Supporting Information

An injectable thermogel with high radiopacity

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Materials

Methoxy poly(ethylene glycol) (mPEG; M_n=550 and 750), poly(ethylene glycol) (PEG; M_n=1000) and stannous 2-ethylhexanoate (stannous octoate, 99%) were purchased from Sigma-Aldrich. D,L-lactice (LA) was acquired from Purac and 2,3,5-triiodobenzoic acid (TIB) was obtained from Aladdin. All chemicals were analytical grade and used without further purification.

Synthesis of PEG/PLA Copolymer

Diblock copolymer mPEG-PLA was obtained by ring-opening polymerization of LA using mPEG as the initiator and $Sn(Oct)_2$ as the catalyst. For example, to synthesize the mPEG-PLA diblock copolymer (P2 in Table 1), 10.0 g of mPEG (M_n=550) was

firstly added into a dry four-neck flask and was heated under vacuum at 120 °C for 3 h to remove residual water in the polymers. Then, 21.0 g of LA was added under argon atmosphere and heated under vacuum at 100 °C for 0.5 h. Afterwards, 1 mL of $Sn(Oct)_2$ toluene solution (30 mg/mL) was added and the reaction system was keep at 150 °C with continuous stirring for 12 h under argon atmosphere. After the reaction was completed, a vacuum was used for 1 h to remove unreacted monomers in the reaction mixture. Next, crude product was dissolved in dichloromethane and then precipitated with cool ethyl ether. The precipitated polymer was separated from the supernatant by decantation. The final product was dried under vacuum for 48 h to remove the residual solvent. The other mPEG-PLA diblock copolymers and PLA-PEG-PLA triblock copolymer were synthesized using a similar procedure.

Synthesis of TIB-capped mPEG-PLA

TIB-capped mPEG-PLA was prepared by esterification between the end hydroxyl group of mPEG-PLA and the carboxylic group of TIB. In brief, 8.00 g of mPEG-PLA (0.005 mol) was dissolved in 150 mL toluene in a 250 mL bottle and then azeotropic distillation was performed to remove the residual moisture in the polymers. Next, anhydrous tetrahydrofuran (THF, 30 mL), TIB (5.03g, 0.01 mol), DMAP (0.65 g, 0.005 mol), and DCC (5.15g, 0.025 mol) were introduced in sequence. The reaction was proceeded in the dark under an argon atmosphere for 48 h. After the completion of reaction, a few drops of water was added to remove unreacted DCC. The clear solution was obtained via filtration and precipitated with cool ethyl ether. The precipitated polymer was separated from the supernatant by decantation and re-

dissolved in dichloromethane. After filtration again, the solvent was removed using a rotary evaporator and the obtained product was dissolved in unionized water. Finally, the polymer aqueous solution was centrifuged at 12,000 rpm for 30 min and the desired product was obtained by lyophilizing the supernatant. TIB-capped PLA-PEG-PLA was synthesized using a similar procedure.

NMR Characterization

Chemical structure, composition of polymers and TIB substituted percentage of TIB-capped samples were confirmed by ^{1}H-NMR measurements uisng a 400 MHz proton NMR spectrometer (Bruker, AVANCE III HD FT-NMR spectrometer). $CDCl_3$ was chosen as the solvent and tetramethylsilane was used as the internal standard.

The TIB substituted percentage of TIB-capped mPEG-PLA was calculated based on ratio between the intergral of atomatic protons of TIB (8.40~7.80 ppm) and that of -OC$\underline{H}_3$ of mPEG (3.45~3.35 ppm,). For TIB-capped PLA-PEG-PLA, the TIB substituted percentage was calculated from ratio between the intergral of atomatic protons of TIB (8.40~7.80 ppm) and that of -C$\underline{H}_2$C$\underline{H}_2$O- of PEG (3.75~3.60 ppm). The extent of acylation exceeded 90% for all the TIB-capped derivatives, indicating the successful introduction of TIB moieties into mPEG-PLA. Meanwhile, it is worth pointing out that the purification process of TIB-capped derivative led to a bit increase of length of PLA chain compared with mPEG-PLA diblock copolymer itself.

Gel Permeation Chromatography

Molecular weights and their distributions of the polymers were determined by the gel permeation chromatography system (GPC, Agilent 1260) equipped with a differential

refractometer. THF was used as eluting solvent at a flow rate of 1.0 mL/min at 35 °C and mono-disperse polystyrene was used to obtain the standard curve.

Sol-Gel Transition

Sol-gel transition temperature was determined via the vial inverting method.[1, 2] Each sample with a given concentration was prepared by dissolving polymers in unionized water and stored at 4 °C. Then, the 2 mL vials containing 0.5 mL polymer solutions were immersed in a water bath with predetermined temperature for 15 min to reach equilibrium. The sol-gel transition was monitored by inverting the vials with a temperature increment of 1 °C per step. It was regarded as gel state in case of no visual flow within 30 s after inverting the vials.

Rheological Analysis

Rheological analysis was also performed to invesitgate the sol-gel transition of the polymer aqueous solutions using a dynamic stress-controlled rheometer (Malvern, Kinexus) equipped with a cone plate (diameter, 60 mm; conical degree, 1°). After the samples were loaded, the edge of the plate was overlaid with a layer of low viscosity silicone oil to minimize the evaporation of solvent. In the analysis, the complexed viscosity η* was collected as a function of temperature from 15 to 50 °C with a heating rate of 0.5 °C/min and a frequency ω of 1 rad/s. During the test, an appropriate mode was applied according to the linear viscoelastic regions. Specifilly, an oscillation single frequency stress mode was first set at targeted stress of 5.0 Pa with a trigger. As temperature ramped, it would skip to the oscillation single frequency strain mode once the complex shear strain was less than 1%. The results

showed that the viscosity of both 35% and 40% P2-TIB thermogels reached 130 Pa•s at body temperature.

***In vitro* Micro-CT Measurement**

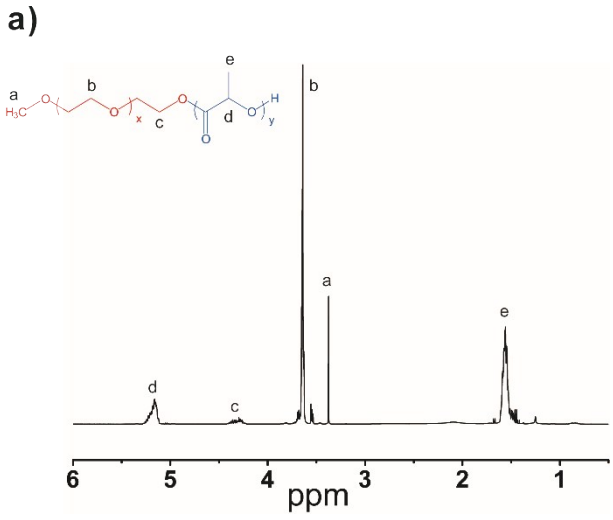
The 2 mL vials containing the polymer aqueous solutions or Al_2O_3 powder were scanned using a Micro-CT scanner (Bruker, Skyscan 1176). The parameter of scans were set as follow: spatial resolution of 35 μm pixel size; X-ray tube voltage of 65 kV; X-ray tube current of 370 mA; 1 mm Al filter; 0.70° rotation step over 198°. In the experiment, Al_2O_3 powder and 40% P2 aqueous solution were chosen as the control group and the blank group, respectively. Two kinds of uint were used to represent the radiopacity of object. One is attenuation coefficient which is one of the intrinsic value of the objects calculated according to Beer's law.[3] Another is grayscale which is converted from attenuation coefficience by reference to the intensity window.

***In vivo* Micro-CT Measurement**

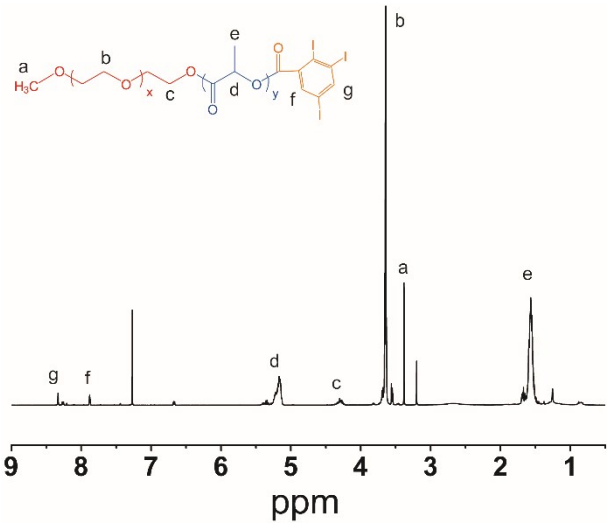
Fifteen male ICR mices (20~23 g; SLAC Laboratory Animal CO. Ltd, Shanghai, China) were raised in room at 23℃ and 55% relative humidity with a 12 h light-dark regimen. The mice were fed with standard chow diet and tap water *ad libitum*. All animal experiments were conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and approved by the ethics committee of Fudan University.

The mice were randomly divided into five groups (three animals per group): (a) s.c. injection group of 40% P4; (b) s.c. injection group of 35% P2-TIB; (c) s.c. injection group of 40% P2-TIB; (d) i.p. injection group of 40% P4 and (e) i.p.

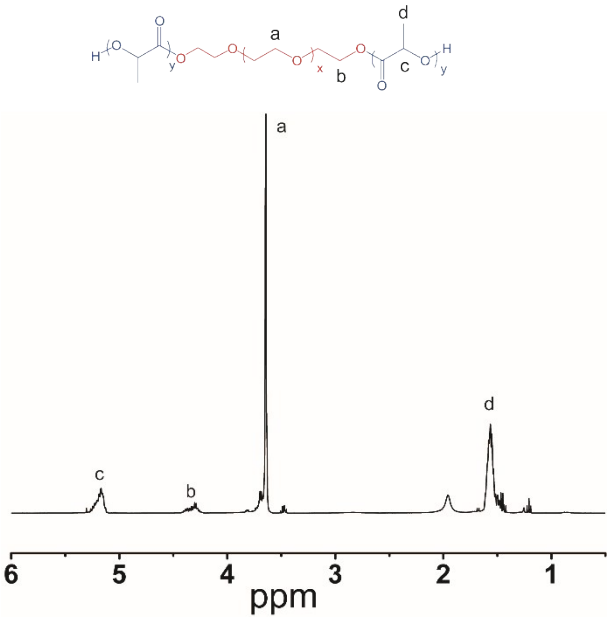
injection group of 40% P2-TIB. The volume was 0.24 mL each injection. Before injection of differernt samples, mices were anesthetized by i.p. administration of 0.25 mL chloral hydrate aqueous solution (4%, w/v). The scan was performed after the injection of different samples for 0.5 h and the parameters of scan were set as follow: spatial resolution of 35 μm pixel size; X-ray tube voltage of 65 kV; X-ray tube current of 370 mA; 1 mm Al filter; 0.70° rotation step over 180°. After the accomplishment of Micro-CT measurement, some aninals were scarificed and the injection area was carfully dissected. The photographs of in situ formed gel were recored using a digital camera. The other animals were recovered from general anesthesia and the observation of adverse reaction for the animals was performed. No sign of acute toxicity was observed for the animals after the injection of P2-TIB thermogels at different administration sites.



b)



c)



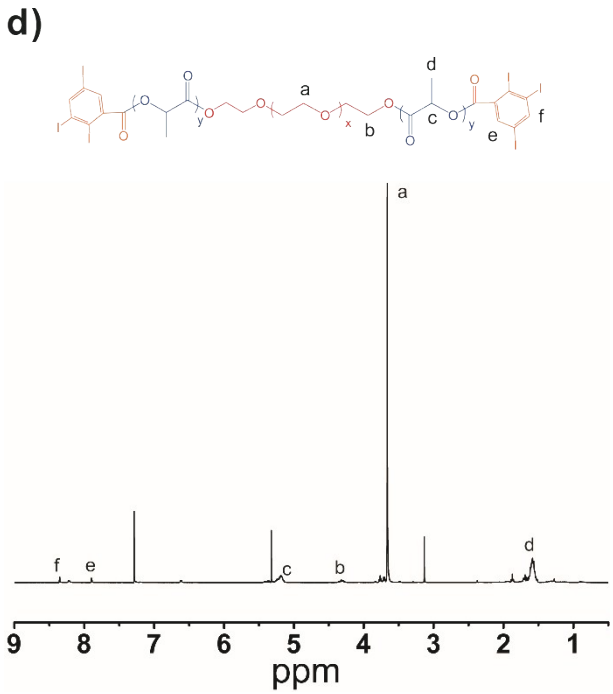


Fig. S1. ^{1}H NMR spectra of P2 (a), P2-TIB (b), P5 (c) and P5-DiTIB (d) polymers in $CDCl_3$.

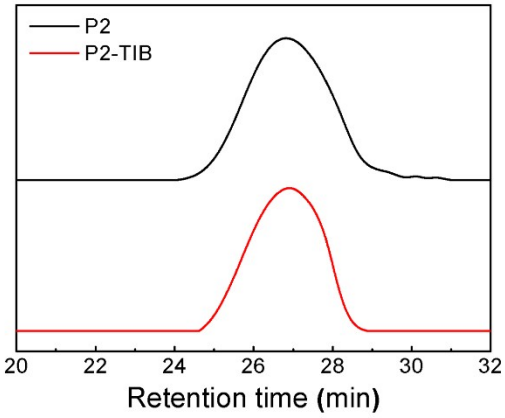


Fig. S2. GPC traces of P2 and P2-TIB

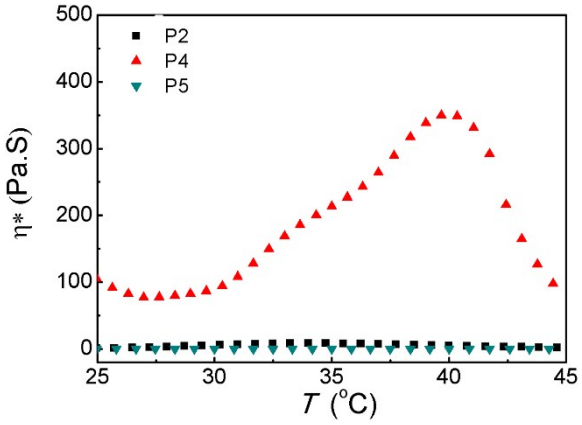


Fig. S3. Viscosity η* of 40% P2, P4 and P5 aqueous solutions as a function of temperature.

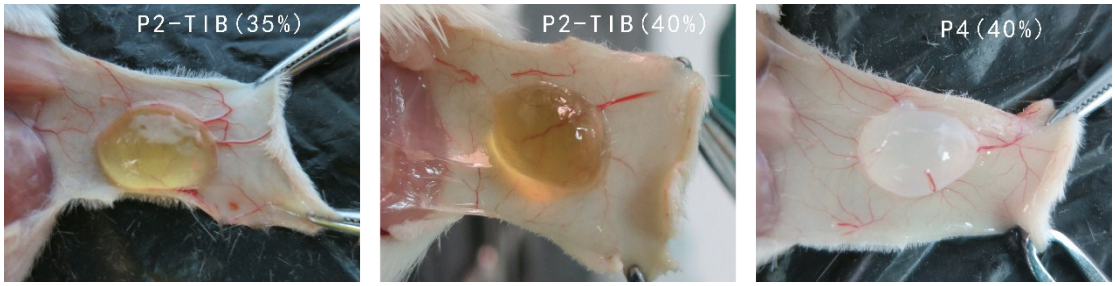


Fig. S4. Global views of 35%, 40% P2-TIB and 40% P4 thermogels under the skin in ICR mice 30 min post-injection.

References

1. B. Jeong, Y. H. Bae and S. W. Kim, *Macromolecules*, 1999, **32**, 7064-7069.
2. C. Chen, L. Chen, L. Cao, W. Shen, L. Yu and J. Ding, *RSC Adv.*, 2014, **4**, 8789-8798.
3. M. H. McKetty, *Radiographics*, 1998, **18**, 151-163.