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Supporting Information

Injectable Hydrogel Dressing for Controlled Release of Hydrogen Sulfide Pleiotropically Mediates Wound Microenvironment

Junwei Yang, [a] Xianzhen Dong, [a] Wenying Wei, [a] Kun Liu, [a] Xiaopei Wu, [a] Honglian Dai *[a b]

Junwei Yang, Xianzhen Dong, Wenying Wei, Kun Liu, Xinyue Liang, Yuanfang Huo, Zhiqiang Li, Xiaopei Wu, Honglian Dai

State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Biomedical Materials and Engineering Research Center of Hubei Province, Wuhan University of Technology, Wuhan 430070, China.

Honglian Dai

Wuhan University of Technology Advanced Engineering Technology Research Institute of Zhongshan City, Zhongshan 528400, ChinaEmail: daihonglian@whut.edu.cn

1. Synthesis of PMet

2 g mPEG4000-NH_2 (1 equiv.) were introduced into a single-necked flask and subjected to vacuum drying at 80 °C in an oil bath for a duration of 2 h. Following the cooling to r.t., 60 mL of anhydrous DMF was added, along with 2.1901 g of Met NCA (28 equiv.) were added and the reaction was stirred under argon protection for three days. The reaction was settled with 600 mL ice ether, the solid was vacuum filtration and dissolved in DMF, dialyzed with deionized water for three days (MwCO 3500 Da), and the white solid was obtained by freeze-dried. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.11 (s, 10H), 3.64 (s, 360H), 2.57 (s, 20H), 1.62 (s, 120H).

2. Synthesis of Amino-terminated F127 (F127-NH2) and Amino-teminated mPEG4000

(mPEG4000-NH_2) were prepared by the following method: F127 (5 mmol), potassium hydroxide (100 mmol), and paratoluensulfonyl chloride (50 mmol) were added to 300 mL of DCM and stirred at r.t. for 7 days. Then the resultant mixture was washed with brine (5 ×100 mL), dried over $MgSO_4$, concentrated using rotary evaporator, settled with ether, the sediment was filtered and dried in vacuo to receive a white solid. The product (10 g) was dissolved in $NH_3 \cdot H_2O$ (100 mL), then NH_4Cl were added to the solution and stirred for 7 days at r.t. The mixed solution was extracted with DCM until the solution was clear. The organic layer was

collected, washed with brine, dried over $MgSO_4$, concentrated, and settled with ice ether. Then, the sediment was collected with suction filtration and dried in vacuo to obtain F127-NH_2. The procedure for preparing mPEG4000-NH_2 was the same, except that F127 was substituted with mPEG4000 (5 mmol).

3. Synthesis of L-aspartic Acid 4-benzyl ester N-carboxy Anhydride (Asp NCA) and L-methionine N-carboxy Anhydride (Met NCA)

A mixture of amino acid (10 g, 3 equiv.), THF (150 mL), and triphosgene (1.25 equiv.) were added to the 250 mL flame-dried three-necked flask and reacted under argon bubble in a 65 °C oil bath. After the solution was clarified, the excess solvent was removed with argon, and the concentrated solution was poured into cold n-hexane to settle. The product was dissolved in EA, washed with cold saturated salt water, dried with $MgSO_4$, and the solution was vacuum-dried after filtration to obtain white solid (Asp NCA) and yellow liquid (Met NCA). Asp NCA ^{1}H NMR (500 MHz, Chloroform-d) δ 7.44 – 7.32 (m, 5H), 5.19 (s, 2H), 3.15 – 2.83 (m, 2H). Met NCA ^{1}H NMR (500 MHz, DMSO-d6) δ 5.09 – 4.94 (m, 1H), 3.10 – 3.01 (m, 2H), 2.56 – 2.45 (m, 5H).

4. PTCM@PMet NPs release PTCM upon stimulation with different concentrations of H_2O_2

Characterization of the release of PTCM from PTCM@PMet NPs using dialysis method. Firstly, PBS (pH7.4, 40 mL) buffer and Tween80 (0.2 mL) were added to a 50 mL centrifuge tube, which was then placed in a constant temperature shaker and incubated for 30 minutes. Subsequently, PTCM@PMet NPs (effective drug concentration: 50 μM) were weighed and added to 2 ml PBS solutions containing H_2O_2 (50 μM). The solution was poured into a dialysis bag, sealed, and placed in the preheated centrifuge tube. At 2, 8, 24, and 48 h time points, 3 mL of the solution from the centrifuge tube was withdrawn and an equal amount of fresh PBS buffer was added. Subsequently, the decomposition by-products of PTCM in hydrogen peroxide were characterized by high-performance liquid chromatography (HPLC), and the

absorption peak of p-hydroxy benzyl alcohol at 275 nm was measured to calculate the cumulative release of PTCM under different concentrations of hydrogen peroxide stimulation.

5. Skin Scalded Wound Model Building and Treatment

Male SD rats (180−220 g, Experimental Animal Research Center, Hubei Province, China) were used in this study. All rats were randomly divided into three groups (n=12), including control (saline) F127-P(Asp-NHS) and PTCM@PMet NPs/F127-P(Asp-NHS) groups.The rats were administered intraperitoneal injections of 5% pentobarbital sodium at a dosage of 40 mg/kg for anesthesia. The rats underwent a procedure where their back hair was removed and they were immobilized on the operating table in a prone position. The aluminum rod was placed in 100 °C water for 10 min, and rapidly placed on the back of the rats for 20 s, and the ice pack was immediately placed on the scalded site for 1 min to quench the scald. The process was repeated to create the second scald on either side of the spine. The scalded site was injected subcutaneously with 1 mL of saline to prevent dehydration, and all wounds were covered with Tegaderm Film (3M Health Care, USA) to limit infection. Following the surgical procedure, the rats were administered buprenorphine at a dosage of 0.05 mg/kg and saline at a volume of 1ml per mouse on daily basis based on their behavior. Two days after the scald, the rats were anesthetized and all necrotic tissue was demineralized using a 15 mm diameter biopsy punch to create a fresh full-thickness wound. The day of full-thickness wound establishment was recorded as day 0. The wound in control group was washed with saline, and the prepared precursor solution was injected into the wound in F127-P(Asp-NHS) and PTCM@PMet NPs/F127-P(Asp-NHS) and gelled in situ, and the material was added once every three days. On days 0, 5, 10, and 14, the skin tissue samples were obtained, and the images of the wounds were recorded. The area of the wound was analyzed using ImageJ 1.8 software. After washing with PBS, the skin was fixed in 4% paraformaldehyde for 24 h, and then dipped in ethanol and the mixed solution of ethanol and dimethylbenzene. The tissue specimen was immersed in paraffin and subsequently sliced into thin sections with a thickness of 5 μm.

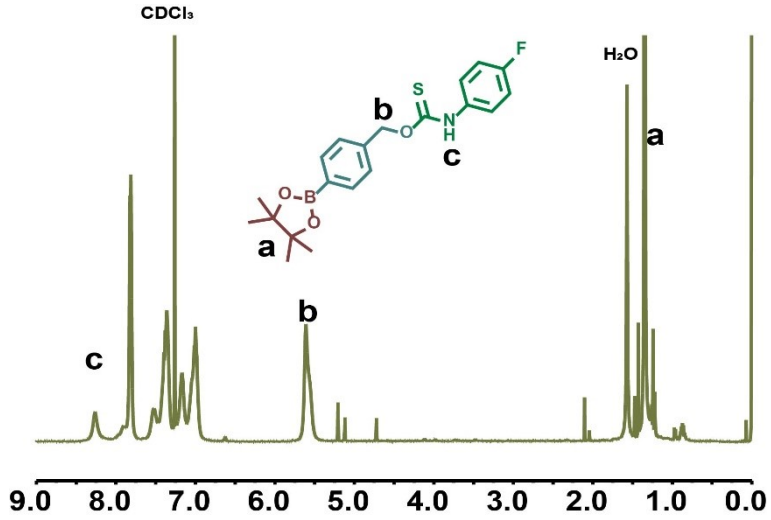


Fig. S1. ^{1}H NMR spectrum of PTCM.

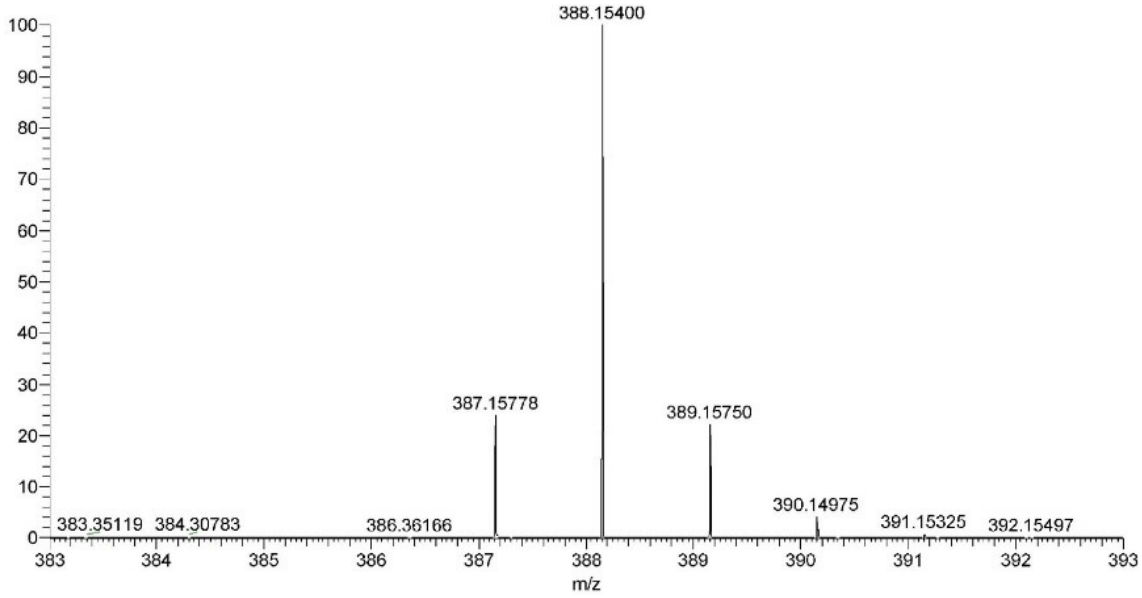


Fig. S2. HRMS results of PTCM.

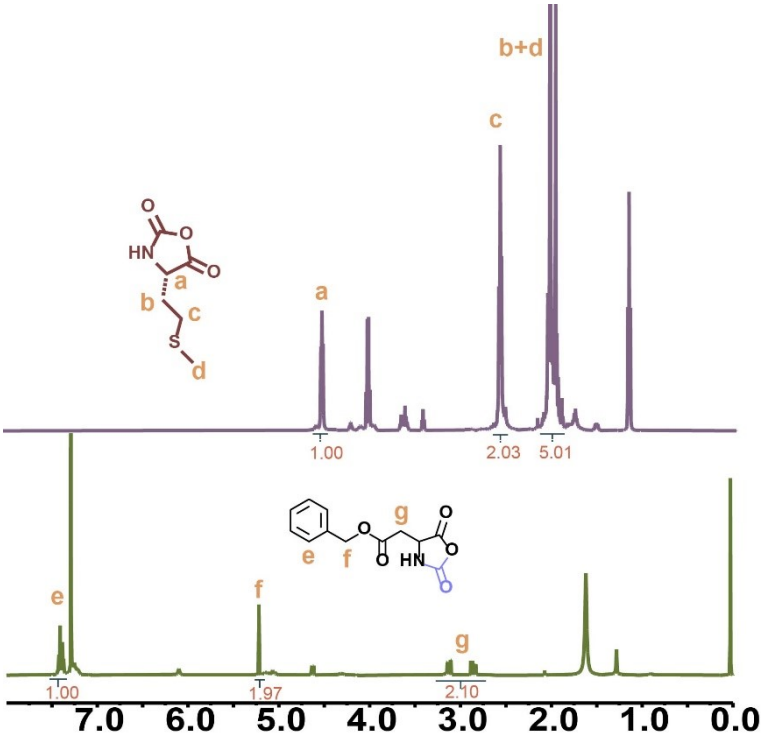


Fig. S3. ^{1}H NMR spectrum of Asp NCA in $CDCl_3$ and Met NCA in DMSO-d6.

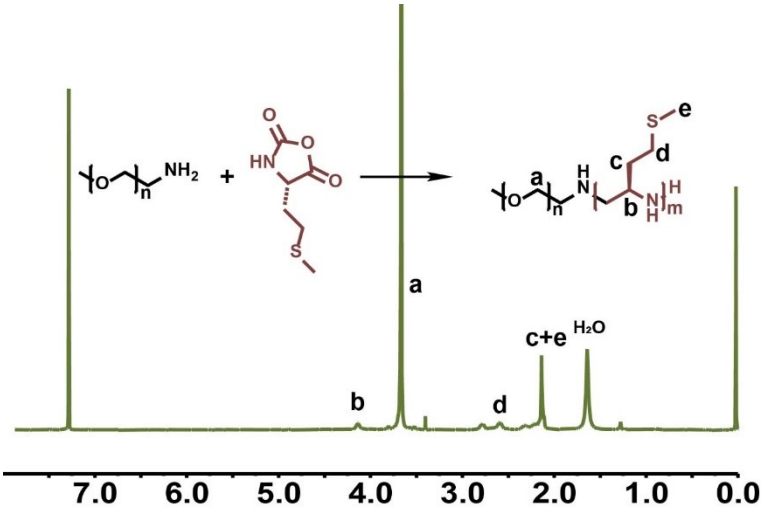


Fig. S4. ^{1}H NMR spectrum of mPEG-PMet in $CDCl_3$.

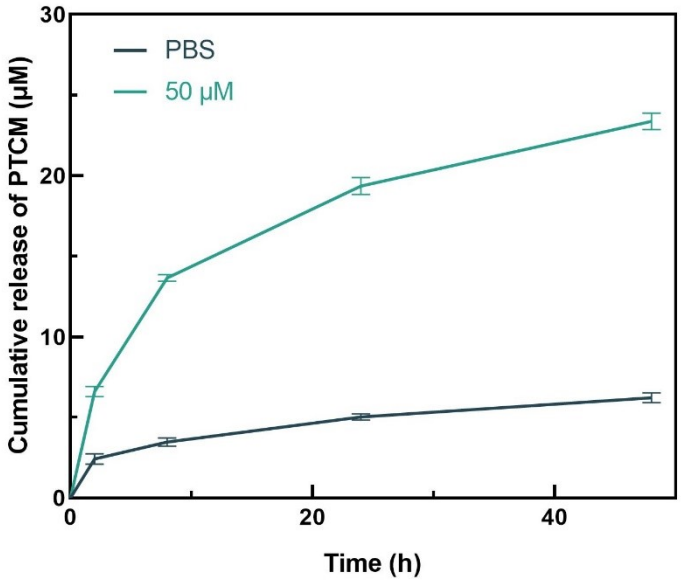


Fig. S5. PTCM release of PTCM@PMet NPs in H_2O_2 (50 μM).

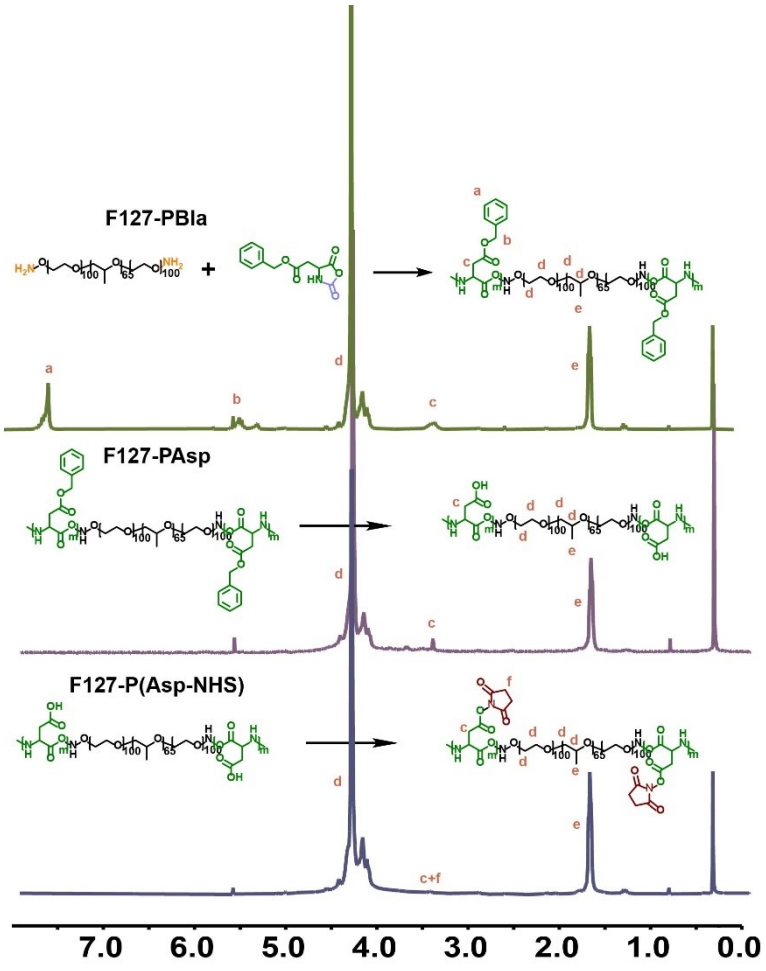
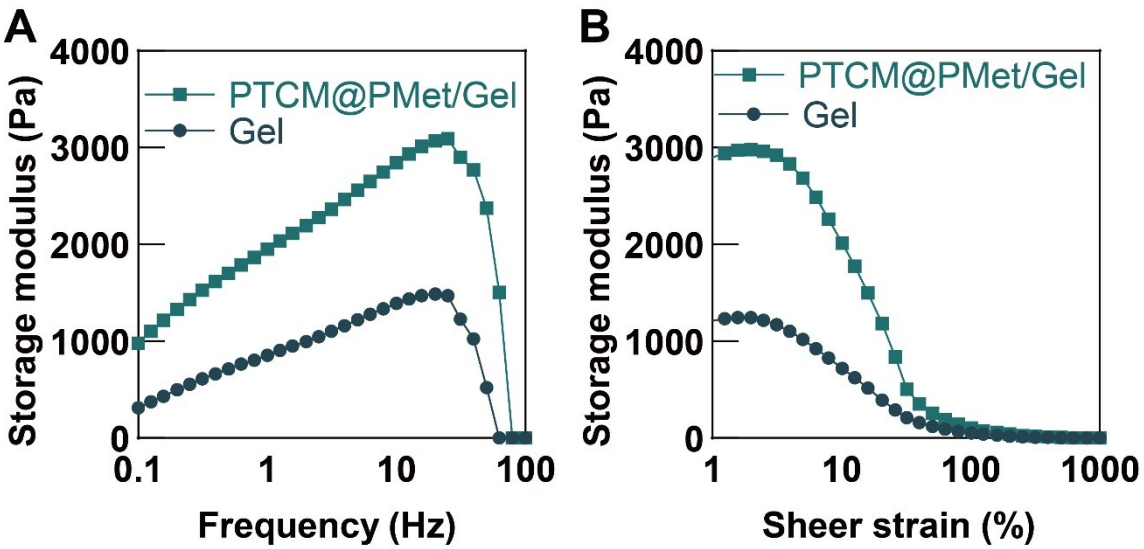


Fig. S6. ^{1}H NMR spectra of the products of each step during the synthesis of F127-P(Asp-NHS) in TFA-d.



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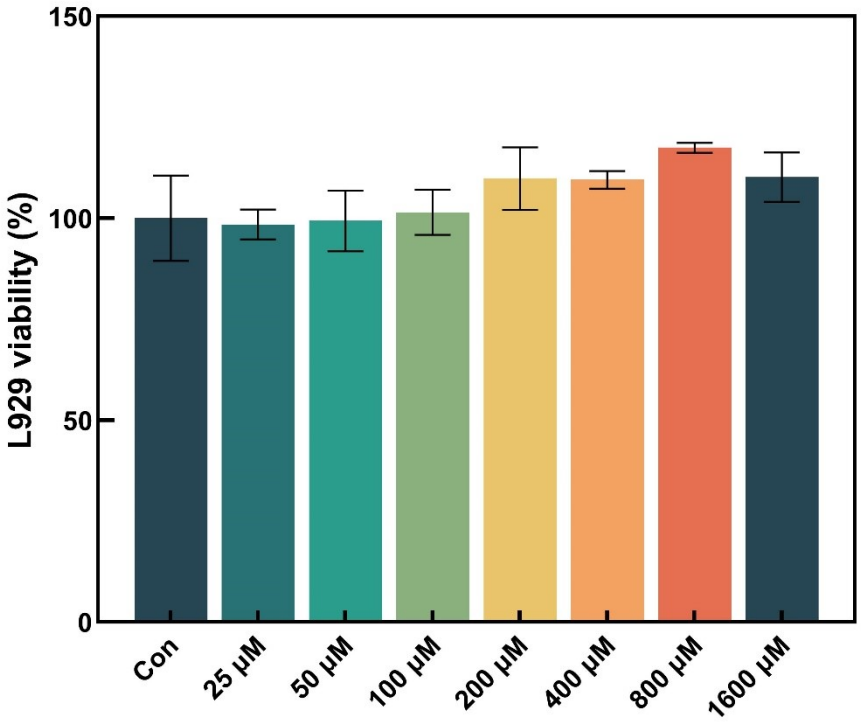
101 **Fig. S7.** (A) Frequency sweep measurement (0.01-100 Hz; 1% strain) of F127-P(Asp-NHS) and PTCM@PMet/F127-P(Asp-NHS)
102 hydrogels. (B) Storage modulus (G') curves from rotational strain sweeps (0.01-1000% strain; 10 Hz).



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104 **Fig. S8.** Cytotoxicity of different concentrations of H_2S on L929 cells at 1 day.