Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2016, Article ID 7597295, 7 pages
http://dx.doi.org/10.1155/2016/7597295

*Research Article*

# Bactericide Effect of Silver Nanoparticles as a Final Irrigation Agent in Endodontics on *Enterococcus faecalis*: An *Ex Vivo* Study

**Pedro-IV González-Luna,[1] Gabriel-Alejandro Martínez-Castañón,[1,2] Norma-Verónica Zavala-Alonso,[2] Nuria Patiño-Marin,[2] Nereyda Niño-Martínez,[3] Javier Morán-Martínez,[4] and Jorge-Humberto Ramírez-González[2]**

[1]*Doctorate Program in Engineering and Materials Science, Autonomous University of San Luis Potosí, Avenida Dr. Manuel Nava No. 2, Zona Universitaria, 78290 San Luis Potosí, SLP, Mexico*
[2]*Doctorate Program in Dental Science, Faculty of Dentistry, Autonomous University of San Luis Potosí, Avenida Dr. Manuel Nava No. 2, Zona Universitaria, 78290 San Luis Potosí, SLP, Mexico*
[3]*Faculty of Science, Autonomous University of San Luis Potosí, Avenida Dr. Salvador Nava S/N, Zona Universitaria, 78290 San Luis Potosí, SLP, Mexico*
[4]*Biology Cell and Ultrastructure Department, Biomedical Research Center, Faculty of Medicine, Autonomous University of Coahuila, 27000 Torreón, COAH, Mexico*

Correspondence should be addressed to Gabriel-Alejandro Martínez-Castañón; mtzcastanon@fciencias.uaslp.mx

Received 16 February 2016; Revised 13 April 2016; Accepted 10 May 2016

Academic Editor: Seunghan Oh

Copyright © 2016 Pedro-IV González-Luna et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The objective of this study was to determine the bactericidal effect of silver nanoparticles as a final irrigation agent in endodontics. This study included 120 uniradicular extracted dental organs inoculated with *Enterococcus faecalis* (*E. faecalis*) and organized into 4 groups: (A) 30 teeth irrigated with a dispersion of silver nanoparticles (537 $\mu$g/mL); (B) 30 teeth irrigated with a sodium hypochlorite solution (2.25%); (C) 30 teeth irrigated with a dispersion of silver nanoparticles (537 $\mu$g/mL) + EDTA (17%); and (D) 30 teeth with a saline solution. After the irrigation protocol, the samples were analyzed through a spectrophotometer to measure the bactericidal effect and scanning electron microscope and atomic force microscope in order to observe the presence of dental smear layer. The results showed that nanoparticles of 10 nm and the sodium hypochlorite at 2.25% were effective for eliminating *E. faecalis*, with no significant difference between them.

## 1. Introduction

Prior to learning any new endodontic technique, precise knowledge of the morphology of the pulp cavity and of the root canals is fundamental; for this reason the professional should have a wide knowledge of the pulp cavity's normal aspect and also of the possible variations, due to age, caries, abrasions, erosions, and periodontal illnesses. The unfamiliarity of this anatomy and the alterations of the original morphology could raise the percentages of failure in the canal therapies [1]. Cleaning the root canal using instrumentation or irrigation is considered one of the most important factors in the prevention and treatment of endodontic infections [2, 3]. Good instrumentation and irrigation combined with a good crowning reconstruction are considered essential in order to have a good long-term forecast [4]. The endodontic irrigation is defined as the insertion of one or more chemicals into the pulp cavity and into the root canal; irrigation is a fundamental addition to the instrumentation necessary to eliminate the residual pulp tissue, bacteria, and dental debris that may still be present in the canal even after a meticulous biomechanical preparation.

*Enterococcus faecalis* is a Gram-positive facultative anaerobic microorganism; this microorganism is a normal

inhabitant of the oral flora, which is associated with the failure in the endodontic treatment as well as in different forms of periradicular lesions including primary and secondary endodontic infections. In the case of primary endodontic infections, *E. faecalis* is associated with periradicular chronic symptomatic abrasions and is found in 40% of these cases, while the secondary periradicular infection is found in higher percentages of 67–77% [5, 6].

The most important material used as an irrigant in root canal treatment is sodium hypochlorite [7]. Although it has several advantages such as its broad bactericide spectrum and its capacity to remove organic debris, its antibacterial activity could be inactivated by dentin and the biomass in the root canal; also, irrigation with sodium hypochlorite leads to decreased bond strength between dentin and resin [8]. Due to this, researchers in the endodontic field have focused their efforts in developing a new nanomaterial-based irrigant. Monzavi et al. used nano-MgO to treat endodontic pathogens and found that nano-MgO aqueous solutions represent a promising antimicrobial activity [9]. Javidi et al. evaluated the bactericide effect of $Ca(OH)_2$ with and without silver nanoparticles on *E. faecalis* from root canals. They found that the number of CFUs observed after the use of $Ca(OH)_2$ plus silver nanoparticles suspension was significantly less than the number observed with $Ca(OH)_2$ alone [10].

On the other hand, silver has been used in various forms, metallic silver, silver nitrate, and silver sulfadiazine, for treating burns and severe bacterial infected wounds and injuries, but, due to the discovery and increase of antibiotics, these silver compounds were no longer used. Silver in the form of nanoparticles has reappeared as a potential antimicrobial agent because of the greatly developed microbial resistance of a large variety of microorganisms. In this scenario the materials in nanoscale have reappeared as a new antimicroorganism agent due to their physical and chemical properties [11].

The objective of this study was to determine, in an *ex vivo* study with instrumented root canals, the bactericidal effect of a dispersion of silver nanoparticles as a final irrigation agent in endodontics.

## 2. Materials and Methods

*2.1. Synthesis and Characterization of Silver Nanoparticles.* Silver nanoparticles were synthesized as follows. 100 mL of a 0.01 M $AgNO_3$ solution was prepared and placed in a reaction vessel; after that and under magnetic stirring, a second solution made of 0.1 g of gallic acid and 10 mL of deionized water was added. The pH value of the reaction mixture was immediately adjusted to 11 using a 1.0 M NaOH solution. Once the pH was changed the solution remained under stirring for 20 more minutes.

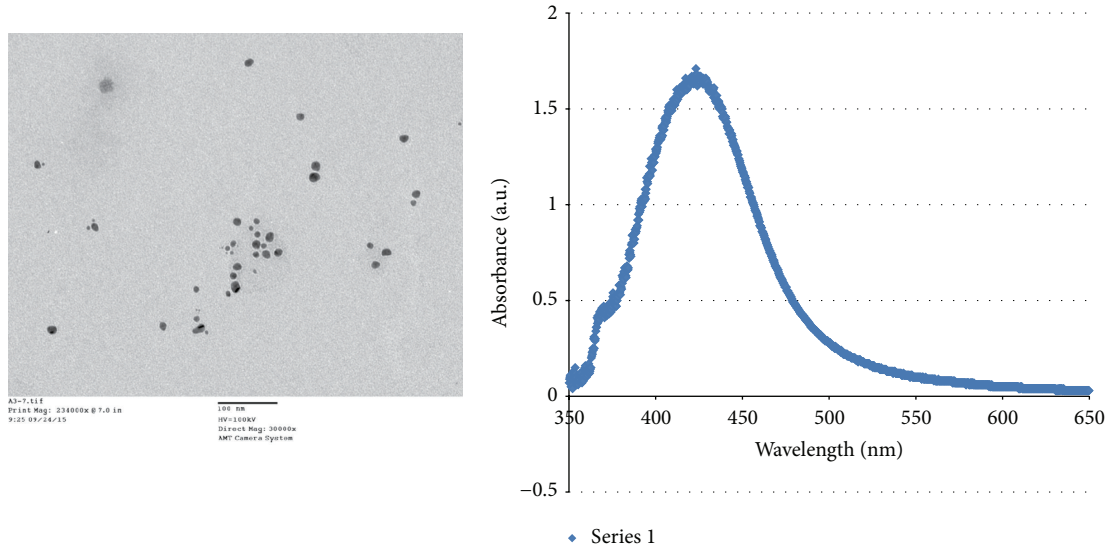
*2.2. Characterization of the Ag Nanoparticles.* The morphology of the synthesized silver nanoparticles was analyzed using a JEOL JEM-1230 TEM (Transmission Electron Microscope). The surface plasmon resonance was measured using a CHEMUSB4-VIS-NIR (Ocean Optics) spectrophotometer. Both analyses were performed using the aqueous dispersion of the nanoparticles.

*2.3. Preparation of the Teeth.* 120 uniradicular extracted teeth were obtained from the Surgery Clinic of the Faculty of Dentistry at the Autonomous University of San Luis Potosí and from the Surgery Clinic at the Autonomous University of Coahuila. The teeth were stored after extraction and divided into 4 study groups ($n = 30$ for each group). Thereafter they were stored in thymol at 0.1% for 24 h; after that time, the teeth were placed in sodium hypochlorite at 2.25% for another 24 h to eliminate soft tissue residue as well as bacteria. For their future use, the teeth were washed with running water and air-dried. Before being prepared, the clinical crowns were cut with a low speed handpiece with a diamond disk without irrigation; longitudinal furrows were made on the vestibular and lingual sides of the root without perforating it. The odontometry was determined 1 mm before the radiographic apex using a manual file 15 k; for the rotatory instrumentation a low speed motor was used until the file 40/04 k3; roots were irrigated with sodium hypochlorite at 1% after every instrumentation step and dried with paper points number 40.

Every tooth was placed in a microtube and all of them were sterilized in autoclave (SM32 Yamato, New Jersey, USA) for 20 min at 120 lb. and 120°C. In order to determine the efficiency of the sterilization treatment each tube received 1 mL of BHI (brain-heart infusion) and was incubated for 24 h at 37°C. Afterwards, the teeth were transferred to a laminar flow hood (LABCONCO Purifier Class II, Biosafety Cabinet, Ohio, USA) for its inoculation with 100 $\mu$L of an *E. faecalis* dispersion at $10^8$ UFC/mL.

The teeth were incubated at 37°C for 3 days and were resupplied with fresh broth every 8 hours. The tubes in which they were stored were marked individually with a letter (A, B, C, and D). They were then placed in one collective recipient to permit unbiased irrigation at the time of assignment. Every tooth was assigned to each group at random and was irrigated with 5 mL during 5 min with one of the following solutions or dispersions: (A) 30 teeth were irrigated with silver nanoparticles (537 mg/mL); (B) 30 teeth were irrigated with sodium hypochlorite (2.25%); (C) 30 teeth were irrigated with silver nanoparticles (537 mg/mL) + EDTA (17%); and (D) 30 teeth were irrigated with a saline solution (control). All the teeth were handled with sterilized forceps and gloves to avoid contamination from other bacteria. Once irrigation was completed, the roots were dried and then split longitudinally with a previously sterilized manual clipper. One-half was selected at random and put into 1 mL of BHI and mixed for 15 s and then incubated for 18 h. To determine the bactericidal effect of the irrigant, 200 $\mu$L of each solution was placed on a 96-well microplate and the absence or presence of turbidity was determined by using a microplate absorbance reader (iMark, Bio-Rad) at a wavelength of 585 nm which is specific for *E. faecalis* with no interference of the testing solution.

*2.4. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) Observations.* By means of the AFM and SEM the presence of smear layer was observed on the teeth




(a) (b)

Figure 1: (a) TEM image and (b) surface plasmon resonance of the nanoparticles used in this work.

samples after being irrigated with the testing solutions. For each group and after the tooth had been instrumented, irrigated, and divided with manual pliers, it was placed on a double-sided adhesive tape at the base of the AFM. For this we used a Nanosurf Easyscan 2 atomic force microscope with the following configuration: the tip was made of silicon nitride, 2 nm in thickness, 450 nm in length, and 50 nm in width, with resonant frequency of 13 kHz and a constant of 0.2 N/m, and operated by contact mode.

After their observation in AFM, samples were fixed, dehydrated, and gold-sputtered in order to observe them in the scanning electron microscope (JEOL JSM-1650) at 10 or 5 kV.

*2.5. Statistical Analysis.* In order to evaluate difference between groups we used the one-way ANOVA test with an alpha of $p < 0.05$. The analysis was made using the JMP V. 4.0 and Stat-View programs.

## 3. Results

Silver nanoparticles prepared in this work have spherical morphology and size around 10 nm with a narrow size distribution (Figure 1(a)). The surface plasmon resonance is narrow and located at 420 nm (Figure 1(b)).

Figure 2 shows the bactericidal effect of the solutions measured by turbidity; the height of the bar is directly related to the quantity of bacteria in the roots after the irrigation process. All of the solutions were more effective than the physiological solution in the elimination of *E. faecalis*. The sodium hypochlorite solution proved to be the most effective bactericidal material followed by the silver nanoparticles and lastly the nanoparticle/EDTA mixture. The bactericidal effects of silver nanoparticles and the sodium hypochlorite at 2.25% had no significant difference between them (Table 1).

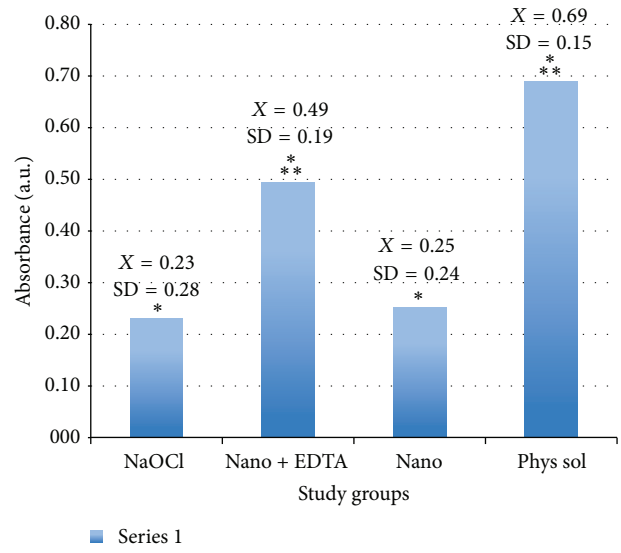



Figure 2: Bactericide effect of the silver nanoparticles compared against different solutions; results with the same number of asterisks have no statistical difference. a.u. = arbitrary units; $X$ = mean; SD = standard deviation.

Figures 3 and 4 show the evaluation of the capacity for removal of the smear layer by the silver nanoparticles and EDTA under an atomic force microscope and scanning electron microscopy, respectively; EDTA solution, which is the best irrigator for this use, left the dentinal tubules completely open and dentin without debris (Figures 3(b), 4(c), and 4(d)). Silver nanoparticles present a good effect in the removal of the smear layer leaving the dentinal tubules open without dentin erosion (Figures 3(a), 4(a), and 4(b)).

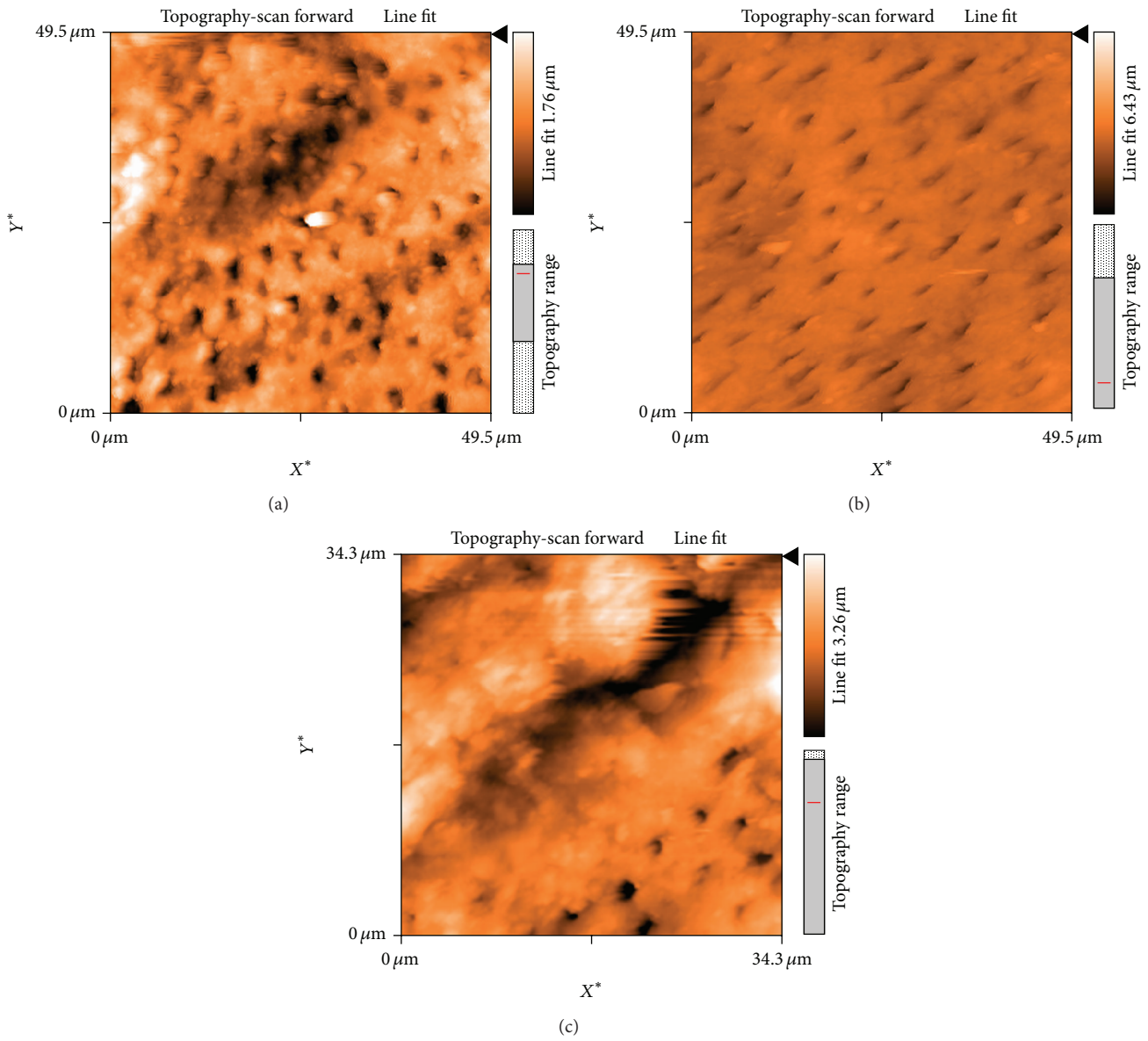



Figure 3: AFM images showing the capacity of smear layer removal of (a) silver nanoparticles, (b) EDTA, and (c) saline solution. This images show that the silver nanoparticles are more effective in removing smear layer than saline solution but show less smear layer removal when compared with EDTA.

## 4. Discussion

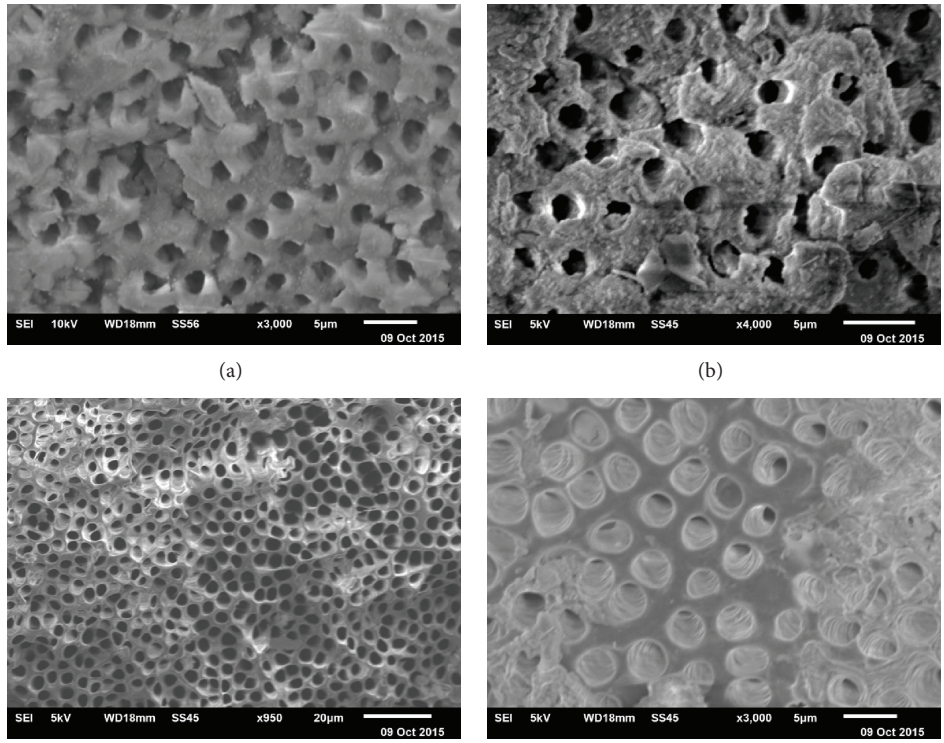
The clinical isolates of *E. faecalis* recovered from infections of the root canal offer the opportunity to do studies *in vitro* in which the mechanics of virulence, genetic structure, and defense are not altered. This allows us to efficiently study the bactericidal effect of components that need to be evaluated [8].

In this study, the bactericidal effect of various irrigators of the root canal on strains of *E. faecalis* was evaluated. *E. faecalis* has certain factors of virulence including enzymes, cytolysin, and lipoteichoic acid. It was selected for this study because it has proven to be resistant to numerous antimicrobial agents and has been associated with resistant apical periodontitis [12]. The study was performed on root canals, which permits an improved observational study of the behavior of the bacteria in clinical conditions very similar

Table 1: Results of the one-way ANOVA of the turbidity results.

| Irrigant comparison | $p$ value |
|---|---|
| Sodium hypochlorite versus Ag nanoparticles/EDTA | 0.0001 |
| Ag nanoparticles versus Ag nanoparticles/EDTA | 0.0001 |
| Physiological solution versus Ag nanoparticles/EDTA | 0.0001 |
| Physiological solution versus Ag nanoparticles | 0.0001 |
| Physiological solution versus sodium hypochlorite | 0.0001 |
| Ag nanoparticles versus sodium hypochlorite | 0.6787 |

Statistical differences were found when $p < 0.05$ between groups.




(a) (b) (c) (d)

Figure 4: SEM images showing the capacity of smear layer removal of (a and b) silver nanoparticles and (c and d) EDTA solution.

to actual conditions. These clinical isolates allowed us to know the bactericidal activity of the endodontic irrigators used on the root canal. The irrigation protocol, that is, the protocol to evaluate the bactericide effectiveness of the silver nanoparticles, was made inside the root of the teeth; when teeth were immersed into the sodium hypochlorite solution (in order to eliminate soft tissue residue as well as bacteria) they were not instrumented yet; then, the root was not exposed to this solution and the cleaning procedure does not affect the bactericide results.

This study demonstrates a positive bactericidal effect from the silver nanoparticles solution as an endodontic irrigator on *E. faecalis*; the same effect occurred with the sodium hypochlorite at 2.25%. Wu et al. obtained similar results when they evaluated the antibacterial efficacy of silver nanoparticles against *E. faecalis* [7]; they prepared dentin cubes and then infected them with bacteria to perform the bactericide test; the main difference with our study is that we prepared and instrumented the entire teeth in a way which is very similar to that in clinical treatment. Estrela et al. studied the bactericidal effect of 2% chlorhexidine and 2% sodium hypochlorite on different strains of reference, *E. faecalis* among them, and their results showed a greater bactericidal effect of chlorhexidine in comparison to sodium hypochlorite [13]. The authors explained that maybe the hypochlorite solution does not penetrate into the smallest confines of the root canal and also its reaction with the organic components of the smear layer deactivates it, which reduces its bactericidal effect. Silver nanoparticles are capable of penetrating the dentinal tubules and they are not deactivated with organic tissues thus having several advantages over NaOCl [7]. We used sodium hypochlorite as control because as reported by Haapasalo et al., sodium hypochlorite is the most important irrigant in root canal treatment. This solution that can dissolve organic matter is of vital importance in removing necrotic tissue remnants as well as biofilm [14].

In this study a diminished bactericidal effect was identified when the nanoparticles were mixed with EDTA; this could be because EDTA is a chelating agent. That is, it takes ions from other compounds; in this case it could interact with $Ag^+$ ions and, by this means, reduce their bactericidal capacity [15, 16].

The capacities of NaOCl 1% and NaOCl 2.25% have been evaluated on removal of the smear layer, obtaining similar results without a statistical difference. It is believed that the sodium hypochlorite effect upon inorganic material is due to the formation of hypochloric acid that reacts to insoluble proteins forming soluble products that would allow the removal of the superficial smear layer [17]. The effect of the removal of the smear layer by EDTA 17% and CHX 2% was studied by Mohammadi and Shahriari and a significant difference was discovered between EDTA 17% and CHX 2%; the latter removed less smear layer [18].

In our study the capacity of the silver nanoparticles and EDTA 17% to remove the smear layer was evaluated (generally EDTA at 17% is used to compare the capacities of different irrigators that eliminate smear layer); silver nanoparticles showed good smear layer removal and this property could

be a result of a physical interaction between nanoparticles and the debris. Sodium hypochlorite is not an effective smear layer removal agent and this is an advantage of silver nanoparticles over sodium hypochlorite; they have the same bactericide effect but, in addition to that, silver nanoparticles present a smear layer removal capacity.

As for the cytotoxicity of different root canal irrigants, different authors studied the effects on fibroblasts; sodium hypochlorite 2.25%, citric acid 15%, and phosphoric acid 5% were used and, as a result, a greater cellular viability with sodium hypochlorite at 2.25% was obtained [19, 20]. The toxicity of the silver nanoparticles has been studied *in vitro* on different mammalian cells, including rat liver [21], keratinocytes, and human fibroblasts [22], and on stem cells and spermatogonia [23]. It has been discovered that high doses of silver nanoparticles can induce oxidative stress as a mechanism of cytotoxicity [9]. Nevertheless, in small doses and sizes of the particles at 10 nm, the silver nanoparticles appear to have anti-inflammatory characteristics and speed up the healing process of wounds, modulation of cytokines, and induction and production of peripheral blood cells [10], inhibit dermatitis through contact, suppress the signs of TNF-$\alpha$ and IL-12, and also induce apoptosis in inflammatory cells. Also, Gomes-Filho et al. [24, 25] published results on the response of rat connective tissue to tubes filled with fibrin embedded in AgNPs dispersion in comparison to sodium hypochlorite 2.5%; they found that silver nanoparticles are biocompatible and could be used as an endodontic irrigant.

## 5. Conclusion

Our principal finding is that silver nanoparticles of 10 nm prepared using gallic acid as the reducing agent present a bactericide effect against *E. faecalis* in an *ex vivo* study and this effect is the same compared with sodium hypochlorite and, in addition to that, silver nanoparticles present a good smear layer removal capacity. This is why silver nanoparticles could represent a good option in the eradication of *E. faecalis* in root canals. Future studies are required to evaluate the nanoparticles against other endopathogenic microorganisms as well as their endotoxin effect, specially the bacterial LPS.

## Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

## Acknowledgments

This work was partially supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT Grant CB-169020); PRODEP 2015 (Grant DSA/103.5/15/6988); Fondo de Apoyo a la Investigación (FAI, UASLP 2015, Grant C15-FAI-04-77.77); Fondo de Recursos Concurrentes (Grant C13-FRC-01-12.12). Pedro-IV González-Luna would like to thank CONACYT for its support with Scholarship no. 213888.

## References

[1] M. R. Leonardo, *Endodontics: Root Canal Treatment. Technical and Biological Principles*, Artes Médicas Latinoamerica, Sao Paulo, Brazil, 1st edition, 2005 (Spanish).

[2] E. S. Reddy, D. Sainath, M. Narenderreddy, S. Pasari, S. Vallikanthan, and G. Sindhurareddy, "Cleaning efficiency of anatomic endodontic technology, profile system and manual instrumentation in ovalshaped root canals: an in vitro study," *Journal of Contemporary Dental Practice*, vol. 14, no. 4, pp. 629–634, 2013.

[3] B. P. F. A. Gomes, M. E. Vianna, A. A. Zaia, J. F. A. Almeida, F. J. Souza-Filho, and C. C. R. Ferraz, "Chlorhexidine in endodontics," *Brazilian Dental Journal*, vol. 24, no. 2, pp. 89–102, 2013.

[4] L. Giardino, E. Ambu, C. Becce, L. Rimondini, and M. Morra, "Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic," *Journal of Endodontics*, vol. 32, no. 11, pp. 1091–1093, 2006.

[5] M. Haapasalo, U. Endal, H. Zandi, and J. M. Coil, "Eradication of endodontic infection by instrumentation and irrigation solutions," *Endodontic Topics*, vol. 10, no. 1, pp. 77–102, 2005.

[6] B. Athanassiadis, P. V. Abbott, and L. J. Walsh, "The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics," *Australian Dental Journal*, vol. 52, supplement 1, pp. S64–S82, 2007.

[7] D. Wu, W. Fan, A. Kishen, J. L. Gutmann, and B. Fan, "Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm," *Journal of Endodontics*, vol. 40, no. 2, pp. 285–290, 2014.

[8] R. D. Morgental, A. Singh, H. Sappal, P. M. P. Kopper, F. V. Vier-Pelisser, and O. A. Peters, "Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants," *Journal of Endodontics*, vol. 39, no. 3, pp. 406–410, 2013.

[9] A. Monzavi, S. Eshraghi, R. Hashemian, and F. Momen-Heravi, "In vitro and ex vivo antimicrobial efficacy of nano-MgO in the elimination of endodontic pathogens," *Clinical Oral Investigations*, vol. 19, no. 2, pp. 349–356, 2015.

[10] M. Javidi, F. Afkhami, M. Zarei, K. Ghazvini, and O. Rajabi, "Efficacy of a combined nanoparticulate/calcium hydroxide root canal medication on elimination of *Enterococcus faecalis*," *Australian Endodontic Journal*, vol. 40, no. 2, pp. 61–65, 2014.

[11] K. C. Bhol and P. J. Schechter, "Topical nanocrystalline silver cream suppresses inflammatory cytokines and induces apoptosis of inflammatory cells in a murine model of allergic contact dermatitis," *British Journal of Dermatology*, vol. 152, no. 6, pp. 1235–1242, 2005.

[12] A. Afzal, V. R. Gopal, R. Pillai, A. Jacob, S. U-Nu, and S. Shan, "Antimicrobial activity of various irrigants against *E. faecalis* biofilm: an *in vitro* study," *Journal of Interdisciplinary Dentistry*, vol. 3, no. 2, pp. 103–108, 2013.

[13] C. Estrela, R. G. Ribeiro, C. R. A. Estrela, J. D. Pécora, and M. D. Sousa-Neto, "Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods," *Brazilian Dental Journal*, vol. 14, no. 1, pp. 58–62, 2003.

[14] M. Haapasalo, Y. Shen, Z. Wang, and Y. Gao, "Irrigation in endodontics," *British Dental Journal*, vol. 216, no. 6, pp. 299–303, 2014.

[15] A. Travan, C. Pelillo, I. Donati et al., "Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity," *Biomacromolecules*, vol. 10, no. 6, pp. 1429–1435, 2009.

[16] I. Chopra, "The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern?" *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 4, pp. 587–590, 2007.

[17] C. C. R. Ferraz, B. P. F. A. Gomes, A. A. Zaia, F. B. Teixeira, and F. J. Souza-Filho, "Comparative study of the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution and sodium hypochlorite as endodontic irrigants," *Brazilian Dental Journal*, vol. 18, no. 4, pp. 294–298, 2007.

[18] Z. Mohammadi and S. Shahriari, "Residual antibacterial activity of chlorhexidine and MTAD in human root dentin *in vitro*," *Journal of Oral Science*, vol. 50, no. 1, pp. 63–67, 2008.

[19] M. Georgopoulou, E. Kontakiotis, and M. Nakou, "Evaluation of the antimicrobial effectiveness of citric acid and sodium hypochlorite on the anaerobic flora of the infected root canal," *International Endodontic Journal*, vol. 27, no. 3, pp. 139–143, 1994.

[20] S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss, and J. J. Schlager, "In vitro toxicity of nanoparticles in BRL 3A rat liver cells," *Toxicology in Vitro*, vol. 19, no. 7, pp. 975–983, 2005.

[21] A. Burd, C. H. Kwok, S. C. Hung et al., "A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models," *Wound Repair and Regeneration*, vol. 15, no. 1, pp. 94–104, 2007.

[22] L. Braydich-Stolle, S. Hussain, J. J. Schlager, and M.-C. Hofmann, "In vitro cytotoxicity of nanoparticles in mammalian germline stem cells," *Toxicological Sciences*, vol. 88, no. 2, pp. 412–419, 2005.

[23] S. Arora, J. Jain, J. M. Rajwade, and K. M. Paknikar, "Cellular responses induced by silver nanoparticles: *in vitro* studies," *Toxicology Letters*, vol. 179, no. 2, pp. 93–100, 2008.

[24] J. E. Gomes-Filho, F. O. Silva, S. Watanabe et al., "Tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution," *Journal of Endodontics*, vol. 36, no. 10, pp. 1698–1702, 2010.

[25] J. E. Gomes-Filho, F. O. Silva, S. Watanabe et al., "Evaluation of silver nanoparticles as irrigating solution," *Dental Press Endodontics*, vol. 3, no. 2, pp. 16–23, 2013.

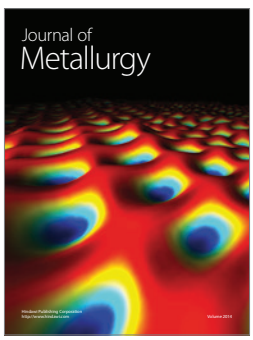

Journal of
Nanotechnology

International Journal of
Corrosion

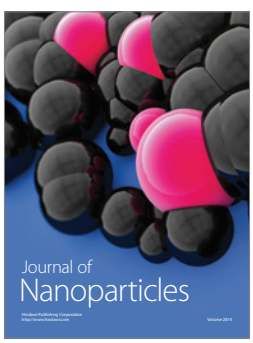
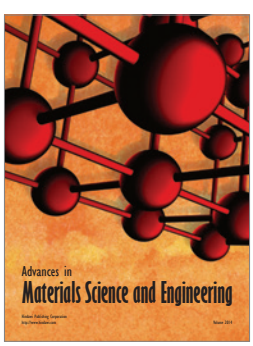
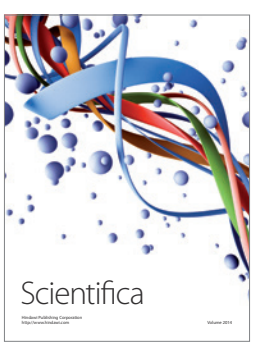
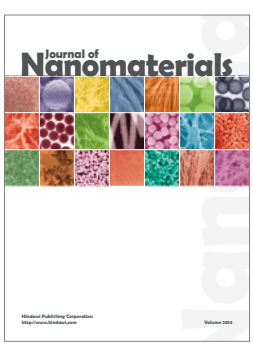
International Journal of
Polymer Science

Smart Materials
Research

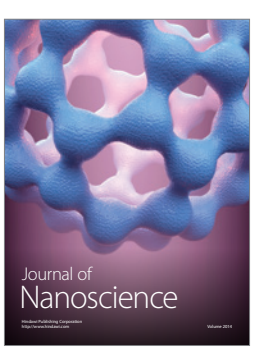
Journal of
Composites

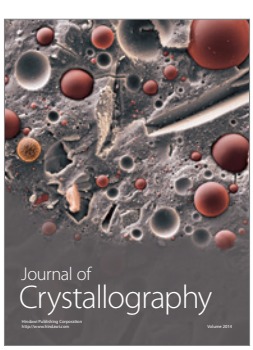
BioMed
Research International

Journal of
Metallurgy

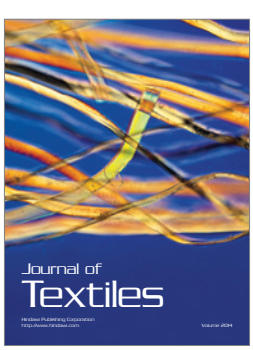
Journal of
Materials

Hindawi
Submit your manuscripts at
http://www.hindawi.com

Journal of
Nanoparticles

Advances in
Materials Science and Engineering

Scientifica

Journal of
Nanomaterials

The Scientific
World Journal

International Journal of
Biomaterials

Journal of
Nanoscience

Journal of
Coatings

Journal of
Crystallography

Journal of
Ceramics

Journal of
Textiles