Supporting Information

Synthesis of Oxidant Prone Nanosilver to Develop H_2O_2 Responsive Drug Delivery System

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This file includes:

1. Experimental materials, characterization and methods.
2. Figure S1 to S6 and Table S1.

MATERIALS:

Chemical reagents used in this study are of analytical grade and used as received. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), Cetyltrimethylammonium bromide (CTAB) and Pluronic F127were purchased from Sigma-Aldrich. Ibuprofen, 3-aminopropyltriethoxysilane (APTES), tetraethyl orthosilicate (TEOS, 99.98%), hexane, rhodamin 6G, citric acid, sodium citrate, and absolute ethanol were purchased from Aladdin reagent Company.

CHARACTERIZATION:

The powder XRD patterns were acquired using Rigaku D/Max 2550 X-ray diffractometer with Cu-Kα radiation (λ = 1.5418 Å). The morphologies and detailed structure of the samples were investigated by JEOL JSM-6700F field-emission scanning electron microscope (SEM) and FEI Tecnai G2 F20 S-TWIN transmission electron microscope (TEM). (λ=1.5418 Å). Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 410 FTIR spectrometer in the range of 400-4000 cm^{-1}. Elemental analysis was carried out on Perkin-Elmer ICP-OES Optima 3300DV. X-ray photoelectron spectroscopy (XPS) was done using an ESCALAB 250 spectrometer. The nitrogen adsorption and desorption isotherms were measured at liquid N_2 temperature by using a Quantachrom Autosorb-iQ, after degassing the sample for 12 h at 120°C. Surface area was calculated according to the conventional BET method and the adsorption branches of the isotherms were used for the calculation of the pore parameters using the BJH method.

2. EXPERIMENTAL SECTION

2.1 Synthesis of citrate stabilized Silver Nanoparticles (Ag NPs): Ag NPs are prepared using a newly developed synthetic protocol. At the outset, 3 g of pluronic F127 was dissolved in 100 mL of Millipore water at 4 C and then $AgNO_3$ (130 mg, 0.75 mmol) was added into surfactant solution. After 30 min stirring, 3 mL of $NaBH_4$ solution (40 mg/mL) was rapidly introduced into light yellow silver-F127solution. Color of solution was quickly changed into brown after the addition of strong reducing agent. In order to provide conjugating sites, minute amount of citric acid (300 μg) aqueous solutions was rapidly introduced into as-synthesized silver solution. **Note:** Compared to previous synthetic strategies, this method generates ultrasmall, water soluble and 10-times more concentrated solution of Ag NPs. Freshly prepared Ag NPs solution can be used within few, however, if long time stability is required then dilution of Ag NPs solution can further increase the stability. Moreover, fresh as-synthesized Ag NPs solution should be used in case of citrate stabilized Ag NPs.

2.2 Synthesis of Mesoporous Silica Nanosphere: Mesoporous silica nanoparticles (MSNs) were prepared via using previously established approach. CTAB surfactant (1.0 g, 1.35 mmol) was first dissolved in 240 mL of water. Then, aqueous solution of sodium hydroxide (2.00 M, 1.7 mL) was introduced into the CTAB solution and temperature of the mixture was elevated to 78°C. After reaching the desired temperature, silica precursor TEOS (2.5 mL, 11.2 mmol) and amine source APTES (250 μL) were successively added into alkaline surfactant solution under vigorous stirring. The solution was stirred for 2 h to obtain a white precipitated product. The resulting product was filtered, washed with nanopure water, ethanol and then dried at 60°C.

2.3 Post-grafting of MSNs (MSNs-NH_2): To further functionalize the outer and inner surfaces of MSNs with amine group, as-prepared MSNs sample (500 mg) was dispersed in 30 mL of dry toluene, containing 500 µL of APTES. Solution was stirred under reflux conditions for 12 h and then washed with ethanol and distilled water. Subsequently, acid treatment was performed to remove CTAB by dispersing MSNs-NH_2 nanoparticles in 100 mL of methanol solution having 3 mL of 37%HCl. The suspension was refluxed at 80°C for 12 h. The final product was centrifuged and washed with ethanol and water.

2.4 Loading, Capping and Release Experiments: For loading hydrophobic Ibuprofen (IBU) and rhodamin 6G cargo molecules in the nanopores of MSNs, amine functionalized nanoparticles (50 mg) were introduced into ethanolic solution of ibuprofen (10 mL, 3 mg mL^{-1}) and rhodamine 6G and stirred the samples for 12 h. After 12 h stirring, both solutions were centrifuged and washed with ethanol and water. In order to cap IBU and rhodamine 6G loaded MSNs, respective samples were dispersed separately in water and then 1mg EDC and 20 mL of freshly prepared aqueous solutions of citrate- functionalized Ag NPs were added into both MSNs solutions under sonication. Samples were quickly centrifuged and washed with water. To remove extra cargo molecules from uncapped nanopores, wet powders were stirred in water for one day and then centrifuged and dried at room temperature. The loading quantity of IBU (95 mg g^{-1}) was calculated by using UV/Visible spectroscopy, whereas the loading amount rhodamin 6G was found to be around 32.5 mg g^{-1}. Capping protocol was investigated by studying the release profiles of both Ag@MSNs nanoformulations in H_2O_2 oxidizing medium at various concentrations, using a dialysis bag diffusion technique. Briefly, 10 mg of IBU and/or Rhd 6G loaded Ag@MSNs samples were dispersed in 3 mL of Tris buffered (5 mM) hydrogen peroxide solutions. Samples were then sealed in a dialysis bag (molecular weight cutoff = 8000). The

dialysis bag was later submerged in 20 mL of water and stirred for 3 days. The released cargo molecules in the buffer were collected at predetermined time intervals and monitored by UV/Vis spectroscopy.at 265nm and 525 nm respectively.

2.5. Bacteria culture: Escherichia coli DH5a (E. coli), as the Gram-negative model was used to determine bactericidal activity. Bacteria cells were incubated overnight at 37 C in Luria-Bertani (LB) medium containing tryptone (50 mg), yeast extract (25 mg) and NaCl (50 mg) in 5 mL sterile distilled water at pH 7.0. These fresh overnight cultures of bacteria were diluted to obtain a required concentration before every experiment.

2.6. Bacteria kinetic test: For the growth-curve experiments, a starter culture of each strain was inoculated with fresh colonies and incubated for overnight in LB Lennox media. Bacterial growth rates were evaluated by measuring the optical density at 600 nm using a spectrophotometer (Eppendorf BioPhotometer). Fresh media were inoculated with the starter culture and grown to an OD_{600} of 0.1 at 37 C with continuous agitation at 200 rpm. LB broth containing bacteria was used as a positive control. Various concentrations of Ag@MSNs nanostructures were then added to the culture and the turbidity of the solutions were measured in the course of time. The experiments were performed in triplicate.

2.7. LB-agar plates: Different concentrations of Ag@MSNs nanostructures were mixed with molten LB-agar at varying final concentrations (2.5, 5 and 10 μg/mL).Serial dilution (1/104) of late log phase bacteria (OD600¼ 1.0) were then spread onto solidified LB agar plates. Lastly, plates were incubated at 37 C for 12 h, and the viable cells were counted. The experiments were performed twice.

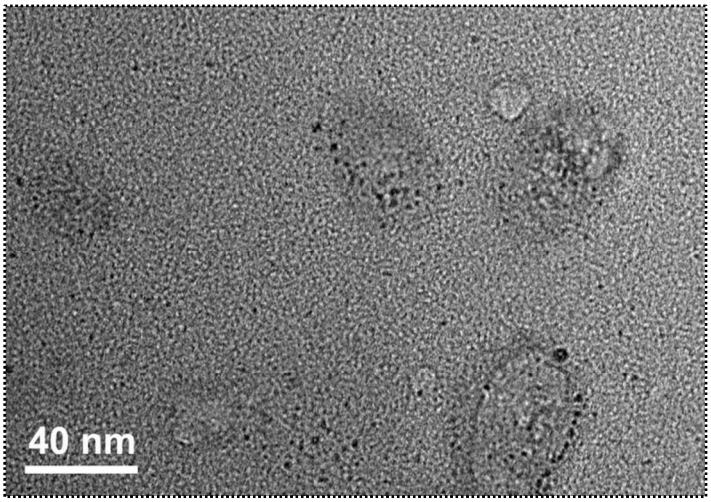

Figure S1. (a) TEM images of Ag NPs generated in the micelles of F127

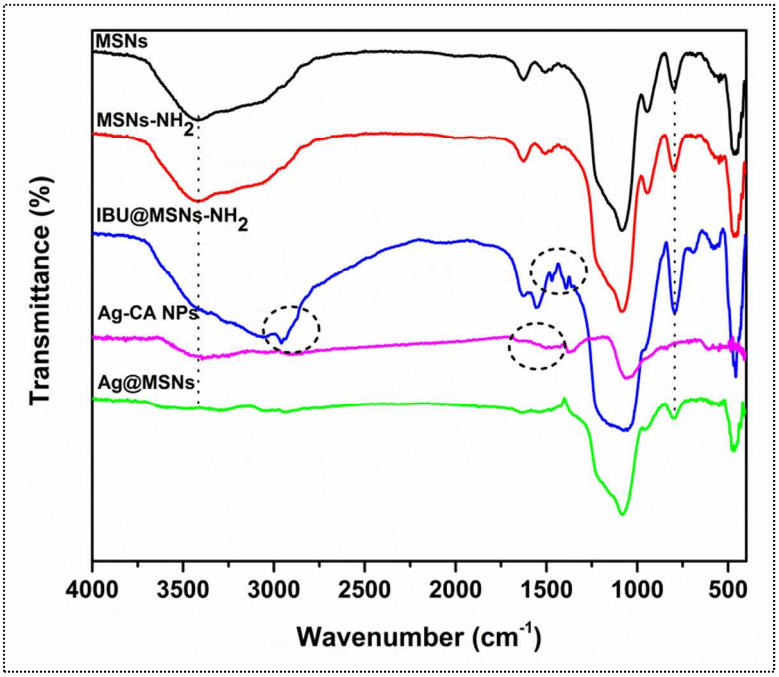

Figure S2. FTIR spectra of MSNs, MSNs-NH_2, IBU@MSNs-NH_2, Ag-CA NPs, Ag@MSNs

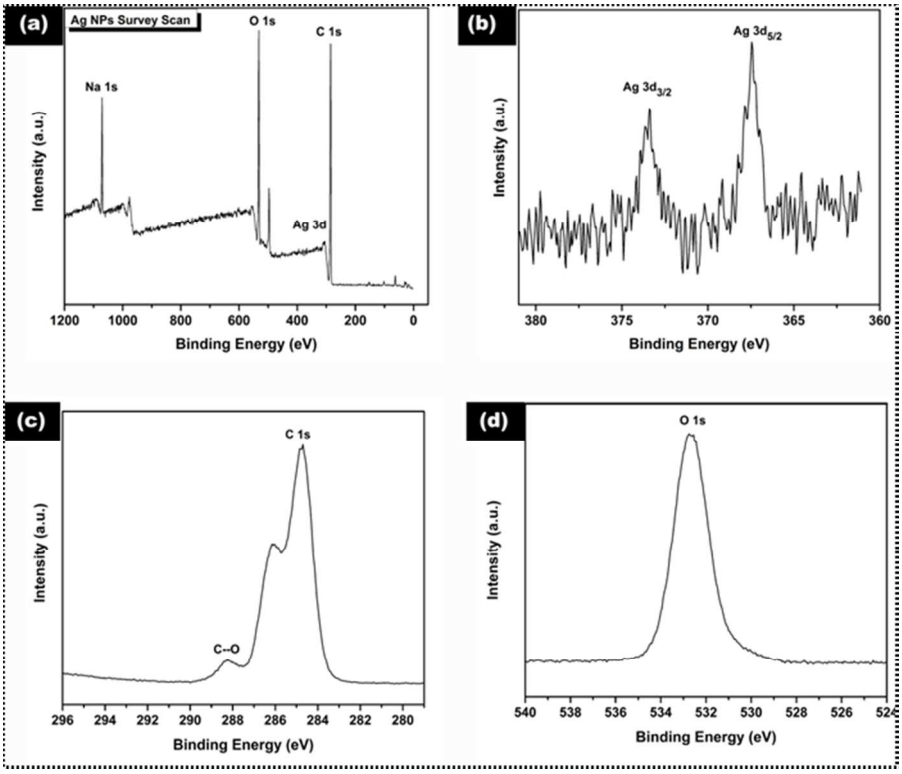

Figure S3. (a) XPS survey scan spectrum of Ag NPs (b) spectrum of silver (c) Spectrum of Carbon (d) Spectrum of Oxygen.

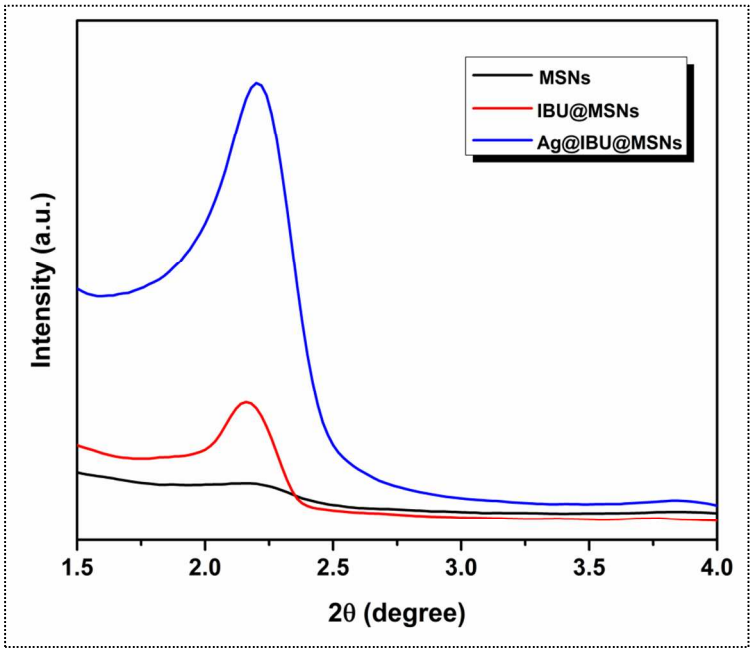

Figure S4. Low-angle X-ray diffraction (XRD) patterns of MSNs-NH_2, IBU@MSNs-NH_2, Ag@IBU@MSNs The PXRD demonstrates an obvious decrease in the intensity of the MCM-41 type peaks after drug loading and capping Ag nanolids.

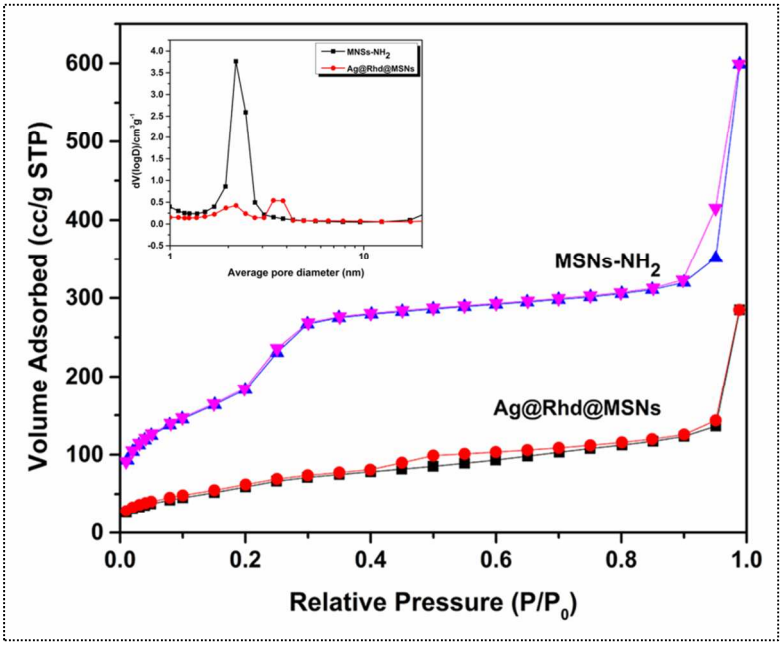

Figure S5. Nitrogen adsorption−desorption isotherms for MSNs-NH_2 and Ag@Rhd@MSNs samples, revealing the clogging of nanochannels after drug loading and pore capping. Inset: pore size distribution of respective samples.

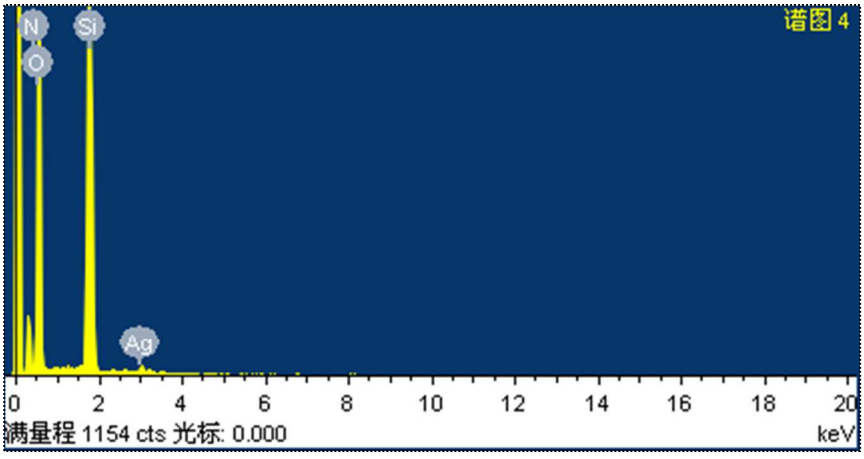

Figure S6. EDX spectrum of Ag@MSNs, which suggests the conjugation of Ag NPs onto the surface of MSNs.

Materials	ζ-potential value
Ag-CA	-10.2 mV
MSNs	-9.45 mV
MSNs-NH_2	30.8 mV
Ag@MSNs	27.3 mV

Table S1. Zeta Potential of various synthesized nanoparticles to reveal surface modifications.