UTTAR PRADESH JOURNAL OF ZOOLOGY

42(12): 25-30, 2021
ISSN: 0256-971X (P)

SYNTHESIS, CHARACTERIZATION AND APPLICATION OF GREEN SYNTHESIZED Ag NPs IN ENDODONTIC THERAPY TO REDUCE MICROBIAL BIOFILM

SUDHA MATTIGATTI[1] AND KAILAS DATKHILE[2*]

[1]Department of Conservative and Endodontics, School of Dental Sciences, Krishna Institute of Medical Sciences "Deemed to be University", Karad, India.

[2]Department of Molecular Biology and Genetics, Krishna Institute of Medical Sciences "Deemed to be University", Karad, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Authors SM and KD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KD managed the analyses of the study whereas author SM managed the literature searches. Both authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Faranak Behnaz, Shahid Beheshti University of Medical Sciences, Iran.
(2) Raji Sundararajan, Purdue University, USA.

Received: 20 March 2021
Accepted: 27 May 2021
Published: 01 June 2021

Original Research Article

ABSTRACT

Introduction: Green synthesis of Silver Nanoparticles (Ag NPs) by aqueous extract of *Nophapodytes foetida* plant offers biocompatible and cost-efficient substitute for costly and non-biocompatible chemical reducing agents.

Methods: The crude aqueous extract of *Nophapodytes foetida* plant bark was used with silver nitrate in the volume ratio of 1:2 for synthesis of Ag NPs. The mixture was incubated at 40 °C for 1 hr results into synthesis of Ag NPs, which was further confirmed by characterization. The characterization of Ag NPs was carried out by UV-Vis Spectroscopy, Scanning Tunneling Microscopy (STM) and Scanning Electron Microscopy (SEM) to study optical and morphological characteristics, respectively. Studies were also performed for determination antibacterial activity of Ag NPs against inoculum of *Enterococcus faecalis* (ATCC: 29212) containing 1×10^5 CFU/ mL by anti-well diffusion assay and broth dilution method.

Results: The absorption spectrum of Ag NPs and band gap was found to be at 355 nm and 3.49 eV respectively. Ag NPs showed cluster morphology on surface at 47.2 nm under STM and rough nuggets like morphology of Ag NPs with average size range of 25 to 55 nm under SEM.

Conclusion: Ag NPs was found to be effective antibacterial agent at the concentration of 1000 µg/mL against *Enterococcus faecalis* predominantly present in the root canal and therefore the Ag NPs based intracanal medicaments may have applications in endodontics.

**Corresponding author: Email: drkailasdatkhile@outlook.com;*

Keywords: Green synthesis; Ag NPs; intracanal medicaments; *Enterococcus faecalis*; *Nophapodytes foetida.*

1. INTRODUCTION

The accomplishment of endodontic treatment relies upon legitimate instrumentation, powerful water system and explicit intra-channel prescription and 3-Dimensional obturation [1]. Intracanal medication is a crucial step in determining the peri-apical healing in severely affected cases [2]. Central goal of an endodontic treatment is to reduce bacterial invasion, which subsequently promotes normal healing process of the periapical tissues [3]. The most resistant microorganism, which is often encountered in failure of root canal in primary and secondary endodontic infections is *E.faecalis* which is a normal inhabitant of oral flora [4]. The bacterial cells can penetrate into dentinal tubules and forms the biofilm which is very difficult to eleminate either by chemomechanical methods or by irrigation [5]. It resists intracanal medicaments by maintaining pH homeostasis.

The endodontic sickness is biofilm-intervened contamination and consequently, end or critical decrease of bacterial biofilm is a fundamental component for fruitful endodontic therapy. Nonetheless, clinical investigations have shown that even after fastidious chemo-mechanical sanitization, bacterial biofilm may, in any case, persevere in the root canal system [6].

Conventional strategies for water system and medicaments are not successful against *E. Faecalis*. Sodium hypochlorite is the most normally utilized intracanal drug during root trench techniques. The antimicrobial movement of Sodium hypochlorite can be inactivated by dentin, exudate from the periapical territory, and microbial biomass. Likewise, it doesn't generally wipe out *E. faecalis* biofilms from the root waterway framework. Hence, it is compulsory to create compelling elective medicaments.

As of late, nanoparticles have been utilized in numerous medical services applications as a result of having an expansive range of antimicrobial movement. Nanoparticles show different antibacterial components, for example, adherence and infiltration into the bacterial cell divider, prompting the deficiency of honesty of bacterial cell member and cell wall porousness [7].

The silver nanoparticles have broad-spectrum antibacterial potential and can effectively inhibit the bacterial biofilm [8]. Silver nanoparticles (AgNPs) found to have antibacterial viability by harming the cell layers, invigorating the arrival of ROS (responsive oxygen species), shaping free revolutionaries with an amazing bactericidal activity, and destabilizing the outer membrane composition [9,10]. AgNP arrangement has been recommended as an option in contrast to endodontic treatment for its bactericidal potential as well as for its biocompatibility, especially in lower focuses. Considering this the point of the current investigation was to assess the antibacterial and antibiofilm movement of Ag NPs in contrast with sodium hypochlorite against *E. faecalis*.

2. MATERIALS AND METHODS

2.1 Synthesis and Characterization of Silvernano Materials

1mM silver nitrate was used to synthesize the Ag NPs. The crude aqueous extract of *Nophapodytes foetida* plant bark was used as a reducing agent for reduction of silver nitrate in the volume ratio of 1:2. The one-part extract was added into the silver nitrate solution and incubated at 40°C for 1 hr. The resultant precipitate was centrifuged at 5000 rpm for 15 minutes followed by washing and drying the pallet at 60°C for 24 hrs. The optical, structural and morphological features of resultant Ag NPs were characterized by UV-Vis spectroscopy, STM and SEM for identifying their optical and morphological information.

2.2 Qualitative Estimation of Antibacterial Activity of NPs

The antibacterial action of AgNPs was tried by agar well dispersion examine (AWDA) [11], on Enterococcus faecalis (ATCC: 29212) found in the root channels. The supplement agar plates were spread immunized with for the time being developed bacterial culture. The blended AgNPs were scattered in sterile DI water to make a colloidal arrangement of nanomaterials. On the outside of agar plates, wells of 8 mm in distance across and of 25 µL in limit were shaped by utilizing a sterile gel drill. The 20 µL of NPs suspension was set in each well and brood all plates at 37 °C for 24 hours.

2.3 Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC & MBC)

MIC and MBC are the most reduced centralization of AgNPs repress development > 3 logs of bacterial cells. The bactericidal exercises of Ag NPs were performed by plate tally technique on supplement

agar plates (in the fixation scope of 125 µg/mL - 1000 µg/mL). The test culture of conclusive cell thickness of 1⍰105 CFU/mL was utilized for spread immunization. The plates were hatched at 370 C for 24 hours and ensuing development hindrance of bacterial culture was resolved.

2.4 Bacterial Inoculation of Specimens

E. faecalis (ATCC 29212) was vaccinated on a supplement agar plate and hatched at 37°C for 24 hours. A solitary settlement of *E. faecalis* was suspended in clean supplement stock at 37°C for 24 hours. Cleaned dentin examples were set in sterile cylinders containing 3 mL of *E. faecalis* culture of conclusive cell thickness of 1⍰105 CFU/mL in supplement stock. The examples were brooded at 37 °C for about fourteen days. The supplement stock was supplanted each week to eliminate dead cells and to guarantee bacterial reasonability. Followed by brooding, the examples delicately flushed with sterile phosphate-supported saline (PBS) in aseptic conditions to eliminate the way of life medium and non-disciple microscopic organisms. Four dentin segments haphazardly chose were seen by a confocal laser examining microscopy (CLSM) to confirm the presence of biofilms on the dentin surfaces.

3. RESULTS AND DISCUSSION

3.1 Characterization of AgNPs

The characterization of synthesized powder was carried out to scrutinize the optical and morphological properties of Ag NPs. UV-Vis range of orchestrated nanomaterials was acquired by UV-Vis spectroscopy (Thermo Scientific UV-10) and its optical band hole was determined [12]. The surface morphology of the powder was observed by Scanning Tunneling Microscopy (STM) [13]. For understanding morphology size and shape of the material Scanning Electron Microscopy (SEM) was used [14]. UV-visible spectroscopy has been extensively used to understand optical properties of materials. It was evident that the maximum absorption (λ_{max}) of Ag NPs found to be at 355 nm and band gap calculated was 3.49 eV (Fig. 1).

The STM picture of AgNPs shows the packed surface morphology at 47.2 nm distance (Fig. 2). The line graph of Ag NPs indicates the sample mounting and tip position was properly achieved and had good tunneling contact.

SEM of Ag NPs clearly shows the rough nuggets like morphology of Ag NPs with average size range of 25 to 55 nm (Fig. 3).

3.2 Qualitative Estimation of Antibacterial Activity of Ag NPs

The antimicrobial movement of Ag NPs suspension of 1000 µg/mL focus was tried on *E. faecalis* and found to have antibacterial action (Fig. 4). In present study, Ag NPs showed a significant zone of inhibition against *E. faecalis* due to the proper diffusion of nanoparticles in the surrounding medium. The significant boundary of AgNPs antibacterial movement against bacterial cell is the surface territory which can reasonably deliver Ag+ all through microscopic organisms [15]. The arrival of Ag+ particles from Ag NPs is answerable for the antibacterial action [16,17].

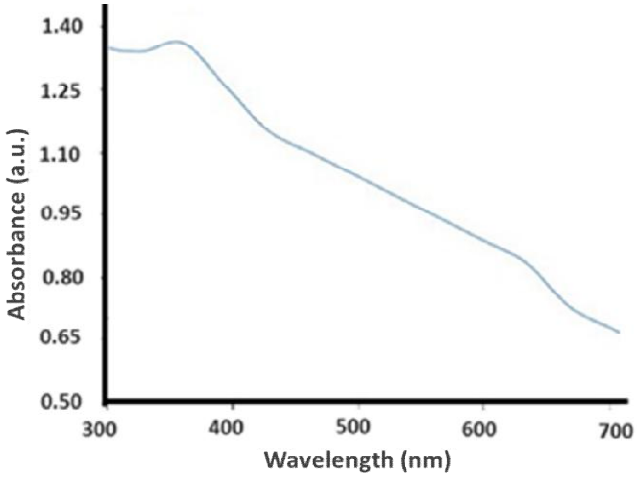

Fig. 1. UV-Vis spectroscopy of Ag NPs

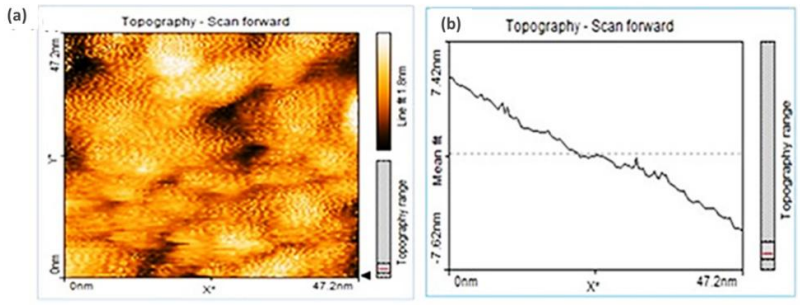

Fig. 2. STM Image of Ag NPs indicates the bunched surface morphology

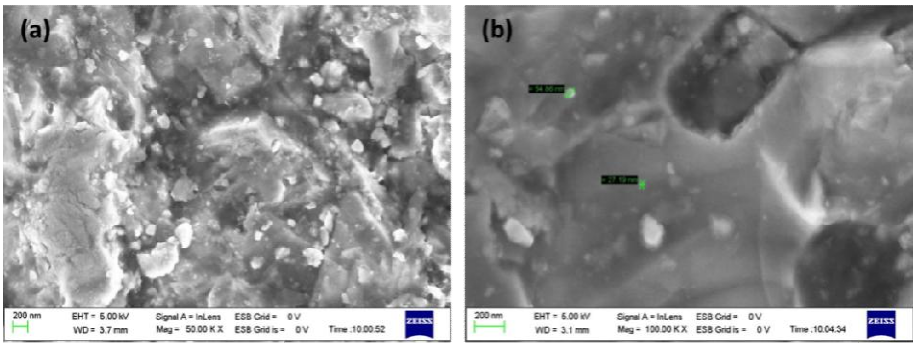

Fig. 3. SEM images of Ag NPs at (a) low and (b) high magnification shows nuggets shaped nanoparticles

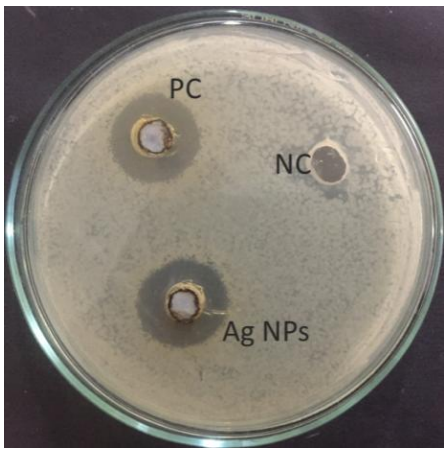

Fig. 4. Antagonistic activity ofAg NPs against *Enterococcus faecalis*

(Note: PC- Positive control (2% sodium hypochlorite), NC- negative control and Ag NPs- silver nanoparticles)

Table 1. Quantitative determination of antibacterial activity of Ag NPs against *E. faecalis*

Conc. of NPs	*Enterococcus faecalis* (CFU)
Culture Control	$2.21X10^4$
125 µg/mL	$1.1\ X10^3$
250 µg/mL	Nil
500 µg/mL	Nil
1000 µg/mL	Nil

3.3 Determination of MIC & MBC

The MIC and MBC of Ag NPs were determined by treating *Enterococcus faecalis* with different concentration of nanomaterial suspension in the range of 125 µg - 1000 µg/mL (Table 1).

The MIC and MBC of Ag NPs was discovered to be 125 µg/mL and 250 µg/mL, individually for

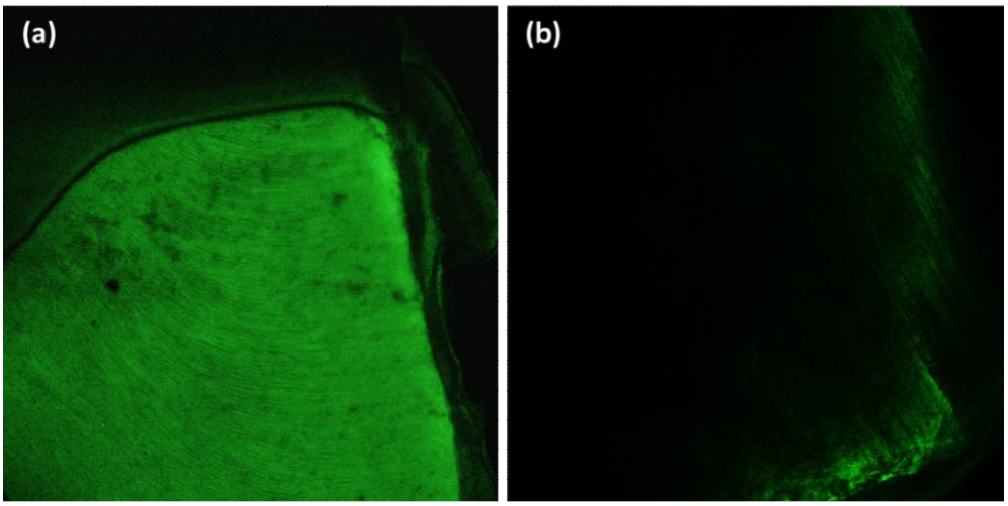

Fig. 5. CLSM images of *E. faecalis* biofilms before and after exposure of Ag NPs (a) Control and (b) Ag NP solution (green fluorescence is indicating biofilm)

E. faecalis. As per the reports the antibacterial action of AgNPs is because of the expanded creation of Reactive Oxygen Species (ROS) and free extremist of H2O2, O^-_2 , - OH, HOCl and[O] [15,18,19], which prompts lipid peroxidation, consumption of glutathione (GSH) and DNA harm [20,21]. The aftereffects of this investigation might be relevant to define intracanal medicament with AgNPs or into the irrigant arrangements and intracanal medicaments for oral wellbeing.

3.4 CLSM of Biofilm Reduction

The heavy biofilm grown by *E. faecalis* on dentin surface was detected in control group (Fig. 5(a)). There was significant biofilm reduction was observed in specimen treated with Ag NPs suspension (Fig. 5(b)). Mallya L, Jathanna V (2019), reported the parallel method for evaluation of endodontic microflora [22]. Ambulkar S. et al, (2019) used crystal violet staining method for evaluation of biofilm inhibition [23]. The nano silver-graphene oxide system has been reported to use to inhibit biofilm on tooth surfaces [24].

4. CONCLUSION

The Ag NPs orchestrated by green amalgamation strategy was discovered to be powerful antibacterial specialist against *E. faecalis*. The MIC and MBC of Ag NPs was discovered to be 125 µg/mL and 250 µg/mL separately for *E. faecalis*. In this way, Ag NPs can be utilized in irrigant answers for better forecast of root trench treatment against *E. faecalis*.

ACKNOWLEDGEMENT

We acknowledge the continuous support of University and our Departments in carrying the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pasricha SK, Makkar S, Gupta P. Pressure alteration techniques in endodontics-a review of literature. Journal of Clinical and Diagnostic Research: JCDR. 2015;9(3):ZE01.
2. Madarati AA, Zafar MS, Sammani AM, Mandorah AO, Bani-Younes HA. Preference and usage of intracanal medications during endodontic treatment. Saudi Medical Journal. 2017;38(7):755.
3. Kumar A, Tamanna S, Iftekhar H. Intracanal medicaments–their use in modern endodontics: a narrative review. Journal of Oral Research and Review. 2019;11(2):94.
4. Zargar N, Marashi MA, Ashraf H, Hakopian, R, Beigi P. Identification of microorganisms in persistent/secondary endodontic infections with respect to clinical and radiographic findings: bacterial culture and molecular detection. Iranian Journal of Microbiology. 2019;11(2): 120.
5. Neelakantan P, Romero M, Vera J, Daood U, Khan AU, Yan A, Cheung GSP. Biofilms in endodontics—current status and future

directions. International Journal of Molecular Sciences. 2017;18(8):1748.
6. Siqueira JF, Rôças IN, Ricucci D. Biofilms in endodontic infection. Endodontic Topics. 2010; 22(1):33-49.
7. Slavin YN, Asnis J, Häfeli UO, Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology. 2017;15(1):1-20.
8. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20(5):8856-8874.
9. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. Journal of Endodontics. 2014;40(2): 285-290.
10. Mirzajani F, Ghassempour A, Aliahmadi A, Esmaeili MA. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Research in Microbiology. 2011;162(5):542-549.
11. Qureshi N, Lee S, Chaudhari R, Mane P, Pawar J, Chaudhari B, Shinde M, Rane S, Kim T, Amalnerkar D. Hydrothermal Generation of 3-Dimensional WO Nanocubes, Nanobars and Nanobricks, Their Antimicrobial and Anticancer Properties. J Nanosci Nanotechnol. 2021;21(10):5337-5343.
12. Shirsat S, Pawar D, Jain N, Pawar J, Tale VS, Henry R. Synthesis of copper oxide nanoparticles by chemical precipitation method for the determination of antibacterial efficacy against *Streptococcus* Sp. and *Staphylococcus* Sp. Synthesis. 2019;12(5).
13. Jain N, Bhosale P, Tale V, Henry R, Pawar J. Hydrothermal assisted biological synthesis of silver nanoparticles by using honey and gomutra (Cow Urine) for qualitative determination of its antibacterial efficacy against Pseudomonas sp. isolated from contact lenses. Eur Asian Journal of Bio Sciences. 2019;13(1):27-33.
14. Viswanathan P, Patel AK, Pawar J, Patwardhan A, Henry R. Fabrication of tin oxide nanoparticles for CO2 gas sensing layer. IETE Journal of Research. 2020;66(4):460-465.
15. Qing Y, Cheng L, Li R, Liu G, Zhang Y, Tang X, Wang J, Liu H, Qin Y. Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. International Journal of Nanomedicine. 2018; 13:3311–3327.
16. Kim T, Braun GB, She ZG, Hussain S, Ruoslahti E, Sailor MJ. Composite Porous silicon–silver nanoparticles as theranostic antibacterial agents. ACS Applied Materials & Interfaces. 2016;8(44):30449-30457.
17. Jin JC, Wu XJ, Xu J, Wang BB, Jiang FL, Liu Y. Ultrasmall silver nanoclusters: Highly efficient antibacterial activity and their mechanisms. Biomaterials Science. 2017;5(2): 247-257.
18. Zhao R, Lv M, Li Y, Sun M, Kong W, Wang L, Hao R. Stable nanocomposite based on PEGylated and silver nanoparticles loaded graphene oxide for long-term antibacterial activity. ACS Applied Materials & Interfaces. 2017;9(18):15328-15341.
19. Siritongsuk P, Hongsing N, Thammawithan S, Daduang S, Klaynongsruang S, Tuanyok A, Patramanon R. Two-phase bactericidal mechanism of silver nanoparticles against Burkholderiapseudomallei. PloS One. 2016; 11(12):e0168098.
20. Korshed P, Li L, Liu Z, Wang T. The molecular mechanisms of the antibacterial effect of picosecond laser generated silver nanoparticles and their toxicity to human cells. PLoS One. 2016;11(8):e0160078.
21. Lee W, Kim KJ, Lee DG. A novel mechanism for the antibacterial effect of silver nanoparticles on *Escherichia coli.* Biometals. 2014;27(6):1191-1201.
22. Mallya L, Jathanna V. Confocal LASER Scanning Microscopy (CLSM) for evaluation of endodontic microflora-a review. Indian Journal of Public Health Research & Development. 2019 Jan 1;10(1).
23. Ambulkar S, Tale V, Khilari S, Pawar J. Antibacterial and antibiofilm activity of *Quercus infectoria* Galls on *Rothia dentocariosa* isolated from Dental Caries. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(10).
24. Ioannidis K, Niazi S, Mylonas P, Mannocci F, Deb S. The synthesis of nano silver-graphene oxide system and its efficacy against endodontic biofilms using a novel tooth model. Dental Materials. 2019;35(11):1614-29.