Electronic Supplementary Material (ESI) for Polymer Chemistry.
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Electronic Supplementary Information for "Injectable cationic hydrogels with high antibacterial activity and low toxicity"

Experimental

Materials

Diamino poly(hexamethylene guanidine) hydrochloride (PHMG, M_n=2200, Hubei Xinyuanshun Pharmaceutical Chemical Co., China), glycidyl methacrylate (GMA, Adamas), dimethyl sulfoxide (DMSO, Sinopharm Chemical Reagent Co., China) were used as received. Poly(oligo(ethylene glycol) mercaptosuccinate) (POEGMS) was synthesized by the polycondensation of oligo(ethylene glycol) (OEG, M_n = 600) and mercaptosuccinic acid (MSA) based on our previous work.

Synthesis of PHMGDMA

The synthesis of PHMGDMA was as follows: in a 100 mL round-bottomed flask equipped with a magnetic stirrer, PHMG (4.4 g, 2 mmol) was dissolved in 30 mL of DMSO at 65 °C, then GMA (5.68 g, 0.04 mol) was added. The reaction was carried out for 72 h at 65 °C. Afterwards, the product was precipitated into acetone, washed three times by acetone and dried in a vacuum to a constant weight at 40 °C. Yield: 4.2 g (89.7%).

Preparation of hydrogels

PHMGDMA (0.11 g, 0.09 mmol of double bonds) and POEGMS (0.065 g, 0.09 mmol of thiol groups) were separately dissolved in phosphate buffered saline (PBS, 0.02 mol/L, pH=7.4) at a certain concentration (10 wt%, 20 wt%, 30 wt%). These two solutions of precursor were mixed well to form the PEG-PHMG hydrogels at 37 °C in a few minutes.

Measurement of Gelation time

Vial-inversion method was employed to test the gelation time of hydrogels. The gelation time was from the point that the solutions of hydrogel precursors were mixed well in a vial at 37 °C to the time that there was no flowing fluid in the vial.

Degradation studies

Hydrogels with different solid contents were prepared for degradation experiments. Each hydrogel was immersed in 20 mL of PBS solution (0.02 mol/L, pH=7.4) in a vial at 37 °C. At appropriate intervals, after removing the PBS solution, the hydrogels were washed with distilled water, lyophilized and weighed (W_t). The weight loss fraction was calculated by the equation:

$$\text{Weight loss fraction (\%)} = (W_0-W_t)/W_0\times 100\%,$$

W_0 is the weight of initial dry hydrogel, W_t is the weight of residual dry hydrogel.

Antibacterial assays

Antibacterial activities of hydrogels were traced and detected by OD600 method. *S. aureus* and *E. coli* were used as the typical gram-positive bacteria and gram-negative bacteria. In detail, the bacteria were grown in lysogeny broth (LB) overnight in an incubator at 37 °C. The optical density (OD) of the bacterial= solution at 600 nm was measured by a Microplate Spectro-photometer (SpectraMax® i3, America), and adjusted to OD_{600nm} = 0.1 by diluting with LB. The hydrogels (100 μL) were *in situ* formed in 96-well culture plates, and then the diluted bacterial solution (100 μL) was added onto each hydrogel. Tissue culture 96-well plate was used as control. The plates were then incubated at 37 °C, and the optical density of bacterial solutions was monitored at certain time points.

Hemolysis assays

Human red blood cells (hRBCs) were obtained by venous-puncture from the healthy non-

smoking human volunteer using standard blood drawing procedures (normal blood flow and no pressure), and informed consent was signed. Fresh hRBCs were washed 3 times with PBS and were diluted to 4 % in volume by PBS. 100 μL of the diluted red blood cell suspension and 100 μL of PBS were introduced onto each hydrogel (100 μL) prepared in 96-well culture plates in turn. The hRBC suspension without hydrogel and treated with 100 μL of 0.1 % Triton X-100 were used as the negative control and the positive control, respectively. The plates were incubated for one hour at 37 °C. The hRBC suspension in each well was centrifuged at 3000 *rcf* for 5 min. 100 μL of the supernatant for each centrifugation was transferred to a new 96-well plate and its OD value at 576 nm was detected by a Microplate Spectro-photometer (SpectraMax® i3, America). The hemolysis ratio was calculated by the following equation:

$$Hemolysis(\%) = \frac{OD_{576sample} - OD_{576blank}}{OD_{576Triton} - OD_{576blank}} \times 100,$$

where $OD_{576samples}$ is the absorbance of the hRBCs treated with hydrogels, $OD_{576blank}$ is the absorbance of hRBCs without hydrogel, and $OD_{576Triton}$ is the absorbance of hRBCs treated with 0.1 % Triton X-100. Data are presented as the average ± SD (n= 5)

***In vitro* cytotoxicity assays**

The cytotoxicity of PEG-PHMG hydrogels was evaluated by the CCK-8 (Dojindo, Japan) assays. In detail, PEG-PHMG hydrogels (100 μL) were placed in 1.0 mL of PBS solution (0.01 M, pH = 7.4) and soaked at 37 °C for 24 h. The hydrogels were then removed, and the extracts were sterilized by filtration (0.22 μm) before tests. HepG2 cells (Cell bank of the Chinese Academy of Science, China) were pre-incubated in a 96-well plate (5×10^3 cells per well) with a culture medium of 10 % FBS/α-MEM (Invitrogen Co., Carlsbad, CA) in a humidified 5 % CO_2 containing atmosphere at 37 °C for 24 h. Then the cells were further incubated with the extracts of hydrogels for 24 h. Subsequently, the media was aspirated and replenished with 100 μL of fresh culture medium. 10 μL of CCK-8 reagents were added into each well, and the cells were incubated in the dark for another 1 h. The absorbance at a wavelength of 450 nm of each well was measured using a Microplate Spectro-photometer (SpectraMax® i3, America). Non-treated cells were used as a negative control; wells without cells but culture medium were used as the blank. The relative cell viability (CV) was calculated by the following equation:

$$CV(\%) = \frac{OD_{450sample} - OD_{450blank}}{OD_{450control} - OD_{450blank}} \times 100,$$

where $OD_{450samples}$ is the absorbance of the cells treated with extracts, $OD_{450control}$ is the absorbance of the control group and $OD_{450blank}$ is the absorbance of blank group. Data are presented as the average ± SD (n= 5)

Characterization

A Bruker Avance DMX400 NMR spectrometer (400 MHz) was used to record ^{1}H NMR spectra with deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$)as solvent. FT-IR spectra were recorded using a VECTOR 22 spectrometer. Rheological analysis was performed with a TA AR-G2 stress-controlled rheometer (TA Instruments, US), using a parallel plate (20 mm diameter) at 37 °C. The storage modulus (G') and loss modulus (G'') were recorded as a function of angular frequency with a strain of 2%. The morphologies of

the hydrogels were observed using a scanning electron microscope (SEM, TM-1000, Hitachi, Japan) operated at an accelerating voltage of 15.0 kv.

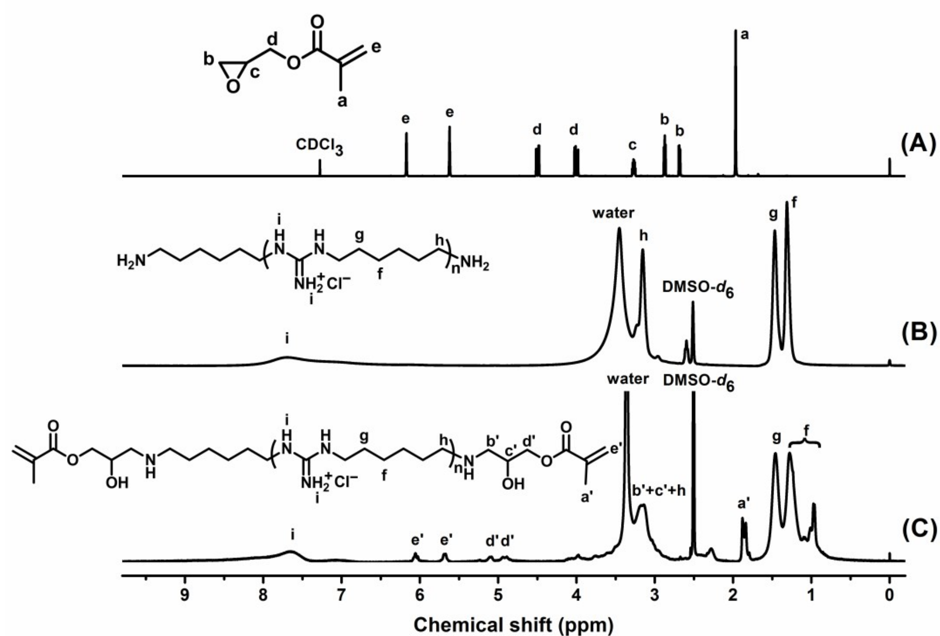

Fig. S1 ^{1}H NMR spectra of GMA (A), PHMGDA (B) and PHMGDMA (C).

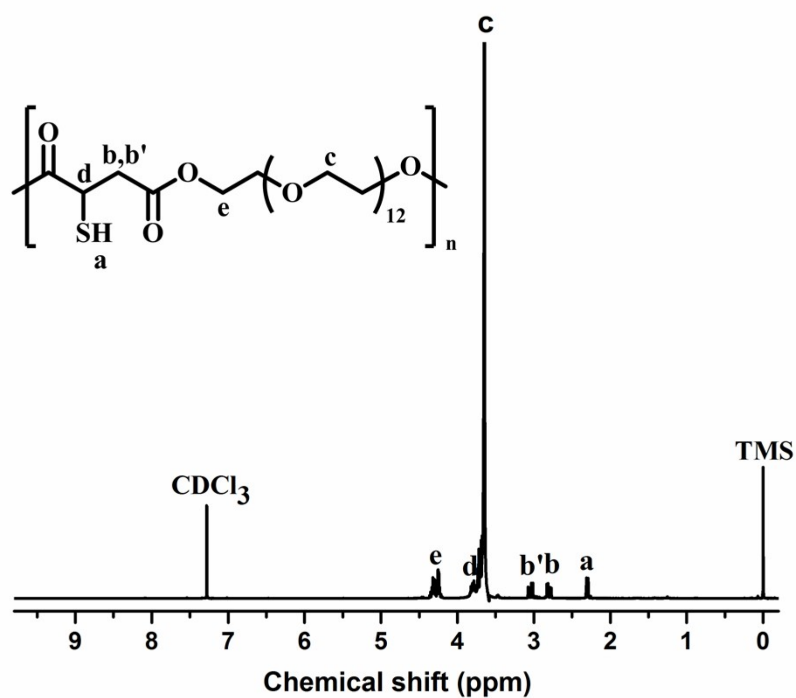

Fig. S2 ^{1}H NMR spectrum of POEGMS.

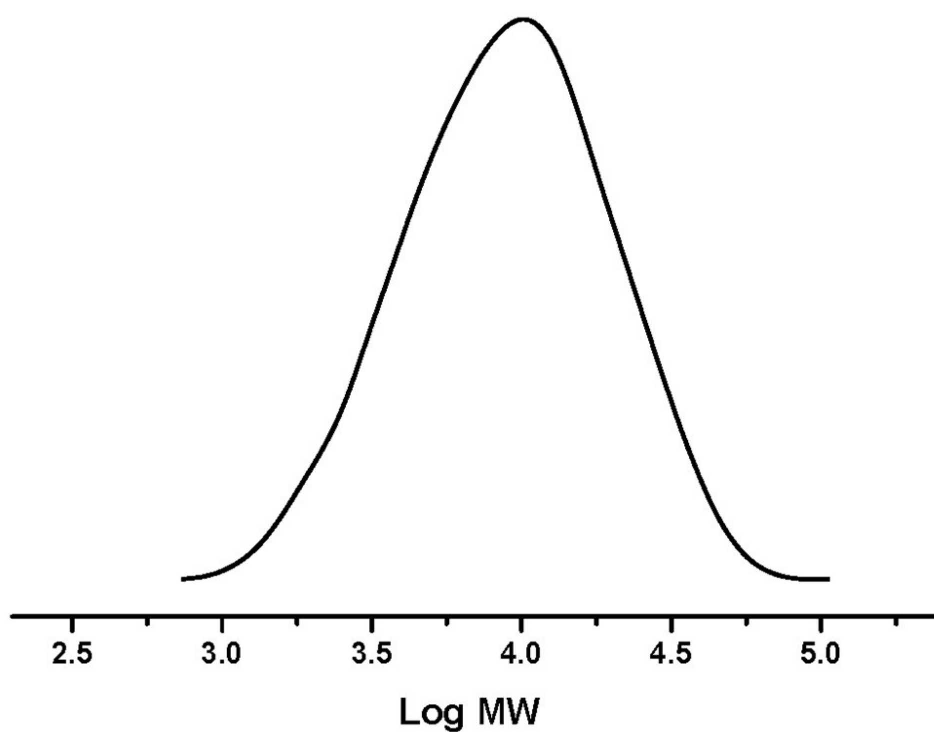

Fig. S3 GPC trace of POEGMS.

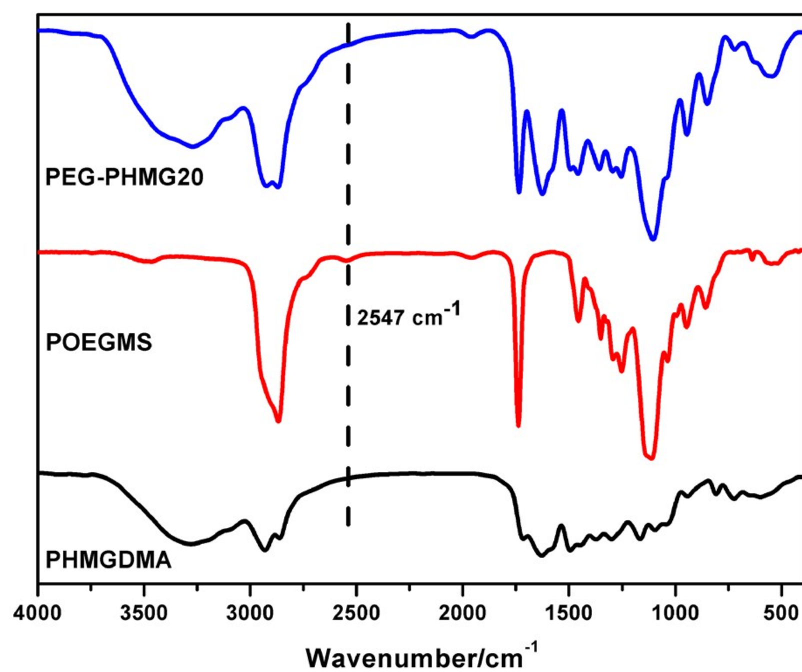

Fig. S4 FT-IR spectra of PEG-PHMG hydrogel and its precursors.

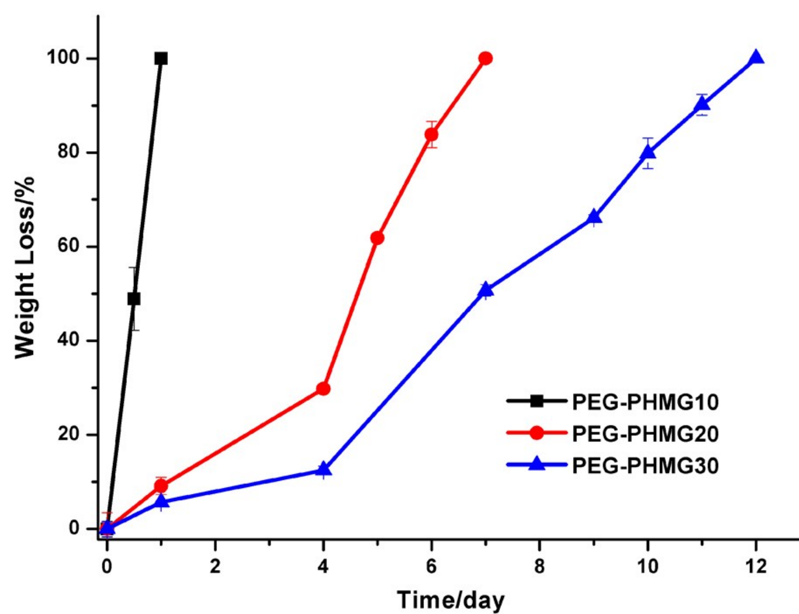

Fig. S5 Degradation curves of PEG-PHMG hydrogels.