Supporting Information

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All-small-molecule dynamic covalent hydrogels with multistimuli responsiveness

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Experimental section

Materials. Tobramycin and neomycin sulphate were purchased from Meilun Biotech. (Dalian, China). Lithium chloride, sodium chloride, potassium chloride, rubidium chloride, sodium fluoride, sodium bromide, sodium iodide, lithium iodide, ammonium acetate, and sodium hydroxide were purchased from Macklin (Shanghai, China). Formaldehyde solution (37%, w/w), acetic acid, acetyl acetone, *o*-phthalaldehyde, boric acid and mercaptoacetic acid were purchased from Aladdin (Shanghai, China). The chemicals were used as received without further purification.

Preparation and characterization of aminoglycoside/formaldehyde hydrogels. Tobramycin was dissolved in 1.6 mL double-distilled water at a concentration of 250 mg/mL. 0.6 mL formaldehyde solution (37%, w/w) was added into the above solution. The mixture was then heated at 85 ^{o}C for 4 h. A light yellow and transparent hydrogel was obtained when the mixture solution was slowly cooled to room temperature. The neomycin/formaldehyde hydrogel was prepared by the same method. ^{1}H NMR and COSY spectra of the gel were conducted on a Varian 699.804 MHz NMR spectrometer. The hydrogel was freeze-dried and then dissolved with 0.5 mL D_2O. The sample concentration was about 10 mg/mL. The freeze-dried hydrogel was also characterized by Fourier transform infrared spectroscopy (FT-IR). Free tobramycin and the tobramycin/formaldehyde mixture were also characterized by ^{1}H NMR and FT-IR.

Temperature-responsive behavior of the aminoglycoside/formaldehyde hydrogels. The aminoglycoside/formaldehyde hydrogels were prepared as described above. Rheological properties of the hydrogels at a continuous step temperature between 25 ^{o}C and 65 ^{o}C were measured by a rheometer (TA Instrument, USA). The temperature of the rheometer was heated from 25 ^{o}C to 65 ^{o}C at 5 ^{o}C/min at 1% strain and 10 rad/s angular frequency. The storage modulus (G') and loss modulus (G") of the hydrogel during the process was recorded to reveal the temperature-responsive behavior.

To monitor the gel degradation at different incubation temperatures, 200 μL

hydrogel gels were prepared in glass vials, and then added with 1 mL double distilled water. The vials were then incubated at 5 ^{o}C, 25 ^{o}C, 37 ^{o}C, 45 ^{o}C and 65 ^{o}C, respectively. At scheduled time intervals, the water in the vials were removed and the weight of residual gels were weighted to monitor the gel degradation.

Acid-responsive behavior of the aminoglycoside/formaldehyde hydrogels. 200 μL hydrogels were prepared in glass vials, and then 1 mL water at different pH values was added into the vials. The vials were incubated at 25 ^{o}C for different time. The solutions in the vials were removed and the weight of residual gels were weighted to monitor the gel degradation. For the release of aminoglycoside and formaldehyde from the gel matrix, the collected solutions at scheduled time intervals were analyzed as follows. The amount of released aminoglycosides in the collected samples was measured by a derivatization method using o-phthalaldehyde as described before.[1] Generally, 268 mg *o*-phthalaldehyde was dissolved in 10 mL methanol. The solution was then mixed with 1.4 mL mercaptoacetic acid and 38.6 mL boric acid (0.2 M, the pH of the solution has been adjusted to 10.5). 0.6 mL of the above solution was added into 0.3 mL collected samples, and incubated at room temperature for 15 min. Absorbance of the samples at 333 nm was recorded for colorimetric analysis. The standard curve was A=0.0282C+0.2069, C is the tobramycin concentration, mg/mL, A is the absorbance of samples, R^2=0.996. The amount of released formaldehyde was measured by an acetyl acetone colorimetric method. Generally, 50 g ammonium acetate and 6 mL acetic acid were dissolved in 60 mL double-distilled water. 0.5 mL acetyl acetone was added, and the solution was fixed to 100 mL with distilled water. 1 mL of the prepared solution and 1.8 mL double-distilled water were added into 0.2 mL collected samples, and incubated at 60 ^{o}C for 15 min. Absorbance of the samples at 414 nm was recorded for colorimetric analysis. The standard curve was A=16.801C+0.2153, C is the formaldehyde concentration, μg/mL, A is the absorbance of samples, R^2=0.9997.

Rheological properties of the aminoglycoside/formaldehyde hydrogels in the presence of alkali ions and halogen anions. The aminoglycoside/formaldehyde hydrogels in the presence of 20 mM LiCl, NaCl, KCl, RbCl, NaF, NaBr, NaI, or LiI

were prepared as described above. The solutions were heated to 85 ^{o}C for 4 h, and cooled to room temperature to allow gel formation. Rheological properties of the formed hydrogels were measured using the rheometer.

1. J. Hu, Y. Quan, Y. Lai, Z. Zheng, Z. Hu, X. Wang, T. Dai, Q. Zhang and Y. Cheng, *J. Control. Release*, 2017, **247**, 145-152.

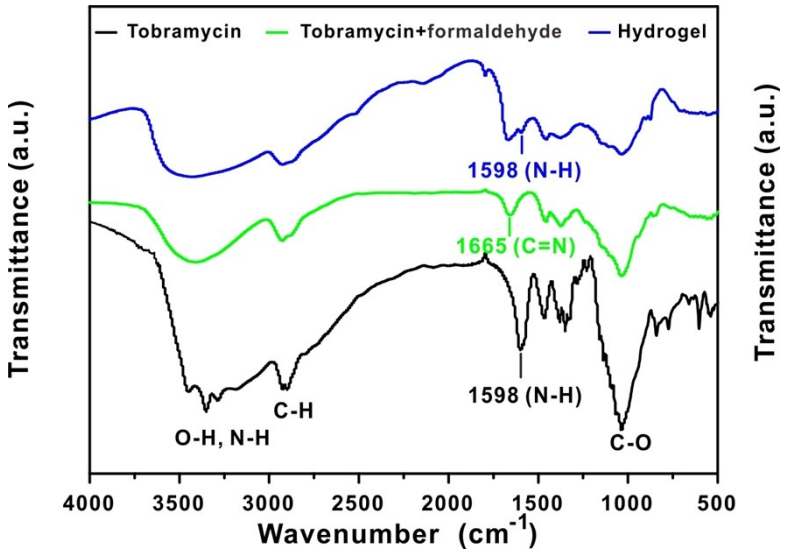

Figure S1. FT-IR spectra of tobramycin, the mixture of tobramycin and formaldehyde, and the prepared tobramycin/formaldehyde hydrogel.

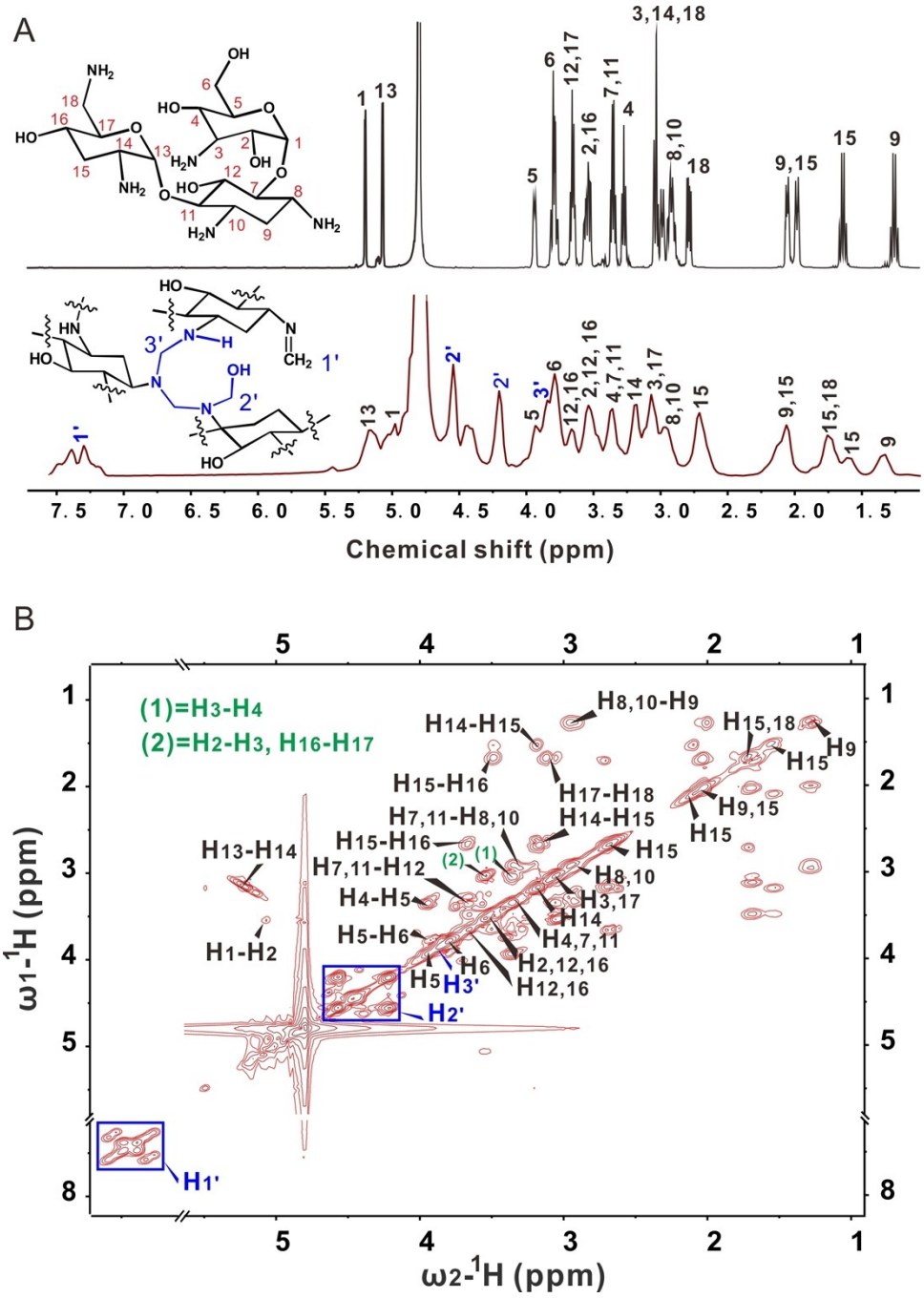

Figure S2. (A) ^{1}H NMR spectra of tobramycin (upper, black) and the tobramycin/formaldehyde gel (down, red). Chemical Structures of the hemiaminal dynamic covalent networks with proton labeling was shown. (B) ^{1}H- ^{1}H COSY spectrum of the tobramycin/formaldehyde sample dissolved in D_2O.

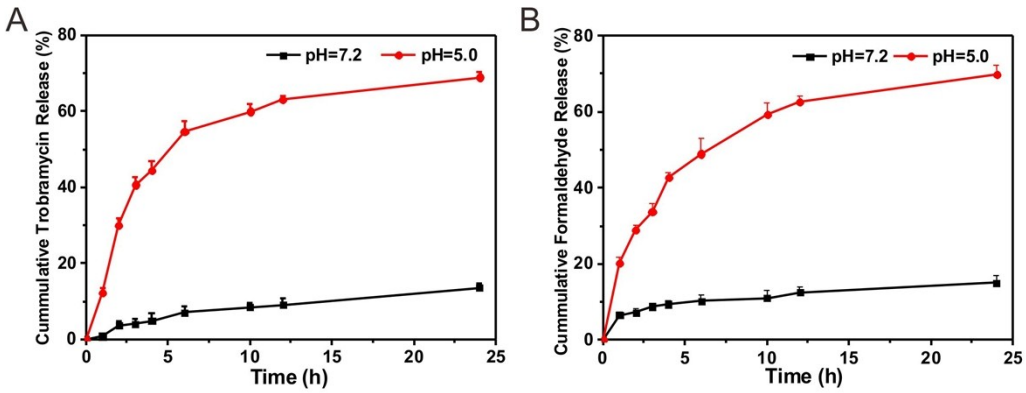

Figure S3. Release profiles of tobramycin (A) and formaldehyde (B) from the tobramycin/formaldehyde hydrogel at pH 7.2 and pH 5.0, respectively.

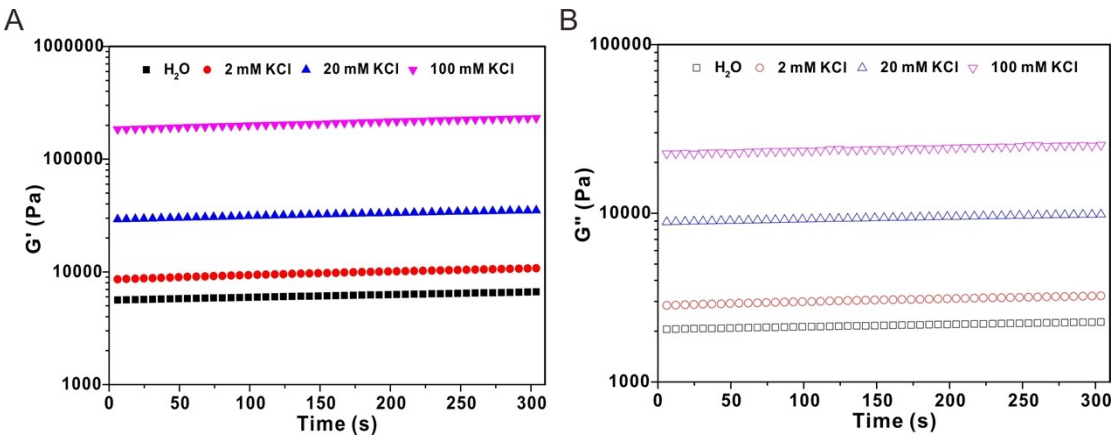

Figure S4. Effect of KCl concentration on G' (A) and G" (B) of the tobramycin/formaldehyde hydrogel.