Cytocompatibility and Antimicrobial Efficacy of Pluronic F-127 Coated Colloidal Silver Nanoparticles as an Endodontic Irrigant

MS Rama Rao, Sai Harshini Pindiprolu, Mahendra Varma Nadimpalli, Sudhakar Naidu, Gowtam Dev Dondapati, TBVG Raju

Department of Conservative Dentistry and Endodontics, Sree Sai Dental College and Research Institute, Srikakulam, Andhra Pradesh, India

ABSTRACT

Aim and objective: The aim and objective of present study are to prepare and characterize Pluronic F-127 coated colloidal silver nanoparticles and to evaluate its cytocompatibility and anti-microbial efficacy in comparison to 5.25% NaOCl. **Materials and Methodology:** The cytocompatibility was evaluated on L929 human fibroblast cell line by MTT assay at five different concentrations of Pluronic F-127 coated colloidal silver nanoparticles [0.01µg/mL; 0.1µg/mL; 1µg/mL; 10µg/mL; 100µg/mL] and antimicrobial activity was evaluated by Agar well diffusion method on Enterococcus faecalis (ATCC 29212) using 100µL Pluronic F-127 coated colloidal silver nanoparticles [as test solution], 100µL 5.25%NaOCl [as standard solution], 100µL water [as control]. **Statistical Analysis:** Statistical analysis was performed using one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (Graph Pad Prism, Version 6, Graph Pad Software Inc., La Jolla, USA). **Results:** The values with $p < 0.05$ were considered significant. MTT assay showed Pluronic F-127 coated colloidal silver nanoparticles are more cytocompatible on healthy fibroblast cells. In antimicrobial activity there is more significant difference among groups. **Conclusion:** Pluronic F-127 coated colloidal silver nanoparticles showed more zone of inhibition compared to NaOCl. Pluronic F-127 coated colloidal silver nanoparticles proved to be more cytocompatible and has highest antimicrobial activity compared to NaOCl.

KEYWORDS: *Anti-microbial activity, cytocompatibility, NaOCl, Pluronic F-127 coated colloidal silver nanoparticles*

Received: 07-September-2019.
Revised: 17-September-2019.
Accepted: 27-September-2019.
Published: 23-December-2019.

INTRODUCTION

Endodontic reinfection or failures are major concerns in endodontics. A compelling body of evidence suggests that the colonization of various kinds of bacteria in biofilm, formation of smear layer during instrumentation, and complex anatomy of the root canal system are its main causes.[1] The final irrigation is necessary to improve and complement the mechanical debridement procedure.[2]

Sodium hypochlorite (NaOCl), chlorhexidine, MTAD, ethylenediaminetetraacetic acid, phenol derivatives, etc., are the irrigating solutions used till date.[3] However, there are limitations for each of these irrigants. NaOCl is the most commonly used irrigant due to its antimicrobial action and tissue dissolving ability. However, NaOCl is associated with cytotoxicity of periapical tissues. It acts as a proteolytic agent on collagen matrix and decreases the elastic modulus and flexural strength of dentin.[4,5] Therefore, there is a need for novel endodontic irrigant to overcome the limitations associated with conventional irrigating solutions.

Access this article online

Quick Response Code:

Website: www.jicdro.org

DOI: 10.4103/jicdro.jicdro_27_19

Address for correspondence: Dr. Sai Harshini Pindiprolu, Department of Conservative Dentistry and Endodontics, Sree Sai Dental College and Research Institute, Chapuram, Srikakulam - 532 001, Andhra Pradesh, India.
E-mail: drharshini93@gmail.com

How to cite this article: Rao MS, Pindiprolu SH, Nadimpalli MV, Naidu S, Dondapati GD, Raju TB. Cytocompatibility and antimicrobial efficacy of pluronic F-127 coated colloidal silver nanoparticles as an endodontic irrigant. J Int Clin Dent Res Organ 2019;11:96-100.

In recent years, silver nanoparticles (AgNPs) gained much attention due to their antimicrobial properties and biocompatibility.[6,7].

AgNPs act on microbial cells by inactivating essential microbial enzymes, increasing the permeability of the membrane, and damaging the cytoplasm. The Ag+ ions released from AgNPs catalyze the production of oxygen radicals, inhibiting multiplication of the microorganisms.[8] In addition, the smaller particle size increases the area of contact with the microbial membrane, providing a greater bactericidal effect.

Successful synthesis of AgNPs is, however, challenging due to physicochemical changes in the biological media in which these AgNPs are dispersed. Thus, polymers or proteins are used to encapsulate these nanoparticles to stabilize them and to minimize their toxicity.

Such a polymer is Pluronic F 127, a synthetic triblock copolymer, consisting of poly (ethylene oxide) (PEO)–poly (propylene oxide) (PPO)–PEO chains having amphiphilic characteristics (PPO being lipophilic, while PEO is hydrophilic).[9]

In this study, Pluronic F-127 coated colloidal silver nanoparticles are prepared, characterized, and cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles at five different concentrations (0.01, 0.1, 1, 10, 100 μg/mL) is measured. Five different concentrations of Pluronic F-127 coated colloidal silver nanoparticles are included in the study to evaluate their effectiveness and to measure concentration-dependent effect.

The main aim of the study is to evaluate the antimicrobial efficacy and cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles as endodontic final irrigating agent.

Materials and Methods

Preparation of Pluronic F-127 coated colloidal silver nanoparticles

10 mg Pluronic F127 added to 10 mL ultrapure water dissolved with continuous stirring at 60°C for 12 h. The resultant solution was incubated at 60°C for 24 h, then 100 μL of NaOH (0.1 M) was added to Pluronic F-127 to form reducing environment. After 20 min, AgNO3 8 millimoles were added to the above solution with stirring slowly.

Characterization of Pluronic F-127 coated colloidal silver nanoparticles

The formation of PAgNPs was confirmed by UV. The particle size of PAgNPs was measured by dynamic light scattering Nanotrac Wave™ (Microtrac, San Diego, CA, USA). Before measurement, the samples were suspended in deionized water. All measurements were carried out at 25°C and performed in triplicate. The morphology was analyzed using transmission electron microscope (TEM).[10]

Cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles

Cell and culture conditions

L929 fibroblast cell line (ATCC) was maintained as a continuous cultured in Dulbecco's Modified Eagle's medium (DMEM; Sigma-Aldrich, Inc.), supplemented with 10% fetal bovine serum (FBS; Himedia, Mumbai, Maharashtra, India), 100 μg/mL penicillin, and 100 μg/mL streptomycin. The cells were grown at 37°C in humidified atmosphere of 5% CO_2.

Before the experiment, the cells were detached by trypsinization at 37°C. The supernatant was centrifuged (1000 rpm for 10 min), the pellet was resuspended in DMEM, and the cells were counted in a hemocytometer.

Cytotoxicity assessment

The cells were seeded in 96-well plates at a density of 10,000 viable cells per well and incubated for 24 h to allow cell attachment. Then, cells were treated with Pluronic F-127 coated colloidal silver nanoparticles at five different concentration (0.01, 0.1, 1, 10, 100 μg/mL). After 24 h of treatment and incubation, fresh DMEM containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylt etrazolium bromide (MTT) (250 μg/mL) was added to replace the formulations, and again cells were incubated for additional 3 h. MTT was aspirated, and dimethyl sulfoxide was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader (Infinite® 200 Pro-Tecan, Switzerland). Untreated cells were taken as control with 100% viability.

Antimicrobial efficacy

Enterococcus faecalis was used for the evaluation of the antimicrobial activity of Pluronic F-127 coated colloidal silver nanoparticles. Freshly prepared and cooled (45°C–50°C) molten agar medium of about 25 ml was inoculated with respective standardized inoculum (0.5–1.0 ml) aseptically in laminar airflow unit. The medium was then transferred aseptically into sterilized Petri plate to occupy a depth of about 4 mm. The plates were then left at room temperature to allow solidification. Under aseptic conditions, wells of 5 mm diameter were made in each plate using a sterile borer. Accurately, 100 μL of test and standard solutions and vehicles were transferred to cups aseptically and labeled accordingly. The plates were left undisturbed for 1 h to allow preincubation diffusion of the solution into the medium to minimize the effects of time variation between

applications of a different solution. After incubation of the plates at 37°C ± 10°C for 24 h, the diameters of the zones of complete inhibition surrounding each of the wells were measured with a millimeter scale.

Statistical analysis

Statistical significance was determined by one-way analysis of variance, followed by Dunnett's multiple comparison test (Graph Pad Prism, Version 6, Graph Pad Software Inc., La Jolla, USA). The values with $P < 0.05$ were considered statistically significant.

Results

Preparation and characterization of Pluronic F-127 coated colloidal silver nanoparticles

The surface modification with Pluronic F-127 confers PAgNPs, its stability, biocompatibility by reducing toxicity and immune response.

The prepared Pluronic F-127 coated colloidal silver nanoparticles were found to be spherical, as observed by TEM. The particle size was found to be 20 nm with narrow size distribution [Figure 1].

Cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles

The cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles with L929 (fibroblast) cells was assessed by MTT assay. Untreated cells with 100% cell viability were taken as control. Pluronic F-127 coated colloidal silver nanoparticles at five different concentrations have high cell viability, and there is no significant ($P > 0.05$) difference observed when compared to control. These results ensure the Pluronic F-127 coated colloidal silver nanoparticles for *in vivo* applications [Figure 2].

Antimicrobial efficacy

The antimicrobial efficacy of Pluronic F-127 coated colloidal silver nanoparticles against *E. faecalis* was evaluated by agar well diffusion method, and zone of inhibition (ZOI) was measured [Figure 3]. From this method, it was evident that Pluronic F-127 coated colloidal silver nanoparticles (22.03 ± 2.06) and NaOCl (11.10 ± 2.06) have significantly greater ZOI compared to control. However, ZOI when treated with PAgNPs was significantly higher than NaOCl.

Discussion

An objective of endodontic treatment is the removal of diseased tissue, elimination of bacteria from the canal system, and prevention of recontamination.[11] Irrigation is key in reducing the number of bacteria within the root canal, it reduces friction between the instrument and dentine, improves the cutting effectiveness of the files, dissolves tissue, it has a washing effect and an antimicrobial effect.[12]

Numerous solutions have been recommended for use as root canal irrigants. NaOCl is the most commonly used

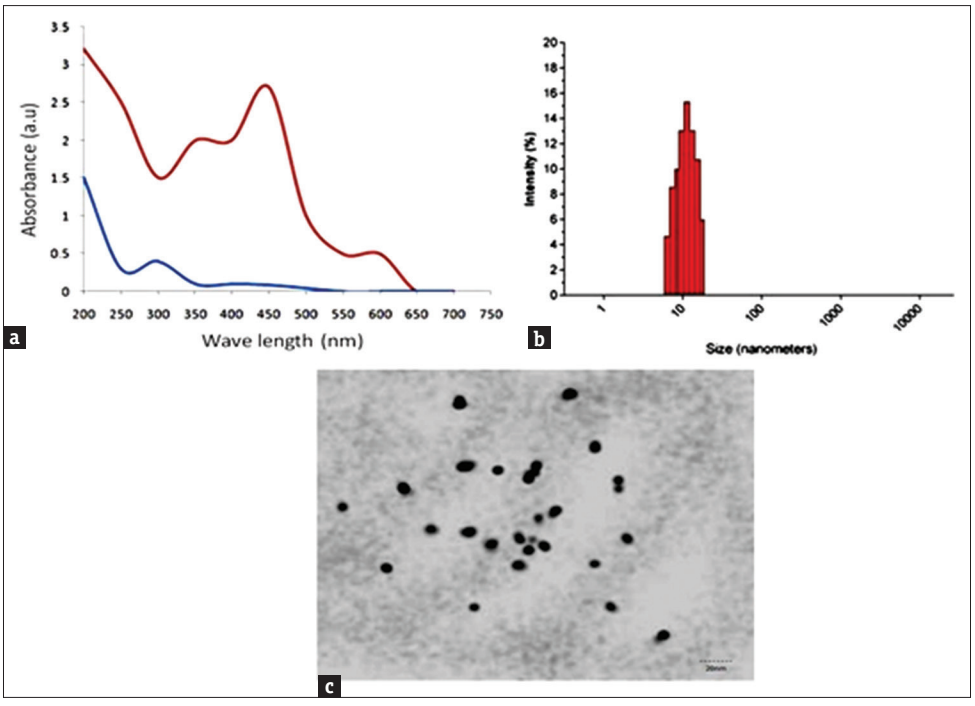

Figure 1: (a) UV-absorbance of Pluronic F-127 coated colloidal silver nanoparticles (650 nm). (b) Particle size distribution of Pluronic F-127 coated colloidal silver nanoparticles using zetasizer. (c) TEM image of Pluronic F-127 coated colloidal silver nanoparticles (×30,000)

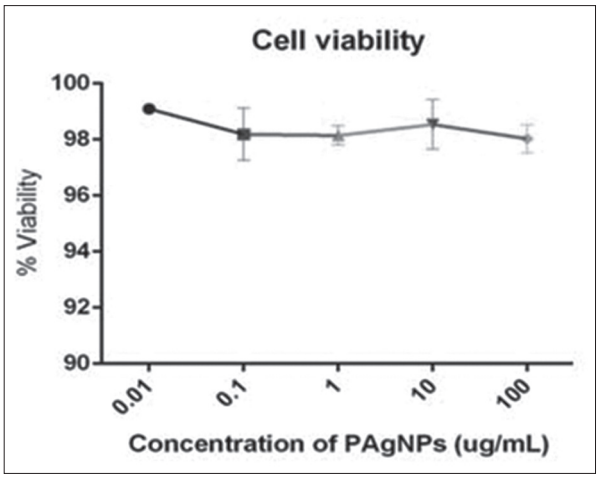

Figure 2: concentration of Pluronic F-127 coated colloidal silver nanoparticles

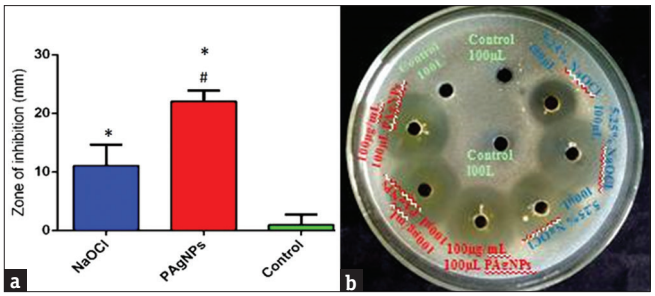

Figure 3: zone of Inhibition of 5.25% NaOCl, Pluronic F-127 coated colloidal silver nanoparticles and control. (a) bar diagram showing zone of inhibition 5.25% NaOCl(*), Pluronic F-127 coated colloidal silver nanoparticles (#) and control. (b) agar well diffusion method showing zone of inhibition 100 µL 5.25% NaOCl, 100 µL of 100 µg/mL Pluronic F-127 coated colloidal silver nanoparticles and 100 µL control

Table 1: Concentrations of Pluronic F-127 coated colloidal silver nanoparticles (PAgNPs)

Concentration of PAgNPs(µg/mL)	Mean	Standard error mean(SEM)
0.01	99.098	0.03083
0.1	98.1833	0.9295
1	98.1476	0.3409
10	98.5412	0.8872
100	98.0306	0.4990

irrigant. NaOCl acts as a lubricant for instrumentation and can flush loose debris from root canals.[13] NaOC1 is an effective antimicrobial agent with the capability of detoxifying the root canal system. In addition, NaOCl is effective in dissolving both vital and nonvital tissue.[14,15]

NaOCl usage is associated with cytotoxicity of periapical tissues. It acts as proteolytic agent on collagen matrix and decreases the elastic modulus and flexural strength of dentin.[4,5] Therefore, there is a need for novel endodontic irrigant to overcome the limitations associated with conventional irrigating solutions.[5,16]

The AgNPs solution has been recommended as a root canal irrigant solution because of its bactericidal potential, and biocompatibility. An AgNPs-based irrigant solution has been evaluated against *E*. faecalis.[17]

Some polymers are active agents, having antibacterial properties, others allow certain groups to attach to them, and hence, they can be used for specific targeting. Therefore, combining polymers and nanoparticles is very promising since the polymer can add new properties and enhance the effect of nanoparticles.[18] Such a polymer is Pluronic F-127, a synthetic triblock copolymer, consisting of PEO–PPO–PEO chains having amphiphilic characteristics (PPO being lipophilic, while PEO is hydrophilic) and can self-assemble into micelles to form a variety of close-packed structures. The micellization process is influenced by the concentration of Pluronic and the temperature; making it suitable for medical applications such as drug delivery, gene therapy, or tissue engineering to their surface and forming a thin protective layer by the hydrophobic association of the PPO blocks. In addition, the capping Pluronic layer can be further exploited to integrate multiple functionalities by encapsulating small molecules, such as drugs, fluorescent labels, or Raman reporters.[19,20] Pluronic F-127 can also be used in the steric stabilization of nanoparticles, by binding. Considering the interesting optical and biological properties of Pluronic F-127, we focused on the preparation of Pluronic F 127 coated AgNPs (PAgNPs) and investigation of their cytocompatibility and antimicrobial activity.

The cytocompatibility of Pluronic F-127 coated colloidal silver nanoparticles with L929 (fibroblast) cells was assessed by MTT assay. This is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria, where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g., isopropanol), and the released, solubilized formazan reagent is measured spectrophotometrically. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.[21] In this study [Table 1], untreated cells with 100% cell viability were taken as control. Pluronic F-127 coated colloidal silver nanoparticles at 0.01 µg/mL has 99% cell viability; at 0.1 µg/mL has 98% cell viability; 1 µg/mL has 98% cell viability; 10 µg/mL has 98% cell viability; 100 µg/mL has 98% cell viability; Pluronic F-127 coated colloidal silver nanoparticles at five different concentrations, have high cell viability and there is no significant ($P > 0.05$) difference observed when compared to control and among different concentrations.

The presence of *E. faecalis* was found to be the major (4%–40%) cause of endodontic infection. In addition, the presence of this strain is nine times higher in failed root canal treatment cases. Hence, *E. faecalis* was chosen in this study. The antimicrobial efficacy of Pluronic F-127 coated colloidal silver nanoparticles against E. *faecalis* was evaluated by agar well diffusion method, and ZOI was measured. From this method, it was evident that PAgNPs (22.03 ± 2.06) and NaOCl (11.10 ± 2.06) have significantly greater ZOI compared to control. However, ZOI when treated with Pluronic F-127 coated colloidal silver nanoparticles was significantly higher than NaOCl; the effectiveness of Pluronic F-127 coated colloidal silver nanoparticles on inactivation of microorganisms was, therefore, higher than NaOCl.

Conclusion

Pluronic F-127 coated silver nanoparticles at five different concentrations (0.01, 0.1, 1, 10, 100 µg/mL) proved to be cytocompatible. 100 µg/mL of Pluronic F-127 coated silver nanoparticle has the highest antimicrobial activity compared to 5.25% NaOCl. Pluronic F-127 coated silver nanoparticles, therefore, exhibit the highest potential for use as a irrigant in endodontics. However, further studies are required to test its applicability as endodontic irrigant.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lin LM, Skribner JE, Gaengler P. Factors associated with endodontic treatment failures. J Endod 1992;18:625-7.
2. Del Carpio-Perochena A, Bramante CM, Duarte MA, de Moura MR, Aouada FA, Kishen A, *et al.* Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod 2015;40:195-201.
3. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010;54:291-312.
4. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 1992;18:605-12.
5. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. Int Endod J 2004;37:272-80.
6. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al.* Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3:95-101.
7. Greulich C, Kittler S, Epple M, Muhr G, Köller M. Studies on the biocompatibility and the interaction of silver nanoparticles with human mesenchymal stem cells (hMSCs). Langenbecks Arch Surg 2009;394:495-502.
8. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod 2014;40:285-90.
9. Pandit N, Trygstad T, Croy S, Bohorquez M, Koch C. Effect of salts on the micellization, clouding, and solubilization behavior of pluronic F127 solutions. J Colloid Interface Sci 2000;222:213-20.
10. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 2007 4;18(22):1-9.
11. Iqbal A. Antimicrobial irrigants in the endodontic therapy. Int J Health Sci (Qassim) 2012;6:186-92.
12. Vande Visse JE, Brilliant JD. Effect of irrigation on the production of extruded material at the root apex during instrumentation. J Endod 1975;1:243-6.
13. Good M, El KI, Hussey DL. Endodontic 'solutions' part 1: A literature review on the use of endodontic lubricants, irrigants and medicaments. Dent Update 2012;39:239-40, 242-4, 246.
14. Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. Oral Surg Oral Med Oral Pathol 1980;49:175-7.
15. Türkün M, Cengiz T. The effects of sodium hypochlorite and calcium hydroxide on tissue dissolution and root canal cleanliness. Int Endod J 1997;30:335-42.
16. Cotter JL, Fader RC, Lilley C, Herndon DN. Chemical parameters, antimicrobial activities, and tissue toxicity of 0.1 and 0.5% sodium hypochlorite solutions. Antimicrob Agents Chemother 1985;28:118-22.
17. Priyaragini S, Sathishkumar S, Bhaskararao K. Biosynthesis of silver nanoparticles using actinobacteria and evaluating its antimicrobial and cytotoxicity activity. Int J Pharm Pharm Sci 2013;5:709-12.
18. Medina-Ramirez I, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. Colloids Surf B Biointerfaces 2009;73:185-91.
19. Marta B, Jakab E, Potara M, Simon T, Imre-Lucaci F, Barbu-Tudoran L, *et al*. Pluronic-coated silver nanoprisms: Synthesis, characterization and their antibacterial activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2014;441:77-83.
20. Bohorquez M, Koch C, Trygstad T, Pandit N. A study of the temperature-dependent micellization of pluronic F127. J Colloid Interface Sci 1999;216:34-40.
21. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: The MTT assay. Methods Mol Biol 2011;731:237-45.