Supporting Materials

Materials

Chemicals and kits

Fmoc-amino acids and short peptides (Phe-Phe-DOPA) were obtained from GL Biochem (Shanghai, China). The other chemicals were purchased from Sangon Biotech (Shanghai, China). The cell counting kit (CCK-8) were obtained from Dojindo (Kumamoto, Japan). The viability/cytotoxicity assay kit for live and dead animal cells was supplied by (Biotium, USA). Nanopure water was used for all the tests of hydrogelation.

Enzyme and cells

Metalloprotease from *Bacillus cereus* WQ9-2 was isolated and characterized by ourself. The strain of *Bacillus cereus WQ9-2* is currently deposited at the China Type Culture Collection Center (Wuhan, China) under CCTCCM2010010. Adult human dermal fibroblasts (Supplied by Dr. Ge of Nanjing Medical University) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (fetal bovine serum, Gibco, USA) and 1% penicillin/streptomycin solution. Before 2D culturing, the cells were treated with a trypsinase (0.25%)-EDTA (0.02%) solution and resuspended in complete medium (DMEM plus 10% FBS and 1% penicillin/streptomycin solution).

General methods

HPLC analysis

The reaction products obtained after different reaction times were dissolved in a fixed volume of methanol (0.1% TFA). This solution also effectively denatured the enzyme and dissolved the hydrogel. HPLC analysis was conducted with a Dionex P680A HPLC system using a Kromasil 100-5C18 column (4.6 mm × 250 mm, Kromasil, Sweden) and a UVD170U detector at 254 nm. A methanolic (solvent A)/water (solvent B) mixture was

used as the mobile phase in the following gradient method: 0–5 min, 20% B; 5–30 min, 20–100% B; 30–40 min, 100% B. The flow rate was 1.0 $mL \cdot min^{-1}$, and the oven temperature was 30°C.

ESI+ TOF MS analysis

The molecular weights of the synthesized product was determined using a TOF mass spectrometer (Micromass) equipped with an electrospray ion source. The spectra were obtained in positive-ion mode.

Scanning electron microscopy

SEM studies were performed with a Hitachi S3400N system using a 15 kV accelerating voltage. For the preparation of the samples for the SEM experiments, the gels were cast onto silicon slices and subsequently freeze-dried under vacuum for 1 day. Afterward, the samples were coated with gold.

Environmental scanning electron microscopy

The microstructure of the gels in their native enviroment was examined qualitatively using ESEM (model: FEI/Phillips XL30). Cross-sections (n = 6) of the transverse plane were collected every 1 mm through the depth of the samples by slicing them on a frozen- stage microtome, and for all samples, the images were captured at the same predetermined, standardized coordinates, to eliminate any bias caused by choosing the image and to ensure uniformity among the samples. Each sample was imaged in its hydrated state using a cooled stage (1.5°C) at 0.7 torr and using an accelerating voltage of 20 kV.

Fluorescence spectroscopy

The interactions between the fluorenyl groups of the peptide molecules were investigated using fluorescence spectroscopy. A solution containing 40 mM Fmoc-Leu **1** and 40 mM Phe-Phe-DOPA **2** was pipetted into a 1.0 cm quartz cuvette and mixed with an equal volume of enzyme solution to induce gelation. The emission data for the self-assembled hydrogels were recorded on a Hitachi F-7000 FL using a mixture of substrates in H_2O as

the background. The data between 285 nm and 450 nm were collected using excitation at 265 nm and a 5 nm bandwidth.

Rheology

The rheology tests were performed with a Thermo RheoStress 6000 rheometer using a 20 mm/2 cone-plate geometry. The gels for the rheological experiments were prepared on the bottom plate of the rheometer by loading a 0.5 mL sample of the gelator immediately after protease addition. The evaporation of water from the hydrogel was minimized by covering the sides of the plate with low-viscosity mineral oil, and an integrated temperature controller was used to maintain the temperature of the sample stage at 37°C. The dynamic time sweep tests were as follows: strain = 1.0%, frequency = 10 $rads^{-1}$, 10 points per decade. The dynamic frequency sweep tests were as follows: initial frequency = 0.1 $rads^{-1}$, final frequency = 100 $rads^{-1}$, and strain =1.0 %.

Biocompatibility experiments

2D-culture of human adult dermal fibroblasts

Adult human dermal fibroblasts (Supplied by Dr. Ge of Nanjing Medical University) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (fetal bovine serum, Gibco, USA) and 1% penicillin/streptomycin solution. Before 2D culturing, the cells were treated with a trypsinase (0.25%)-EDTA (0.02%) solution and resuspended in complete medium (DMEM plus 10% FBS and 1% penicillin/streptomycin solution).

Before the hydrogel was prepared, the powdered substrates were weighed into separate vials and sterilized under UV light for 60 min. Equimolar amounts (40 mmol) of **1** and **2** were mixed to provide a suspension that was dissolved when 0.5 mol NaOH was added to adjust the final pH to 7.4. Subsequently, metalloprotease with a final activity of 2000 U/mL was added. The solution was then vortexed, added to each well in a non-coated 96-well plate, and then incubated at 37°C. One hour after the hydrogel formation, 100 μL of human adult dermal fibroblast suspension (30,000 cells/mL) was pipetted into each well of a 96-well plate for incubation.

Cell viability test

The cellular viability was measured every 24 h for three times using cell counting kit (CCK-8 Kit). Highly water-soluble tetrazolium salt, WST-8, is reduced by dehydrogenase activities in cells to give a yellow-color formazan dye, which is soluble in the tissue culture media. The amount of the formazan dye, generated by the activities of dehydrogenases in cells, is directly proportional to the number of living cells. After 15min, the absorbance of the resulting solution was measured at 450 nm. The result of the cell viability on the hydrogel was shown in Fig. S4, the cell viability in the DMEM was as the control.

Evaluation of cell adhesion and growth proliferation

To investigate the application of the hydrogel as a bioadhesive scaffold for cells, another hydrogel based on Fmoc-Leu-Phe-Phe without the DOPA caps was used as a control. We first focused on quantitatively analyzing the cells attached to the hydrogels' surfaces after they were gently washed with PBS buffer when the cells had been incubated for 2 h. The remaining cells were treated with a trypsinase (0.25%)-EDTA (0.02%) solution and resuspended in physiological saline. An assay solution containing 4 mM EthD-1 (ethidium homodimer-1) and 2 mM calcein AM was added. After 15 min of incubation at room temperature, the cell count and viability were detected using a Nikon Eclipse fluorescence microscope with excitation filters for 494 nm (green, calcein) and 528 nm (red, EthD-1).

Cell proliferation

Cell proliferation on the gels was assessed with CCK-8. The cell numbers at predetermined intervals (1, 2, and 3 days) were measured. Premixed CCK-8 and media (10 μL:100 μL) were added into 96-well plates, and the cells were incubated for 1 h at 37°C. The A450 values of the supernatants were obtained.

4 Characterizations

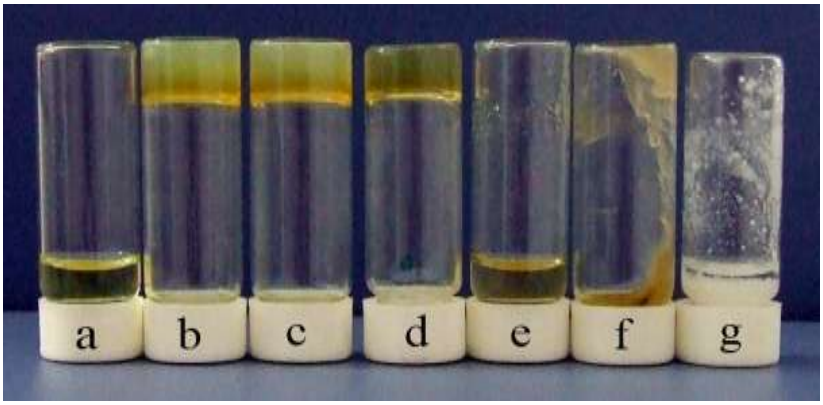

Fig. S1 Optical images of the hydrogels: **a,** 40 mM Fmoc-Leu/Phe-Phe-DOPA and inactivated protease WQ9-2 (control); **b,** 40 mmol·L^{-1} Fmoc-Leu/Phe-Phe-DOPA, protease WQ9-2 (2000 U/mL); **c, d, e,** Fmoc-Leu/Phe-Phe-DOPA (the concentrations of both substrates were 20 mM, 10 mM, and 5 mM, respectively), protease WQ9-2 (2000 U/mL); **f,** 40 mmol·L^{-1} Fmoc-Leu/Phe-DOPA, protease WQ9-2 (2000 U/mL); **g** 40 mmol·L^{-1} Fmoc-Leu/Phe-Phe-Phe, protease WQ9-2 (2000 U/mL).

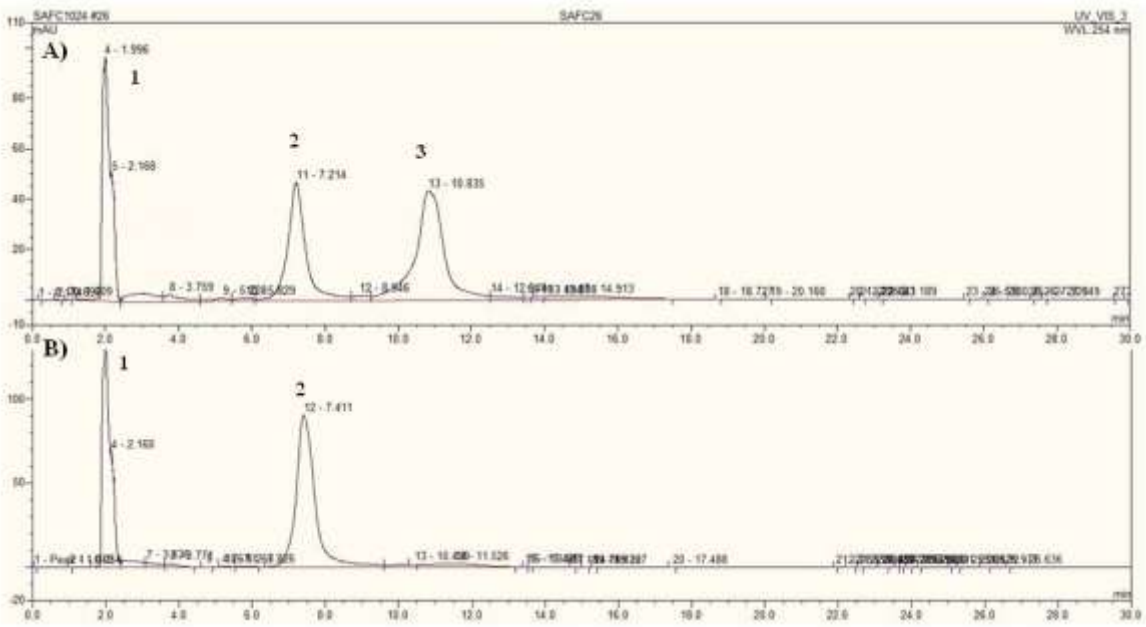

Fig. S2 HPLC chromatograms of **a,** metalloprotease-catalyzed peptide synthesis between Fmoc-Leu and Phe-Phe-Dopa (40 mM) for 6 h and **b,** the control group after the addition of a corresponding inactivated enzyme. Peak 1 is attributed to Phe-Phe-Dopa, peak 2 is attributed to Fmoc-Leu, and peak 3 is attributed to Fmoc-LFFDopa.

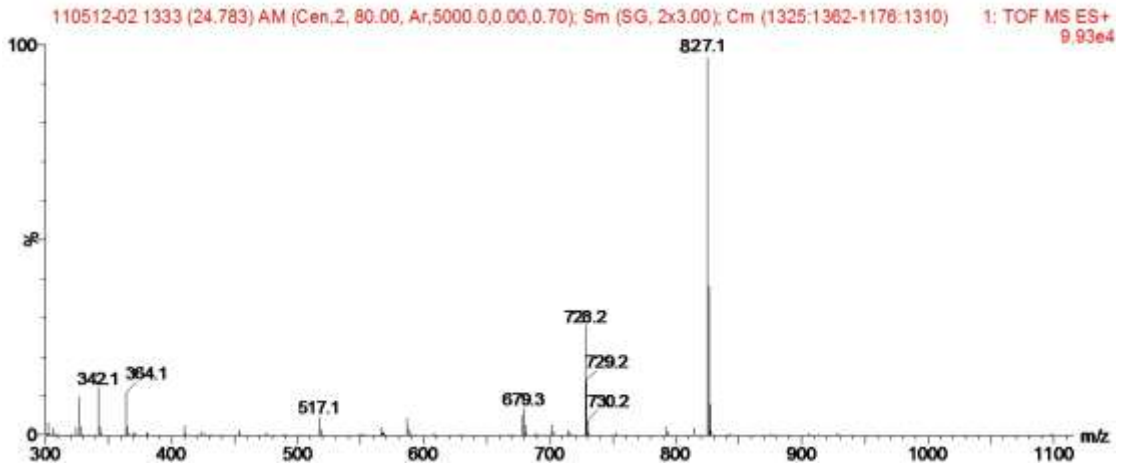

Fig. S3 MS analysis of **3** Fmoc-Leu-Phe-Phe-Dopa {827.1 ($M+H^+$)}.

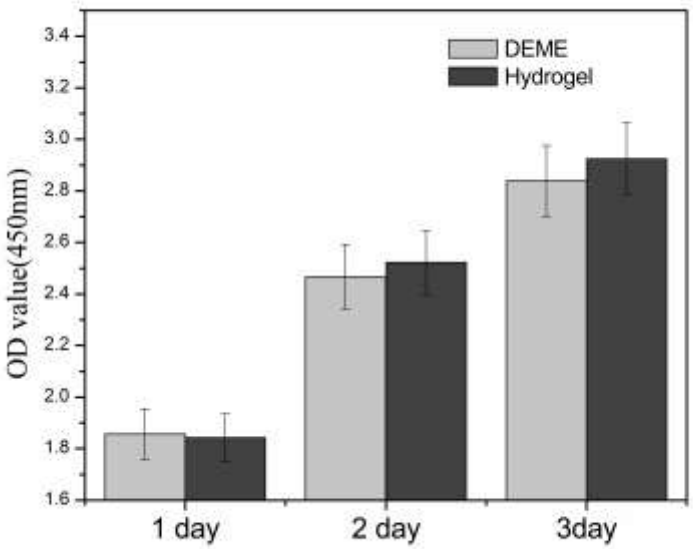

Fig.S 4 Cell viability test on the hydrogel