Electronic Supplementary Information (ESI) for:

Onsite formation of small Ag nanoparticles on superhydrophobic mesoporous silica for antimicrobial application

Yang Zhu,[a] Chunhua Wu,*[b] Kazuyoshi Kanamori,[a] Toshiyuki Kamei,[c] Toyoshi Shimada,[d] Kazuki Nakanishi*[d,e]

[a] Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto, 606-8502, Japan.
[b] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[c] Nara National College of Technology, 22-Yata-cho, Yamatokoriyama, Nara 639-1080, Japan.
[d] Institute for Integrated Cell-Material Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan.
[e] Institute of Materials and Systems for Sustainability, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan.

1. Experimental section

1.1. Chemicals and reagents

Hydrochloric acid and acetone were purchased from Kishida Chemicals Co., Ltd. (Japan). Silver acetate (AgOAc), poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) ($EO_{20}PO_{70}EO_{20,}$ P123) and octadecylsilane were obtained from Sigma-Aldrich Co. (USA). Tris(pentafluorophenyl)borane ($B(C_6F_5)_3$) and palladium(II) chloride ($PdCl_2$) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Dichloromethane (DCM, dehydrated, 99.5%) was obtained from Kanto Chemical Co., Inc. (Japan). Tetraethoxysilane (TEOS) was purchased from Shin-Etsu Chemical Industry Co., Ltd. (Japan). Distilled water was acquired from Hayashi Pure Chemical Ind., Co., Ltd. (Japan). All chemicals were of analytical grade or above, and used as received without further purification.

1.2. Surface functionalization of SBA-15-type mesoporous silica particles

SBA-15-type mesoporous silica was synthesized using a supramolecular self-assembly synthetic method, as described by Zhao et al.[1] The supermolecular templates in the materials were removed by calcination of the as-synthesized material at 600 °C for 2 h. Modification of the silica surface with octadecylsilane was performed according to our previous report.[2] Typically, 500 mg of SBA-15 (dried at

200 °C for 4 h before use) was mixed with 15 mL of anhydrous DCM. Into this dispersion a mixture of 1 mL of octadecylsilane and 25 mg of the $B(C_6F_5)_3$ catalyst was added. The formation of H_2 gas bubbles was quickly observed. The solution was kept shaking at room temperature until the formation of bubbles was no longer observed. The ODS/hydrido-modified SBA-15 (denoted hereafter as ODS-H-SBA-15) was then recovered by centrifugation and washed with plenty of DCM and dried at room temperature for more than 24 h.

For the introduction of Ag metal NPs, 100 mg of the dried ODS-H-SBA-15 was soaked in a 100 mL H_2O/acetone (*V*/*V* 1/99) solution of AgOAc with various concentrations (0.1, 0.5 and 1 mmol L^{-1}). The mixture was sonicated for 30 min. The resulting material (denoted hereafter as *X*Ag-ODS-SBA-15, where *X* represents the concentration of AgOAc used, for instance, 1Ag-ODS-SBA-15 for 0.1 mmol L^{-1} of AgOAc) was retrieved by centrifugation, washed with plenty of hot water to remove the unreacted AgOAc, and finally dried at 50 °C for more than 24 h.

The plausible reaction mechanism for the surface functionalization of octadecylsilane on silica surface and the following reduction of Ag^+ into Ag NPs by the residual hydrido groups is shown in **Scheme 1**.

1.3. Antimicrobial tests

Two typical pathogen bacteria, a Gram-negative bacteria *Escherichia coli O157:H7* (ATCC25922) and a Gram-positive bacteria *Staphylococcus aureus* (KCTC 1928), provided by Hope Bio-Technology Co. Ltd. (Qingdao, Shandong, China), were used in the present study.

Minimum inhibitory concentration (MIC) of the obtained SBA-15-based materials was determined by a broth dilution method. Briefly, Log phase *E. coli* or *S. aureus* cells (10^5 CFU) was mixed thoroughly with the SBA-15-based materials with different concentrations, and incubated at 37 °C for overnight. To investigate the antimicrobial activity of the SBA-15-based materials on the bacterial growth, the MIC of SBA-15-based materials for *S. aureus* and *E. coli* was determined by optical density (OD_{600}) of the bacterial culture solution after 24 h. The experiments were carried out in triplicate.

The colony-forming count method was performed according to the previously study[3] with slight modifications. Briefly, The SBA-15-based materials were mixed with molten LB-agar medium at double MIC concentrations. PBS was used as controls. After the agar was cooled to room temperature, Log phase *E. coli* and *S. aureus* cells (10^6 CFU) were plated onto the above agar plates and incubated at 37 °C, followed by counting the number of bacterial colonies after 24 h incubation.

2. Characterizations

The grafting of octadecylsilane on the surface of silica was investigated by Fourier transform infrared spectroscopy (FT-IR, IR Affinity-1, Shimadzu Co., Japan) using the KBr method and thermogravimetry-differential thermal analysis (TG-DTA, Thermal Plus TG 8120, Rigaku Co., Japan) with a continuous air supply at 100 mL min^{-1}. The presence of Ag NPs on the surface of silica was confirmed by X-ray diffraction (XRD, RINT Ultima III, Rigaku, Co., Japan) using Cu Kα (λ = 0.154 nm) as an incident beam and ultraviolet-visible absorption spectroscopy (UV-Vis, V-670， JASCO Co., Japan). The size, morphology and spatial distribution of Ag NPs were characterized by transmission electron microscopy (TEM, JEM-1400 Plus, JEOL, Japan). The valence state of metal species in the obtained materials was investigated by X-ray photoelectron spectroscopy (XPS, ESCA 3057, ULVAC-PHI, Inc., Japan). In the XPS spectra, the peak at 284.6 eV corresponding to C 1s was used as the reference. The loading of Ag NPs in the SBA-15 materials were determined by inductively coupled plasma-mass spectroscopy (ICP-MS, NexION 300X, PerkinElmer Inc., USA). Ag NPs in the samples were first dissolved in concentrated nitric acid and then diluted for the measurement. The final value of Ag loading for each sample was determined by the average value of four measurements. The meso- and micropore structure was characterized by nitrogen adsorption-desorption measurement (BELSORP-mini II, MicrotrackBEL Corp., Japan). The samples were degassed at 120 °C for 6 h before each measurement. The water contact angle was measured by Drop Master DM-561Hi, Kyowa Interface Science Co., Ltd., Japan. Volume of the water droplet was fixed at 6.0 μL and the contact angle was determined as the average of 20 times of measurements in 20 s of time interval.

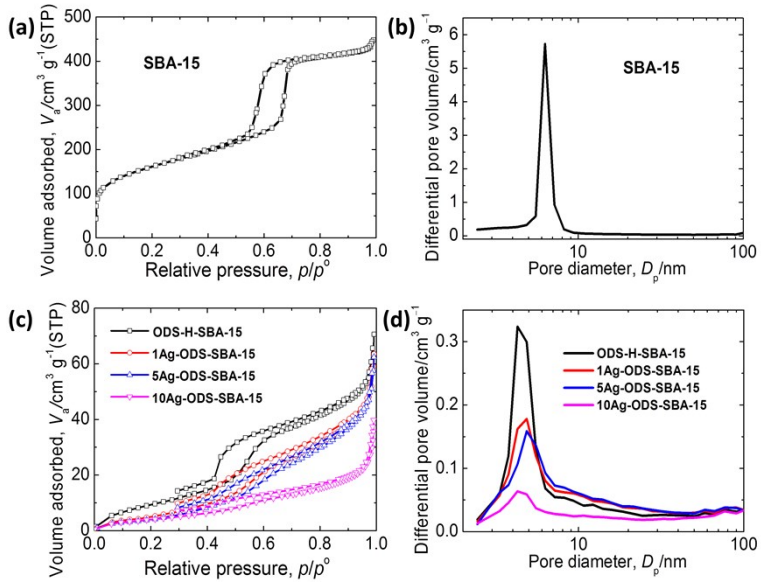

Fig. S1 (a) N_2 adsorption desorption isotherm and (b) BJH pore size distribution

(obtained from the adsorption branch) of SBA-15, (c) N_2 adsorption desorption isotherm and (d) BJH pore size distribution (obtained from the adsorption branch) of ODS-H-SBA-15, 1Ag-ODS-SBA-15, 5Ag-ODS-SBA-15 and 10Ag-ODS-SBA-15.

Table S1 Pore Characteristics of SBA-15, ODS-H-SBA-15, 1Ag-ODS-SBA-15, 5Ag-ODS-SBA-15 and 10Ag-ODS-SBA-15.

	S_{BET}*1 / m^2 g^{-1}	V_p*2 / cm^3 g^{-1}	D_p*3 / nm
SBA-15	560	0.60	6.3
ODS-H-SBA-15	40	0.11	4.3
1Ag-ODS-SBA-15	19	0.10	4.8
5Ag-ODS-SBA-15	15	0.10	4.8
10Ag-ODS-SBA-15	17	0.06	4.2

*1 BET surface area, *2 specific pore volume, *3 modal pore diameter from the BJH method.

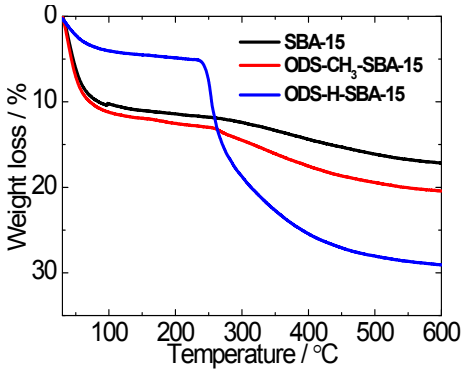

Fig. S2 TG curves of SBA-15, ODS-CH_3-SBA-15 (dimethylosctadecylsilane-modified SBA-15) and ODS-H-SBA-15.

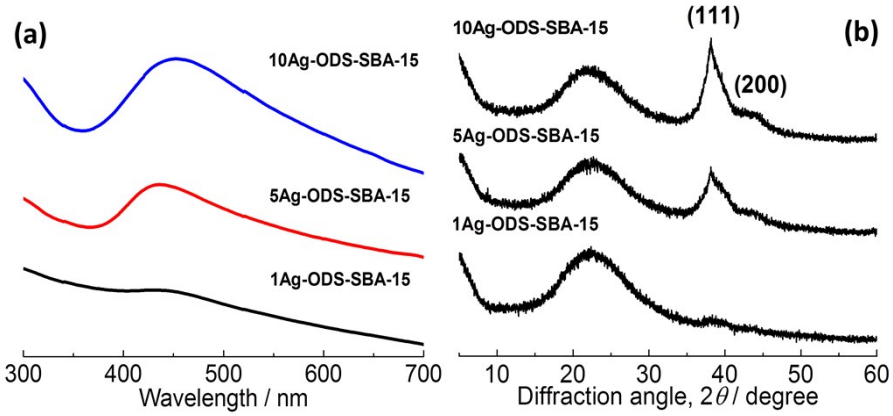

Fig. S3 (a) UV-Vis spectra and (b) XRD patterns of 1Ag-ODS-SBA-15, 5Ag-ODS-SBA-15 and 10Ag-ODS-SBA-15.

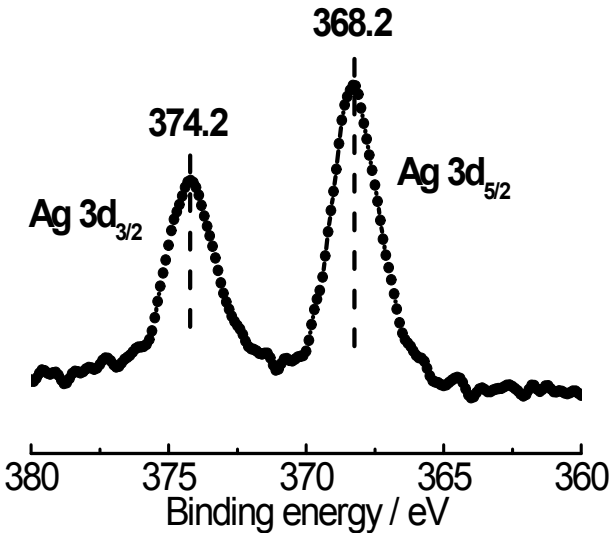

Fig. S4 XPS spectrum of Ag 3d peaks of 10Ag-ODS-SBA-15.

References for ESI Section

1. Zhao, D.; Feng, J.; Huo, Q.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. F.; Stucky, G. D., Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores. *Science* **1998,** *279* (5350), 548-552.

2. Moitra, N.; Ichii, S.; Kamei, T.; Kanamori, K.; Zhu, Y.; Takeda, K.; Nakanishi, K.; Shimada, T., Surface functionalization of silica by Si-H activation of hydrosilanes. *J. Am. Chem. Soc.* **2014,** *136* (33), 11570-3.

3. Tian, Y.; Qi, J.; Zhang, W.; Cai, Q.; Jiang, X., Facile, one-pot synthesis, and antibacterial activity of mesoporous silica nanoparticles decorated with well-dispersed silver nanoparticles. *ACS Appl. Mater. Interfaces* **2014,** *6* (15), 12038-45.