Supplemental Information

Shell crosslinked nanoparticles carrying silver cation and silver carbene antimicrobials as therapeutics

Yali Li,[a] Khadijah Hindi,[b] Kristin M. Watts,[b] Jane B. Taylor,[b] Ke Zhang,[a] Zicheng Li,[a] David A. Hunstad,[*b,c] Carolyn L. Cannon,[*b,c] Wiley J. Youngs,[d] and Karen L. Wooley[*a,e]

[a] *Departments of Chemistry and Radiology, Washington University, St. Louis, Missouri, 63130-4899, USA*
[b] *Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, 63110-1002, USA. Fax: +1 314 286-2895; Tel: +1 314 286-2954; E-mail: cannon_c@kids.wustl.edu*
[c] *Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, 63110-1002, USA. Fax: +1 314 286-2895; Tel: +1 314 286-2874; E-mail: hunstad_d@kids.wustl.edu,*
[d] *Department of Chemistry and Center for Silver Therapeutics Research, University of Akron, Akron, Ohio, 44325-3601, USA. Fax: +1 330 972-7370; Tel: +1 330 972-5362; E-mail: youngs@uakron.edu*
[e] *Department of Chemistry and Department of Chemical Engineering, Texas A&M University, College Station, Texas, 77842-3012, USA Fax: +1 979 862-1137; Tel: +1 979 845-4077; E-mail: wooley@mail.chem.tamu.edu*

Materials

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used without further purification unless otherwise noted. SCC10[1] and SCK (30% crosslinked, 0.265 mg/mL, prepared from PAA_{130}-*b*-PS_{40}, D_{av} = 10 ± 1 nm (TEM))[2,3] were synthesized as previously reported. Amicon® ultra centrifugal filter devices (100 kDa MWCO) were purchased from Millipore Corp. (Bedford, MA). Nanopure water (18 MΩ·cm) was acquired by means of a Milli-Q water filtration system (Millipore Corp., Bedford, MA).

Measurements

UV-vis spectra were collected on a Varian Cary 100 Bio UV-visible spectrophotometer (Varian, Inc., Palo Alto, CA) using PMMA cuvettes.

Dynamic light scattering (DLS) measurements were conducted with a Brookhaven Instrument, Co. (Holtsville, NY) DLS system equipped with a model BI-200SM goniometer, BI-9000AT digital correlator, and a model EMI-9865 photomultiplier, and a model Innova 300 Ar ion laser (Coherent Inc., Santa Clara, CA), operated at 514.5 nm. Measurements were made at 25 ± 1 °C. Prior to analysis, solutions were filtered through a 0.45 μm Millex®-GV PVDF membrane filter (Millipore Corp., Medford, MA) to remove dust particles. Scattered light was collected at a fixed angle of 90 °. The digital correlator was operated with 522 ratio spaced channels, and initial delay of 5 μs, a final delay of 50 ms, and a duration of 2 minutes. A photomulitplier aperture of 400 μm was used, and the incident laser intensity was adjusted to obtain a photon counting of between, 200 and 300 kcps. The calculations of the particle size distributions and distribution averages were performed with the ISDA software package (Brookhaven Instruments Company), which employed single-exponential fitting, cumulants analysis, non-negatively constrained least-squares (NNLS) and CONTIN particle size distribution analysis routines. All determinations were average of values from five measurements.

Inductively coupled plasma-mass spectrometry (ICP-MS) was performed on a 7500ce Agilent, quadrupole mass spectrometer, equipped with an octapole reaction cell for removal of polyatomic interferences, and using 2% HNO_3 as the matrix and Tl (III) as internal standard.

High-resolution transmission electron microscopy (HRTEM) measurements were conducted using a JEOL 2010F field-emission TEM with a 200 kV acceleration voltage. The TEM instrument has a point resolution of 1.9 Angstrom, and it's therefore capable of imaging the (111) and (100) lattice spacings of metallic Ag. The Bruker XFlash Energy-Dispersive Spectroscopy (EDS) detector is also installed in the TEM. The EDS capability, which has a detection limit for most elements of about 0.5 wt%, was used to confirm the presence of Ag element. The samples were supported on carbon-coated copper grids, which were prepared by ethanol treatment to make the surface hydrophilic. The sample solution was deposited onto a grid, after 1 minute excess solvent was wicked away.

Transmission electron microscopy (TEM) measurements were conducted using a Hitachi H-600 TEM with a 80 kV acceleration voltage. The samples were supported on carbon-coated copper grids, which were prepared by ethanol treatment to make the surface hydrophilic. The sample solution was deposited onto a grid, after 1 minute excess solvent was wicked away. A drop of 1% phosphotungstic acid (PTA) stain was then added, and left for 1 minute before excess stain was wicked away. Particle diameters and standard deviations were calculated from measurements of a minimum of 100 particles from three TEM micrographs from different regions.

General procedure for the preparation of $AgNO_3$-SCK

A solution of $AgNO_3$ (10 mg/mL in water) was added to the SCK solution (1 mL). The solution was protected from light and stirred overnight at room temperature. The solution was transferred to a centrifugal filter device (100 kDa MWCO), and washed extensively with nanopure water to remove free small molecules. Sodium chloride was added to the filtrate until no precipitation formed to confirm the removal of any free Ag^+. The resulting $AgNO_3$-SCK solution was then reconstituted to a final volume of 1 mL. The Ag^+ capacities of the nanoparticles were measured by ICP-MS. $D_{av} = 9 \pm 1$ nm (TEM)

General procedure for the preparation of SCC10-SCK

A solution of SCC10 (10 mg/mL in $CHCl_3$) was added to the SCK solution (1 mL). The solution was protected from light and stirred vigorously to form a water/oil emulsion at room temperature. After evaporation of the organic solvent overnight, the solution was transferred to a centrifugal filter device (100 kDa MWCO), and washed extensively with nanopure water to remove unincorporated SCC10. The filtrate was analyzed by UV-vis spectroscopy to confirm the removal of any free SCC10. The resulting SCC10-SCK solution was then reconstituted to a final volume of 1 mL. The Ag^+ capacities of the nanoparticles were measured by ICP-MS. $D_{av} = 9 \pm 2$ nm (TEM)

Loading capacity measurements

A series of $AgNO_3$-SCK and SCC10-SCK solutions with different silver loading percentage (20%, 50%, 100%, 150%, 200% loading with respect to moles of acrylic acid residues in the polymer for $AgNO_3$-SCK, and with respect to the polymer weight of the

SCK solution for SCC10-SCK, respectively). $AgNO_3$-SCK nanoparticles reached [Ag^+] of *ca*. 370 μg/mL at 200% feed, while SCC10-SCK nanoparticles had a capacity of *ca.* 75 μg/mL at 200% feed. At higher than 200% feed, for silver-loaded SCKs, cloudiness and precipitation of the nanoparticles were observed.

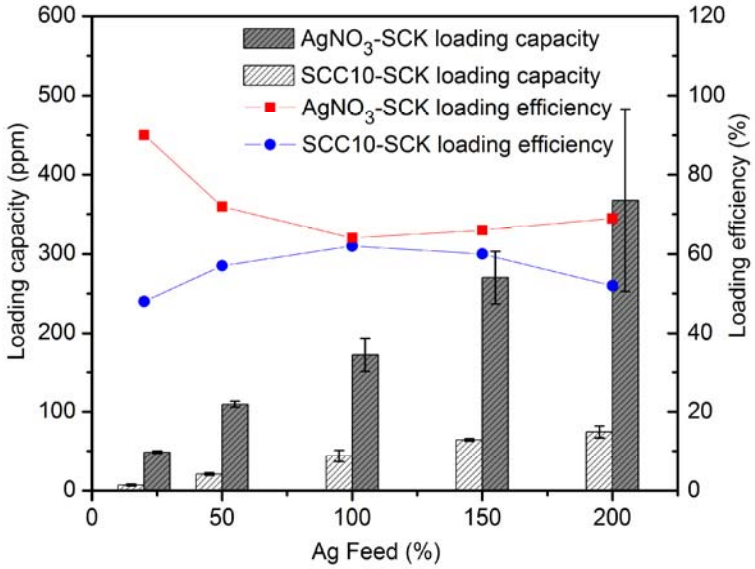

Figure S1. Ag loading capacities (left axis) and efficiencies (right axis) of $AgNO_3$-SCK and SCC10-SCK with different silver feed (20%, 50%, 100%, 150%, 200% with respect to the combined total theoretical moles of acrylic acid and amide residues in the polymer for $AgNO_3$-SCK, and with respect to the polymer weight of the SCK solution for SCC10-SCK, respectively).

General procedure for the preparation of sequential silver-loaded SCKs, $AgNO_3$-SCC10-SCK and SCC10-$AgNO_3$-SCK

A solution of $AgNO_3$ (65 μL, 10 mg/mL in water, 150% loading) was added to the SCK solution (1 mL). The solution was protected from light and stirred for 1 h at room temperature. A solution of SCC10 (41 μL, 10 mg/mL in $CHCl_3$, 150% loading) was then added to the above solution, and stirred vigorously to form a water/oil emulsion at room temperature. After evaporation of the organic solvent overnight, the solution was transferred to a centrifugal filter device (100 kDa MWCO), and washed extensively with nanopure water to remove free small molecules. The filtrate was analyzed by UV-vis spectroscopy to confirm the removal of any free small molecules, and this process was repeated until no further small molecule release into the filtrate was observed. The resulting $AgNO_3$-SCC10-SCK solution was then reconstituted to a final volume of 1 mL. For the preparation of SCC10-$AgNO_3$-SCK, $AgNO_3$ solution was added 1 h after the addition of SCC10 solution. Sequential loaded SCKs with 200% feed were observed to become cloudy and some precipitations were found. The Ag^+ capacities of the nanoparticles were measured by ICP-MS to be 250 μg/mL for $AgNO_3$-SCC10-SCK, and 210 μg/mL for SCC10-$AgNO_3$-SCK, respectively. D_{av} = *ca.* 9 nm (TEM)

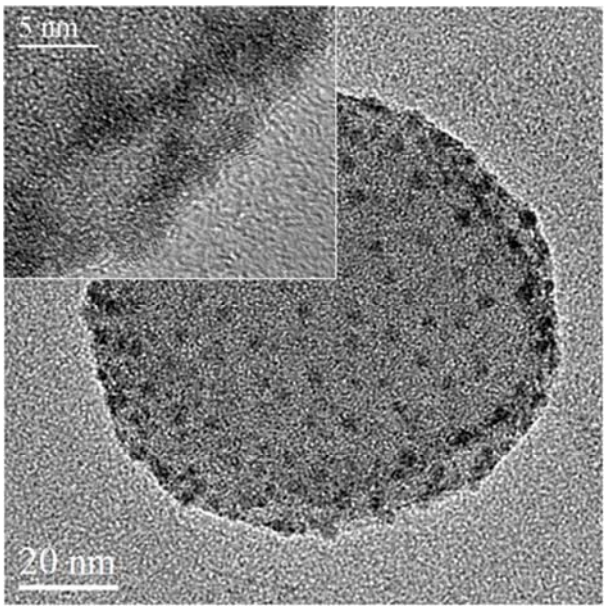

Figure S2. High-resolution TEM image of $AgNO_3$-SCK nanoparticles with elemental silver on the surface, which were measured to have metallic Ag lattice spacing = 2.36 Å = $d_{Ag(111)}$. (For clarity reason, a relatively larger than average nanoparticle was selected to identify the lattice spacing of the elemental silver).

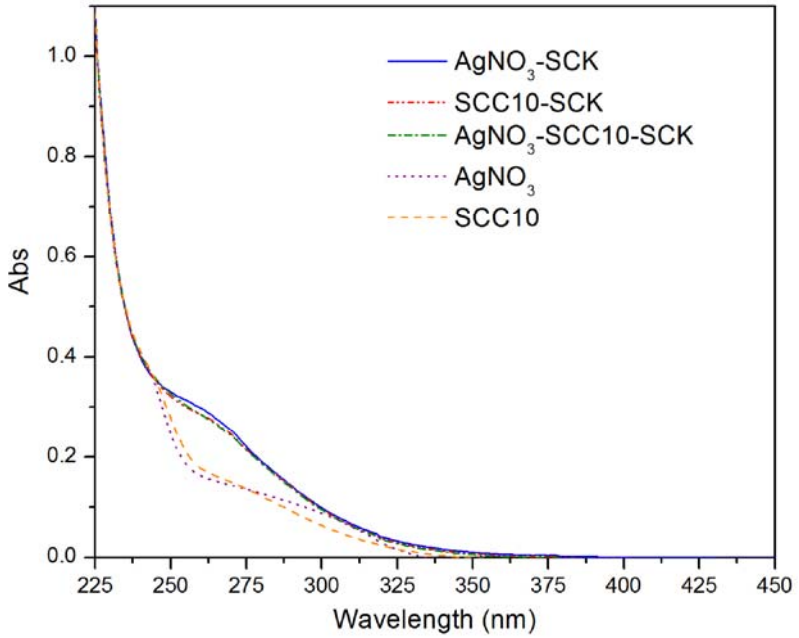

Figure S3. UV-vis spectra of silver-bearing nanoparticles after purification, and control samples, $AgNO_3$ and SCC10. Depletion of a UV-vis silver signal in the filtrates, following centricon centrifugal purification, combined with measurement of silver content by ICP-MS confirmed the purification of the Ag-loaded nanoparticles. The absence of any signal from 380-440 indicates the absence of large silver particles (*ca*. >10 nm).

General procedure for the release of silver from SCK nanoparticles

Silver-bearing SCKs (2 mL) was transferred to a presoaked dialysis cassette (Slide-A-Lyzer, 10 kDa MWCO, Pierce Biotechnology, Rockford IL, USA). The cassette was incubated in PBS (5 mM, pH 7.4) at 37 ºC for 7 days. Aliquots (*ca.* 100 μL) were taken at different time intervals, and analyzed by ICP-MS to determine the concentration of silver.

Minimum inhibitory concentration (MIC) determinations

Escherichia coli and *Pseudomonas aeruginosa* were streaked on tryptic soy agar plates and incubated overnight at 37 ºC. The next day, colonies were picked, subcultured into 2 mL of Mueller-Hinton broth, and grown to OD_{650nm} = 0.4. This culture was diluted further in Mueller-Hinton broth such that 100 μL (*ca.* 5×10^5 CFU) could be allocated into wells of a 96-well plate. Silver carbene SCC10 (from stock in water with 1% DMSO) was added to wells at final [SCC10] of 0.5–20 μg/mL. Microplates were incubated overnight in a 37 ºC warm room and visually evaluated for growth the next day. The MIC was interpreted as the lowest concentration of SCC10 that inhibited growth. MIC values for an array of clinical isolates of *E. coli* and *P. aeruginosa* are shown in Supplementary Table 1.

Bacterial growth assays

E. coli and *P. aeruginosa* were streaked on tryptic soy agar plates and incubated overnight at 37 ºC, then subcultured, diluted, and allocated into wells of a 96-well plate as described above. Silver constructs were diluted in water and added to the plate wells at the indicated final Ag^+ concentrations. Plates were placed in an incubated (37 ºC), shaking microplate reader overnight, with readings every 15 min. The OD_{650nm} in each well after 6 h of incubation is reported in Figure 3 of the paper.

Table S1. MIC values of SCC10 against *E. coli* and *P. aeruginosa* strains

Species/strain	**MIC (μg/mL)**	**Origin**
E. coli		
MG1655	4	Laboratory strain[4]
ASB1	2	Asymptomatic bacteriuria[5]
ASB5	2	Asymptomatic bacteriuria[5]
UTI89	2	Acute cystitis[6]
Acute1	2	Acute cystitis[5]
rUTI2	2	Recurrent cystitis[5]
CFT073	1	Pyelonephritis[7]
Pyelo2	4	Pyelonephritis[5]
P. aeruginosa		
PA 2192	2	Mucoid CF clinical isolate[8]
PA 324	1	Mucoid CF clinical isolate[8]
PA N6	6	Nonmucoid CF clinical isolate[8]
PA JG3	2	Nonmucoid CF clinical isolate[8]
PA N13	4	Nonmucoid CF clinical isolate[8]
PA 1061	2	Nonmucoid CF clinical isolate[8]
PA N8	1	Nonmucoid CF clinical isolate[8]

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