Original Article

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Comparing the Antibacterial Effect of Green-Synthesized Nano-Silica with Calcium Hydroxide and Tannic Acid in Canals Infected by Enterococcus faecalis

Amirhosein Hoseini[1], Mehdi Shakibaie[2], Shima Bijari[3]✉, Maryam Mortazavi[4]

[1] Department of General Dentistry, Birjand university

[2] Department of Medical Nanotechnology, School of Medicine, Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

[3] Department of Endodontics, Faculty of Dentistry and Dental Research Center, Birjand University of Medical Sciences, Birjand, Iran

[4] Department of Endodontics, Faculty of Dentistry and Dental Research Center, Birjand University of Medical Sciences, Birjand, Iran

✉**Corresponding Author:** Tel: +989106794221; Email: shima.bijari223@gmail.com

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Abstract

Background and Objective: The antibacterial properties of nanoparticles have been recently considered in the treatment of root canals. Therefore, this study aimed to compare the antibacterial effect of green-synthesized nanoparticles of silica, calcium hydroxide and tannic acid as intracanal medicaments against *Enterococcus faecalis* bacteria.

Methods: In this in-vitro study, 58 single-rooted teeth were first treated, silica nanoparticles (SiNPs) were synthesized by the green method, and then minimum inhibitory concentration (MIC) and minimum bacterial concentration of tannic acid, calcium hydroxide, and green nano-silica were measured using the microbroth dilution method. Then, the minimum bactericidal concentration was measured in extracted teeth prepared for each material, and this concentration obtained for each material was placed in three groups of teeth (green nano-silica, tannic acid, and calcium hydroxide) to count the number of colonies. Brunauer-Emmett-Teller, dynamic light scattering, and transmission electron microscopy analyses were also performed for the nanoparticles. Data were analyzed using SPSS software (version 20) and the Kruskal–Wallis test and Mann–Whitney test. The level of statistical significance was set at $P<0.05$.

Results: The results of the microbroth dilution test for green-synthesized nano-silica, tannic acid, and calcium hydroxide showed MIC values of 0.2, 0.8, and 1.56 mg/mL, respectively. Green-synthesized silica had the highest antibacterial activity against *Enterococcus* among different materials. The results obtained from the evaluation of minimum bactericidal concentration in teeth and colony counting also showed a greater effect of green-synthesized nano-silica compared to other materials.

Conclusion: Using SiNPs can effectively clean root canals of bacteria; however, further in vivo studies and assessments of long-term safety and efficacy are necessary before clinical application.

Key words: Silicon dioxide, Nanoparticles, Tannic acid, Calcium hydroxide, *Enterococcus faecalis*

Introduction

Bacteria and their products are pathogens of endodontic diseases (1). Complete biomechanical cleansing of the root canal system is an accepted principle in endodontic treatment. If cleansing is not properly performed, necrotic soft tissue debris acts as a feeding source for bacteria and causes reinfection in the canal and apical periodontitis (2). *Enterococcus faecalis* is a normal oral flora bacterium with a significant role in endodontic infections, and is one of the most important bacteria in the failure of endodontic treatments (3).

In addition to its antimicrobial properties, calcium hydroxide is the most common intracanal medicament that can dissolve tissue, prevent tooth decay and induce hard tissue formation. On the other

hand, calcium hydroxide can make dentin fragile and cause inflammation and cell necrosis due to its high pH as well (4, 5). Also, some studies have indicated that *Enterococcus faecalis* is resistant to calcium hydroxide, therefore, using a material with properties capable of eliminating *Enterococcus faecalis* can significantly improve the treatment of teeth with periradicular infection (6).

The antibacterial properties of nanoparticles have been recently considered in the treatment of root canals (7). Research has indicated that this property is due to the small size and high surface and mass ratio of these materials (8).

Among a wide range of nanoparticles, silica nanoparticles (SiNPs) represent a unique class of mineral particles with a wide range of functional properties (9). Given the special properties of these materials, such as large area, adjustable pores, particle size, controllable morphology, high mechanical and thermal stability, biocompatibility, easy preparation even on large scales, and their selective functions, much attention has been paid to them in science and fundamental technology (10, 11).

There are different methods for making nanoparticles, out of which physical and chemical methods often involve difficult techniques and are very expensive and time-consuming and are also not environmentally friendly (12). While today, using green methods in preparing nanomaterials has received more attention due to biosecurity, non-toxicity, cheapness and the presence of a wide variety of metabolites, such as polyphenols, and alkaloids (13).

Tannins are derived from polyphenols, and tannic acid is a hydrolyzable tannin (14) and is found naturally in a variety of plants and fruits (15, 16). Anti-tumor, anti-biofilm, antibacterial, and anti-viral activities are important properties of tannic acid leading to its widespread use in medicine. It also has anti-inflammatory and antioxidant properties (17). Given that calcium hydroxide cannot completely eliminate *Enterococcus faecalis* and also has adverse effects, such as dentin fragility and tissue toxicity (18), this study was conducted to compare the effect of nano-silica synthesized by a green method as an intracanal medicament with calcium hydroxide and tannic acid against *Enterococcus faecalis*.

Materials and Methods

Sampling and Data Sources

In this in vitro study, extracted 58 single-rooted human maxillary anterior teeth, with closed apices, single and straight root canals, without cracks, caries, restorations, resorption, or previous root canal treatment, were selected. This study was approved by the Ethics Committee of Birjand University of Medical Sciences, Birjand, Iran (IR.BUMS.REC.1399.539). Until experiment time, these teeth were kept in 0.9% normal saline (Samen, Mashhad, Iran) to maintain moisture and prevent crack formation and bacterial growth.

Preparing the Teeth

The selected teeth had healthy roots with an average length of 18-20 mm. Firstly, tissue debris was removed from the outer surface of the root by a curette. Then, all teeth were placed in 5.25% sodium hypochlorite solution (Shamin Chemical Company, Tehran, Iran) for 24 hours and then they were placed in saline solution until they were used. Then, the crowns were removed from the crowns of the tooth by a turbine diamond mill. Teeth with apical stop equivalent to k-file number 15 were selected after examining the apical teeth with k file number 10 (Mani, Tochigi, Japan). To determine the working length, K-file number 15 was inserted into the canal until it was observed from the apical foramen. This length was then reduced by one millimeter. To enlarge the canals and create a space to inoculate bacteria to the canal, preparing the coronal area of the canal was performed with Gates-Glidden (Mani, Tochigi, Japan) No. 2, 3, and 4 and preparing the middle and apical areas with a rotary file (Denco Gold) was performed using the crown down method. Afterwards, 5 mL of 2.5% sodium hypochlorite solution was used with a special endo wash needle (30 gauge) between each instrumentation. At the end of cleaning and preparing the canals, the smear layer was removed using 3 mL of 17% ethylenediaminetetraacetic acid (Master Dent,

Billings, United States) for 5 minutes and then 3 mL of 5.25% sodium hypochlorite for 5 minutes. The final wash was performed with 10 mL of normal saline and the canals were dried using paper points (Meta Dental Corp., Seoul, Korea). To prevent bacterial leakage when inoculated into the canal, the apical end of the roots was sealed with resin and the lateral canals were sealed with nail polish (20).

Sterilizing the Teeth

The teeth were sterilized in an autoclave (Eastern Weber, Milan, Italy) for 20 minutes at 121°C and 15 PSI pressure, and to ensure sterilization five channels were randomly sampled and cultured using a Hedstrom file (Mani, Tochigi, Japan) No. 35. Each Hedstrom file was then transferred to a test tube containing 1 mL of sterile normal saline solution. After 20 seconds, 10 microliters of the vortexed solution was cultured from the test tube, and the plates were incubated at 37°C for 24 hours, in which no bacterial growth was observed (21).

Study Implementation

Single-rooted teeth were first treated, SiNPs were synthesized by a green method, and then the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of tannic acid, calcium hydroxide, and green nano-silica were measured using the microbroth dilution method. Then, the minimum bactericidal concentration was measured in extracted teeth prepared for each material, and this concentration obtained from each material was placed in three groups of teeth (green nano-silica, tannic acid, and calcium hydroxide) to count the number of colonies. Brunauer-Emmett-Teller (BET), dynamic light scattering (DLS), and transmission electron microscopy (TEM) analyses were also performed for the nanoparticles.

Green Synthesis

Green-synthesized SiNPs were prepared using a previously reported method (19). Briefly, a tannic acid solution was prepared by dissolving 408 mg of tannic acid powder in 300 mL of ethanol. This solution was heated to 40°C and stirred for 30 minutes. Then, 150 mL of 32% ammonia solution was added and stirred for 30 minutes. Then, 1.8 mL of tetraethyl orthosilicate was added dropwise to this solution. The synthesized nanoparticles were then centrifuged after stirring for 2 hours. The precipitate was washed five times with an ethanol-hydrochloric acid solution.

Characterization

The morphology of green-synthesized SiNPs was assessed using TEM (Zeiss EM10C, Zeiss, Germany) at an accelerating voltage of 100 kV. Size distribution of synthesized nanoparticles was determined by DLS method (Brookhaven, USA). The surface area and pore size distribution of the prepared nanoparticles were obtained by the BET method. The pore diameter distribution and total pore volume were calculated from the adsorption branch of the isotherm using the Barrett-Joyner-Halenda method (BJH) and from the nitrogen adsorption-desorption isotherms.

Investigating the Antibacterial Properties

To evaluate the antibacterial properties of calcium hydroxide, tannic acid, and SiNPs synthesized by different methods, first the bacterial strain *Enterococcus faecalis* (ATCC: 29212) was purchased from the Pasteur Institute of Iran. Then, the MIC and MBC of the studied materials were investigated, using the microbroth dilution method (8).

Microbroth Dilution Method

In this method, the antibacterial properties of the material are investigated with the help of a 96-well plate. For this purpose, different concentrations of calcium hydroxide, tannic acid, and green-synthesized SiNPs were prepared using Mueller-Hinton broth culture medium and also the 0.5 McFarland suspension of *Enterococcus faecalis* was diluted to a ratio of 1:150 with Mueller-Hinton broth culture medium. Afterwards, 100 µL of bacterial suspension and test materials were added into wells of the plate. The plate was incubated at 37°C for 24 hours.

After 24 hours, the lowest concentration of the material in which no turbidity was observed was considered the MIC.

From the MIC concentrations and higher concentrations, 10 µL was cultured on blood agar medium, to evaluate the MBC and it was incubated

in an incubator for 24 hours. Then, the minimum concentration of materials in which no growth was observed on blood agar medium was considered the MBC.

To ensure that the work was done, the steps were performed with three repetitions.

Investigating Minimum Bactericidal Concentration in Teeth

To determine the minimum bactericidal concentration of calcium hydroxide, tannic acid, and green-synthesized SiNPs in teeth, 10 µL of *Enterococcus faecalis* suspension was inoculated into 18 teeth (this group of teeth was in addition to the studied groups). Then, 10 µL of different concentrations of calcium hydroxide, tannic acid and green-synthesized SiNPs were added into the teeth. The canals of the teeth were closed using wax and placed in an incubator for 24 hours. After 24 hours, 10 µL of physiological serum was poured into the canal to investigate the bacterial growth inside the teeth and then the teeth were sampled from the canal using a paper point and the suspension was cultured on blood agar medium. Then, the blood agar medium was incubated at 37°C for 24 hours, after which the lowest concentration of material that prevented the growth of bacteria was considered as the minimum bactericidal concentration inside the tooth.

Investigating the Number of Colonies

The teeth were randomly divided into 5 groups, including green-synthesized nanosilica (16 teeth), tannic acid (16 teeth), calcium hydroxide (16 teeth), negative control (5 teeth), and positive control (5 teeth), to count the number of bacterial colonies in different teeth. To perform the antibacterial test on the teeth, first, 10 µL of *Enterococcus faecalis* suspension was inoculated into the root canal of the teeth for one week, and every 24 hours, then 10 µL of material with the minimum bactericidal concentration obtained from the previous step, was added into the teeth of each group. The canal orifice of the teeth was sealed with paraffin and then the teeth were kept in an incubator at 37°C for one week. Then, to evaluate the number of bacterial colonies inside the teeth, 10 µL of physiological serum was poured into the canal and the solution inside the canal was activated by a Hedstrom file No. 25 and then the samples were collected from the canals of the teeth using a No. 35 paper point and cultured on blood agar medium. Then, blood agar media was kept in an incubator at 37°C for 24 hours and the number of colonies in each group was counted. All steps were performed by the flame using sterile equipment.

Data Analysis and Interpretation

Data were analyzed using SPSS (version 20). Descriptive statistics were first calculated to summarize the data. The Shapiro–Wilk test was used to assess the normality of the data, which indicated a non-normal distribution. Therefore, the Kruskal–Wallis test was applied to compare the groups at a significance level of $P \leq 0.05$. Post hoc pairwise comparisons were performed using the Mann–Whitney test with Bonferroni correction.

Results

The average size of the synthesized nanoparticles measured by the DLS method was 222±10 nm with an appropriate polydispersity of 0.073±0.005. Figure 1 shows the morphology of green-synthesized SiNPs assessed by TEM. The calculated average size of the nanoparticles was 181±11 nm with a spherical shape.

Figure 2 shows the adsorption–desorption isotherm and the classical type-IV hysteresis, implying the presence of well-defined mesoporous structure. Based on the BET and BJH methods, the surface area was 275 m2/g and the mean pore diameter was 7.45 nm, respectively.

Table 1 presents the results of the microbroth dilution method against *Enterococcus faecalis*. The results of the microbroth dilution method showed that among the used materials, green-synthesized silica (MIC=0.2 mg/mL), tannic acid (MIC=0.8 mg/mL) and calcium hydroxide (MIC=1.56 mg/mL) had the highest antibacterial activity against *Enterococcus faecalis*, respectively.

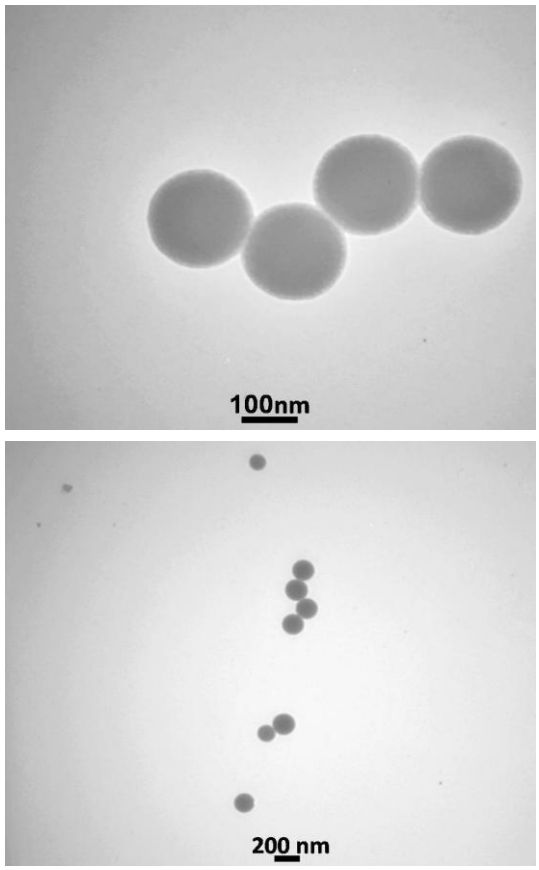

Figure 1. Brunauer-Emmett-Teller (BET) Nitrogen Adsorption/Desorption Isotherms of Tannic Acid-Synthesized Silica Nanoparticles

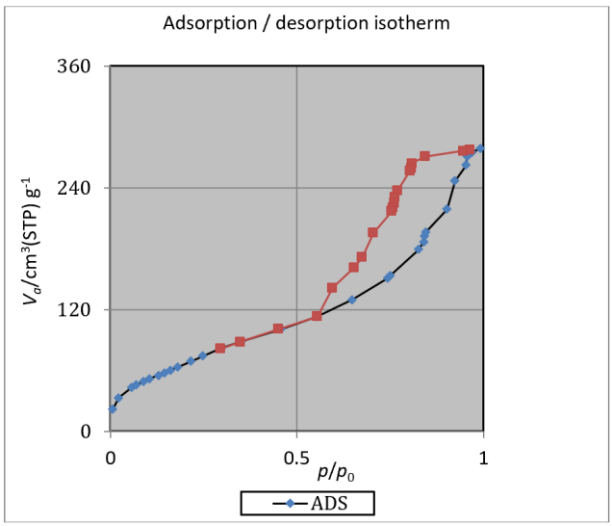

Figure 2. Transmission Electron Microscopy (TEM) Image of Tannic Acid-Synthesized Silica Nanoparticles

Table 1. Antibacterial Results of Microbroth Dilution Method

	MIC (mg/mL)	**MBC (mg/mL)**
Green-synthesized silica	0.2	0.3
Tannic acid	0.8	1
Calcium hydroxide	1.56	6.25

Table 2 presents the results of the study of antibacterial properties of various materials against *Enterococcus faecalis* inoculated in teeth. The results indicate an increase in the concentration required to eliminate bacteria in the teeth relative to the microplate in all materials used. The antibacterial results of using materials in teeth, such as microplates, showed a greater impact of green-synthesized silica. Also, in this method, the minimum bactericidal concentration in teeth was equal for the two substances of tannic acid and calcium hydroxide.

Table 2 presents the results of examining the number of bacterial colonies in 16 different teeth in each group. The results showed a significant reduction in the number of bacteria within the dental canals compared to the positive control group (P<0.05). Green-synthesized SiNPs and calcium hydroxide had similar effects on root canal cleansing at concentrations equivalent to the minimum bactericidal concentration. A significant difference (P<0.05) in root canal cleansing was observed between the SiNPs and calcium hydroxide groups compared to tannic acid group, indicating that tannic acid is not as effective as SiNPs and calcium hydroxide in root canal cleansing.

Table 2. Antibacterial Results of Using Materials in Teeth

Intracanal Medicament	**Minimum Bactericidal Concentration in Teeth** (mg/mL)	**Number of *Enterococcus faecalis* Colonies** (Mean ± SD) (CFU/mL)
Green-synthesized silica	10	12.31±10.47*#
Tannic acid	25	41.19±32.09*
Calcium hydroxide	25	17.25±14.41*#

*significant difference (P<0.05) with positive control group.
#significant difference (P<0.05) with tannic acid group

Due to the non-normality of the data, the Kruskal-Wallis analysis test was used, and no significant difference in mean was observed between the studied groups (P>0.05).

Discussion

The results of this study showed that green-synthesized SiNPs exhibit strong antibacterial activity overall, although their effectiveness may be influenced by limited tannic acid release from the particle surface and reduced surface area. Furthermore, higher concentrations were required to eliminate bacteria in the tooth than in microplate conditions, likely due to material absorption by enamel and the dry intra-tooth environment.

The bacteria in the root canal are the most important factor in the formation and spread of root lesions during the root canal treatment process (22). In the present study, *Enterococcus faecalis* was used, which is often associated with unsuccessful endodontic treatment and root infections (23).

Nowadays, hydroxide, as the most common intracanal medicament today, eliminates bacteria by a variety of mechanisms, including damage to the cytoplasmic membrane, DNA damage, and denaturation of proteins (24, 25). However, its antibacterial activity is affected by the buffering properties of dentin. Safavi et al. (26) indicated that exposure to calcium hydroxide for one day eliminates the bacterium *Enterococcus faecalis* from infected dentin. Residual bacteria from mechanical cleansing in the study of Sjögren et al. (27) were effectively eliminated after one week of exposure to calcium hydroxide.

In the present study, exposure of bacteria to calcium hydroxide for one week did not eliminate the bacteria completely; however, it led to a significant reduction in the number of bacteria in the canal compared to the positive control group. The reason for the difference in the response of bacteria to calcium hydroxide in different studies can be due to the study of this substance at different concentrations and times or the study of different species of *Enterococcus faecalis*.

Many studies have proven the antibacterial effect of tannic acid. The results of a study by Guofeng Dong et al. showed that tannic acid directly interferes with the peptidoglycan wall of bacteria and destroys the integrity of the wall, which ultimately leads to the death of bacteria (28). The results of the present study also confirm the antibacterial effects of tannic acid on *Enterococcus faecalis* in teeth, although its antimicrobial effects were less compared to green-synthesized silica and calcium.

Despite introducing different methods for the production of silica nano-particles, in the present study, the production of these particles was performed by the green method, which is a low-cost, safe, and environmentally friendly approach to the production of nanomaterials in which the tannic acid antibacterial properties are used along with silica (29-31).

Given the results of this study, the two materials, calcium hydroxide and green-synthesized nanosilica, in spite of not being able to completely remove *Enterococcus faecalis* from the canal within a week, showed a strong antibacterial effect. According to other studies and the results of this study, it is recommended that the time of placing the medicament into the canal should be more than 24 hours (32).

Regarding SiNPs, it should be noted that their antibacterial properties are different in different studies, which can be due to differences in the size and morphology of the nanoparticles used, so that the smaller the particles, the greater the antibacterial effect due to the increased contact surface. The type of surfactant and stabilizing compound used can affect its antimicrobial properties as well (33, 34).

Although *Enterococcus faecalis* is one of the most common species in causing dental infections, infected canals usually contain more than one species of bacteria, and the oral environment, including pH and saliva, can also affect the material. Moreover, the most important limitation in using different materials is their toxicity, which must be thoroughly investigated before use in vivo.

However, this study demonstrates the potential properties of green-synthesized SiNPs as an antibacterial agent in teeth and as a possible alternative to calcium hydroxide.

Conclusion

The findings of this study indicate that green-synthesized SiNPs can effectively reduce the *Enterococcus faecalis* bacterial load in the root canal compared to tannic acid and calcium hydroxide under the tested conditions. These results suggest that SiNPs have potential as an adjunctive intracanal medicament; however, further in vivo studies and assessments of long-term safety and efficacy are necessary before clinical application.

Ethics Approval and Consent to Participate

The Ethics Committee of Birjand University of Medical Sciences, Birjand, Iran, approved this study under code IR.BUMS.REC.1399.539. The informed consent form was filled out by the patients before taking part in the study, and they were assured that their information would not be published individually.

Consent for Publication

Not applicable.

Data Availability Statement

Not applicable.

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Authors' Contribution

Conceptualization: Mehdi Shakibaie and Shima Bijari and Maryam Mortazavi; Data gathering, data synthesis, and final approval: All authors.

Conflict of Interest

The authors declared no conflicts of interest.

Declaration of Generative Artificial Intelligence in Scientific Writing

We have not used any AI tools or technologies to prepare this manuscript.

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