rate of 3 liters per minute with an intensity level of 4, which is 2 L/min lower than that in previous studies.[17] According to the results, there was no significant reduction in the number of *S. sanguinis* colonies within the 60-second treatment window. This aligns with the findings of S. Gorynia et al,[17] where a minimum CAP application time of 90 seconds was required to achieve a 0.3 log10 reduction in the colony count.

In the groups treated with CAP, both the PLASMA and PLASMA + PDT groups showed no significant decrease in bacterial colonies after 60 seconds compared to the control group, which may be attributed to the short exposure time of the plasma. In contrast, the study by Idlibi et al[29] demonstrated that with an extended CAP application time of 196 seconds, CAP therapy could be more effective and even preferable to other interventions such as PDT.

In the final comparison, the most significant reduction in bacterial colonies was achieved with the combined treatment of CAP therapy and PDT using a 660 nm laser with MB. The synergistic effect of these two methods proved to be highly effective. One of the strengths of this study was the simultaneous evaluation of these interventions, providing a comprehensive analysis of their efficacy. Additionally, PDT with MB, which demonstrated a substantial decrease in bacterial colonies, is recommended as an adjunct or even alternative to conventional treatments, offering a significant complementary approach to enhancing therapeutic outcomes.[24]

Among the key findings, the positive effect of PDT with MB stood out, showing better performance compared to MB alone and CAP. This result aligns with the findings of Hafner et al,[30] although it should be noted that their research was conducted on *Staphylococcus aureus*, and the intervention environment differed from that used in this study. Given these differences, further research is needed, particularly in dry environments or on artificial surfaces, to fully explore the potential of PDT in varying conditions.

Using MB alone to harness its antibacterial properties for *S. sanguinis* may be a viable option, especially since laser application can induce temperature changes in the target tissue, potentially enhancing its effectiveness.[31] However, considering the side effects and limitations associated with MB, particularly its staining effect on dental surfaces,[27] further research is needed to fully assess its safety and efficacy in this context. This substance is widely used therapeutically today in appropriate concentrations for infectious diseases.[32] Moreover, as previously mentioned in the discussion, the combination of MB with PDT is significantly more effective than using MB alone. Furthermore, as highlighted in Table 1 and Table 5, which are thoroughly detailed in the results section, a significant reduction in bacterial colonies is observed when PDT with MB is combined with plasma therapy. This combination demonstrates enhanced efficacy, reinforcing the therapeutic potential of integrating these modalities. Additionally, our experimental results revealed that MB alone exhibited notable efficiency in reducing *S. sanguinis* colonies. This aligns with previous studies, which have shown that while PDT or PS like MB alone can reduce bacterial colonies, the combination of PDT with a PS provides the most pronounced therapeutic effects.[23]

Finally, within a 60-second treatment window, the optimal therapeutic approach for reducing *S. sanguinis* bacterial load, particularly as an adjunct therapy in patients requiring bacterial load reduction, is the simultaneous application of PDT with a 660 nm laser in combination with MB, complemented by CAP. This dual-modality

treatment is highly effective in minimizing the presence of this pathogen, especially in cases where its natural balance has been disrupted, offering a powerful strategy for restoring oral microbial health.

Conclusion

In conclusion, a comparison of the various interventions in this study demonstrates that the combined use of PDT with a 660 nm laser and MB, alongside CAP, produces the most significant reduction in *S. sanguinis* colonies. This dual-approach therapy proves to be highly effective in targeting and reducing the bacterial load, offering a powerful and innovative method for combating this pathogen.

Authors' Contribution

Conceptualization: Salma Ghaiyoomi, Arash Azizi.
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Investigation: Salma Ghaiyoomi, Shirin Lawaf.
Methodology: Seyyed Khalil Shokouhi Mostafavi, Arash Azizi.
Project administration: Salma Ghaiyoomi.
Resources: Salma Ghaiyoomi, Seyyed Khalil Shokouhi Mostafavi.
Supervision: Arash Azizi.
Validation: Salma Ghaiyoomi, Arash Azizi.
Visualization: Arash Manavi.
Writing-original draft: Seyyed Khalil Shokouhi Mostafavi, Arash Manavi.
Writing-review & editing: Salma Ghaiyoomi, Arash Manavi, Shirin Lawaf, Arash Azizi.

Competing Interests

The authors declared that they have no conflict of interest.

Ethical Approval

This study was registered and approved by the ethical committee of Islamic Azad University of Tehran Medical Sciences, with the number IR.IAU.PS.REC.1402.160.

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