The antibacterial properties and mechanism of nanosilver and nanozinc incorporated mesoporous calcium-silicate nanoparticles

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Research

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Abstract

Background: Silver (Ag) and/or zinc (Zn) incorporated mesoporous calcium-silicate nanoparticles (MCSNs) have good physicochemical characteristics and can be advanced materials for root canal filling. This study was to analyze the antibacterial properties and mechanism of Ag/Zn-MCSNs with different percentage of Ag and Zn.

Methods: The antibacterial properties and the cytotoxicity of them were evaluated. Human root canals were inoculated with *E. faecalis* for 4 weeks to establish bacterial biofilm model. The *E. faecalis* biofilms were treated with MCSNs, Ag-MCSNs, Zn-MCSNs, Ag/Zn-MCSNs, calcium hydroxide (CH) and mineral trioxide aggregate (MTA) for 7 days. The ultrastructure and distribution of viable bacteria of the specimens were evaluated using SEM and CLSM. Human root canals were pretreated with CH, MCSNs, Ag-MCSNs, Zn-MCSNs and Ag/Zn-MCSNs, then the root canals were immersed in *E. faecalis* suspension for 7 days. The adhesion and colonization of *E. faecalis* on the root canal walls were observed using SEM and CLSM. Endocytosis of *E. Faecalis* treated by Ag-MCSNs and Ag/Zn-MCSNs were observed using TEM.

Results: The MCSNs containing Ag showed better antibacterial properties than MCSNs and Zn-MCSNs ($P<0.05$). MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(1:9)-MCSNs showed no obvious cytotoxicity ($P>0.05$), while Ag-MCSNs and Ag/Zn(9:1)-MCSNs showed cytostatic effects. Zn-MCSNs slightly promote cell proliferation ($P<0.05$). The MCSNs containing Ag showed antibacterial abilities against *E. faecalis* biofilm in different degree, and can adhere to dentin surfaces to get a continuous antibacterial effect, but MTA, MCSNs and Zn-MCSNs could not disrupt the bacterial biofilm obviously. The Ag-MCSNs and Ag/Zn-MCSNs release Ag^+ and destroy the cell membranes to kill bacteria.

Conclusions: The antibacterial effects and cytotoxicity of Ag/Zn-MCSNs are related to Ag content, while Zn reduce cytotoxicity and promote cell proliferation.

Background

Intracanal medication is an important disinfection way used between appointments of endodontic treatment, because bacteria and bacterial biofilm can persist in the root canal system over a long period of time even after thoroughly chemo-mechanical preparation [1]. Calcium hydroxide (CH) is the commonly used intracanal medication, because CH releases hydroxy ions in liquid, resulting in antimicrobial and endotoxin neutralizing effects, which will be beneficial for regeneration of the periapical hard tissues [2]. However, dentin, exudates from the periapical area, microbial biomass and residual necrotic pulp can inactivate the antimicrobial activities of CH [3]. CH has low solubility and diffusibility, which make it difficult to eliminate the bacteria located within dentinal tubules and anatomical variations of canals [4]. In addition, dentin exposed to CH over time may show reduced flexural strength and low fracture resistance, which will increase the risk of tooth fracture and endodontic treatment failure [5–6].

Mesoporous calcium silicate nanoparticles (MCSNs) are newly synthesized advanced materials with multi-functions for root canal filling because of their special nanostructure, injectability, mineralization of apatite, antibacterial capability and drug delivery [7]. They can release Ca and Si ions and show great ability to deliver various biomolecules in a sustained manner [8–9]. The potential antimicrobial mechanisms of MCSNs are they release SiO_4^{4-} and Ca^{2+} , creating a weak alkaline microenvironment, and the nano-size MCSNs could infiltrate the bacteria [7]. They are considered as excellent platforms for the efficient delivery of drugs and osteogenesis [8–9].

Silver ions (Ag^+) are substitution to antibiotics because they have broad spectrum antimicrobial abilities and show no resistance to target bacteria [10]. Zinc (Zn) is an important trace element for protein and DNA synthesis, cell mitosis and proliferation, and it can increase the proliferation and differentiation of osteoblasts [11]. Zinc ions (Zn^{2+}) can inhibit enzymatic activities and prevent cell metabolism above the threshold ion concentration [12]. Ag and Zn can incorporate with MCSNs and release in a sustained manner. Zhu et al reported that Ag/Zn-MCSNs did not reduce the mechanical properties of dentin for an extended period [5]. Fan et al reported that Ag-MCSNs possess good antibacterial ability and had low cytotoxicity [8]. In our previous studies, we incorporated Ag and Zn into MCSNs by template methods, and found these nanoparticles had good physicochemical characteristics and could adhere to the root canal walls well and infiltrate into the dentinal tubules [5, 8]. However, there are few studies focus on the synergistic antibacterial action of Ag and Zn incorporated MCSNs (Ag/Zn-MCSNs). The aim of this study was to analyze the antibacterial properties of Ag/Zn-MCSNs with different percentage of Ag and Zn.

Materials And Methods

Synthesis of the nanoparticles

MCSNs, Ag-MCSNs, Zn-MCSNs and Ag/Zn(1:1)-MCSNs were synthesized and characterized in our previous studies [5]. Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs were synthesized by a template method according to our previous methods [5,8]. Different weight of silver nitrate and zinc nitrate (Reagent No.1 Factory of Shanghai Chemical Reagent Co., Ltd, China) were added (Table 1).

The prepared Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs were characterized by transmission electron microscopy (TEM, JEM-2100; JEOL, Tokyo, Japan), field emission-scanning electron microscopy (FE-SEM, 1530 VP; LEO, Germany) and energy dispersive spectrometry (EDS, I MCA 300; OXFORD, UK). The surface area, pore volume and pore size distribution according to N_2 adsorption-desorption isotherms (ASAP 2020; Micromeritics, Norcross, GA, USA) were determined using Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analyses. The ions release and pH measurement of the prepared nanoparticles were tested according to our previous methods [5].

Antimicrobial effects of the nanoparticles

E. faecalis (ATCC 29212, Manassas, VA, USA) suspension was diluted into 1×10^4 colony forming units (CFUs)/mL. Then 1 mL suspension was mixed with 10 mg of all prepared nanoparticles and CH respectively, and incubated at 4°C for 24 h. Then 10 μ L inoculums was plated on brain heart infusion (BHI, OXOID, Basingstoke, UK) agar plate and incubated at 37°C for 24 h. Finally, CFUs of *E. faecalis* were counted by Automatic colony counter (Scan 1200, Interscience, France). The test was repeated 6 times for each group.

Cytotoxicity test

The extracts were prepared by adding the nanoparticles to α -MEM medium (Gibco/ Thermo Scientific, Grand island, USA) at 10 mg/mL and incubated at 37 °C for 24 h respectively. Then the supernatant was sterilized using a 0.22 μ m filter (Merck Millipore Ltd., Darmstadt, Germany) after centrifugated at 1000 rpm for 5 min, and supplemented with 10 % fetal bovine serum (FBS, ScienCell, SanDiego, USA).

Mouse pre-osteoblast cell line (MC3T3-E1, ATCC) were cultured in α -MEM medium containing 10% FBS and 100 IU/mL penicillin-streptomycin (HyClone, Utah, USA) at 37 °C in a 5% CO₂ atmosphere. MC3T3-E1 cells were inoculated at density of 2×10^3 cells/well in 100 μ L fresh medium for 48 h. Then, they were treated with different extracts respectively. After 1, 3 and 7 d, the cells were washed with fresh α -MEM. Then they were incubated with 100 μ L α -MEM and 10 μ L CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) in each well at 37 °C and 5% CO₂ for 4h. The absorbance at 450 nm was measured by a spectrophotometer (Spectramax190, Molecular Devices, USA). The cells not treated by extracts were used as controls. The results of optical density (OD) were obtained in sextuplicate.

Antimicrobial activity of the nanoparticles against *E. faecalis* biofilms

Mature human mandibular premolars with single root were collected under the protocol approved by the Ethical Committee Department, the Affiliated Stomatological Hospital of Nanjing Medical University (PJ 2017-055-001). The crowns were removed and roots were standardized to 12 mm long from root apex. The root canals were prepared using ProTaper NiTi rotary instruments (Dentsply Maillefer, Tochigi, Japan) to F3 size according to standard processes. For sterilization, they were autoclaved at 121°C for 20 min. Then they were placed in 3 mL of *E. faecalis* suspension (1×10^8 /mL) and were cultured under anaerobic conditions for 4 weeks at 37°C. The BHI broth was refreshed every second day to remove dead cells and to ensure bacterial viability. CH and MTA pastes (mixed with sterile ddH₂O=1:1.5), MCSNs, Ag-MCSNs, Zn-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs pastes (mixed with sterile ddH₂O=1:3) were prepared. The pastes were introduced into the root canals by lentulo-spirals (Mani Inc, Tochigiken, Japan). Then the roots were placed in sterile tubes. Five specimens were tested in each group. After 7 days, each canal was gently washed with 10 mL phosphate buffer saline (PBS) to clear the intracanal paste and dried with sterilized paper points.

Then the specimens from each group were split into two halves. One root-half randomly selected from each root was scanned with a SEM (HITACHI SU3500, Tokyo, Japan) or FE-SEM (QUANTA 200F, Fei, USA)

according to our previously described method [8]. The other root-half was stained with fluorescent LIVE/DEAD BacLight Bacterial Viability stain (Molecular Probes, Eugene, OR, USA) according to the instructions of manufacturer. Three randomly selected canal wall areas of each root were scanned by a confocal laser scanning microscope (CLSM, LSM 710, Carl Zeiss, Germany) with a 5 μm step size at 20 $\times$ lens. The excitation/emission wavelengths were 488/525 nm for SYTO[®] 9 and 561/642 nm for PI. Simultaneous dual-channel imaging was used to display the green fluorescence (live bacteria) and red fluorescence (dead bacteria) using the ZEN software (Carl Zeiss, Germany).

Bacterial colonization on root canal walls pretreated with the nanoparticles

Forty roots were prepared and autoclaved as described above. Each prepared canal was filled with 10 mg/mL suspension of CH, MCSNs, Ag-MCSNs, Zn-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs and activated by the ultrasonic device (P5XS, Satelec, Cedex, France). The ultrasonic device was set at scale 4, and two 30-second sessions of vibration were applied. All roots were stored in a 100% humid environment at 37°C for 7 days. Then each canal was washed with 10 mL PBS to remove the intracanal medication. All pretreated specimens were immersed in 3 mL *E. faecalis* suspension ($1 \times 10^8/\text{mL}$) at 37 °C for 7 days. Afterwards, each tooth was split into two halves, one root-half randomly selected from each root was observed by the FE-SEM (QUANTA 200F, Fei, USA) and the other root-half was assessed using the CLSM (LSM 710, Carl Zeiss, Germany) according to the methods mentioned above.

Endocytosis of *E. faecalis*

One milliliter *E. faecalis* suspension ($1 \times 10^8/\text{mL}$) was co-cultured with 10 mg of MCSNs, Ag-MCSNs, Zn-MCSNs and Ag/Zn(1:1)-MCSNs for 24 h, respectively. After diluting, the nanoparticle-treated culture material was gained by centrifugation at 3000 rpm for 10 min. Each sample was prepared for TEM observation using standard processes, including fixation, staining, dehydration, infiltration with polymer resin, oven curing, and slicing via ultra-microtome (LEICA EM UC7, Leica, Germany). Ultrathin sections were stained with uranyl acetate and lead citrate and were observed using the TEM (JEOL JEM-1010, JEOL, Japan) at 80 kV.

Statistical analysis

All data were showed as means $\pm$ standard deviation (SD) and were analyzed using One-Way ANOVA with a Post Hoc test by SPSS 22.0 (SPSS Inc., Chicago, IL). The level of significance was set at $P < 0.05$.

Results

Characterizations

Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs possessed spherical morphology with 200-250 nm diameter (Fig. 1 A and B), well ordered nanopores and channel structures (Fig 1. C and D). Ca, Si, Ag and

Zn elements present in both nanoparticles (Fig 1. E and F). The nanoparticles showed type IV isotherms with H1-type hysteresis loops (Fig. 1 G and H). The surface area, pore volume and mean pore size of the nanoparticles were showed in Table 2. Ag/Zn(9:1)-MCSNs showed a faster release of Ag⁺ and a slower release of Zn²⁺ than Ag/Zn(1:9)-MCSNs (Fig. 1 I). The pH values of the nanoparticles gradually increased within 14 days and were stabilized at 10. The addition of Ag did not significantly affect the pH while Zn slightly reduced the pH of the MCSNs (Fig. 1 J).

Antibacterial effect

CH showed the best antibacterial properties among the [experimental groups](#). Ag-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs revealed better antimicrobial capacities than MCSNs and Zn-MCSNs ($P<0.05$) (Fig. 2 and 3).

Cytotoxicity of materials

After 1 d incubation, MCSNs, Ag-MCSNs, Ag/Zn (1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs showed no obvious cytotoxicity ($P>0.05$), while Zn-MCSNs can slightly promote cell proliferation ($P<0.05$). However, CH showed obvious cytotoxicity ($P<0.05$) (Fig. 4 A). After 3d incubation, Ag-MCSNs showed distinct cytotoxicity to MC3T3-E1 cells, Ag/Zn(9:1)-MCSNs also significantly inhibited cell growth ($P<0.05$). MCSNs, Ag/Zn (1:1)-MCSNs and Ag/Zn(1:9)-MCSNs showed no obvious cytotoxicity, while Zn-MCSNs still promote cell proliferation ($P<0.05$) (Fig. 4 B). The results of incubation for 7 d were same as these results of 3 d (Fig 4. C).

Antimicrobial activity of the nanoparticles against *E. faecalis* biofilms

SEM images showed a homogenous and dense *E. faecalis* biofilm on dentin surface (Fig 5. A and a). *E. faecalis* biofilm treated with CH showed deformation and rupture (Fig 5. B and b), while MTA did not destroy the *E. faecalis* biofilm structures (Fig 5. C and c). The biofilms treated with MCSNs, Ag-MCSNs, Zn-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs exhibited different degrees of structural damages (Fig 5. D-I and d-i). Ag-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs can remove most of *E. faecalis* and their biofilms. But there are still intact *E. faecalis* biofilms observed in the MCSNs and Zn-MCSNs groups.

CLSM images displayed a high percentage of live bacteria in the *E. faecalis* biofilm (Fig. 6 A). Most dead bacteria with little live bacteria was observed in CH group (Fig 6 B). The treatment with MTA, MCSNs and Zn-MCSNs revealed a high percentage of green fluorescence in the biofilms (Fig 6. C, D and F). Large areas of red fluorescence and a small amount of scattered green fluorescence were observed in Ag-MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(9:1)-MCSNs groups (Fig 6. E, H and I). The staggered existing red and green fluorescence were observed in Ag/Zn(1:9)-MCSNs group showing a certain antibacterial ability (Fig 6. G).

Bacterial colonization on root canal walls pretreated with the nanoparticles

FE-SEM images showed that innumerable *E. faecalis* colonized on the canal walls in negative control, CH, MCSNs and Zn-MCSNs groups (Fig. 7 A-C, E and a-c, e), and the canal pretreated with CH and Zn-MCSNs seemed to attract more *E. faecalis* than negative group (Fig 7. B, E and b, e). Few bacteria colonization was observed in Ag-MCSNs, Ag/Zn(1:9)-MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(9:1)-MCSNs groups (Fig 7 D, F-H and d, f-h). Many nanoparticles could be found to combine with dentin on root canal walls in experimental groups (Fig 7. C-H and c-h).

CLSM images showed no background fluorescence produced by the root canal wall (Fig 8. A), strong green fluorescence was observed on the canal walls in negative control, CH and MCSNs groups (Fig 8. B-D). Weak green fluorescence and scattered red fluorescence were observed in Ag-MCSNs, Ag/Zn(1:9)-MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(9:1)-MCSNs groups, which indicated very few bacteria survival and colonization (Fig 8. E,G-I). For Zn-MCSNs group, some patches of strong green fluorescence can be seen on the pretreated root canal walls (Fig 8. F).

Endocytosis of *E. faecalis*

Live *E. faecalis* showed typical spherical structure and intact cell membrane in the TEM images (Fig 9. A). For coculture groups, the destruction of cell walls and cell membranes was observed in Ag-MCSNs and Ag/Zn(1:1)-MCSNs groups (Fig 9. B-F).

Discussion

Nano-antibacterial agents have been proposed as a choose for intracanal disinfections because they can disrupt bacterial biofilm and prevent bacterial adhesion to dentin [13–15]. As compared with antibiotics, microorganisms are unlikely to develop resistance against nanoparticles [16]. In this study, the synthesized Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs possess a representative mesoporous structure, and the Ag and Zn are distributed inside the mesoporous structures. The nanoparticles were on the nano-scale and had high surface areas and pore volumes. They release Ca^{2+} and SiO_4^{4-} in aqueous solution, producing a weak alkaline micro-environment and maintaining a high pH value over time.

In this study, MCSNs showed a slight antimicrobial activity, which is agree with our previous study [8]. The released Ca^{2+} and SiO_4^{4-} of MCSNs produce a weak alkaline micro-environment [7], and the nano-dimension of MCSNs may interfere with bacteria metabolism, especially when they are in direct contact with bacteria wall in a liquid environment [17]. Zn^{2+} had antibacterial property that might destroy the bacteria membrane structure, intracellular enzymes and the replicate ability of DNA [18]. However, Zn-MCSNs showed a weak antimicrobial activity. This may be due to the low proportions of Zn contained in Zn-MCSNs and its slow release. Ag-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs showed evident higher antibacterial effects than the MCSNs and Zn-MCSNs groups. Ag incorporated into MCSNs could be released in the form of Ag^+ in liquid environment [8]. Therefore, these phenomena indicated that the antibacterial mechanism of Ag/Zn-MCSNs is main associated with the

release of Ag^+ rather than the Ca^{2+} or SiO_4^{4-} . In addition, the synergistic antibacterial effects of Ag and Zn are not obvious.

In the cytotoxicity test, CH showed very strong cytotoxic while MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(1:9)-MCSNs have no obvious cytotoxicity to MC3T3 cells. However, Ag-MCSNs and Ag/Zn(9:1)-MCSNs significantly inhibited cell growth, and Ag-MCSNs even showed the same strong cytotoxicity as CH. The results indicated that the cytotoxicity of Ag/Zn-MCSNs depends on the amount of Ag, the higher the content of Ag, the greater the cytotoxicity. These results were comparable as previous studies that Ag have adverse effects on human health [16–17, 19]. It was interesting to find that Zn-MCSNs has advantage to promote cell proliferation, the higher the content of Zn in Ag/Zn-MCSNs, the less cytotoxicity of them.

As a cofactor and a structural and regulatory ion, Zn takes major biological roles and involves in homeostasis, immune responses, oxidative stress and apoptosis [20]. Previous studies demonstrated that Zn takes the active part in bone metabolism and has a stimulatory effect on osteogenesis [21–23]. Ag/Zn(1:1)-MCSNs and Ag/Zn(1:9)-MCSNs possess wonderful antibacterial capability without significant cytotoxicity. Therefore, MCSNs containing Zn and low concentrations of Ag can exert excellent antibacterial effects and increase cell proliferation. Further studies will be performed to evaluate the optimal proportions of Zn and Ag.

The antibiofilm activity of the nanoparticles was studied for the potential use in root canal disinfection. MTA is the most representative calcium silicate cements used in clinical, which can induce the generation of hard tissue and achieve ideal apical seal [24]. However, MTA showed no obvious antibacterial effect to *E. faecalis* biofilms. MCSNs showed less antibacterial effective than CH against bacterial biofilms. The possible reason maybe that the changes in interaction between MCSNs and bacteria in bacteria biofilms instead of planktonic bacteria, which compromised the antibacterial effect of MCSNs [8]. Studies reported that nano-sized ZnO exhibit distinct bactericidal mechanisms [12]. However, Zn-MCSNs did not show significant antibacterial biofilm in this study. The possible reasons may be the low Zn^{2+} concentration and slow release of Zn^{2+} of Zn-MCSNs. Ag-MCSNs, Ag/Zn(1:9)-MCSNs, Ag/Zn(1:1)-MCSNs and Ag/Zn(9:1)-MCSNs have improved antibacterial biofilm than MTA, MCSNs and Zn-MCSNs. These results confirm the above-mentioned possible explanations that the antimicrobial mechanism of Ag/Zn-MCSNs is main associated with the released Ag^+ , and the higher the content of Ag, the greater the antibacterial biofilm.

Substantivity on dentin surface is another significant feature of intracanal medications. Fan et al reported that CH paste has very weak substantivity on dentin surface, and there was no residual antimicrobial activity after CH paste being removed [9]. The findings of this study also showed that CH had no substantivity property, and the root canal surface treated with CH seemed to have enrichment effect on *E. faecalis*. MCSNs had no obvious resistance to bacterial adhesion and proliferation, while Ag-MCSNs and Ag/Zn-MCSNs adhered to dentin and infiltrated into dentinal tubules, which will release Ag^+ continuously that get a long-term antibacterial effect. Zn-MCSNs also showed enrichment effect on *E. faecalis* and

seem to encourage *E. faecalis* to form biofilms. This phenomenon may be explained by Zn promote cell proliferation, which is consistent with our cytotoxic results.

Li et al reported that nanoparticles can enter bacterial cells through endocytosis. They may destruct the intracellular structures such as mitochondria, vacuoles, ribosomes and lysosomes, leading to cell lysis [26]. In this study, TEM images showed that little intact *E. faecalis* in the Ag-MCSNs and Ag/Zn(1:1)-MCSNs groups, while more intact *E. faecalis* can be seen in the MCSNs and Zn-MCSNs groups. The results indicated that although the pH and osmotic pressure of solution may rise due to ions releasing of the nanoparticles which made its surroundings harmful to bacterial survival, the Ag⁺ released by Ag-MCSNs or Ag/Zn(1:1)-MCSNs can directly damage bacterial cell walls and membranes and enter the cells, causing cell contents to leak out and destruction of intracellular structures, leading to bacteria death (Fig. 10). It may be the reason why Ag-MCSNs and Ag/Zn-MCSNs showed better antibacterial ability in our experiments.

Conclusions

In this study, we successfully synthesized MCSNs containing different proportions of Ag and Zn. They have ideal physicochemical properties and obvious antibacterial activity. They release Ag⁺ and destroy the cell membranes to kill bacteria. The antibacterial effects and cytotoxicity of Ag/Zn-MCSNs are related to Ag content, while Zn reduce cytotoxicity and promote cell proliferation. Therefore, they have potential for intracanal disinfectant or be developed into sealers for endodontic treatment.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

All authors made substantial contributions to the present study. DL, YL, JZ and RL contributed to acquisition of data, data analysis and writing the paper. CZ, YZ, ML, YW and DR contributed to the selection of the teeth, the literature research and data analysis. DW and JL contributed to the study design, interpretation of the results, revised the paper, supervision. All authors read and approved the final manuscript.

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Availability of data and materials

All data and material are included in this article.

Ethics approval and consent to participate

This study has been approved by the Ethical Committee Department, the Affiliated Stomatological Hospital of Nanjing Medical University and conducted in accordance with ethical guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

MCSNs: mesoporous calcium-silicate nanoparticles; CH: calcium hydroxide; MTA: mineral trioxide aggregate; SEM: scanning electron microscopy; CLSM: confocal laser scanning microscope; TEM: transmission electron microscopy; EDS: energy dispersive spectrometry; BET: Brunauer-Emmett-Teller; BJH: Barrett-Joyner-Halenda; CFUs: colony forming units; BHI: brain heart infusion; FBS: fetal bovine serum; OD: optical density; PBS: phosphate buffer saline; SD: standard deviation;

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Tables

Table 1 The molar ratio of Ag/Zn and the weight of silver nitrate and zinc nitrate added in the synthesis of the nanoparticles

Nanoparticles	Ag: Zn (molar)	Silver nitrate	Zinc nitrate
Ag/Zn-MCSNs 1:9		0.238 g	3.75 g
Ag/Zn-MCSNs 9:1		2.14g	0.416g

Table 2 Surface area (SBET), pore volume (VP), and mean pore size (DP) of the nanoparticles

Nanoparticles	SBET(m ² /g)	VP(cm ³ /g)	DP(nm)
Ag/Zn(9:1)-MCSNs	117.90	0.21	7.05
Ag/Zn(1:9)-MCSNs	225.96	0.40	7.12

Figures

Figure 1

Physicochemical properties of the nanoparticles. A and B: FE-SEM images of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs; C and D: TEM images of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs; E and F: EDS of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs; G and H: Nitrogen adsorption-desorption isotherm test and pore size distribution of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs; I: Ions release of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs; J: pH measurement of Ag/Zn(1:9)-MCSNs and Ag/Zn(9:1)-MCSNs.

Plant Counting

Figure 2

Antibacterial effects of the materials against planktonic *E. faecalis*. Comparisons of CFUs count among the groups, including Negative, CH, MCSNs, Ag-MCSNs, Ag/Zn(9:1)-MCSNs, Ag/Zn(1:1)-MCSNs, Ag/Zn(1:9)-MCSNs and Zn/MCSNs. * $P < 0.05$ when compared with the negative control.

Figure 3

Antibacterial effects of the materials against planktonic *E. faecalis*. A: negative control group; B: CH group; C: MCSNs group; D: Ag-MCSNs group; E: Ag/Zn(9:1)-MCSNs group; F: Ag/Zn (1:1)-MCSNs group; G: Ag/Zn(1:9)-MCSNs group; H: Zn-MCSNs group.

Figure 4

CCK8 test on the MC3T3-E1 cells. A: culture of time for 1 day; B: culture of time for 3 days; C: culture of time for 7 days. * $P < 0.05$ when compared with the negative control (decrease); &# $P < 0.05$ when compared

with the negative control (increase); # $P < 0.05$ when compared with the CCK8 group.

Figure 5

Representative *E. faecalis* biofilms (4-weeks-old) on dentin surface after medication were scanned by SEM or FE-SEM (capital letters: $\times 3,000$ magnification; small letters: $\times 10,000$ magnification). A and a: Negative control group; B and b: CH group; C and c: MTA group; D and d: MCSNs group; E and e: Ag-

MCSNs group; F and f : Zn-MCSNs group; G and g: Ag/Zn(1:9)-MCSNs group; H and h: Ag/Zn(1:1)-MCSNs group; I and i: Ag/Zn(9:1)-MCSNs group.

Figure 6

CLSM 3-dimensional reconstructions of *E. faecalis* biofilms (4-weeks-old) after medication. A: Negative control group; B: CH group; C: MTA group; D: MCSNs group; E: Ag-MCSNs group; F: Zn-MCSNs group; G: Ag/Zn (1:9)-MCSNs group; H: Ag/Zn (1:1)-MCSNs group; I: Ag/Zn (9:1)-MCSNs group (green: live cells; red: dead cells).

Figure 7

Representative FE-SEM images showing colonization of *E. faecalis* on the pretreated root canal walls (capital letters: $\times 3,000$ magnification; small letters: $\times 10,000$ magnification). A and a: Negative control group; B and b: CH group; C and c: MCSNs group; D and d: Ag-MCSNs group; E and e: Zn-MCSNs group; F and f: Ag/Zn(1:9)-MCSNs group; G and g: Ag/Zn(1:1)-MCSNs group; H and h: Ag/Zn(9:1)-MCSNs group.

Figure 8

E. faecalis adherence and colonization on the pretreated root canal walls as observed by CLSM. A: Blank control group (no background fluorescence produced by dentin); B: Negative group; C: CH group; D: MCSNs group; E: Ag-MCSNs group; F: Zn-MCSNs group; G: Ag/Zn(1:9)-MCSNs group; H: Ag/Zn(1:1)-MCSNs group; I: Ag/Zn(9:1)-MCSNs group.

Figure 9

Planktonic *E. faecalis* cocultured with Ag/Zn(1:1)-MCSNs was observed by TEM. A: *E. faecalis* (×30,000); *E. faecalis* cocultured with Ag/Zn(1:1)-MCSNs. B: ×30,000; C and E: ×100,000; D and F: ×200,000.

Figure 10

Antibacterial property, Bacterial biofilm, Mesoporous calcium-silicate nanoparticles, Silver, Zinc