SUPPORTING INFORMATION FOR

Injectable drug-loaded hydrogel using "clickable" amphiphilic triblock copolymer as precursor

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Experimental

Materials

Oligo(ethylene glycol) (OEG, Fluka, M_n = 400) was dried by azeotropic distillation in the presence of dry toluene. ε-Caprolactone (CL, 99 %, Acros) was distilled under reduced pressure in the presence of calcium hydride before use. Mercaptosuccinic acid (MSA, 98 %, Aladdin) was used without further purification. Scandium triflate was synthesized according to previous report.[1] Poly[oligo(ethylene glycol) mercaptosuccinate] (POEGMS) were synthesized according to our previous reports[2]. All other reagents were purchased from Shanghai Chemical Reagent and used as received.

Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Advance DMX500 spectrometer in $CDCl_3$ with tetramethylsilane as internal standard.

Gel permeation chromatography (GPC) curves were recorded on a Waters Breeze 1525 GPC analysis system with two PL mix-D column and a Waters 2414 RI detector, using THF as eluent and polystyrene as calibration sample at the flow rate of 1.0 mL/min at 40 °C.

Fourier transform infrared (FT-IR) spectra were recorded using a PE Paragon 1000 spectrometer (KBr disk).

X-ray diffractomer (XRD) measurements were performed on a PANalytical X'Pert PRO X-ray diffractometer. Copper was used as the source of X-rays at 40 kV and 40 mA. The diffraction angle 2θ was recorded from 10 ° to 60 ° at a scan speed of 10 °/min.

Differential scanning calorimetry (DSC) measurements were carried out using a DSC-Q20 (TA, American) differential scanning calorimeter. The DSC thermograms covered a temperature range from 20 to 100 °C at a scanning rate of 10 °C/min.

Quantitative analysis of the release of ibuprofen was performed on High-Performance Liquid Chromatography (HPLC) using an XTerra RP185 μm 4.6 × 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector. Samples

were filtered using 0.22 μm poly(vinylidine fluoride) syringe filters (Fisher). The HPLC method was adapted from previously published methods.[3] The mobile phase was 10 mM KH_2PO4 in 70% acetonitrile (ACN) and 30% water at pH 3.0. Samples (10 μL) were run at 25°C at a flow rate of 1 mL/min. Absorbance was monitored at λ = 222 nm for ibuprofen. The instrument was calibrated using standard 1 mg/mL and 0.1 mg/mL solutions of known concentrations.

Rheological behavior of the samples was characterized with a TA AR-G2 stress-controlled rheometer (TA Instruments, U.S.A.), equipped with a parallel plate of 20 mm in diameter. Elastic (*G*') and loss (*G*'') moduli of the two precursors mixed solution were monitored as a function of frequency to characterize the viscoelastic properties. The frequency sweeping tests were carried out from 0.5 Hz to 50 Hz with 1 % strain. The sample volume was 100 μL in total.

Scanning Electron Microscopy (SEM) was used to visually examine the interior structure of the hydrogels in swollen state, the swollen hydrogel samples equilibrated in deionized water for 24 h were quickly frozen in liquid nitrogen and further freeze-dried in a freeze drier at -40 °C for 2 days until all the solvent was sublimed. The freeze-dried hydrogels were then fractured carefully and then loaded on the surface of an aluminum SEM specimen holder and sputter coated with gold before observation, and then the interior morphologies of the hydrogels were visualized by using a scanning electron microscope (Hitachi S-4700).

Synthesis of PCL-POEGM-PCL triblock copolymer

Anhydrous OEG (16.0 g, 40 mmol) and malic acid (4.2 g, 36 mmol) with a molar ratio of 10: 9 were added in three-necked flask to conduct polycondensation in the presence of $Sc(OTf)_3$ (19.7 mg, 0.4 mmol) for two days, to give POEGM with two hydroxyl end groups. After taking out tiny amount of POEGM under nitrogen atmosphere for ^1H NMR and GPC analyses, the polymerization system was cooled to 70 °C, and anhydrous CL (5.5 g or 5.1 mL, 48 mmol) was added into the flask to conduct ring-opening polymerization (ROP) for 12 hours under nitrogen atmosphere. The crude product was dissolved in CH_2Cl_2 and precipitated into cold diethyl ether for three times. The obtained triblock polymer was dried in vacuum at room temperature for 24 hours. Yield 20.3 g (83 %).

Preparation of ibuprofen-loaded hydrogel

Ibuprofen loaded hydrogel with a solid content of 10 wt% was prepared as follows: 100 mg of PCL-POEGM-PCL1 and 10 mg of ibuprofen were added into 0.9 mL of PBS solution (0.1 M, pH = 7.4) to acquire transparent solution by vigorously stirring. Then PBS solution of POEGMS (10 wt%) with equimolar mercapto group was mixed with the above drug and polymer solution, and ibuprofen-loaded hydrogels were obtained. Hydrogels with other solid contents were prepared via similar method.

***In vitro* drug release**

1 mL of ibuprofen loaded hydrogel was immersed into the PBS solution (10.0 mL, pH = 7.4, 37 °C). At predetermined time intervals, 3.0 mL of the release medium was collected periodically and replenished with an equal volume of fresh medium. The amount of released ibuprofen was analyzed by High-Performance Liquid Chromatography (HPLC) as described above.

Swelling tests

Hydrogels for swelling tests were prepared in a PBS solution (0.01 M, pH = 7.4) with a precursor concentration of 10, 20 and 30 wt%, respectively. Swelling tests were performed by immersing the weighed dry hydrogels (by lyophilization) in PBS solution (0.01 M, pH=7.4) at 37 °C. At regular time intervals, the swollen hydrogels were weighed after removal of the buffer on the surface. The swelling ratio (SR) of the hydrogels was calculated from the equation:

$$\mathrm{SR}(\%) = (\mathrm{W_t} - \mathrm{W_0}) / \mathrm{W_0} \times 100$$

, where W_t is the weight of the swollen hydrogel and W_0 is the weight of the dry hydrogel.

Degradation studies

1 mL of hydrogel was incubated in 10 mL of PBS solution (pH = 7.4) at 37°C in vials to assess the hydrolytic degradation. Masses of wet and dry gels (by lyophilization) were measured at a pre-defined time. The degradation fractions (DF) were calculated as follows:

$$\mathrm{DF}(\%) = (\mathrm{W_0} - \mathrm{W_t}) / \mathrm{W_0} \times 100$$

, where W_0 is the mass of original dry gel and W_t is the mass of undegraded dry gel.

***In vitro* cytotoxicity of precursors and hydrogels**

The cytotoxicities of precursors and hydrogels were evaluated by the CCK-8 (Dojindo, Japan) assay. All the tests were run five times. The precursor solutions were prepared in PBS solution (0.01 M, pH 7.4) and then sterilized by filtration (0.22 μm). Hela 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 precursor solutions and hydrogels for 24 h. Subsequently, the media was aspirated and replenished with 100 μL of fresh culture medium. 10μL 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 reader (Sunrise™ Basic; TECAN, Zurich, Switzerland). Non-treated cells were used as a negative control; wells without cells but culture medium were used as the blank. The relative cell viability was calculated as follows:

$$\text{Cell viablity}(\%) = \left[(\mathrm{OD_{450samples}} - \mathrm{OD_{450blank}}) / (\mathrm{OD_{450control}} - \mathrm{OD_{450blank}}) \right] \times 100$$

, where $OD_{450samples}$ is the absorbance of the cells treated with gel precursor extracts or hydrogels with different concentrations, $OD_{450control}$ is the absorbance of the control group and $OD_{450blank}$ is the absorbance of the well with no cells but culture medium. Data are presented as the average ± SD (n= 5).

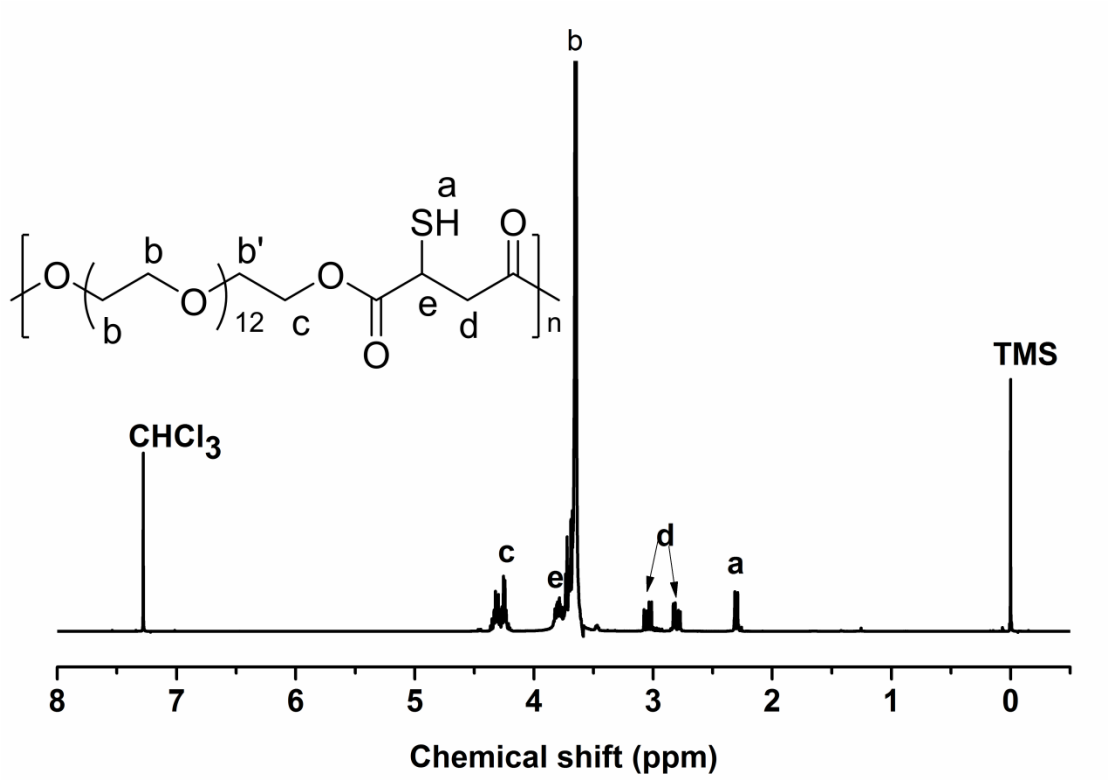

Fig. S1 ^{1}H NMR spectrum of POEGMS

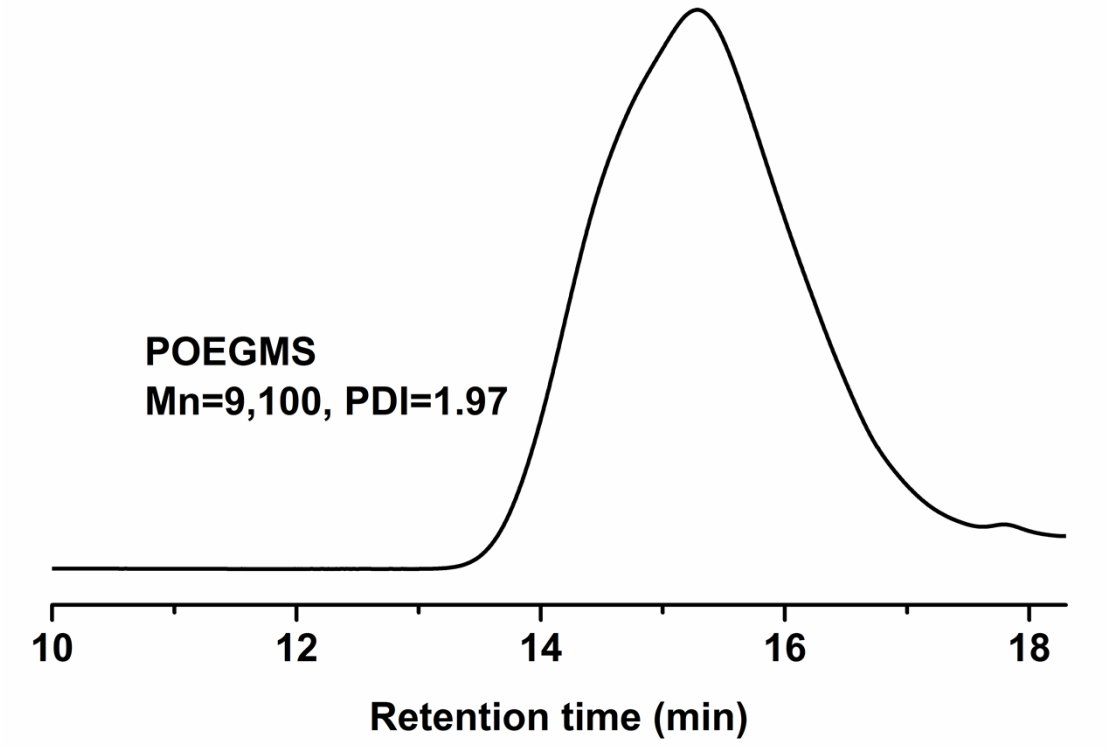

Fig. S2 GPC trace of POEGMS.

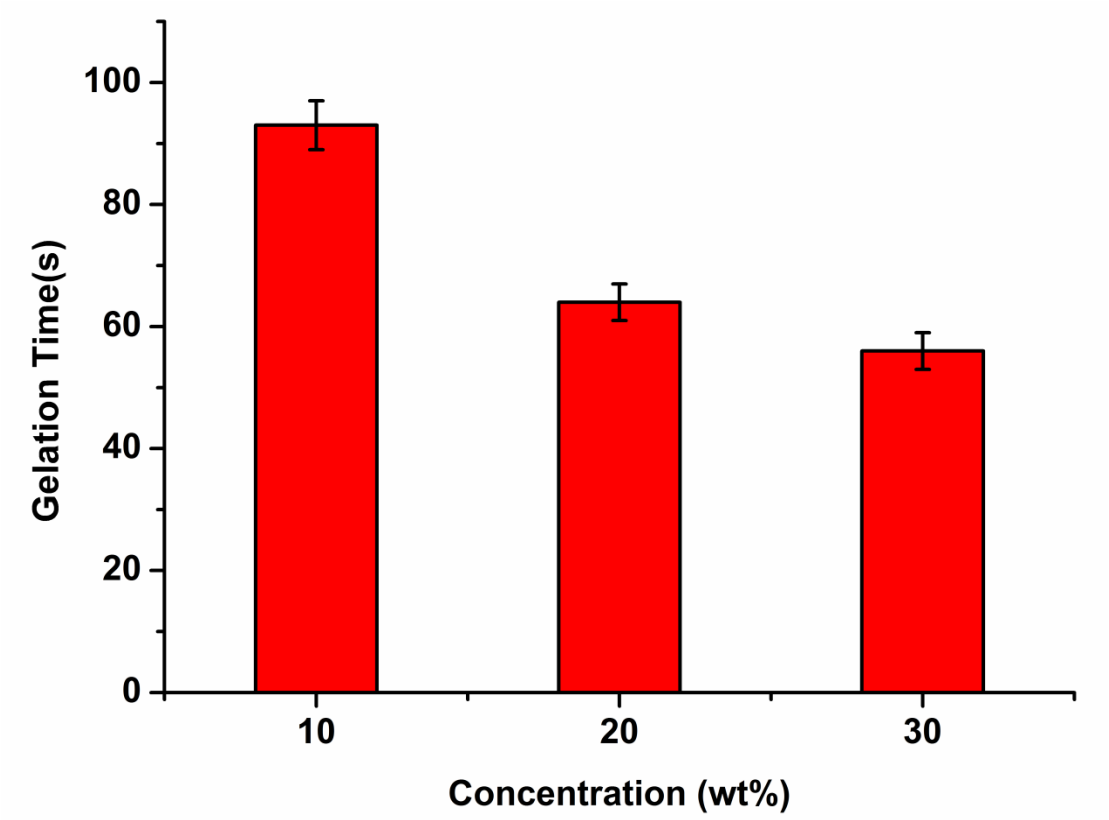

Fig. S3 Gelation time of hydrogels with various precursor concentrations in PBS solution (pH=7.4, 0.1M, 37 ℃).

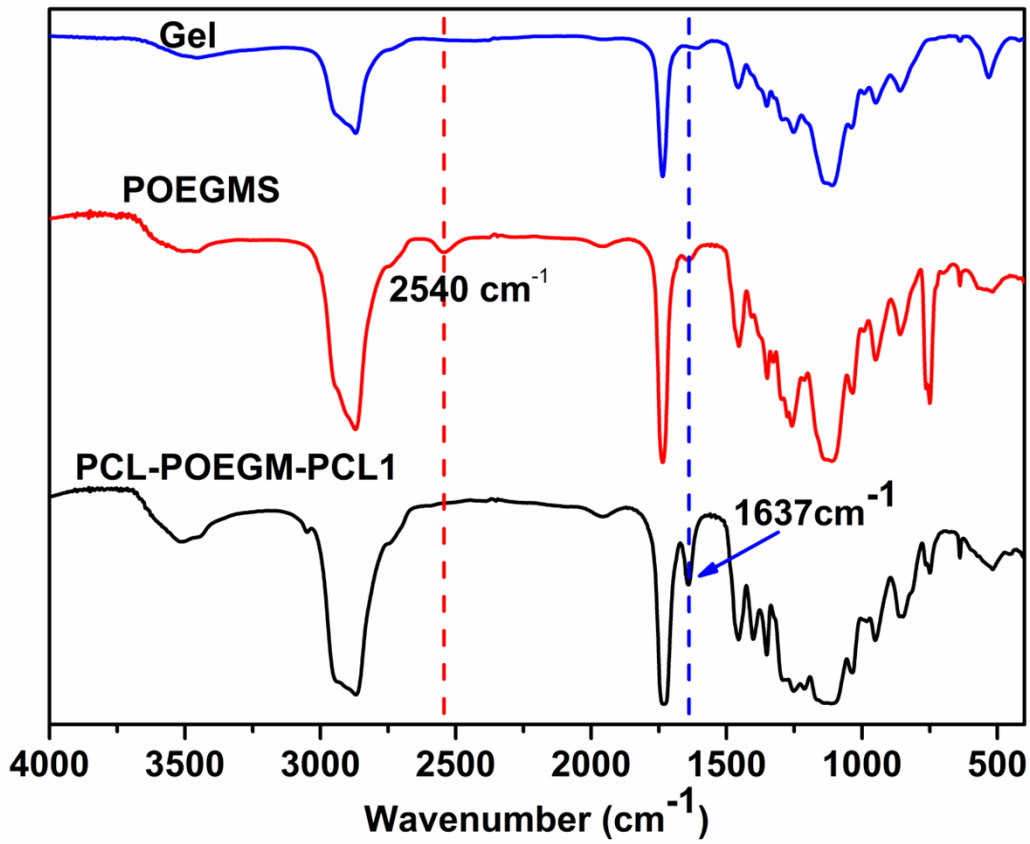

Fig. S4 FT-IR spectra of PCL-POEGM-PCL1, POEGMS and freeze-dried hydrogel.

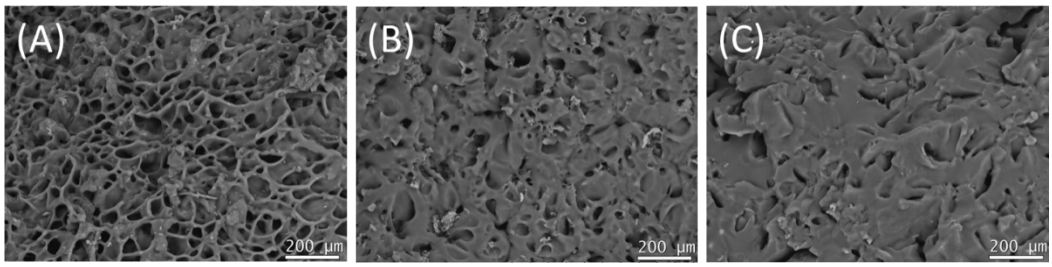

Fig. S5 Typical SEM images of the freeze-dried hydrogels with (A) 10 wt%, (B) 20 wt% and (C) 30 wt% solid contents, the scale bar is 200 μm.

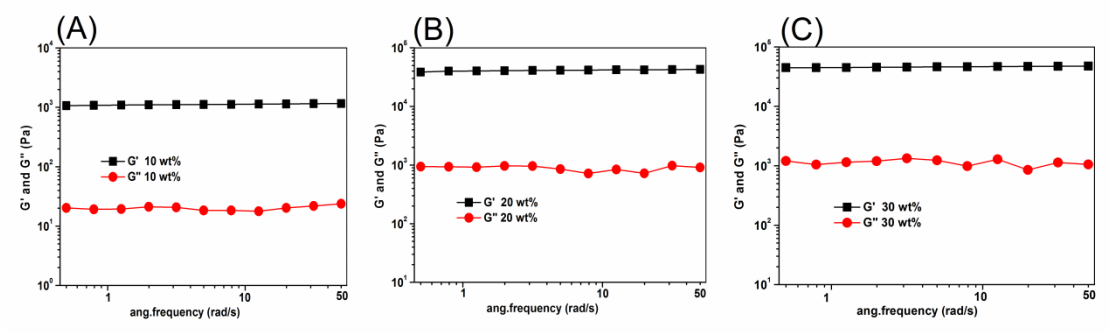

Fig. S6 Frequency dependence of storage modulus (G') and loss modulus (G") of hydrogels at 37 ^{o}C with (A) 10 wt%, (B) 20 wt% and (C) 30 wt% solid contents.

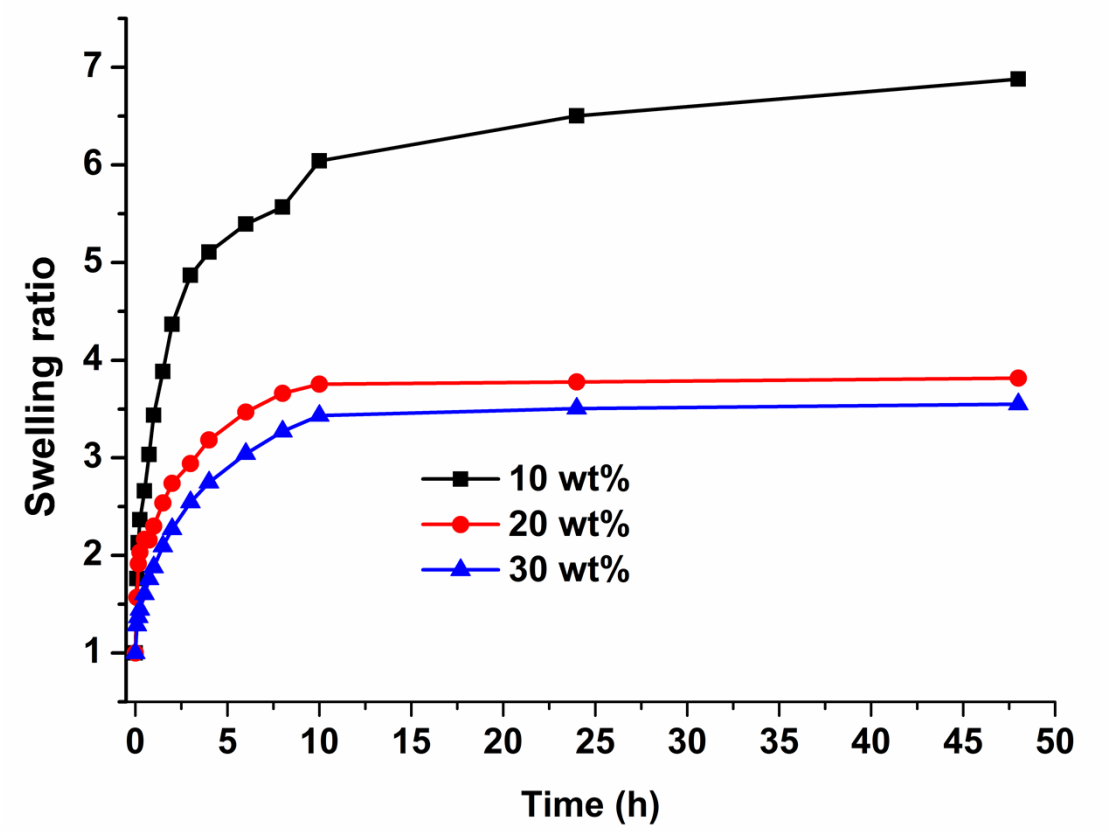

Fig. S7 Swelling curves of freeze-dried hydrogels.

References

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3 R. Rosario-Meléndez, W. Yu and K. E. Uhrich, *Biomacromolecules*, 2013, **14**, 3542-3548.