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RESEARCH PAPER

# Targeting Periodontal Pathogens: The Efficacy of Nano-Enhanced and Photodynamic Therapies Against Porphyromonas gingivalis

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ABSTRACT

*Porphyromonas gingivalis* is a key pathogen in periodontitis that can form resistant biofilms, which complicate treatment. Conventional antimicrobial agents, such as chlorhexidine, are widely used but may be limited by microbial resistance and side effects. This study aimed to evaluate the antibiofilm efficacy of zinc nanoparticles (ZnNP) in combination with ultraviolet-visible (UV) photodynamic therapy and without UV, against biofilms formed by *P. gingivalis*. Clinical isolates of *P. gingivalis* were obtained from patients with periodontitis. Nanoparticles were synthesized using Pulsed Laser Ablation in Liquid (PLAL) and characterized by scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) analysis, UV-Vis spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy. Antibiofilm activity was evaluated using microtiter plate biofilm assays before and after treatment. Statistical analysis was performed using the t-test, with significance set at ($P < 0.05$). The results showed that ZnNPs with (U.V) exhibited superior activity compared to ZnNPs alone, suggesting they are a promising alternative for periodontal treatments. ZnNPs with U.V demonstrated superior antibiofilm activity against *P. gingivalis* and presented a promising alternative to conventional antiseptics like chlorhexidine. These findings support further investigation of nanoparticle-based therapy as a promising approach for combating microbial resistance and biofilm-associated infections.

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## INTRODUCTION

Periodontal diseases, including gingivitis and chronic periodontitis, are among the most common periodontal conditions. Historically, these conditions have been known by several names, such as necrotizing ulcerative gingivitis and scurvy gingivitis; however, modern understanding recognizes them as diseases with bacterial causes, as dental plaque biofilms contain more than 500 species of bacteria. Understanding the pathogenesis of periodontitis highlights the interplay between patient susceptibility and environmental factors [1].

Throughout their life, microorganisms alternate between two modes of development: the planktonic state, in which individual single cells float and proliferate in a liquid environment, and the sessile state, in which they form complex communities embedded in a self-produced extracellular matrix and adhere to biological or

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abiotic surfaces. Biofilm formation is a complex process controlled by physical, chemical, and biological factors, resulting in the cohesion and coexistence of bacteria in a suitable growth environment [2]. *Porphyromonas gingivalis* is one of the most prominent pathogens associated with these membranes, contributing to the development of periodontitis, which causes gradual destruction of the tissues supporting the teeth [3].

Metal nanoparticles (NPs) exhibit potent antimicrobial activity against various bacteria with limited side effects. Rapid, specific, and effective treatment strategies are required to inhibit these noxious pathogens. Zinc nanoparticles are effective in inhibiting biofilm formation in *P. gingivalis* and other strains, and they have also exhibited antiviral properties that differ from those of conventional drugs [6]. In parentheses (NPs) have attracted considerable attention because of their unique physicochemical properties.

In dentistry, these particles have proven effective in several disciplines, such as oral surgery, orthodontics, and periodontics, owing to their biological interactions with dental tissues and bacteria [7,8]. They work by damaging the cell wall, stimulating oxidative stress, and generating free radicals, which lead to the destruction and disintegration of DNA and the extracellular matrix, thereby eliminating biofilm cells [8].

Photodynamic therapy (PDT) is a non-invasive treatment that uses light-activated photosensitizers to target and destroy pathogens. Nanoparticles enhance the efficacy of PDT in treating periodontal diseases by improving photosensitizer delivery, increasing light absorption, and enabling targeted therapy for infected tissues [9]. Furthermore, upconversion nanoparticles enable deeper tissue penetration, addressing the limitations of conventional PDT by allowing for the effective treatment of periodontal pathogens under ultraviolet light [10]. Overall, the integration of nanotechnology with PDT represents a promising advancement in periodontal disease management.

## MATERIALS AND METHODS

*Samples Collection*

This was an in-vitro-experienced study, and samples were collected from patients with periodontitis after obtaining ethical approval and informed consent. This study was conducted at the College of Dentistry, University of Babylon, in November 2024 and February 2025. One hundred patients with periodontitis (male and female) were recruited for this study, with an age range of (18 – 70) years. The exclusion criteria were as follows: patients who had received antibiotic therapy in the last 6 months, patients with systemic diseases (diabetes, hypertension, cardiovascular disease, autoimmune disorders, and asthma), pregnant women, and smokers. Clinical periodontal parameters, including pocket depth (PD), clinical attachment loss (CAL), plaque score (PS), and bleeding on probing (BOP), were documented before the initiation of periodontal treatment.

Samples were collected from four sites from each patient with periodontitis. Sample collection included sites with clinical attachment loss and maximum pocket depth. The sampling sites were dried and maintained dry using cotton rolls. Sterile paper points of size 30 mm were placed in the chosen area (periodontal pockets or gingival sulcus) for approximately 30 s to collect the plaque below the gum line. We disposed of any contaminated paper points containing blood. We precisely matched the paper position from each sampling site to a sterile tube containing 4 ml of transport media (nutrient broth). The samples were then stored at -20°C for bacteriological and

Table 1. The size and sequences of specific primers of *p. gingivalis*.

| Target gene | | Primer sequence (5'- 3') | Product size (bp) |
|---|---|---|---|
| GIN | F | 5`AGG CAG CTT GCC ATA CTG CG-3` | 404 |
| | R | 5`ACT GTT AGC AAC TAC CGA TGT-3` | |

* A. adenine, C. cytosine, G. guanine, T. Thymine

PCR analysis. Polymerase chain reaction (PCR) was used to detect *P. gingivalis* [11]. Specific primer sequences for *P. gingivalis* are listed in Table 1.

*Preparation of Treatments*

Nanoparticles: Zn nanoparticles synthesized via Pulsed Laser Ablation in Liquid (PLAL). The study involved using a zinc chip and zinc oxide inside a beaker with water (DDDW), utilizing a pulsed laser with a wavelength of 1064 nm. The laser was focused on the zinc material at a frequency of (8) Hertz. The laser pulses created a visible cloud of metal particles that were absorbed and scattered, generating light and sound. The color of the solution changed with increasing pulse count. and characterized via scanning electron microscopy (SEM), energy-dispersive X-ray analysis (EDX), UV-Vis Spectroscopy technique, and Fourier Transform Infrared Spectroscopy technique (FTIR) [12].

*Microtiter Plate Assay*

*Pre-treatment Biofilm Formation*

To induce biofilm formation, the wells of a 96-well polystyrene plate were filled with 100 µL of Luria-Bertani (LB) broth and 100 µL of bacterial suspension. The plate was then incubated in an anaerobic incubator at 37°C for 24 hours. Non-adherent cells were removed by inverting the plate onto absorbent paper, gently tapping it, rinsing with distilled water, and then drying at 60°C. The wells were stained with crystal violet (0.5%), and excess dye was washed off. The biofilm was then fixed with an ethanol-acetone solution (20:80), and absorbance density was measured using ELISA. Finally, the optical density (OD) of each well was measured at 490 nm [13]. All tests

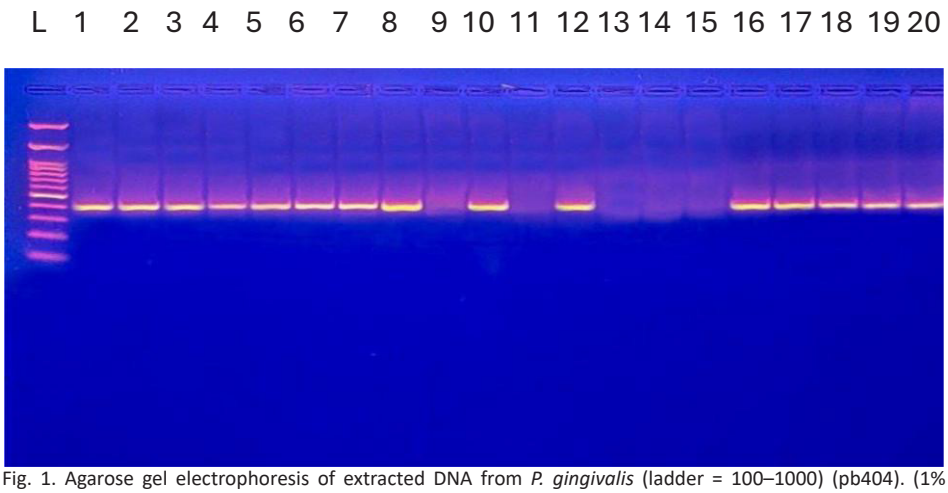


Fig. 1. Agarose gel electrophoresis of extracted DNA from *P. gingivalis* (ladder = 100–1000) (pb404). (1% agarose at 80 volts for 80 min) of 16S rRNA amplicons from the patients. Line L: DNA Ladder 1000bp line: Positive results for *P. gingivalis* appeared at 404bp.

Table 2. Clinical periodontal parameter examination.

| Clinical parameters | PPD | CAL | PS | BOP |
|---|---|---|---|---|
| No. of patients | 100 | 100 | 100 | 100 |
| mean ± S.D | 3.407±0.948 | 3.616±0.643 | 41.269±12.530 | 34.54±9.282 |
| Minimum | 2.18 | 2.4 | 21.2 | 21.9 |
| Maximum | 6.2 | 5.32 | 71 | 49.7 |

*PD: Probing Pocket Depth; CAL: Clinical Attachment Loss; PS: Plaque Score; BOP: Bleeding on Probing; S.D: Standard division.

were performed in triplicate.

*Post-treatment Biofilm Inhibition*

Following biofilm formation, 100 µL of the treatment (ZnNPs) was added to each well. The plates were incubated, and optical density readings were taken at 490 nm to quantify the residual biofilm mass. The plate was then exposed to ultraviolet light (UV-C) at a wavelength of 254 nm for 10 minutes, and the strains were categorized into four groups: no biofilm producers, weak biofilm producers, moderate biofilm producers, and strong biofilm producers [14].

*Data Analysis*

IBM SPSS Statistics 26 was used to analyze all data. The correlation between the nanoparticles and the control was estimated using a t-test, and the results are shown as (mean SD). A *P*-value of less than 0.05 indicated statistical significance.

**RESULTS AND DISCUSSION**

Clinical periodontal parameters, including plaque score (PS), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL), were measured during the intraoral examination. As shown in Table 2, each of these parameters was assessed in a cohort of 100 patients. (PPD): The average depth measured was 3.407 mm with a standard deviation of 0.948 mm. The values ranged from a minimum of 2.18 mm to a maximum of 6.2 mm. (CAL): The mean attachment loss was 3.616 mm (±0.643 mm), with individual results ranging from 2.4 mm to 5.32 mm. (PS): An average score of 41.269% was noted, with a variation of ±12.530%. The lowest and highest recorded scores were 21.2% and 71 %, respectively. (BOP): Bleeding was observed at 34.54% of the sites on average, with a standard deviation of 9.282%. The minimum and maximum values were 21.9% and 49.7%, respectively.

*Molecular Identification of P. gingivalis Using PCR*

Molecular identification using PCR targeting the 16S rRNA gene has proven to be a precise and efficient method for detecting *P. gingivalis*. PCR results showed clear bands at the expected molecular size of 404 bp for the tested isolates. A DNA ladder was used to estimate the band sizes. Fig. 1 showed that out of the 100 collected subgingival plaque samples, 15 pure isolates of *P. gingivalis* were successfully obtained and subjected to PCR and subsequent biofilm experiments. The remaining samples either yielded no growth or mixed growth and were excluded from further molecular analysis.

*Scanning of nanoparticles*

The nanoparticles prepared in the present study were examined using SEM, EDX, UV-Vis spectroscopy, and FTIR to determine the size of the zinc nanoparticles. Scanning electron microscopy

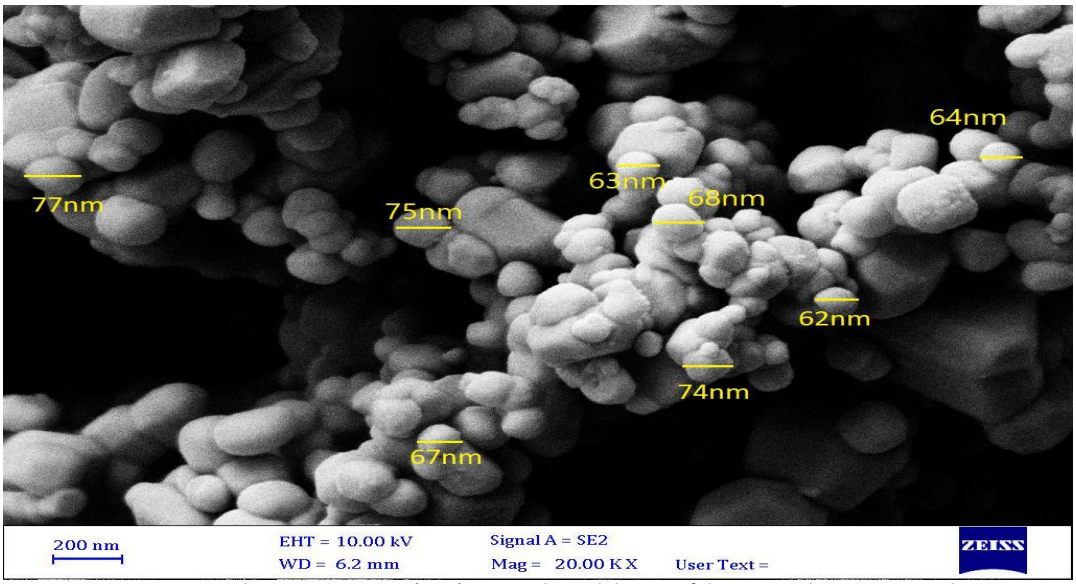


Fig. 2. Scanning electron microscopy (SEM) images showed the size of the prepared zinc nanoparticles.

(SEM) was used to determine the size, distribution, and shape of the particles. High-resolution surface images were produced by this method [15], and Fig. 2 shows the nanoscale aggregates and their aggregation.

Energy Dispersive X-ray Analysis (EDX), linked to the SEM device, revealed that the primary component was zinc (Zn), with additional elements such as oxygen (O) also present. Fig. 3A shows that the particle purity was validated by the EDX results, which also supports their use in biological and medicinal applications [16]. Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the chemical linkages and functional groups connected to the particle surface. The creation of nano-Zn is confirmed by absorption peaks in the 400–4000 $cm^{-1}$ range, which show in Fig. 3B the presence of a Zn–O link. Additionally, this method can detect the presence of contaminants or organic substances that may cover particles [17]. UV-Vis spectroscopy was used to determine the wavelength at which the highest absorbance was observed for Zn. The results showed that all samples exhibited optical activity in the UV region [17].

*Microtiter Plate Assay*

Dental plaque is a hallmark feature of caries, gingivitis, and periodontitis. *Porphyromonas gingivalis* is widely regarded as well-studied and effective pathogenic bacteria. Biofilm, consisting of bacteria and their exopolymeric substances (EPS), acts as a protective niche for the bacteria, preventing them from being cleared internally by the immune response or exposed to antimicrobial agents. The behavioral protection that biofilm confers on bacteria has long been a prominent focus of research in bacteriology [18]. The disease is associated with a group of pathogenic species that are particularly adept at evading the immune system and subverting host-destructive activity, thereby enabling them to withstand and multiply under the immunological storm orchestrated by the innate immune system [19].

In this study, the optical densities of the samples were categorized according to the standards listed in (Table 3) [20].

In the current study, nanomaterials and UV radiation were used as alternatives to conventional antibiotics to inhibit the growth of *P. gingivalis*. The nanoparticles (Zn) are prepared by pulsed laser ablation in liquid (a physical method) where they are characterized by SEM, EDX, UV-Vis, and FTIR techniques. than treated the fifteen isolates of *P. gingivalis* with zinc nanoparticles. PDT was more effective in stabilizing the bacterial biofilm than using nano zinc without UV light (Table 4).

As shown in Table 4, the experimental results highlight a marked reduction in biofilm formation following treatment with zinc nanoparticles (ZnNPs) and their combination with ultraviolet (UV) light. In the untreated condition, the optical density (O.D.) values, which reflect the extent of

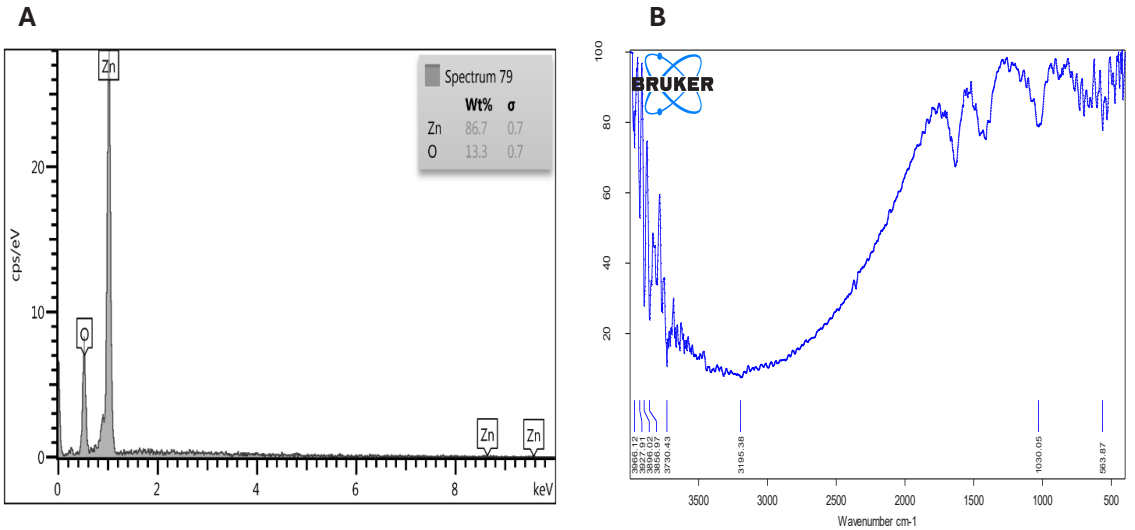


Fig. 3. A, EDX (Energy-dispersive X-ray spectroscopy) analysis of ZnNPs B, FTIR (Fourier Transform Infrared) spectroscopy of ZnNPs.

biofilm formation, ranged from the lowest value (0.078 ± 0.016) in the control (non-bacterial sample) to the highest value (0.286 ± 0.013) observed in isolate 15, which was classified as forming a moderate biofilm. Another relatively high O.D. value was recorded for isolate 12 (0.282 ± 0.064), which also corresponded to moderate biofilm formation. These values represent the natural capacity of the isolates to form biofilms in the absence of antimicrobial interference.

Upon treatment with ZnNPs alone, a substantial decline in O.D. values were observed across all isolates, with the lowest O.D. recorded in isolate 1 (0.056 ± 0.0005) and the highest in isolate 14 (0.101 ± 0.006). Despite this variation, all isolates under ZnNP treatment demonstrated complete inhibition of biofilm formation, as indicated by the uniform classification of "No biofilm" across the board. Compared to the untreated state, where many isolates had O.D. values exceeding 0.2, this result shows the strong antibiofilm activity of ZnNPs, reducing even the densest biofilms to below the threshold of detection.

When ZnNPs were combined with ultraviolet light, the inhibitory effect was maintained and, in some cases, appeared to be slightly enhanced. The lowest O.D. in this treatment was 0.047 (±0.009) in isolate 1, while the highest was 0.074 (±0.023) in isolate 14, mirroring the same isolates that had the extremes under ZnNPs treatment alone. Notably, these values were slightly lower than those in the ZnNP-only group, suggesting a mild additive or synergistic effect from UV exposure. Again, all isolates in this group were categorized as forming "No biofilm."

In terms of statistical significance, both treatment methods produced extremely low *P*-values (<0.001 for ZnNPs alone and ZnNPs with UV), indicating that the reduction in biofilm formation compared to the untreated group is highly significant. These results emphasize the potent inhibitory effect of ZnNPs on biofilm production by *P. gingivalis* and further suggest that the addition of UV light can slightly enhance this effect. Such findings hold promise for the development of advanced antimicrobial treatments targeting persistent biofilm-associated infections.

The nanoparticles showed similar results in some samples, suggesting their potential as a chemical alternative or complement to traditional agents. Nanoparticles showed an inhibitory effect against the isolates. The efficacy varied between isolates. ZnNPs with UV demonstrated marked reduction and inhibition of biofilm formation in vitro. demonstrated that nanoparticles disrupt the cytoplasmic membrane of bacteria through high surface charges and the generation of free radicals (ROS) [21]. nanoparticles had stronger antibacterial activity than root filling agents. This enhanced antibacterial property helps control microbial infections by killing microorganisms and preventing reinfection [22]. These findings resonate with the results of Huang, who observed a significant decrease in biomatrix density, as reflected in optical density readings, compared to control groups [23]. These findings corroborate the results of a previous study, which confirms zinc's antibacterial action against periodontal pathogenic bacteria, such as *P. gingivalis*. Zinc and its compounds suppress bacterial growth and reproduction, potentially contributing to healthier periodontal tissues. Low concentrations do not cause cytotoxic effects on fibroblasts [24].

Table 3. Classification for biofilm producer with optical densities (OD).

| Optical Density (OD) Range | Classification | Optical Density (OD) value |
|---|---|---|
| OD ≤ ODc | Non-biofilm producer | OD ≤ 0.126 |
| ODc < OD ≤ 2 × ODc | Weak biofilm producer | 0.127 < OD ≤ 0.252 |
| 2 × ODc < OD ≤ 4 × ODc | Moderate biofilm producer | 0.252 < OD ≤ 0.504 |
| OD > 4 × ODc | Strong biofilm producer | OD > 0.504 |

* ODc: Optical Densitycut

Ultraviolet showed significant results in reducing the biofilm of *P. gingivalis* when combined with ZnNPs. The first isolation recorded the lowest value of light density (0.047±0.009), as shown in Table 3. The results of our current study are consistent with those of previous studies [25, 26, 27]. Nanoparticle-based treatments are a promising alternative due to their multiple mechanisms of action, which include the generation of reactive oxygen species (ROS) and the physical disruption of bacterial membranes.

Zinc nanoparticles were particularly effective, largely due to their small size and increased reactive surface area. Notably, the use of UV light in conjunction with the nanoparticles completely prevented biofilm formation, providing results superior to those achieved by the nanoparticles alone. These results suggest the potential for

Table 4. Effect of Nanoparticles and Ultraviolet (UV) radiation on the Growth and Biofilm Formation of *P. gingivalis*.

| Isolates | Without treatment | | Treatment by Zn NP | | *P*-value | Treatment by ZnNPs with Ultraviolet | | *P-value* |
|---|---|---|---|---|---|---|---|---|
| | O.D±SD | Biofilm response | O.D±SD | Biofilm response | | O.D±SD | Biofilm response | |
| control | 0.078±0.016 | No biofilm | | | | | | |
| 1. | 0.18±0.026 | weak | 0.056±0.0005 | No biofilm | | 0.047±0.009 | No biofilm | |
| 2. | 0.226±0.0162 | weak | 0.062±0.006 | No biofilm | | 0.054±0.007 | No biofilm | |
| 3. | 0.192±0.016 | weak | 0.056±0.0005 | No biofilm | | 0.059±0.003 | No biofilm | |
| 4. | 0.185±0.019 | weak | 0.064±0.005 | No biofilm | | 0.063±0.015 | No biofilm | |
| 5. | 0.209±0.098 | weak | 0.066±0.002 | No biofilm | | 0.063±0.022 | No biofilm | |
| 6. | 0.128±0.007 | Weak | 0.086±0.019 | No biofilm | | 0.062±0.017 | No biofilm | |
| 7. | 0.129±0.010 | Weak | 0.085±0.008 | No biofilm | | 0.074±0.017 | No biofilm | |
| 8. | 0.128±0.005 | Weak | 0.079±0.002 | No biofilm | | 0.062±0.023 | No biofilm | |
| 9. | 0.128±0.004 | Weak | 0.144±0.026 | weak | <0.001 | 0.051±0.007 | No biofilm | <0.001 |
| 10. | 0.139±0.017 | Weak | 0.075±0.003 | No biofilm | | 0.052±0.005 | No biofilm | |
| 11. | 0.222±0.031 | Weak | 0.08±0.001 | No biofilm | | 0.057±0.009 | No biofilm | |
| 12. | 0.282±0.064 | Moderate | 0.074±0.003 | No biofilm | | 0.059±0.012 | No biofilm | |
| 13. | 0.243±0.054 | Weak | 0.090±0.007 | No biofilm | | 0.064±0.01 | No biofilm | |
| 14. | 0.230±0.015 | Weak | 0.101±0.006 | No biofilm | | 0.074±0.023 | No biofilm | |
| 15 | 0.286±0.013 | Moderate | 0.094±0.0005 | No biofilm | | 0.055±0.018 | No biofilm | |

* S.D: standard deviation; OD: optical density

developing more effective treatment strategies. Future studies should include validation of in vivo efficacy and long-term safety assessments.

## CONCLUSION

One intriguing and cutting-edge method for treating periodontal infections, particularly those caused by *P. gingivalis*, is photodynamic therapy (PDT). By adding a photosensitizing chemical and then activating it with light, the technique produces cytotoxic reactive oxygen species that specifically target and destroy bacterial cells. PDT is considered less prone to resistance development compared to conventional antibiotics, in contrast to traditional antibiotics. It is a useful supplement to periodontal therapy regimens due to its ability to reduce bacterial levels and improve clinical outcomes. The study suggests that incorporating zinc nanoparticles into periodontal therapy can improve antibacterial efficacy and reduce side effects. Zinc nanoparticles exhibit potent antibacterial properties through the generation of reactive oxygen species, membrane penetration, and enzymatic disruption. These nanoparticles are promising candidates for replacing or enhancing conventional agents, such as chlorhexidine. Further clinical studies are recommended to evaluate their long-term efficacy, safety, and practical dental applications. Future studies are recommended to validate the efficacy of these materials through in vitro experiments. These findings suggest that both agents have potential applications in periodontal therapy, with nanoparticles that offer U.V. protection exhibiting a novel effect, and the synthesized nanoparticles displaying significant antimicrobial activity against *P. gingivalis*. Among the tested agents. The findings highlight the promising role of as an effective alternative to traditional antiseptics, which can be used periodically.

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## CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest. This research was carried out with complete independence and objectivity, without any financial or personal ties to individuals or organizations influencing the study.

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