Electronic Supplementary Information (ESI) for

Silver(I)-Glutathione Biocoordination Polymer Hydrogel: Effective Antibacterial Activity and Improved Cytocompatibility

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Part S1. Detailed materials and methods.

Materials: Silver perchlorate monohydrate ($AgClO_4 \cdot H_2O$), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and sodium hydroxide (NaOH) were purchased from Alfa Aesar. L-glutathione was purchased from Sigma Aldrich with highest purity. All the reagents were used as received. 1% silver sulfadiazine cream was purchased from China Shenghuo Pharmaceutical Holdings, Inc.

Gel Preparation: The hydrogel was prepared with two concentrations of silver: One is equal to that of 1% SSD cream, 0.3%, and the other is 0.45%. Except the Oxford cup test, in which 0.3% hydrogel was used, all the other experiments were done using 0.45% hydrogel. Typically, the 0.45% gel was prepared as follows: (1) Dissolving 0.21 mmol GSH and 0.21 mmol $AgClO_4 \cdot H_2O$ in 5 mL deionized water under vigorous stirring with final pH value of around 5.2 (adjusted by 2 M NaOH carefully); (2) After the mixed solution was placed under ambient condition for several hours, the viscous transparent Ag(I)-GSH BCP hydrogel was formed; (3) Adding 1.25 mL 0.2 M aqueous solution of $Ca(NO_3)_2$ (in excess) into the viscous solution to make Ag(I)-GSH BCP hydrogel cross-linking. The translucent hydrogel was obtained after 4 hours.

Compositional and Structural Characterizations: The gels before and after cross-linking were characterized by scanning electron microscopy (SEM, Hitachi S-4800), energy-dispersive X-ray spectra (EDX, Horiba EMAX7593-H), Fourier transform infrared spectroscopy (FT-IR, PE2000), and X-ray diffraction (XRD, Rigaku D/max2500).

Rheological Measurement: Strain sweep measurement was collected with a HAAKE MARS rheometer operating in a 35 mm parallel plate configuration. For the uncross-linked state, 1 mL Ag(I)-GSH viscous solution was measured with a 0.5 mm gap distance. For the cross-linked state, the hydrogel was made directly on the plate to ensure good contact between the hyrogel and the upper and lower plates, as described previously by Stupp and coworkers.[1] 1 mL Ag(I)-GSH viscous solution was loaded onto the rheometer, and then gelled with 0.25 mL Ca^{2+} aqueous solution for 1 h before lowering the top plate. Strain sweep was measured at angular frequency of 10 rad/s.

Antibacterial Activity Assay: Oxford cup methods were used to examine the antibacterial activity against four bacterial strains: *P. aeruginosa* wild-type strain PAO1, *E. coli* (ATCC 11775), *S. aureus* (ATCC 6358P) and *S. epidermidis* (ATCC 12228). The Ag(I)-GSH hydrogel was directly cross-linked in the Oxford cups with the aqueous solution of Ca^{2+} ions. Detailed methods were as follows: 290 μL of the uncross-linked gel was added into each Oxford cup (inner diameter 6 mm), then these cups of gel were cross-linked by Ca^{2+} ions. Because the uncross-linked hydrogel had equivalent content of silver (0.3%) with SSD cream, the same weight of SSD cream was added into Oxford cups. In antibacterial tests, 1 mL freshly grown bacteria (about 1×10^8 cfu/mL (cfu, colony-forming unit)) was added into 100 mL warm LB agar broth, and then the mixed solutions were poured into aseptic Petri dishes before solidification. Oxford cups with the cross-linked gel and SSD cream were placed on

the solid agar plates. The plates were incubated at 37 °C overnight. Colonies were visualized and digital images were captured on the next day.

Minimum Inhibitory Concentration Testing: The MIC values of the cross-linked hydrogel, $AgClO_4$ and GSH were measured using a broth microdilution method. Firstly, 100 μL of LB broth with various concentrations of the cross-linked hydrogel, $AgClO_4$ and GSH was injected into each well of 96-well plates. Then, 100 μL of microorganism solution (~ 1×10^6 cfu/mL) was added to each well. The optical density readings of microorganism solutions were measured after 24 h of incubation. The concentration at which no growth was observed with the naked eye and microplate reader (Tecan Infinite M200) was recorded as MIC. Negative control wells contained only inoculated broth. The tests were repeated three times.

Silver Release: The silver release experiments were carried out in PBS solution. Briefly, 1 g 0.45% Ag(I)-GSH hydrogel was cross-linked with Ca^{2+} ions and then immersed in 20 mL PBS for different time periods. For comparison, 1.5 g SSD cream was incubated in 20 mL PBS for 24 h. After filtration, the concentration of silver was determined by inductively coupled plasma (ICP) spectrophotometer (Leeman PROFILE SPEC).

Cytotoxicity Testing: NIH 3T3 fibroblast cells were used to test the cytotoxicity of the cross-linked hydrogel and SSD cream. Cells were seeded in 96-well plates (1×10^4 cells/200 μL of growth medium/well) followed by 3 h incubation. Supernatants from the wells were aspirated out, and fresh aliquots of culture medium (containing the cross-linked gel or SSD cream with silver concentrations in the range of 25-300

μg/mL) were added. After 24 h, supernatants were removed and the cell monolayers in the wells were washed with 200 μL of PBS solution. For quantitative analysis of cell viability, 200 μL culture medium containing 20 μL Cell Counting Kit-8 (Dojindo) was added in each well and incubated for 1.5 h, and absorbance at 450 nm was recorded using the microplate reader. The IC_{50} of the hydrogel was then calculated.

Part S2. Additional experimental data.

Table S1. Comparison of the EDX data of Ag(I)-GSH hydrogel before and after cross-linking with Ca^{2+} ions. The background signal from silicon substrate is deducted and the data are normalized.

Element	Atomic percentage (%)	
	Uncross-linked hydrogel	Cross-linked hydrogel
C K	39.41	54.28
O K	39.36	34.76
Na K	7.75	0.41
S K	3.49	4.60
Cl K	6.84	0.20
Ca K	/	1.41
Ag L	3.14	4.33
Total	100.00	100.00

Table S2. FT-IR assignments of freeze-dried GSH, Ag(I)-GSH precipitate, uncross-linked gel and Ca^{2+} cross-linked gel in the region of 4000-400 cm^{-1}.

Assignment	GSH	Ag(I)-GSH precipitate	Uncross-linked gel	Cross-linked gel
ν_s(OH) of H_2O	3424	3436	3411	3390
ν_s(NH)	3347, 3251	3284	3277	3280
$\nu_s(NH_3)$	3127, 3027	3074	3069	3080
ν_s(SH)	2525	\	\	\
ν_s(C=O) of –COOH	1713	1722	\	\
amide I	1661	1638	1632	1635
$\nu_{as}(CO_2)$	1601	\	\	\
amide II	1559, 1538	1533	1523	1522
$\nu_s(CO_2)$	1397	1409, 1385	1410	1412, 1385
ν(CH)	1334, 1280, 1249	1306, 1228	1308, 1231	1307, 1232
$\nu(ClO_4)$	\	1115	1121, 627	1110

Table S3. Assignment of the XRD reflections of Ag(I)-GSH precipitate.

(hkl)	*d* spacing (Å)
(010)	14.92
(020)	7.55
(100)	8.56
(120)	5.67
(101)	3.64
(201) or (310)	2.82
(231)	2.47

[a] The reflections are assigned based on the monoclinic unit cell with artificial symmetry (a = 8.70 Å, b = 15.01 Å, c = 4.35 Å, and β = 100.2°), which are derived from the slab dimensions Ag-S = 2.56 Å. The interlayer (axial) spacing d is calculated from (010) and (020) reflections to be 15.01 Å.

Table S4. Amount of silver released from the cross-linked Ag(I)-GSH hydrogel and SSD cream in PBS solution after 24 h of incubation at 37 °C.

	Silver released (μg/mL)
Ag(I)-GSH gel	88.5
SSD cream	0.595

Figure S1. Rheological properties of Ag(I)-GSH hydrogel (a) before and (b) after cross-linking with Ca^{2+} ions. When the strain is larger than 2%, the cross-linked hydrogel is broken and the data are not collected.

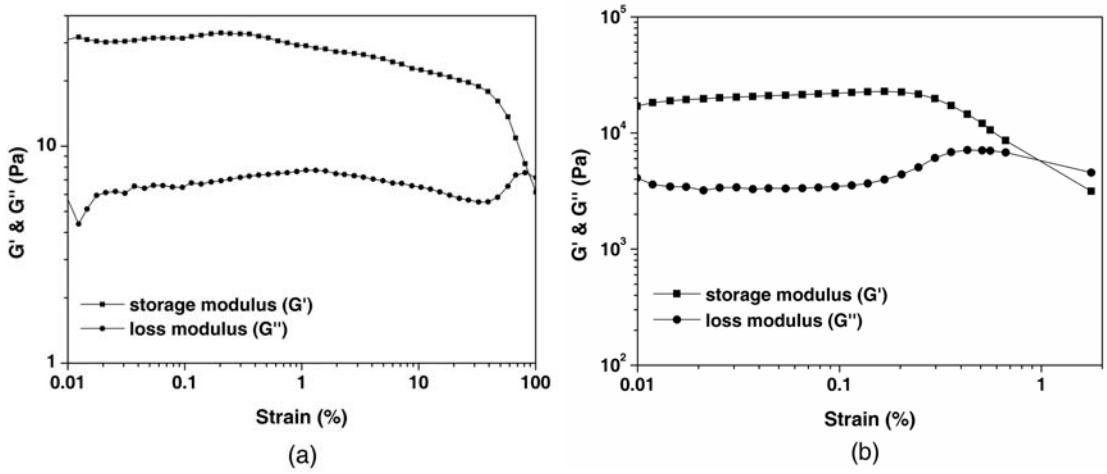


Figure S2. SEM images of Ag(I)-GSH xerogels (a) before and (b) after cross-linking with Ca^{2+} ions.

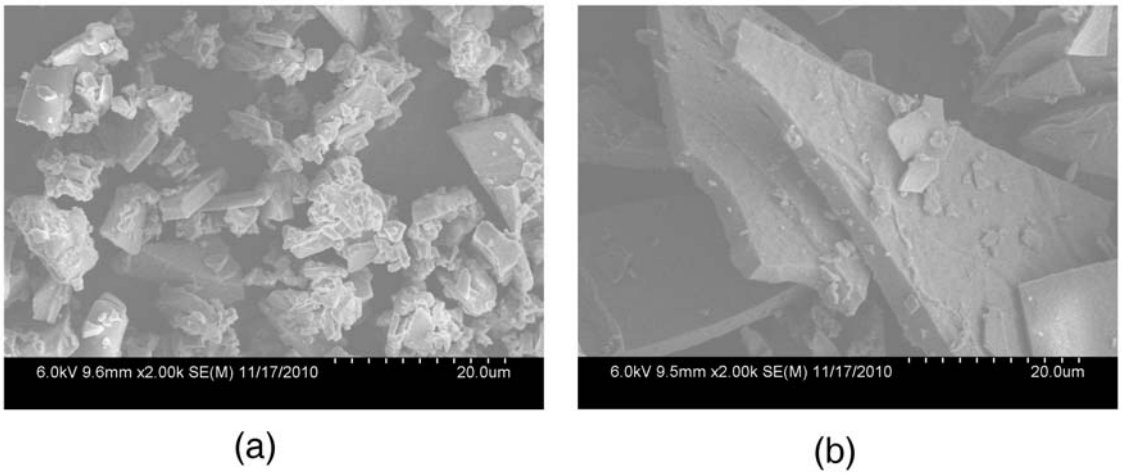
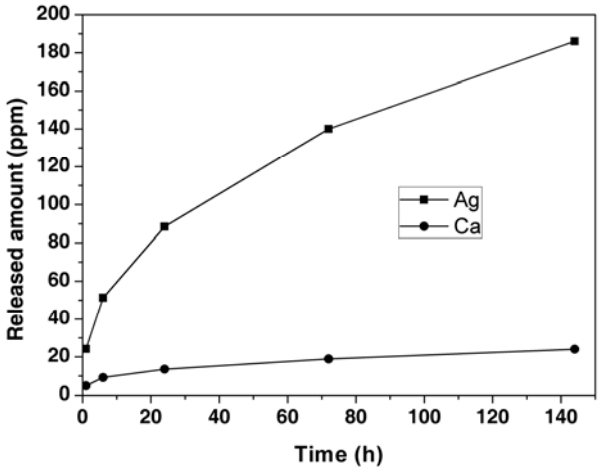


Figure S3. Amount of silver and calcium released from the cross-linked Ag(I)-GSH hydrogel in PBS solution as a function of immersion time.



1 M. A. Greenfield, J. R. Hoffman, M. O. de la Cruz, S. I. Stupp, *Langmuir*, 2010, **26**, 3641.