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Antibacterial Effects of a 940 nm Diode Laser With/Without Silver Nanoparticles Against *Enterococcus faecalis*

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Abstract

Introduction: The final goal of root canal therapy is to remove of the most bacteria from the root canal. This study aimed at comparing the antibacterial effects of a diode laser with a wavelength of 940nm and silver nanoparticles and the synergic effects of both techniques on *Enterococcus faecalis*.

Methods: Ninety single-rooted human teeth were decoronated and prepared with rotary files. The samples were irrigated with sodium hypochlorite and EDTA17%. Then they were autoclaved and contaminated with *E. faecalis* suspension (1.5×10^8 CFU/mL) for 21 days. The samples were fixed in a microtube and were randomly divided into 4 experimental (n=20) groups and a negative control group (n=10) as follows: Group 1: hypochlorite sodium 5%, Group 2: silver nanoparticle, Group 3: diode laser, and Group 4: diode laser and silver nanoparticle. The samples were obtained from dentin chips before and after the intervention. The data were analysed using the Kruskal-Wallis nonparametric test. Furthermore, alterations in bacterial colonies were entered using the Wilcoxon signed ranks test ($\alpha=0.05$).

Results: There was a significant decrease in colony counts for all groups after interventions (*P* value<0.05). Also, all groups showed more reductions in colony counts compared with the negative control group (*P* value<0.004). There was a significant reduction for group 1 in comparison with other groups (*P* value<0.001) and this group had an extreme decrease of colony counts (RCC=100%). There was an important differential between silver nanoparticles and diode laser groups in bacterial counts (*P* value<0.001) and silver nanoparticles (RCC=83.15%) had more efficiency than the diode laser (RCC=41/33%). RCC of group 4 was 68/52%.

Conclusion: Followed by sodium hypochlorite 5%, silver nanoparticles were the most effective antibacterial substances. The 940 nm laser diode had less antibacterial effect compared to its use with silver nanoparticles.

Keywords: Root Canal Therapy, Laser Diode, Silver Nanoparticles, Hypochlorite Sodium, *Enterococcus faecalis*.

Introduction

The ultimate purpose of root canal treatment is to remove all microorganisms, particularly bacteria which are regarded as major causes of pulp and periapical diseases in the root canal system. Bacteria within the biofilms have become a major challenge in the root canal treatment process due to their high resistance to antimicrobial agents.[1-3] When the bacteria penetrate into the deeper layers, it will be more difficult to clean the root canal.[4,5] One of the most common bacteria related to treatment failure is *Enterococcus faecalis*. This facultative anaerobic bacterium is able to survive without food for a long time and infiltrate into dentin tubules.[1,6]

Sodium hypochlorite serves as the main irrigator in the root canal treatment, but it may have toxic effects upon its contact with periapical tissue cells[7,8] and it also decreases

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bond strength between dentin and resin. Given the weakness of conventional root canal therapy, new means like lasers have been introduced in recent years.[9] Among the types of lasers, diode lasers have been widely used due to their small size and low price.[10] Diode lasers can easily penetrate into curved canals due to their flexible and small size fibers,[11,12] better distribution of laser light in the root canal system and thus better disinfection of the canal. Diode lasers interfere slightly with water and hydroxyapatite, and enhances its bactericidal effect with thermal photo disruptive activity in inaccessible areas of dentin. Therefore, these properties have led to their use in disinfecting the canal.[13]

Although most studies have shown improved disinfection of dentin tubules, the complete removal of bacterial species, especially pathogenic species of the root canal, has not been observed using lasers alone. Therefore, it is recommended that lasers be used along with another disinfectant.[1]

The technology of nanoparticles has attracted much attention due to their antibacterial and anti-inflammatory effects.[14] Silver nanoparticles have been shown to have a destructive effect on about 650 microorganisms causing resistant diseases. They are used to prevent recurrent infection, prevent microbial colonization and remove debris from the root canal system.[5]

The purpose of our study was to investigate the antibacterial effect of silver nanoparticles alone and simultaneous with the 940 nm laser diode and sodium hypochlorite as the best material in washing the root canal and determine the decrease in the number of *E. faecalis* colonies in each of these interventions.

This study was carried out at the Faculty of Dentistry (Endodontic and Laser Department), Medical Microbiology and Molecular-Cellular laboratories at Hamadan University of Medical Sciences (2018-19), Hamadan, Iran.

Materials and Methods

The study population consisted of bacterial biofilm formed in dentin tubules of anterior single root teeth after filing and rinsing with 5% sodium hypochlorite, 0.9% normal saline, 100 ppm silver nanoparticles suspension and radiating of 940 nm diode laser.

In this study, the number of bacterial colonies before and after the treatment was calculated and reported as CFU/mL. Accordingly, 90 freshly extracted human anterior teeth were collected, and it was confirmed by radiography that they were single-canal. The samples were selected from among the teeth that had long roots (at least 14 mm) and had no cracks, caries or fracture

Tooth Preparation

The teeth crowns were removed with the help of a diamond disk (KaVo Kerr, Germany) in a way that the remaining root had a length of 14 mm. The working length was determined 1 mm shorter than the end of the canal (13 mm) (Figure 1). The root canals were first cleaned with the help of manual files (MANI INC, Japan) up to number 20 and then continued by rotary files (V-Taper Gold, Shanghai Fanta Dental, China) to the main file. The tooth was then fixed inside a 2 mL microtube using acryl (Acropars, Iran) and autoclaved at 121°C for 15 minutes (Figure 2).

Preparation of the Pomegranate Peel Extract

In order to prepare pomegranate peel extract (PPE) solution, the black peel of pomegranate fruit was prepared from the local gardens of Saveh city (Iran). The peels were powdered after being dried in 50°C of an oven. 50 g/L of the powder was mixed with ethanol 70% and then came to spin with a rotary shaker (150 rpm) for 24 hours and finally filtered. The remaining part of it was re-extracted using 200 mL of new solvent, followed by filtration, and the concentrate was pooled. By using a rotary at 40°C, the extracted solvent was evaporated and then boiled

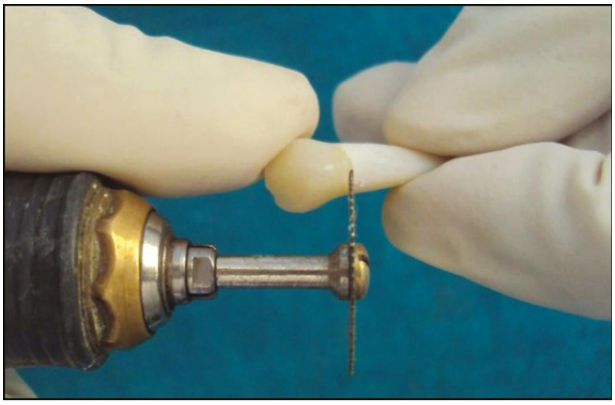

Figure 1. Removing the Roots by Disk and Hand-Piece.

Figure 2. A Tooth Root Fixed in Microtube.

in deionized water and allowed to cool. Eventually, after being cooled, the extract was filtered and held in a refrigerator (4°C) to be used as PPE solution.[12]

Cell Toxicity Evaluation of PPE and AgPPENPs

To examine suspicion toxicity of Argentum *Pomegranate Peel Extract nano partichles* (AgPPENPs) and PPE against a cell line, the RMPI medium covering 10% of bovine fetal serum, 50 µg/mL streptomycin and 50 IU/mL penicillin in 5% CO2 of atmosphere with the temperature of 37 degrees was employed and fibroblast cell line L929 was cultured inside. Different concentrations of PPE and AgPPENPs (100, 200, 300, 400, and 500 µg/mL) were applied to cultured cells. MTT assay was used to assess cell viability. The cells were cultured in 96 well plates and incubated for 3 hours with MTT solution. DMSO solution was also added to solubilize formazan grains in each well and absorption value was studied using the ELISA reader in 580 nm.[12]

Bacterial Inoculation in the Root Canal

Enterococcus faecalis (ATCC: 29212), as a standard strain, was inoculated in 5 mL of Trypticase soy broth (TSB) (HiMedia Laboratories, India) and then incubated at 37°C for 24 hours. Microbial cells were diluted to reach the concentration of 1.5×10^8 CFU/mL (a suspension equivalent to 0.5 McFarland standard). The teeth were fixed inside a 2-mL microtube using acryl (Acropars, Iran) and autoclaved at 121°C for 15 minutes, and they were filled with 0.5 mL of *E. faecalis* suspension (1.5×10^8 CFU/mL) and then incubated at 37°C for 21 days for biofilm formation. Every 24 hours, one-third of the microtube suspension was replaced with sterile TSB.[4]

Silver Nanoparticles Preparation

PPE was used to prepare silver nanoparticle suspension. The pomegranate skin was dried in an oven for 2 days, and to prepare the hydroalcoholic extract, 50 g of it was immersed in 500 mL of 70% alcohol for 72 hours at room temperature. The extract was then separated from the pomegranate skin by Whatman filter paper 40. The silver nanoparticles were settled down by a centrifuge and 100 ppm nanoparticle suspension was made with sterile water.[15]

The samples were randomly divided into four experimental groups and one control group as follows:

Group 1: Washing with 5% sodium hypochlorite: Cleaning of root canals was done with 5 ml of 5% sodium hypochlorite solution for 5 minutes (Golrang, Iran). Next, 1 mL of 5% thiosulfate solution (Samchun Pure Chemical, Korea) was injected with an insulin syringe to neutralize the sodium hypochlorite solution and remained for 30 seconds.

Group 2: The root canal was cleaned with 5 mL of 100 ppm silver nanoparticle suspension (average particle size 20 nm) by an insulin syringe for 5 minutes.

Group 3: Diode laser: The teeth were irradiated with a 940 nm diode laser (Epic 10-Biolase, USA) and 1-watt power with continuous mode. The laser end tip with a diameter of 200 micrometers (E2, USA, Biolase, E200) and 1 mm shorter than the apex was applied with spiral motions in epicocoronally direction for 15 seconds (1 second per mm). Because silver nanoparticles solution will moisten the canals, the canals in third group were moistened with normal saline to standardize with the fourth group (silver nanoparticles and diode laser). The irradiation process was performed 3 times, each time for 15 seconds with a 15-second interval between each irradiation.[7,16]

Group 4: Diode laser and silver nanoparticles: The root canal was cleaned with 5 mL of 100 ppm silver nanoparticle suspension by an insulin syringe. After 5 minutes, the diode laser was irradiated at 940 nm for 15 seconds for 3 times similar to the diode laser group.[6,7]

Group 5: Negative control group: Washing of the root canal was done with 5 mL of normal saline solution and sampled after 5 minutes of being left untouched.

The samples were collected from each canal once before and after the intervention, and the formation of colonies was counted before and after the intervention and then turned into real numbers.

Initial Sampling

In order to standardize the model of bacteria after removing TSB and cleaning the canal with 5 mL normal saline with file 40 (MANI INC, Japan), 15 filing movements were obtained from the canal. Then the file was moved to a sterilized microtube containing 1 mL of Tween 80 solution (Samchun Pure Chemical, Korea) and vortexed for 30 seconds to separate the biofilm of bacteria. One percent dilution was created from the first sample and cultured on the blood agar medium and the incubation of plates was done at 37°C for 24 hours and colony counts were performed in the manner of CFU/mL.[5,17]

Final Sampling

The canals were cleaned with 5 mL of normal saline to standardize all groups and the solution was kept in the root canal for 30 seconds. Then, the sampling was performed like the method of initial sampling and colony counts were reported in the manner of CFU/mL.

Accordingly, SPSS software (version 21) was utilized to analyze the data. For this purpose, the central dispersion indices for the number of live bacterial colonies were counted before and after the interference. Reduction in bacterial colonies after the interference in different groups was compared by the Kruskal-Wallis nonparametric test. Also, alterations in bacterial colonies before and after the interference were examined by applying the Wilcoxon

signed ranks test. Because the data did not have normal distribution, nonparametric analyses were applied in the study. The type I error rate was set at 0.05 (α = 0.05).

Results

To assess the possible toxicity of synthesized nanoparticles, cell viability evaluation was performed. The results showed that AgPPENPs could lessen cell viability in the concentration of 400 and 500 µg/mL (as pointed in Figure 2). Given that PPE could diminish the toxicity of the particles alone, they became absolutely safe in the concentration of 400 µg/mL without any notable toxicity (Figure 3).

According to the results of the Wilcoxon test, there was a significant decrease in the number of bacterial colonies after the intervention, *P* value < 0.05 (see Table 1).

According to the number of bacterial colonies before and after the interventions, a parameter known as RCC can be introduced. The value of this variable was calculated for all study groups and it is shown in Table 2. Regarding the RCC values, the highest bacterial elimination (100%) belonged to the sodium hypochlorite group, while the lowest reduction was reported in the diode laser group (RCCmean = 41.33), as shown in Figure 4.

The Kruskal-Wallis nonparametric test was used to compare the performance of different interventions in terms of RCC mean values. The results of this test revealed that there was a significant difference between the reduction of bacterial colonies in different groups (RCC *P* value = 0.03). According to this result, the Mann-Whitney test was used for pair comparison of the two groups. In typical cases, the type I error rate (α) is usually considered to be 0.05, but here in the Mann-Whitney test, depending on the number of groups, it is 0.004. As a result, if the *P* value is less than 0.004, the difference is significant, and if it is bigger, there is no significant difference between the groups (see Table 3).

The results of the Mann-Whitney test with Bonferroni adjustment indicated that there was a significant difference between all groups and the negative control group (*P* value < 0.004). The number of bacterial colonies did not show any significant difference between the diode laser group and the negative control group (*P* value = 0.549). There was also a significant difference between the effectiveness of sodium hypochlorite in the decline of bacterial colonies with the other groups (*P* value < 0.001). There was a significant difference between the diode laser (RCC = 41/33%) and silver nanoparticles (RCC = 83/15%) groups (*P* value < 0.001) and between "diode laser" and use of "diode laser and silver nanoparticles simultaneously" (RCC = 68/52%) (*P* value = 0.001). Moreover, there was no significant difference between the silver nanoparticles and the laser group in terms of the number of bacterial colonies (*P* value = 0.017). The results of the diode laser group in the dry canal (n = 5) showed a higher rate of removal of bacterial colonies than in the wet canal (RCC = 66.6). There was also no significant difference between this group and the silver nanoparticle group (*P* value = 0.237). There was also no significant difference between the silver nanoparticles and laser diode groups (*P* value = 0.284).

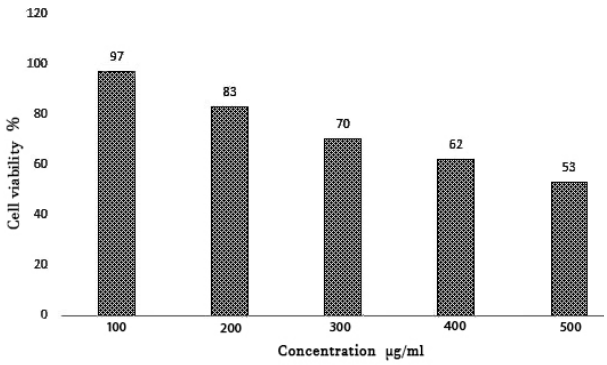

Figure 3. Result of Toxicity Effect of Various Concentration AgPPENPs on Cell Line.

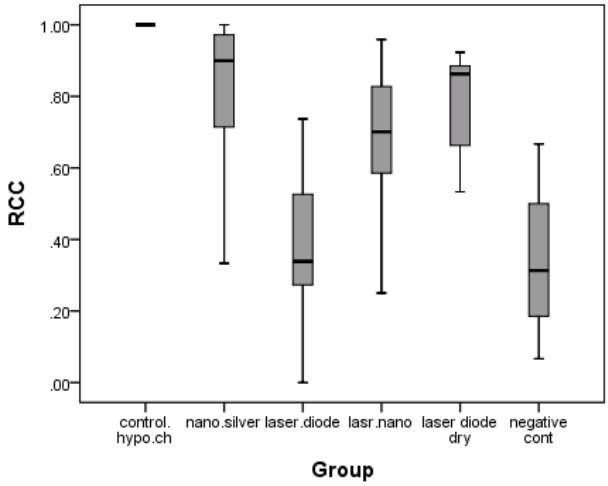

Figure 4. RCCmean Values of the Different Groups.

Discussion

Root canal disinfection is commonly performed through the simultaneous use of mechanical devices and disinfectant solutions as well as the placement of the medical pack in the root canal between treatment sessions.[1] However, the wash solutions have a limited penetration depth and cannot kill bacteria in the deeper layers. Therefore, lasers may increase the rate of bacterial removal due to greater penetration depth along with the wash solutions.[13,18].In addition, silver nanoparticles, as a new technology, have different medical applications because of their antibacterial and anti-inflammatory features, and they can also be used in root canal therapy.[1,10,12] Hence, the main goal of our study was to investigate the antimicrobial effects of simultaneous application of the diode laser and silver nanoparticles on *E. faecalis* compared to sodium hypochlorite as the gold

Table 1. The Values of Descriptive Variables, Mean, Median, Standard Deviation, Amplitude of *Enterococcus faecalis* Colonies Before and After the Intervention and Percentage of Decrease in Bacterial Colonies for Each Group

Group	Time	Mean	Median	Lower Most	Upper Most	Standard Deviation	P_{value} RCC
Sodium hypochlorite	Before	10^3×30	10^3×27	10^3×12	10^3×27	10^3×17	<0.001
	After	0	0	0	0	0	
Silver nanoparticles	Before	10^3×18	10^3×16.5	10^3×5	10^3×60	10^3×12.9	<0.001
	After	10^3×2.4	10^3×1.5	0	10^3×8	10^3×2.5	
Laser diode	Before	10^3×30	10^3×28	10^3×15	10^3×62	10^3×13.5	<0.001
	After	10^3×18.8	10^3×16.5	10^3×4	10^3×39	10^3×9.6	
Silver nanoparticles and laser diodes	Before	10^3×17	10^3×14	10^3×4	10^3×49	10^3×12.3	<0.001
	After	10^3×3.9	10^3×3.5	10^3	10^3×8	10^3×2.1	
Negative control (normal saline wash)	Before	10^3×19.3	10^3×17	10^3×3	10^3×58	10^3×17	<0.005
	After	10^3×14.4	10^3×14	10^3	10^3×41	10^3×13.2	

Table 2. RCC Values of the Different Groups (%RCC_{mean})

Group	RCC%
Sodium hypochlorite	100
Silver nanoparticles	83.15
Laser diode	41.33
Silver nanoparticles and laser diodes	68.52
Negative control (normal saline wash)	33.48

Table 3. Mann-Whitney U Test Results With Bonferroni Adjustment for Pair Comparison of the Groups

Variables/Groups	RCC_{mean} *P* Value
Sodium hypochlorite, silver nanoparticles	0.00
Sodium hypochlorite, diode laser	0.00
Sodium hypochlorite, diode laser and silver nanoparticles	0.00
Silver nanoparticles, laser diodes	0.00
Silver nanoparticles, laser diodes and silver nanoparticles	0.017
Diode laser, silver nanoparticles and diode laser	0.001
Sodium hypochlorite, negative control group	0.00
Silver nanoparticles, negative control group	0.00
Laser diode, negative control group	0.549
Laser diode and silver nanoparticles, negative control group	0.001

standard.

The results exhibit that the reduction in bacterial colonies after diode laser irradiation at 940 nm was significantly different from the bacterial colonies before laser irradiation (*P* value < 0.001). However, the reduction of bacterial colonies was lower than the sodium hypochlorite group (RCC = 41.33). Moreover, a comparison of the data of the same group with the negative control group data showed no significant difference between the two groups (*P* value = 0.549). Generally, laser irradiation should be done in the dry canal. In fact, a powerful diode laser eliminates the bacterium by increasing the temperature, and the moisture of the canal can reduce the temperature. This could also reduce the power of the laser to kill the bacteria. However, in this study, the canal did not dry before laser diode irradiation in order to standardize the conditions for comparing the results in the diode laser group and the silver nanoparticles group in which the canal was moistened with silver nanoparticle solution.

Castelo et a1[16] used a 940-nm diode laser to remove *E. faecalis*. They reported 70% elimination for bacterial colonies, which is more than that of the present study. The laser power was 3.5 watts and irradiation duration were 1 minute in pulse form. In the present study, since the laser was irradiated in a wet canal for standardization (unlike Castelo et al[16]) and laser power (1 W) and irradiation duration (45 seconds in total) were less than those of Castelo et al, the number of eliminated bacterial colonies was less than that of Castelo et al.

Gutknecht et al[19] found that the elimination rate of bacterial colonies was 74% after drying the samples. They used a diode laser with a wavelength of 810 nm and power of 3 watts for 30 seconds and continuous irradiation. However, it should be noted that the laser power in Gutknecht et al was higher than that of the present study, which could increase the capability of the diode laser to kill the bacteria through heat. On the other hand, the laser wavelength in Gutknecht et al was 810 nm, which is shorter than that of the present study. This wavelength (810 nm) penetrates deeper than higher wavelengths and is more desirable for the removal of bacterial colonies in the root canal.

Given the decrease in the diode laser group after drying the canal, it can be said that if the diode laser is used alone, it would be better to dry the canal. However, the radiation should not be exceeded because of the possibility of damage to the root and surrounding tissues.

The results of the silver nanoparticles group data showed a significant decrease in the number of bacterial colonies after the suspension, compared to the preceding one (*P* value < 0.001). The decrease in the number of bacterial colonies in this group was statistically significantly

different from that in the negative control group (*P* value < 0.001). The percentage of bacterial colonies in this group was 84%, which is similar to that of Afkhami et al. They also used silver nanoparticles suspension at 100 ppm for 5 minutes and reported a decrease of 94.42% in bacterial colonies. The difference between the reported values for the percentage of bacterial colonies in Afkhami et al and the present study can be related to the sampling method before and after the intervention. They prepared the initial sample with the manual file and the final sample with the rotary file, which is not appropriate for standardization.[7]

In another study, Rodrigues et al[20] investigated the antimicrobial properties of silver nanoparticles with a concentration of 94 ppm in dentine tubules against Enterococcus faecalis. In the mentioned study, the specimens were prepared as dentin blocks and kept in a silver nanoparticle solution for 5 minutes. It was found that the percentage of reduction for the bacterial colonies was 31.19 after 5 minutes of keeping in silver nanoparticle solution, and this percentage increased with increasing the shelf life of silver nanoparticles. For example, the percentage of reduction for bacterial colonies was 86.85 after 15 minutes.

The difference in reduction percentage of bacterial colonies between our study and Rodrigues and colleagues' study may be attributed to the amount or volume of the suspension of silver nanoparticles; in the present study, the percentage reduction of bacterial colonies with silver nanoparticles was 83.15 after 5 minutes. The volume of the suspension of silver nanoparticles in Rodrigues and colleagues' study was 1 mL, and in the present study, it was 5 mL.

Moghadas and colleagues' study[21] showed that silver nanoparticle-based wash solution was as effective as 5.25% sodium hypochlorite wash solution in the removal of *E. faecalis*. The decrease of bacterial colonies has been reported to be 100% after keeping them in wash solution for 3 minutes. However, in the present study, the effect of silver nanoparticles on bacterial colonies was significantly less than hypochlorite (83/15%). This difference can be due to the chemical composition of the wash solution used in Moghadas and colleagues' study, which consisted of three components of silver nanoparticles (as the main component), ethanol and sodium hydroxide, whereas in the present study the suspension of silver nanoparticles consisted of water, silver solvents and PPE.

Litvin and Minaev[22] and Maneerung et al[23] have reported that adding sodium hydroxide to silver nanoparticles reduces the absorption of silver ions in the bacterial cell and subsequently increases the antibacterial effects of silver nanoparticle solution.

According to the present study, the percentage of the reduction of bacterial colonies in the simultaneous application of the silver nanoparticles and diode laser (68.52) was lower than that of silver nanoparticles alone and higher than the diode laser in the wet canal. However, there was no significant difference between the silver nanoparticles group and the laser and silver nanoparticles group in terms of the reduction of bacterial colonies. It can be concluded that the application of silver nanoparticles can reduce the dampening effect of the canal during laser application. However, laser irradiation could not increase the effect of silver nanoparticles. This difference is not significant, but no synergistic effect was observed.

In a recent study, Abdelgawad et al assessed the synergic effects of an 810nm laser diode on antibacterial effects of Chlorhexidine and silver nanoparticles as washer solution of canals. Diode laser and silver nanoparticle had the most antibacterial effect (78%). This rate was 68.52% in our study. However, in Abdelgawad and colleagues' study, the antibacterial effect of silver nanoparticles alone was not investigated to determine whether laser irradiation increased or decreased the antibacterial effect of silver nanoparticles, and it only had a greater antibacterial effect than the other two methods. Moreover, *E. faecalis* was also incubated for 7 days to form a biofilm, but it was done for 21 days in our study.[10]

However, some studies have reported that by adding sodium hydroxide, the silver nanoparticles offer antibacterial effects similar to sodium hypochlorite. Moreover, the use of a light-sensitizing agent can also increase the antibacterial effects of the diode laser,[22,23] but because of insufficient studies in this area, further studies are required to investigate the increased antibacterial effects of silver nanoparticles and the diode laser.

According to the results, the efficacy of sodium hypochlorite solution (5%) was the highest among all groups, eliminating all bacteria. This result is in good agreement with that of Ashofteh et al[24] who compared the antibacterial effect of 5.25% sodium hypochlorite and a diode laser (830 nm).

Therefore, it can be argued that sodium hypochlorite is still the gold standard in root canal disinfection, although this effect depends on the length of time within the canal and the concentration of the solution. Due to its low cost and availability, sodium hypochlorite is also cost-effective. Perhaps the most important drawback is the effect of toxicity. It is, therefore, recommended that better strategies be provided in order to safely deliver sodium hypochlorite to the root end areas, making it more reliable to use.

Conclusion

Although the diode laser showed a significant difference in the number of bacterial colonies before and after the intervention, it alone was not sufficient to remove the bacteria. According to the results, silver nanoparticles along with appropriate concentration and time removed more bacterial colonies than the negative control group.

However, its simultaneous use with diode lasers showed that laser irradiation could not increase the antibacterial effect of silver nanoparticles. In the end, sodium hypochlorite is still the gold standard in root canal disinfection.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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