ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antibacterial Effect of Silver & Gold Nanoparticles and Diode Laser against Lactobacillus acidophilus bacteria

Ali Safan[1], Tareq Youssef[2], Mohmed Hussein Zaazou[3], Sherein I. Abd El-Moez[4], Hanaa El-Shinawy[5], and Doaa Mohamed Sadony[6].

1. Department of dental laser application,
2. Department of photochemistry and photobiology-national institute of laser enhanced sciences-Cairo University.
3. Department of restorative & dental materials research,
4. Department of Microbiology and Immunology,
5. Department of oral surgery & medicine research-National Research Centre, Cairo, Egypt.
6. Department of restorative &dental materials research,

Manuscript Info

Manuscript History:

Received: 18 June 2014
Final Accepted: 25 July 2014
Published Online: August 2014

Key words:
Silver & Gold Nanoparticles , Diode Laser, L.acidophilic bacteria

Corresponding Author

Ali Safan

Abstract

The presence of necrotic tissue and bacteria may cause the persistence of infection in root canals. The aim of this study is to assess the antibacterial effect of diode laser irradiation, silver nanoparticles, gold nanoparticles and combination between them on extracted single rooted teeth. A reference strain of anaerobic L.acidophilic bacteria was irradiated at different time (30 seconds, 1 minute, 2 minutes, and 3 minutes). After the tests were performed, the number of colony-forming units per milliliter (CFU/ml) was counted and the results were statistically analyzed. According to the data evaluated, laser and silver groups showed the lowest mean bacterial count, while no statistical difference between silver groups, gold groups ,and laser + gold groups.

Copy Right, IJAR, 2014,. All rights reserved

Introduction

In endodontics, the main goal of endodontic treatment is disinfection of the root canal system for ensuring successful, long-lasting root canal therapy [(4)]. The contamination of root canals with bacteria and its by products in remnants of necrotic soft tissue is considered one of the main reasons for failure in endodontic treatment [(3, 20)]. Teeth that give a negative culture for bacterial growth at the time of a root canal filling have a higher success rate than teeth that are culture positive [(7)].

In recent years, various laser systems have gained importance in the field of laser assisted endodontics, namely the Nd:YAG, diode, Er:YAG, and Er,Cr:YSGG laser [(10)]. Gomes et al has shown that bacteria present in root canal after endodontic treatment are different from those present in infected root canals before endodontic treatment [(3, 4)].

L. acidophilic has been identified in persistent root canal infections and also related to the failure of endodontic treatment [(7)]. The presence of a smear layer after instrumentation reduces the effectiveness of root canal filling, which should be removed for opening dentinal tubules to obtain successful treatment [(1, 16)].

Hardee et al indicates that, besides the treatment of dental tissue and forming the root canal by using laser radiation, the effects of laser light on endodontic bacteria are postulated [(5,13)]. The laser radiation was transmitted through quartz optical fibers, which facilitate introducing laser light around canal curvatures and irregularities [(15)].

They also focused on the respective wavelength, its specific bactericidal capabilities, and potential usefulness in root-canal disinfection. Silver nanoparticles (Ag-NPs) in the range of 10-13 nm have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem [(6,7)].

The gold nanoparticles (Au-NPs) have remarkable physical and chemical properties in different modifications (e.g., spherical, rods and shells) are the most promising candidates for such a photothermal effect [21]. Since they are photostable, nontoxic and seem to induce varying cellular damage upon irradiation [17].
Finding a method to provide disinfection in root canals without causing a cytotoxic effect on peripheral tissue is necessary. Therefore this study aimed to compare between the effect of diode laser irradiation (980 nm, 1.5 watt) & (Ag-NPs) and (Au-NPs) at different time of laser irradiation (30 seconds , 1 minute, 2minutes ,and 3minutes) on the L.acidophilic bacteria and using (CFU_S) for bacterial count.

2. Materials and methods:

1. Sample selection and preparation:
A total of 45 extracted single rooted human maxillary anterior teeth were collected, scaled to remove all adhering soft tissues and kept in saline solution at room temperature.

2. Root canals Preparation:
The teeth were decapitated at the level of cemento-enamel junction using high-speed diamond disk to facilitate the mechanical preparation of the root canals, which the working length was established by subtracting 0.5 mm from the apical foramen. The canals were shaped using step-back technique to #40 K-file (Denstply-Maillefer). Irrigating solution (17% EDTA) was used for removing smear layers. The apical foramina were sealed with glass ionomer cement from external surface.
The teeth were sterilized in an autoclave at a temperature of 121°C for 15 minutes to remove all pre-existing bacteria. Samples were randomly divided into 3groups: control group (n=5) and two experimental groups, Group I (Ag-NPs) (n=20), Group II (Au-NPs) (n=20). Then each group will be subdivided into 2 subgroups according to laser irradiation, subgroup I (n=20): before laser irradiation and subgroup II (n=20): after laser irradiation, each subgroup will be divided in 4 subdivision according to time of irradiation, subdivision 1: 30 seconds (n=5), subdivision 2: 1 minute (n=5), subdivision 3: 2 minutes (n=5), subdivision 4: 3 minutes (n=5). The teeth in the control group were inoculated with bacterial suspension only. The teeth were covered by wet sterile cotton of saline to provide a moisturized environment during procedure then placed in sterile eppendorfs to be ready for procedure.

3. Preparation of media:
Dehydrated MRS media (De man Rogaso sharp agar media) was prepared according to manufacturer's instructions. Dehydrated media was mixed with distilled water and dissolved by gentle heat to boil. The media was sterilized in an autoclave at 121°C for 15 minutes. The sterile media was poured into sterilized petri-dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37°C for 24hrs [2,14].

4. Bacterial suspension preparation and bacterial inoculation:
The bacterial suspension was prepared from reference strain of L.acidophilus (DSM20079) bacteria, which prepared in MRS broth, adjusted to 0.5 McFarland units, and equivalent to 1.5×10^8 (CFU/mL)**.** Teeth were autoclaved in 1ml sterile saline then 50 µl of the bacterial suspension of L. acidophilus were added to control group. Fifty µl of bacterial suspension in MRS broth were applied to the mechanically enlarged root canals with a sterile micropipette with a sterile needle. Teeth were divided into 2 groups which both of them placed into MRS containing bacterial suspension. Fifty µl of (Ag-NPs) were added in the root canals of group I, and 50 µl of (Au-NPs) were added in the root canals of group II. The two groups were subjected to laser beams at 4 different times for 30 seconds,1 minute,2 minutes, and 3 minutes respectively and the bacterial count was detected before and after subjected to laser beam.

5. Bacterial count:
Enumeration of bacteria was carried out after making serial dilution up till 10^7 of the inoculums and from each diluted inoculation,1ml of the culture was plated onto surface of MRS agar plates and then incubated anaerobically at 37ºC for 24 hours. After incubation enumeration of bacteria was carried out from countable plates (30-300CFU/ml). Suspected colonies were confirmed to be L. acidophilus by Gram staining.

6. Laser irradiation:
The control group, group I ,and group II was irradiated using a diode laser at wavelength 970 nm & power 1.5 Watt which provided through a flexible 320-µm optic fiber with a straight handpiece in noncontact, continuous mode at circular movement at the orifice of root canals. Irradiation was done at different exposure times (30 second, 1 minute, 2 minutes, and 3 minutes).

7. Bacteriological evaluation of the treated root canal:

Colony forming units (CFU_S) will be used to detect bacterial count before and after nanoparticles addition as well as laser irradiation. After laser irradiation, put 50µl of suspension solution was placed on Petri dish, and then incubated anaerobically at incubator for 24hours at 37 ºC. The grown colonies in all groups were identified and counted [13]. Upon irradiation, the specimens were placed into sterile eppendorf tubes and 100µl of physiological saline solution were added. The extracted fluid was diluted in log 10 steps, and then 25µl of each dilution were applied to culture plates then incubated at 37°C for 24hours. The colonies were counted and the total number of bacteria (CFUS / ml) was assessed.

8. Results:

1. Comparison between the groups:

After different laser exposure times 30 seconds, 1 minute and after 2 minutes; there was no statistically significant difference between the different groups.

After 3 minutes; control group showed the statistically significantly highest mean L. acidophilus bacteria counts. There was no statistically significant difference between laser group and (Au-NPs) group; both showed lower mean of bacteria counts. There was also no statistically significant difference between (Ag-NPs) group and laser + (Au-NPs) group; both showed lower mean of bacteria counts. Laser + (Ag-NPs) group showed the statistically significantly lowest mean of bacteria count.

2. Comparison between different times within each treatment:

There was no statistically significant change in mean log10 of L. acidophilus counts with each treatment. Higher mean CFUs/ mL was recorded in laser irradiation+ (Ag-NPs) group.

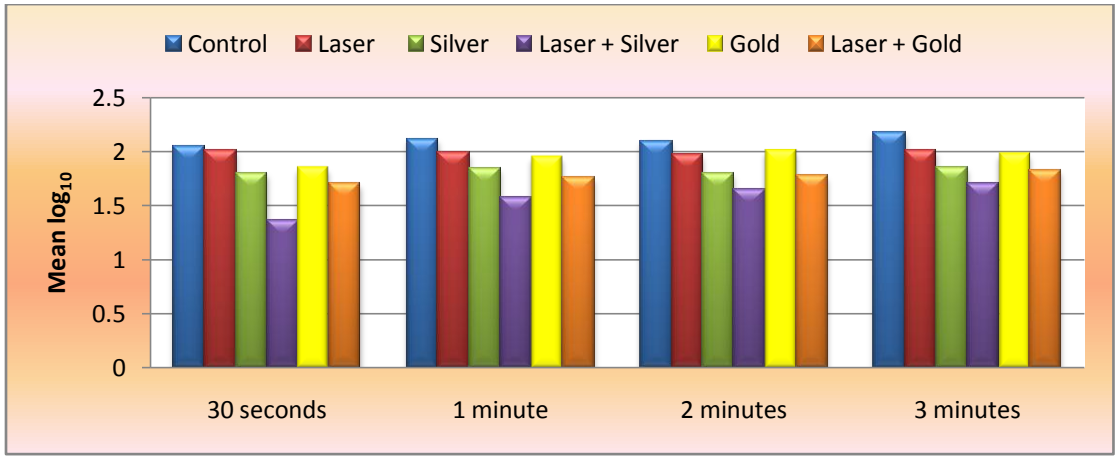

Figure (1): Bar chart representing comparison between mean L. acidophilus bacteria counts with different treatments.

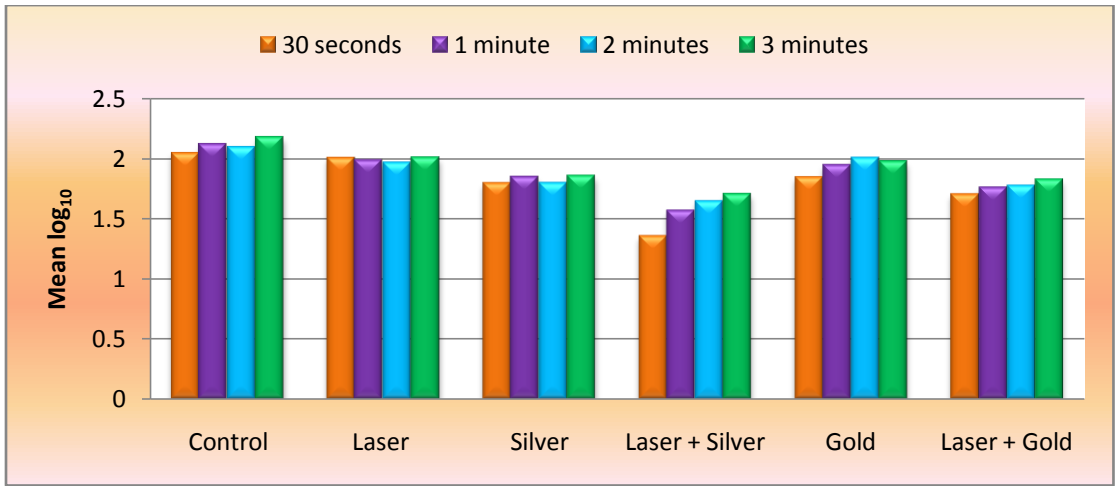

Figure (2): Bar chart representing comparison between mean log_{10} of L. acidophilus counts at different time periods

9. Discussion:

The microbiology examination of the samples had been collected before laser irradiation and after laser irradiation. Analysis of bacteria growth showed that the inhibitory effect of laser + (Ag-NPs) was higher than other groups. The result of the antibacterial test was presented in (fig.1, 2). The different times were active bactericidal agents, with no statistical significant chang when comparing between them.

Infections of the root canal system consist of microbial flora with approximately equal proportions of gram-positive and gram-negative bacteria [(21)] .Studies demonstrated that microorganisms are capable of invading the dentinal tubules to a depth of 1100 micro meter [(9,21)].

In recent years, different laser systems are used in endodontic field, which are effective for root canal disinfection. Initially Nd: YAG laser was first used for root canal disinfection, introduced by Ramskold et al [(18)]. The diode laser is a compact device and now used in different areas of dentistry, Moritz et al introduced use of diode laser for root canal disinfection [(11,12)].

E. fecalis is resistant to high temperatures, which found in cases of therapy resistant infection, so it was preferred to investigate the effect of the laser heat on it. The cell concentration at the time of initial inoculation before the laser treatment was high. After laser irradiation (CFUs) showed that the diode laser irradiation has high level of bactericidal effect than other group without laser irradiation [(19)], this was in agreement with Mortiz et al [(11)].

The high-power diode laser reduces dentine permeability, although it does not provoke the dentine melting unlike Nd: YAG laser [(5)]. The diode laser device is composed of two layers of semiconductor material interlaced with a non-conductive layer. Its light presents a spectrum that allows for greater absorption by water than dental tissues when compared with Nd: YAG laser. This characteristic means greater laser light penetration through the dentin with little interaction on the dentin, making it to act on the microorganisms present inside the dentinal tubules.

The fine diameters of optic fibers (200-320 μm) enable effective delivery of laser light to the root canal to help with reduction of bacterial contamination. The antibacterial effect observed reaches over 1 mm deep into the dentin.Diode laser effectiveness in relation to diverse microorganisms has been demonstrated by many authors [(8)].

Moritz et al, determined that irradiation with a diode laser in two subsequent sessions resulted in almost complete elimination of bacteria and suggested that the diode laser could be considered equal to the Nd: YAG laser in endodontic treatment, they also reported that Nd: YAG and diode lasers at 1 W are effective for E. fecalis; while the power increased to 1.5 W; only the diode laser was effective against the microorganisms [(11,12)]. In the present study, the diode laser was used in both 1.5 W and 3 W. Although complete sterilization cannot be achieved, a significant bacterial reduction was seen.

The parameters used in this study were considered safe in accordance with Radaelli et al [(19)] .However the choice of the essential precautions and the correct laser parameters is crucial for a safe and efficient way of therapy. The diode laser used can be applied by all means as a support in endodontic treatment, thus increasing the success rate through its antibacterial effect in endodontic treatment, which should be confirmed by performing clinical studies [(11)].

In our treatment of root canals with diode laser, results was reached that laser + (Ag-NPs) eliminate 90% of bacteria followed by (Ag-NPs) groups eliminate 80% then laser irradiation only or (Au-NPs) groups eliminate70% of bacteria.

10. Conclusion:

Diode laser + (Ag-NPs) were found that they present antibacterial activity against the bacterial more than other groups. Studies should be done in vivo to check where the radiation can cause cellular damage to periodontal ligament and surrounding structure.

11. Acknowledgment:

This paper was supported by the National Institute of laser and the National Research Center, Cairo, Egypt.

12. References:

1.Berutti ,E., Marini, R.,and Angeretti, A.(1997): Penetration ability of different irrigants into dentinal tubules. J. Endod., 23(12):725-7.
2.Collins, C. H. and Lyne, P.M. (1976): Microbiological Methods[4th] end. Butterworths, London U.K.

3.Gomes, B.P., Lilley, J.D., and Drucker, D.B. (1996): Clinical significance of dental root canal microflora. J. Dent., 24 (1-2):47-55.

4. Haapasalo, M. (1989):Bacteroides spp. in dental root canal infections .Endod. Dent. Traumatol., 5(1):1-10.

5. Hardee, M.W., Miserendino, L.J., Kos, W.,and Walia, H. (1994): Evaluation of the antibacterial effects of intracanal Nd: YAG laser irradiation, J. Endod., 20(8): 377-380.

6. Hardee, J.L., and Halas, N.J.(2000): Applications of nanotechnology to biotechnology commentary. Curr. Opin. Biotechnol.,11 (2):215–217.

7. Hancock, H.H., Sigurdsson, A., Trope M.,and Moiseiwitsch, J. (2001): Bacteria isolated after unsuccessful endodontic treatment in a North American population **.**Oral Surg. Oral Med. Oral. Pathol. Oral Radiol. Endod., 91(5):579-586.

8. Kouchi, Y., Ninomiya ,J., Yasuda, H., Fukui, K., Moriyama, T.,and Okamoto, H.(1980): Location of streptococcus mutans in the dental tubules of open infected root canals. J Dent Res.,59(12):2038-46.
9. Lan, W.H., Chew, K.W., Jeng, J.H., Lin, C.P., and Lin, S.K.(2000):A comparison of the morphological changes after Nd:YAG and Co_2 laser irradiation of dentin surfaces. J. Endod.,26(8):450-3.
10.Myers, T.D., and McDanial, J.D.(1991): The pulse Nd:YAG laser: Review of clinical application. J Calif Dent Assoc., 19(11):25-30.
11. Moritz A., Gutknecht N., Goharkhay K., Schoop U., Wernisch J., and Sperr W. (1997):In vitro irradiation of infected root canals with a diode laser: Results of microbiological examinations. Quintessence Int . 28(3):205-9.
12. Mortiz, A., Doertbudak, O., Gutknecht, N., Goharkhay, K., Schoop G.,and Sperr, U.(1997). Nd: YAG laser irradiation of infected root canals in combination with microbiological examinations. J Am Dent Assoc .,128 (11):1525-30.
13. Monika, C.M. and Froner, I.C.(2006): A scanning electron microscopic evaluation of different root canal irrigation regimens. Braz. Oral Res., 20 (3):235-240.
14. Oxoid Manual,(1982).The Oxoid Manual Culture Media and Other Laboratory Service 5th edition, Oxoid Limited ,Hampshier,R.G.240PW,England.
15. Pearson, G.J., and Schuekert, K.H.(2003):The role of lasers in dentistry: Present and future. Dent Update ., 30 (2):70-4.
16. Porteneier, I., Waltimo ,T.M.T.,and Haapasalo, M.(2003):Enterococcus faecalis– the root canal survivor and 'star' in post-treatment disease .Endodontic Topics., 6 (1):135-159.
17. Pitsillides, C.M., Joe E.K., Wei X., Anderson R.R.,and Lin CP.(2003): Selective cell targeting with light-absorbing microparticles and nanoparticles. Biophys. J., 84(6):4023–4032.
18. Ramskold, L.O., Fong C.D., and Stromberg, T.(1997):Thermal effects and antibacterial properties of energy levels required to sterilize stained root canals with an Nd:YAG laser. J. Endod., 23(2):96-100.
19. Radaelli, C.M., Zezell, D.M., Cai, S., Antunes, A.,and Gouw-Soares, S.C.(2003): Effect of a high power diode laser irradiation in root canals contaminated with Enterococcus faecalis. "In vitro study". Int Congr Scr., 1248:273-6.
20. Sundqvist, G. (1994): Taxonomy,ecology and pathogenicity of root canal flora. Oral Surg Oral Med Oral Pathol .,78 (4):522-530.
21. Souza,L.C., Brito,P.R.,de Oliveira,J.C, Alves, F.R, Moreira, E.J, and Sampaio-Filho, H.R. (2010):.Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of enterococcus faecalis. J. Endod .,3(26):292-6.