

## Supplementary Information:

### Enhanced eradication of bacterial biofilm with DNase I loaded silver-doping mesoporous silica nanoparticles

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### Experimental Section

#### Synthesis mesoporous silica nanoparticles (MSN)

MSNs with large cone-shaped pores were synthesized based on previous report [15]. Briefly, Cetyltrimethylammonium chloride (CTAC, 4 mL) and 0.04 g triethanolamine (TEA) were stirred in 6 mL water at 60 °C until well-mixed. Subsequently, 9.1 mL chlorobenzene and 0.9 mL tetraethyl orthosilicate (TEOS) were added to the mixture and stirred at 500 rpm for 12 h at 60 °C. The samples were collected by centrifugation at 15,000 rpm for 10 min and washed with water and ethanol. The samples were calcined at 550 °C for 3 h to remove the surfactant.

#### Synthesis of silver-doped MSN (MSN-Ag)

To synthesize silver-doped MSN, the as synthesized MSNs (200 mg) were first modified with amino group by dispersing in 30 mL toluene prior to addition of 0.19 mL 3-aminopropyltriethoxysilane (APTES). The mixture was then refluxed for 20 h at 110 °C. The samples were collected by centrifugation and dried at room temperature. The obtained amino modified MSNs (200 mg) were first dispersed in 150 mL water and then 20 mL silver nitrate solution (containing 66 mg AgNO<sub>3</sub>) were added to the MSNs solution and then stirred for 24 h at room temperature. The samples were collected by centrifugation at 15,000 rpm for 10 min, washed and calcined (named as MSN-Ag).

#### Characterization of nanoparticles

MSN-Ag were examined by field emission transmission electron microscopy (FE-TEM, Tecnai F20 FEG-S/TEM, Tecnai, USA) equipped with high-angle annular dark field (HAADF) STEM detectors and EDX detector (Oxford SDD thin window X-ray detector) operated at 200 kV. The SEM images were obtained using JEOL JSM 7800 field-emission scanning electron microscope (FE-SEM, JEOL, Japan) operated at 0.8-1.5 kV without any coating using gentle bean mode. For FE-SEM measurement, the nanoparticles were prepared by dispersing the nanoparticles in ethanol, after which they were dropped to an aluminium foil piece attached to conductive carbon film on SEM mount. The nanoparticles were dried in a vacuum oven at 60 °C for 12 h and cleaned with evacutron 25/45 De-contaminator RF plasma cleaning system. The pores were characterized by nitrogen adsorption-desorption measurements conducting at -196 °C with a Micromeritics Tristar II system (Micromeritics Instrument Corp. USA). The nanoparticles were degassed at 100 °C overnight on a vacuum line prior to testing. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure (P/P<sub>0</sub>) of 0.99. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface areas. The Barrett-Joyner-Halenda (BJH) method was used to calculate the pore size of nanoparticles from the adsorption branches of the isotherms.

#### DNase I loading capacity test

The DNase I was loaded into MSN-Ag by immersing method. Briefly, DNase I recombinant (from bovine pancreas, Sigma-Aldrich) were dispersed in PBS solution with a concentration of 0.3 mg/mL and mixed with MSN-Ag (0.6 mg/mL, sterilized with ethanol and UV). The mixture was gently shaken at 4 °C for 3 h and then the nanoparticles were separated by centrifugation at 15,000 rpm for 10 min. The residual protein concentration of supernatant was measured by Qubit™ Protein Assay Kit (Thermo-Fischer Scientific) and the amount of DNase I loaded to MSN-Ag was determined by the following equation.

$$\text{Loading capacity (mg/g)} = \frac{\text{initial DNase I amount (mg)} - \text{residual DNase I amount (mg)}}{\text{amount of MSN - Ag (g)}} \quad (1)$$

Subsequently, MSN-Ag-DNase I were aseptically dispersed in 0.6 mL of phosphate-buffered saline (PBS, Bio Scientific) for further studies.

### **Bacterial strains and media**

*Escherichia coli* DH5- $\alpha$  (*E. coli*) and *Streptococcus mutans* ATCC 25175 (*S. mutans*) were commercially available strains from the American Type Culture Collection (ATCC) used as the Gram-positive and Gram-negative models, respectively. *E. coli* and *S. mutans* aerobically grown in lysogeny broth (LB) and brain-heart infusion (BHI) media at 37 °C, respectively. Strains were plated on LB and BHI agar from glycerol stock (-80 °C) and stored at 4 °C for 1 month for working stock. A single colony was picked for further studies.

### **Bacterial culture**

Bacterial pre-culture was made by inoculating 10 mL liquid media with a bacterial colony and followed by a static 24-h incubation at 37 °C aerobically. Five millilitres of bacterial pre-culture was then well-mixed with 35 mL fresh liquid media as bacterial main-culture. The main-culture was incubated at 37 °C for 18 h. Following the incubation, bacteria were collected by centrifugation at  $3197 \times g$  for 5 min at 10 °C (Eppendorf Centrifuge 5810R) and washed twice with sterile PBS. The bacterial pellet was resuspended in 10 mL of PBS. The optical density of bacterial suspension at 660 nm was measured (Spectro Infinite M2000 Pro Tecan) and adjusted to  $10^7$  CFU/mL for following experiments.

### **Biofilm growth**

Bacterial biofilm formation began with adherence of 200  $\mu$ L  $10^7$  CFU/mL bacterial suspensions in polystyrene 96-well plate for 30 min at room temperature. Bacterial suspensions were then gently replaced by 200  $\mu$ L liquid media prior to 24 h aerobically static incubation at 37 °C to form biofilms.

### **Antibacterial activity assay against biofilms**

Total plate count method was used for antibacterial activity assay. The nanoparticles were ethanol sterilized and dispersed in PBS prior to dilution in appropriate liquid media to the concentration of 50, 100, and 200  $\mu$ g/mL. Established 24-h biofilms were rinsed gently with 200  $\mu$ L PBS prior to the anti-biofilm treatments. Biofilms were treated with the nanoparticles in various concentrations. MSN-Ag, MSN, DNase I (300  $\mu$ g/mL), and AgNO<sub>3</sub> (20  $\mu$ g/mL) were also tested as controls. 0.12% chlorhexidine (CHX) and 0.4% sodium hypochlorite (NaOCl) were presented as positive control groups, while fresh liquid media were used as untreated or negative control group. Treatments were applied for 18 h at 37°C statically. Following the treatment incubation, biofilms were collected from individual well and plated on an agar plate and then incubated at 37 °C for 24 h (*E. coli*) and 48 h (*S. mutans*). Bacterial colonies grown on agar plates were counted to determine the number of viable bacteria cells in CFU/mL.

### **Biofilm dispersal assay**

Following the 18-h-biofilm treatments, biofilms were dried at 60°C for 30 min and stained with 100  $\mu$ L of 0.1% crystal violet for 30 min at room temperature. Stained biofilms were subsequently rinsed twice with distilled water and dried at 37°C for 30 min. Crystal violet on stained biofilms was dissolved by 200  $\mu$ L of 20% acetone (in ethanol) for 30 min at room temperature. The optical density of crystal violet was measured at 590 nm (OD<sub>590</sub>) to indicate biofilm biomass left after treatments. The higher the absorbance reading, the more biomass left after treatments. Percentage of remaining biofilm was measured by the following equation.

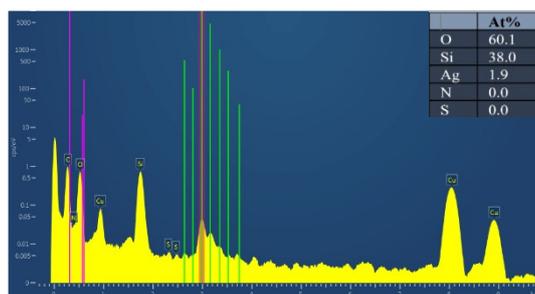
$$\text{Remaining biofilm (\%)} = \frac{\text{OD}_{590} \text{ from treatment group}}{\text{OD}_{590} \text{ from control group}} \times 100 \quad (2)$$

### **Confocal laser scanning microscopy (CLSM)**

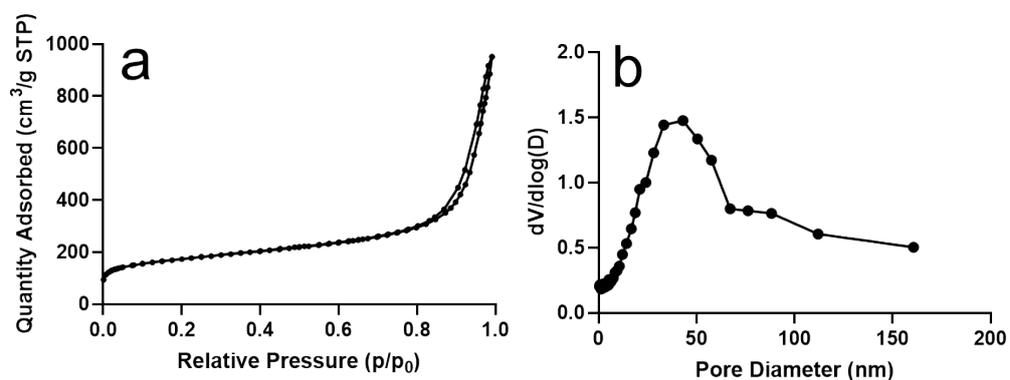
Biofilms were grown on a glass-bottom 24-well plate and treated with the nanoparticles for 18 h. Prior to treatments, 300  $\mu$ L PBS was used to rinse the loosely attached bacteria on individual wells. LIVE/DEAD BacLight™ Bacterial Viability Kit (Thermo-Fischer Scientific) was used to stain biofilms with SYTO 9 and propidium iodide dyes to visualise live and dead bacterial cells, respectively.<sup>41</sup> Staining was done in 20 min under a dark condition at room temperature with 250  $\mu$ L/well of mixed dyes. Random locations of biofilm was imaged by CLSM (Nikon C2+, Nikon, Japan) using NIS-Element Confocal software (Nikon, Japan).

### **Statistical analysis**

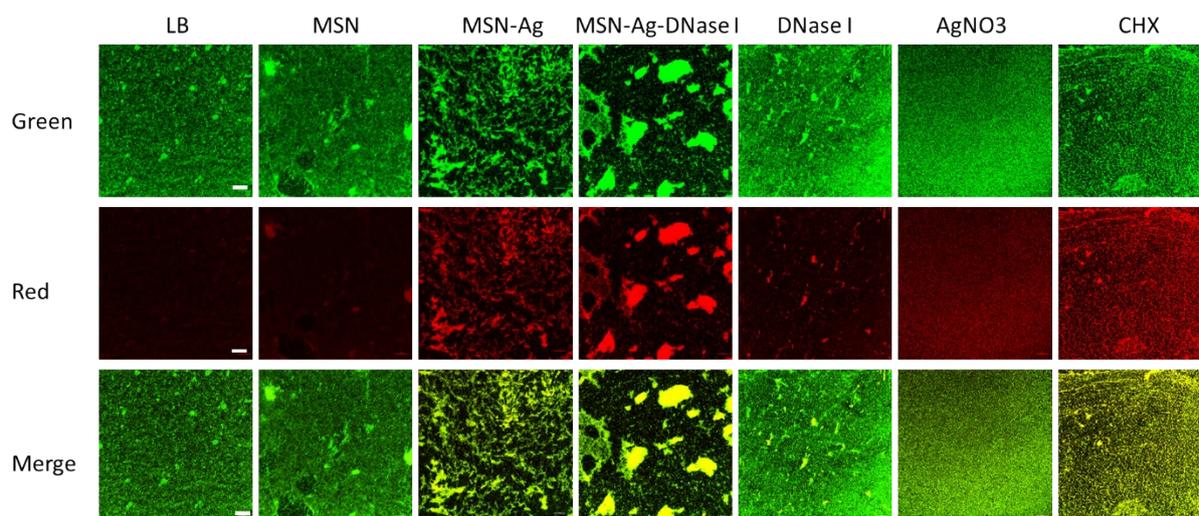
GraphPad Prism 7.00 (GraphPad Software Inc., California, USA) was used to perform statistical analysis. The mean and standard deviation values were calculated and analysed for statistical differences. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests was employed to analyse differences between each treatment group and negative control group.



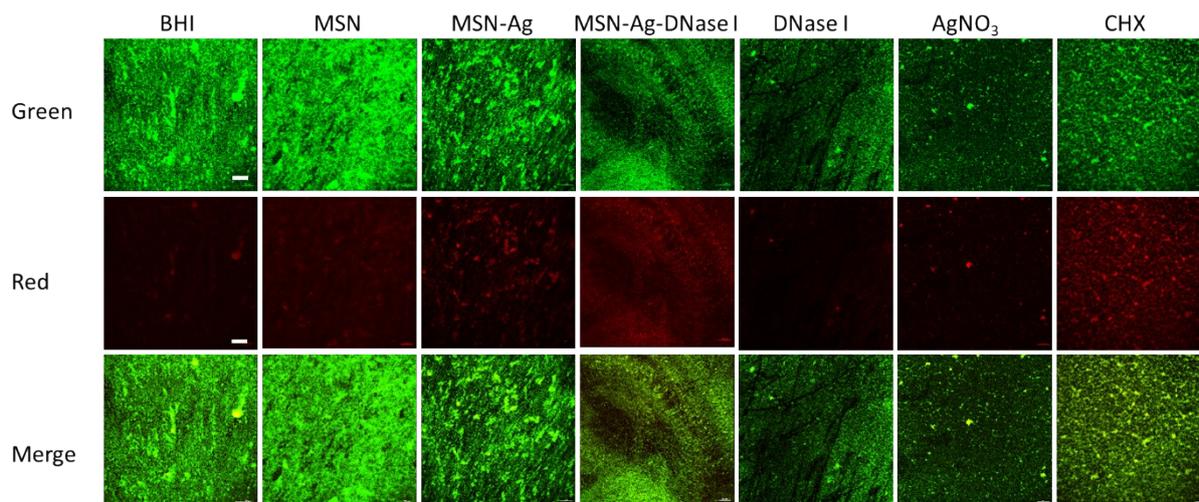
**Figure S1** EDX spectrum of MSN-Ag. Carbon (C), oxygen (O), silicon (Si), and silver (Ag) elements were observed at approximately 0.3, 0.5, 1.8, and 3 keV, respectively. The atom percentage (At %) of Ag was 1.9%, while Si was 38% and O dominated by 60 %.



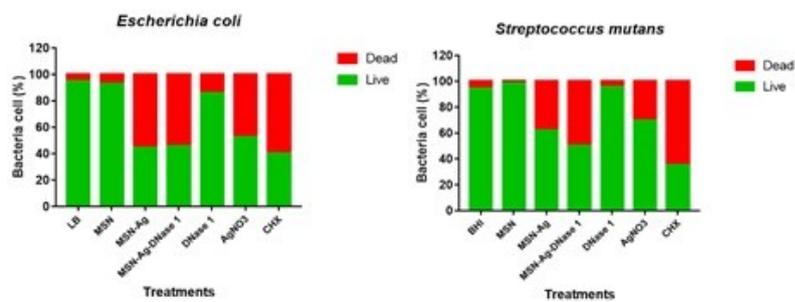
**Figure S2** N<sub>2</sub> sorption isotherms and pore size distribution curve of MSN.



**Figure S3** Bacterial biofilms (*Escherichia coli*) visualization under CLSM after 18 h treatments at 37 °C. Scale bar represented 200  $\mu$ m.



**Figure S4** Bacterial biofilms (*Streptococcus mutans*) visualization under CLSM after 18 h treatments at 37 °C. Scale bar represented 200  $\mu$ m.



**Figure S5** Ratio of live and dead bacteria cells on biofilms analysed from CLSM images.

**Table S1** Physical properties of MSN-Ag and MSN

Sample	BET surface area ( $m^2/g$ )	Pore volume ( $cm^3/g$ )	Pore size (nm)
MSN-Ag	531.4	1.00	41.8
MSN	552.4	1.40	43.1