Supporting Information

An Injectable All-Small-Molecule Dynamic Metallogel for Suppressing Sepsis

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Chemicals and Materials

Ethanol (99%), 2-methoxyethanol (99%) and $CrCl_3 \cdot 6H_2O$ (99%) were purchased from Titan Technology Co. Ltd (Shanghai, China). Tobramycin (TOB, 95%), paromomycin sulfate (95%), kanamycin sulfate (95%), neomycin sulfate (95%), streptomycin sulfate (95%), apramycin sulfate (95%), gentamicin sulfate (95%) were obtained from Meilun Biotechnology Ltd. (Dalian, China). Hydrindantin(98%), ninhydrin (98%), $FeSO_4 \cdot 7H_2O$ (98%) and $NiCl_2 \cdot 6H_2O$ (99%) were purchased from Adamas-beta Chemical Technology Co., Ltd. (Shanghai, China). 2,2'-Bipyridyl-4,4'-dicarboxaldehyde (BIPY, 95%) was purchased from Energy Chemical Co., Ltd (Anhui,

China). Ammonia (~25-28 wt.% aqueous solution) was obtained from Titan Technology Co., Ltd. (Shanghai, China). $MgSO_4$ (99%), $ZnCl_2$ (99%), $CuSO_4 \cdot 5H_2O$ (99.7%), $AgNO_3$ (99.8%), $MnCl_2 \cdot 4H_2O$ (99%), $CoCl_2$ (98%) and phosphate buffered saline (PBS) were obtained from Chron chemicals co. (chengdu, China). Tryptone (99%), yeast extract (99%), and agar (99%) used for bacteria culture were obtained from Oxoid (Basingstoke, UK). Staphylococcus aureus (*S. aureus*)(Gram-positive bacteria) and Escherichia coli (*E. coli*)(Gram-negative bacteria) were obtained from ATCC (American type culture collection, USA). All chemicals were used without further purification.

Methods

Fabrication of the Metallogels

TOB aqueous solution (1 M, 200 μL) was mixed with BIPY (0.3 mol) with the adding of 100 μL ammonia. After 1 minute of ultrasound at room temperature, $FeSO_4$ aqueous solution (1 M, 200 μL) was added into the above solution. It was observed that the solution changes from light pink to dark purple immediately. The metallogel consisting TOB, BIPY and Fe(II) was formed after shaking slightly for 2 seconds. The amount of other kinds of aminoglycosides were kept the same as TOB, and the amount of other types of metal ions were also kept as the same as Fe(II). And there was no difference in other operations.

Dynamic Mechanical Property Testing

Time sweep tests were performed with 1% constant strain and 10 rad s^{-1} constant frequency. Frequency sweep tests were performed from 100 to 0.1 rad s^{-1} with 1% constant strain. When studying its injectability and self-healing behavior, $FeSO_4$ aqueous solution (1 M, 500 μL) was mixed on the metallogels to make the gel more flexible. The thixotropic property was investigated by imposing alternating strain of 1 and 50% at a constant angular frequency of 10 rad/s. The conductivity is characterized by an avometer, copper electrodes (Cu-electrodes) were used to connect the 500 μL metallogel.

In Vitro Release

2 mL PBS buffer of different pH was added into each of the tubes containing samples. The tubes were released at 37 °C and 1 mL of the solution samples were collected at predetermined time intervals and supplemented with 1 mL of equivalent pH PBS buffer. The release behavior of the obtained samples was evaluated by a well-established derivatization method using ninhydrin. To prepare the derivatization reagent, 85 mg ninhydrin and 15 mg reduced ninhydrin were dissolved in 10 mL ethylene glycol methyl ether, and the derivatization reagent was freshly prepared before measurement. The released sample of solution (500 μL) was fully mixed with 500 μL derivatization reagent and 500 μL acetic acid-sodium acetate buffer (pH = 5.4). The mixture was then heated to 100 °C for 15 min and then cooled to room temperature. During this process, the color of the reaction system would change from pale yellow to blue-violet. The Absorbance of the samples at 570 nm was recorded for colorimetric analysis which was tested in a microplate reader. Three repeats were conducted for each sample.

Bacterial Culture

E. coli and *S. aureus* were routinely grown on Luria–Bertani (LB) medium at 37 °C. The counts of bacteria were quantified by measuring the optical density of medium at 600 nm (OD_{600}) using a microplate reader. For all the assays, the bacteria were allowed to grow to the logarithmic phase (OD_{600} = 0.6–0.8) before use.

In Vitro Antibacterial Assay

The antibacterial activity was first evaluated by the LIVE/DEAD BacLight Bacterial Viability Kits (Invitrogen, UK) and *E. coli* and *S. aureus* were seeded in a 12-well plate (200 μL, 10^5 CFU/mL) in LB medium. Then the metallogels were placed into the bacterial solution. After incubation at 37 °C for 24 h, the bacteria in the wells were stained with the dyes (6 μL SYTO 9 and 30 μL propidium iodide, respectively) for 15 min and then visualized by fluorescent microscopy. The unstained samples were

observed by SEM after fixation with glutaraldehyde and gradient dehydration with ethanol.

Zone of Inhibition (ZOI) Test

100 μL of the bacterial solution (10^5 CFU/mL) were distributed evenly and pipetted onto plates containing LB agar culture medium. The metallogels were placed on the plates. After the plates were incubated for 18 h at the temperature of 37 °C, the anti-bacterial activities of the substrates were evaluated by observing ZOI, which was indicated by the clear region around the substrate on the agar surface.

In Vitro Biocompatibility and Cell Proliferation

The cell viability in vitro of each sample was evaluated by Alamar blue assay. Briefly, the sample was soaked in the culture medium for 24 h to obtain a 40 mg mL^{-1} leaching solution. Then, NIH 3T3 cells were incubated in a 96-well plate at a density of 2000 cells per well for 24 h. Then, after being treated with leaching solution in different concentrations (0.1, 1, 5, 20 mg mL^{-1}) for another 24 h, the Alamar blue assay was conducted to evaluate the corresponding cell viability (n = 5). Additionally, NIH 3T3 cells were incubated in a 6-well plate at a density of 100 000 cells per well for 24 h. Then, after treating with the leaching solution for another 24 h, the Live/Dead staining was recorded using an inverted fluorescence microscope to confirm the NIH 3T3 cell viability and morphology.

In Vivo Biocompatibility

Ten rats were randomly divided into three groups. To assess the biological toxicity of metallogels *in vivo*, 50 μL of metallogels were injected into the back of the experimental groups. Untreated blank mice used saline as the control. After feeding for seven days, the injection site was removed from the animals for histopathological examination.

In Vivo Antibacterial Assay

6–8 weeks old female Balb/c mice with an average weight of 20 g were bought from Dashuo Laboratory Animal Co., Ltd. (Chengdu, China). All the studies on these animals were performed by following the animal ethical standard from Animal Ethics Committee in West China Hospital, Sichuan University, Chengdu, China (WCHSIRB-D-2017-263). The mice were randomly allocated into two groups and intraperitoneally injected with *E. coli* (10^8 CFU/mL, 75 μL). After 30 minutes subcutaneous injection, the mice were given a subcutaneous injection of 75 μL PBS or metallogel, the body weights of the mice were monitored. The bacterial counts in major organs were evaluated at 3 days post E. coli induced septic challenge in mice. Meanwhile, other organs were put in paraformaldehyde and sectioned into 4 μm slices. Hematoxylin-eosin (H&E) staining and Gram staining were conducted, and the stained sections were observed by optical microscopy.

Characterization

Scanning Electron Microscope (SEM)

The morphologies were tested by SEM (Phenom Instruments Co. Shanghai) of FEI Quanta 250 (accelerating voltage 10 kV). The samples were treated with the gold spray treatment within 24 h before the test.

X-Ray Photoelectron Spectroscopy (XPS)

The sample was placed in the sample chamber of the Thermo Scientific K-Alpha XPS. The sample was fed into the chamber at a pressure of less than 2.0×10^{-7} mbar, with a spot size of 400 μm, an operating voltage of 12 kV, and a filament current of 6 mA. The fluence energy was 150 eV in steps of 1 eV, and the fluence energy was 50 eV in steps of 0.1 eV for the narrow spectrum.

UV–Vis Spectroscopy

UV-Vis spectroscopy experiments were performed by a PerkinElmer Lambda 650 UV-Vis spectrophotometer to measure the absorption properties of the prepared aqueous solutions. The measured spectra were in the range of 200-800 nm with a slit of 2 nm.

Fourier-transform infrared (FTIR) Spectroscopy

FTIR spectroscopy (Nicolet 670 America) was recorded on a Perkin-Elmer spectrum one B system with a resolution of 4.0 cm^{-1}, using KBr pellets method: Took 1~2mg of lyophilized metallogel powder, 200mg of pure KBr and grinded it evenly, put it in the mold and pressed it into a transparent sheet on the hydraulic press, put the specimen into the infrared spectrometer for testing, the wave number range was 4000~400cm^{-1}, the number of scans was 32, the resolution was 4 cm^{-1}.

Raman Spectrometer

The information on the vibration or rotation of the substances in the samples was obtained by Raman spectroscopy. The samples were used to collect Raman spectra (Horiba, LabRAM Aramis spectrometer) with a 632nm laser line with the spectrometer pinhole.

Near Infrared (NIR) Laser

Metallogel prepared in a glass bottle was irradiated by a NIR laser (808 nm, Tsunami, Spectra-Physics). The temperatures were recorded by an electric coupling thermodetector and an IR camera. Photographs of the metallogels were taken under 2 $W \cdot cm^{-2}$ irradiation.

Torque Rheometer

The viscoelastic properties of the metallogels were monitored by a rheological measurement (MCR320, Austria). The diameter of the parallel plate is 8 mm, and the experimental temperature is constant at 25 °C. The stability constants are obtained from the Lange's Handbook of Chemistry.

Fluorescence Microscope

Placed the samples on the fluorescence microscope (Olympus, Japan) carrier, used the Zen software to operate, found the samples in the field of view, adjusted the aperture so that the display brightness was appropriate.

Microplate Reader

Samples were placed in the microplate reader (Biotek Instruments Co. America) and tested using Gen5 software, using 96-well plates. The released samples concentration was measured at 570 nm; the bacterial concentration was measured at 600 nm.

Table S1. Storage modulus and loss modulus of the metallogels.

Metal ions	Storage modulus (Pa)	Loss modulus (Pa)	logK_1	logK_2	logK_3
Zn^{2+}	34244 ± 3692	6929 ± 693	5.3	9.6	13.1
Mg^{2+}	38423 ± 2489	8128 ± 751	0.5		
Cr^{3+}	50110 ± 3835	7916 ± 1052	4.5	10.5	14.0
Ni^{2+}	55261 ± 3501	7571 ± 315	6.8	13.3	18.5
Cu^{2+}	56969 ± 1973	11636 ± 230	8.0	13.6	17.1
Co^{2+}	64381 ± 3085	6643 ± 197	5.7	11.6	17.6
Ag^{+}	89256 ± 16721	20439 ± 4604	3.7	7.2	
Mn^{2+}	25870 ± 723	3668 ± 171	1.9	2.8	3.4
Fe^{2+}	211850 ± 21503	12514 ± 164	4.4	8.0	17.5

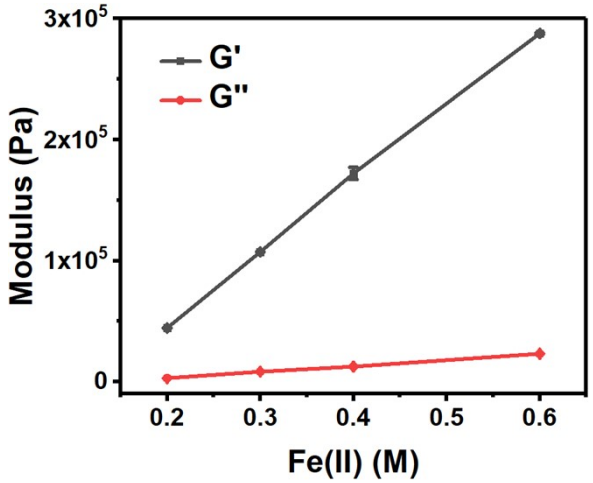

Figure S1. *G′* and *G″* of the metallogel with different Fe(II) concentrations ([TOB] = 0.4 M) measured by a rheometer.

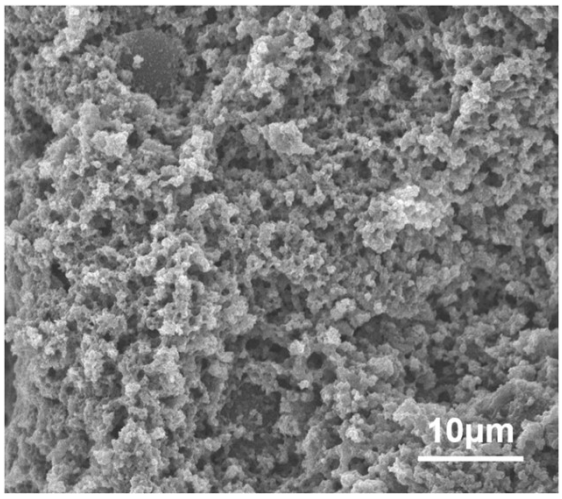

Figure S2. SEM image of the metallogel.

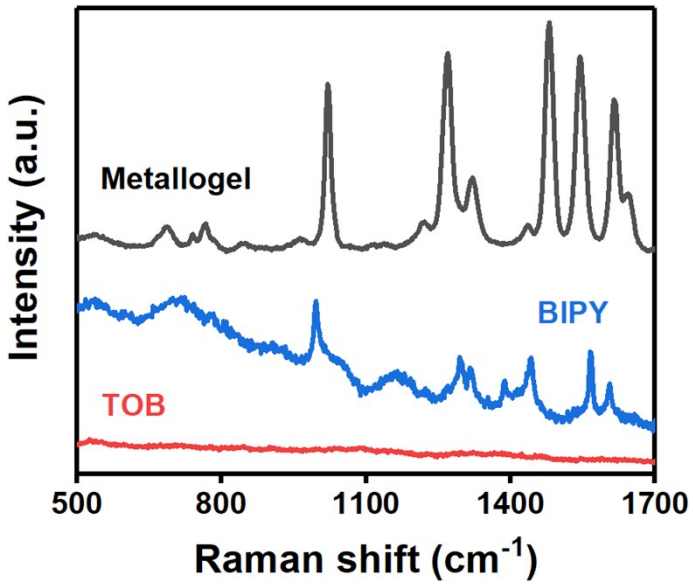

Figure S3. Raman spectra of the metallogel, BIPY and TOB.

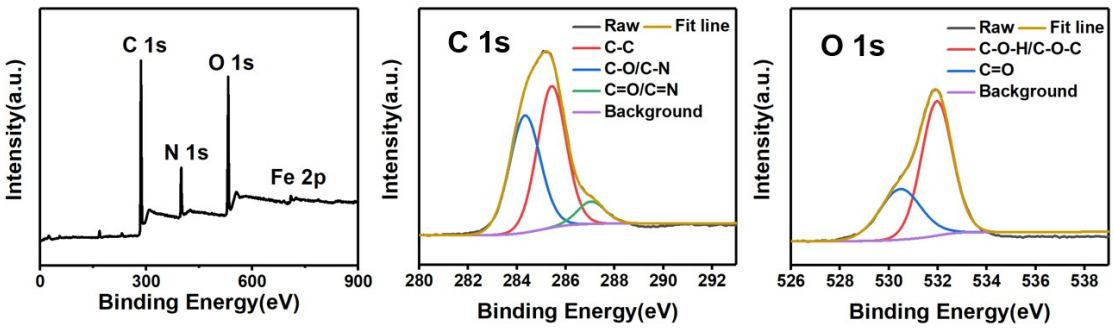

Figure S4. XPS survey spectra of the metallogel.

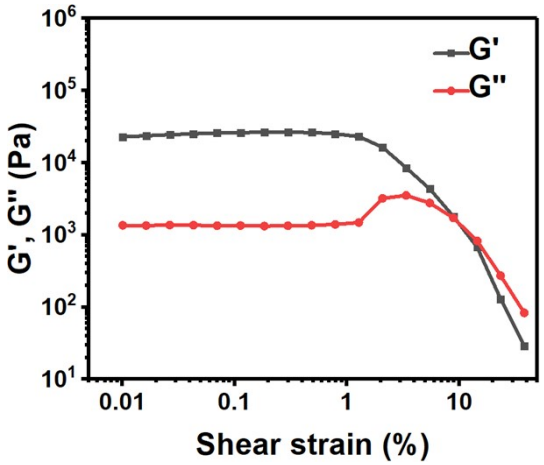

Figure S5. The sol–gel transition point of the metallogel at 25°C.

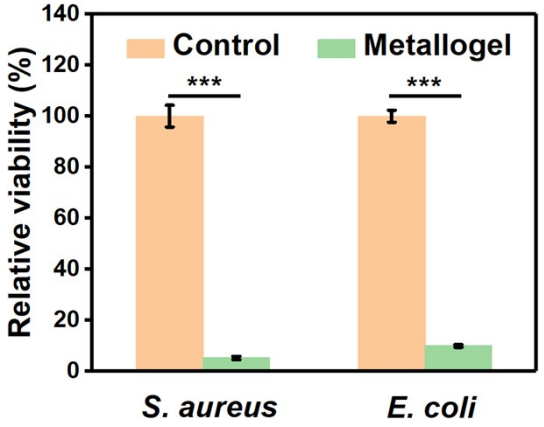

Figure S6. Antibacterial activity of the metallogel against *S. aureus* and *E.coli.*

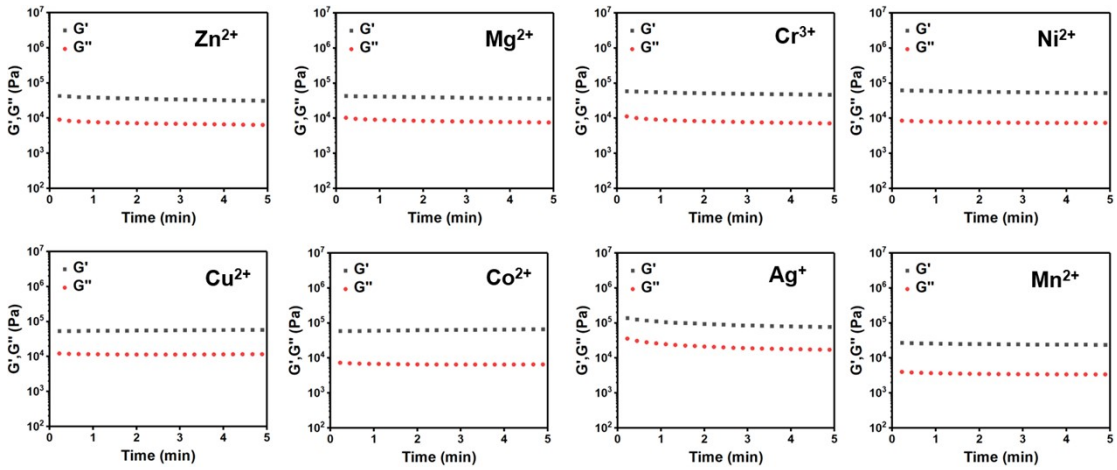

Figure S7. Time-dependent rheology of the metallogels formed by different metal ions.

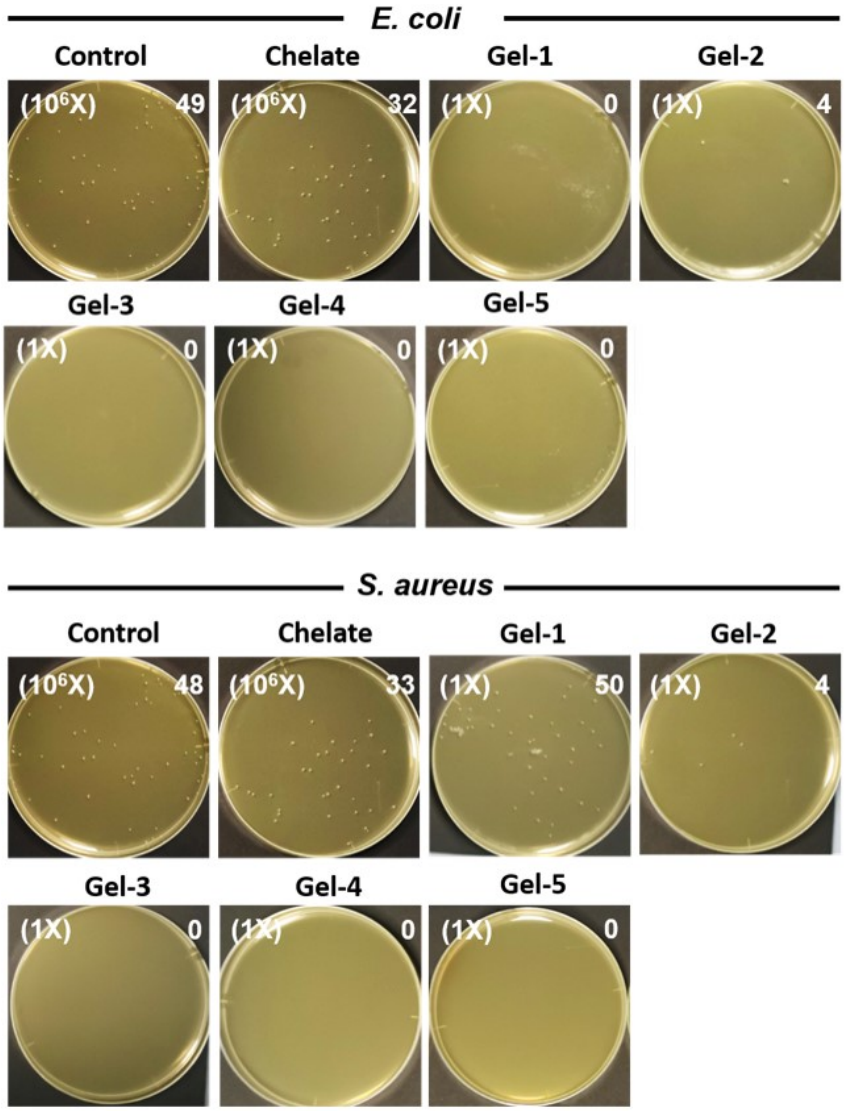

Figure S8. *In vitro* antibacterial activities of the metallogel.

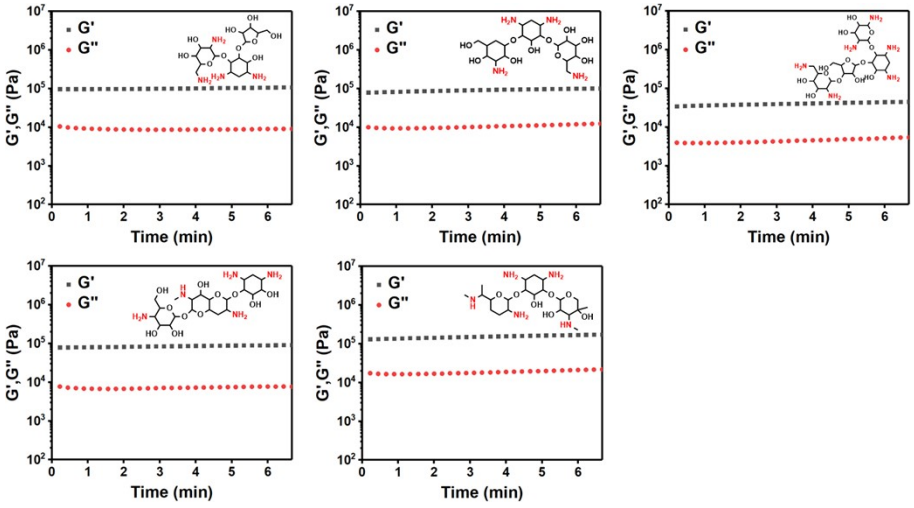

Figure S9. Time-dependent rheology of the metallogels formed by different aminoglycosides.