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Construction of poly(dopamine) doped oligopeptide hydrogel

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Experimental Details

1 Materials

N-Fluorenyl-9-methoxycarbonyl protected L-amino acids (Fmoc-Asp(OtBu)-OH, Fmoc-Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH), 2-chlorotrityl chloride resin (100-200 mesh, loading: 0.911 mmol/g), N-hydroxybenzotriazole (HOBt), benzotriazole-N,N,N',N'-tetramethyluroniumhexa-fluorophosphate (HBTU) and piperdine were purchased from GL Biochem (Shanghai) Ltd. (China) and used as received. Trifluoroacetic acid (TFA) was provided by Shanghai Reagent Chemical Co. (China) and used directly. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was received from Shanghai yuanye Bio-Technology Co. Ltd (China). Dopamine was obtained from Aladdin and Tris-HCl was provided by Shanghai regal Biology Techology Co. Ltd.

2 Synthesis and characterization of PDA

A certain amount of dopamine was oxidized and autopolymerized in vial with 1 mL of Tris-HCl (pH 8.5) at room temperature. To monitor the oxidation process, the fluorescent spectroscopy was exploited to record the fluorescent emission of PDA solutions at different time intervals (2 min, 5 min, 15 min, 30 min, 60 min, 120 min, 180 min, 240 min, 360 min), respectively. The excitation wavelength was set at 400 nm. Particle size of PDA nanoparticles

was measured by Nano-ZS ZEN3600 apparatus (Malvern Instruments) at 25 ^{o}C.

3 Synthesis and characterization of oligopeptide gelator

The oligopeptide gelator was synthesized on 2-chlorotrityl chloride resin using a standard Fmoc chemistry. The product was obtained as a white powder. The molecular structure of peptide was speculated by ^{1}H and ^{13}C NMR on a Mercury VX-300 spectrometer at 300 MHz (Varian) by using dimethyl sulfoxide-d6 (DMSO-d6) as a solvent. ^{1}H NMR: 7.1-8.3: the aromatic hydrogens in Fmoc group, Phe residues, amide hydrogens in peptide backbone and amine hydrogens in guanidine group, 4.0-4.6: the methyne and methylene hydrogens in peptide backbone and Fmoc group, 2.6-3.7: the methylene hydrogens in Phe and Asp residues, 1.0-2.1: the methylene hydrogens in Arg residue (Fig. S2A). ^{13}C NMR: 169-172: the carbonyl carbons in peptide backbone, 157: the carbon in NH_2-*C*(-NH-)=NH group, 156: -O-*C*O-NH- in Fmoc group, 120-144: the aromatic carbons in Fmoc group and Phe residues, 66: the methylene carbon in Fmoc group, 52-56: the methyne carbons in peptide backbone, 13-48: the methylene carbons in Arg and Asp residues, and methyne carbon in Fmoc group (Fig. S2B). The molecular weight of oligopeptide was confirmed by electrode spray ionization mass spectrometry (ESI-MS, LCQ Advantage, Finigan, USA) and time-of-flight mass spectrometry (SELDI-TOF MS, autoflex speed TOF, Bruker, Germany). The m/z value of 1010.5 is observed in ESI-MS spectrum (Figure S3), which was corresponded with the $[M + H]^{+}$ pattern of the oligopeptide with a theoretical molecular weight of 1009.4 g/mol. Meanwhile, molecular weight of 1010.1 g/mol is found in SELDI-TOF MS (Figure S4), indicating the successful synthesis of oligopeptide. The purity of oligopeptide gelator was detected by high-pressure liquid chromatography (HPLC, LC-20AR, Shimadzu, Japan) with a

C18 column and using a linear gradient of DI water containing 0.1% TFA and acetonitrile, and a purity of 92.5% was obtained (Figure S5). The thermal stability of the oligopeptide hydrogel was examined by a thermogravimetric analyzer (NETZSCH Jupiter STA 449C, USA). The measurement was carried out from 40 ^{o}C to 600 ^{o}C at a heating rate of 10 ^{o}C min^{-1}.

4 Preparation of native and hybrid hydrogels and vial inversion test

A certain amount of oligopeptide gelator was dissolved with a good solvent of dimethyl sulfoxide (DMSO) to prepare the stock peptide solution with a concentration of 500 mg/mL. Then a series of oligopeptide solutions with different concentrations (1 mg/mL, 3 mg/mL, 5 mg/mL and 7 mg/mL) were prepared by diluting stock oligopeptide solution with Tris-HCl under the ultrasonic treatment. After that, HCl was used to adjust the pH value of solutions to 7, yielding a series of peptide hydrosols or hydrogels. The vial inversion test was utilized to judge the occurrence of gelation 24 h later.

To prepare the PDA doped hybrid oligopeptide hydrogel, PDA obtained from the oxidization of dopamine for 3 h was exploited to interconnect with oligopeptide gelator at different proportions. Different amounts of the peptide-containing DMSO stock solution were added into the PDA-containing Tris-HCl buffer solution (pH 8.5), which was subjected to ultrasonic treatment for gelator dispersion. Then HCl was also used to adjust the pH value of solution to 7. After 24 h, the vial inversion test was utilized to investigate the gelling ability.

5 Fluorescence experiment

To ascertain the self-assembly mechanism of native peptide hydrogel and hybrid hydrogel, concentration-dependent fluorescent emission property was investigated using Hitachi F-7000

molecular fluorescence spectrometer. Peptide solutions with concentration of 0.05 mg/mL and 0.25 mg/mL were selected to monitor the red shift of fluorescence wavelengths during the self-assembly process. The fluorescent emission spectra were recorded with the excitation wavelength at 285 nm. The fluorescence study was also performed on the PDA-bearing hybrid hydrosol consisting of 0.25 mg/mL of oligopeptide gelator solution and 0.05 mg/mL of PDA.

6 FT-IR experiment

FT-IR spectra of the PDA, self-assembled native oligopeptide hydrogel and hybrid hydrogel were performed on an AVATAR 360 spectrometer. Prior to the measurements, the freeze-dried samples were pressed with potassium bromide (KBr) powder to form pellets.

7 SEM characterization

Scanning electron microscopy (SEM) was conducted on a Nova NanoSEM FEI (Holland) instrument with an accelerating voltage of 30 kV. Before the measurement, the native or hybrid hydrogel was diluted with water and 1 μL of peptide hydrosol was placed on the glass substrate. After the evaporation of liquid in room temperature, the sample was coated with gold for the observation.

8 Assay for free radical scavenging activity of PDA nanoparticles and hybrid hydrogel

The potential antioxidant activity of PDA nanoparticles and hybrid oligopeptide hydrogel was evaluated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay according to the literature method.[S1] In detail, 0.1 mM of DPPH in ethanol was freshly

prepared before the use because of its insolubility in water. A certain amount of DPPH/ethanol solution was incubated with sample for a period of time in the dark. The UV absorbance of DPPH at 516 nm was recorded with a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan). And the radical scavenging activity of samples against DPPH was calculated according to the equation: $I = [1-(A_i-A_j)/A_c] \times 100\%$, where A_i is the absorbance of sample mixed with DPPH, A_j is the absorbance of pure sample, and A_c is the absorbance of pure DPPH solution in the absence of samples.

9 Mechanical property investigation of peptide hydrogel

Mechanical property of the native peptide hydrogel and hybrid hydrogel was assessed using strain-controlled Rotational Rheometer (AR-2000ex, TA Instruments). 2 mL of hydrogel sample was prepared and located on the measuring plate. The gap between the measuring plate and the 2 degree cone-plate was 60-80 μm. In a typical frequency sweep experiment, the variations of storage modulus (G') and loss modulus (G'') were recorded as a function of applied frequency sweep from 0.01 to 100 Hz. In order to establish the ideal viscoelastic property, the constant strain was stabilized at 0.1% in the ambient temperature.

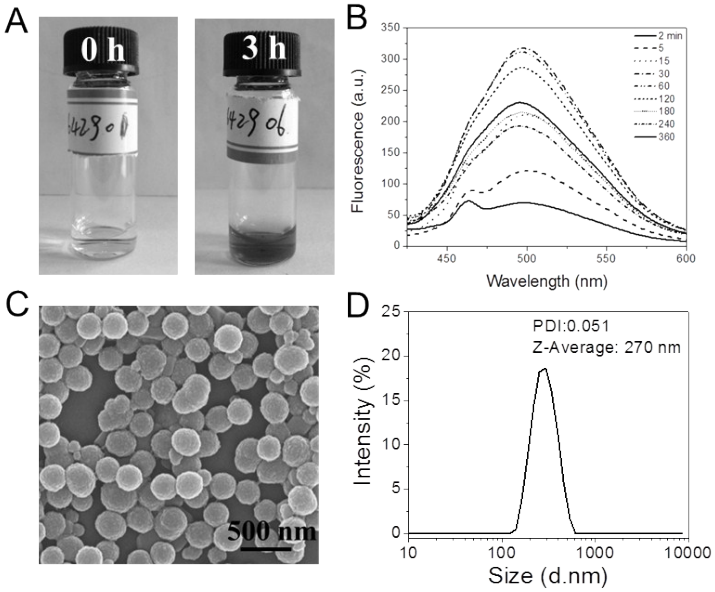

Fig. S1 (A) Photos of the dopamine solution before and after oxidation. (B) Fluorescent spectra of PDA solutions with different oxidation times. (C) SEM image of PDA prepared at the concentration of 0.25 mg/mL. (D) The size distribution of the PDA nanoparticles at room temperature.

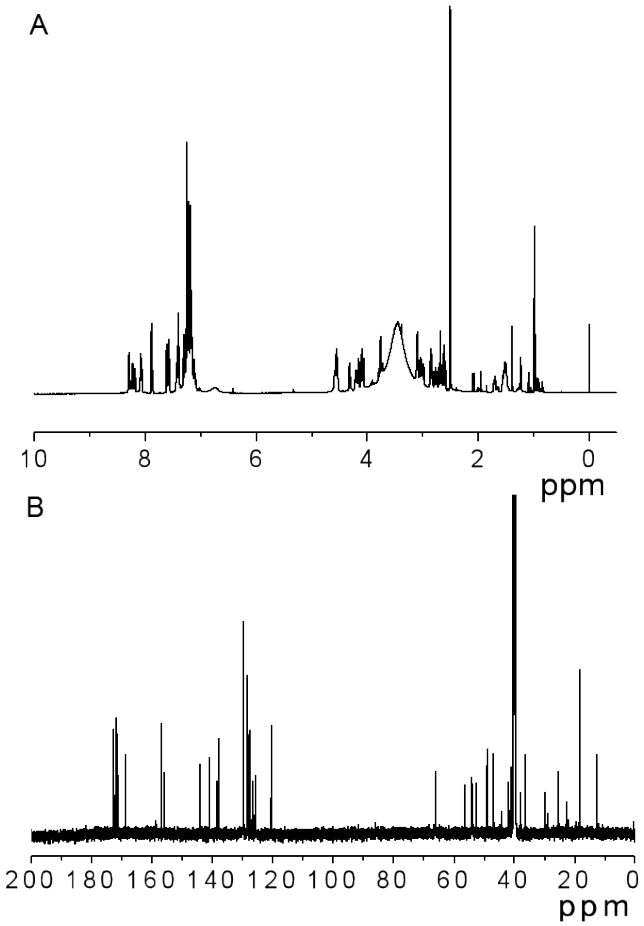

Fig. S2 ^{1}H NMR and ^{13}C NMR spectra of Fmoc-FFFRGD-OH peptide.

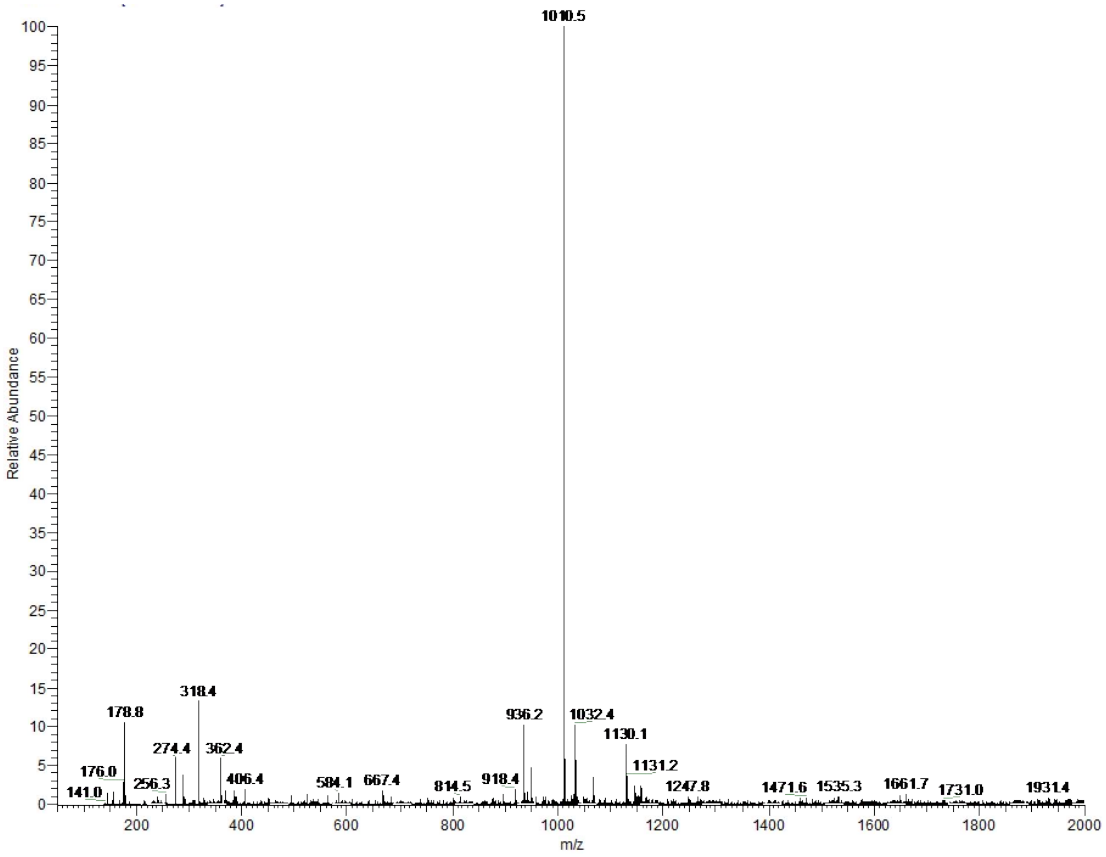

Fig. S3 ESI-MS of Fmoc-FFFRGD-OH.

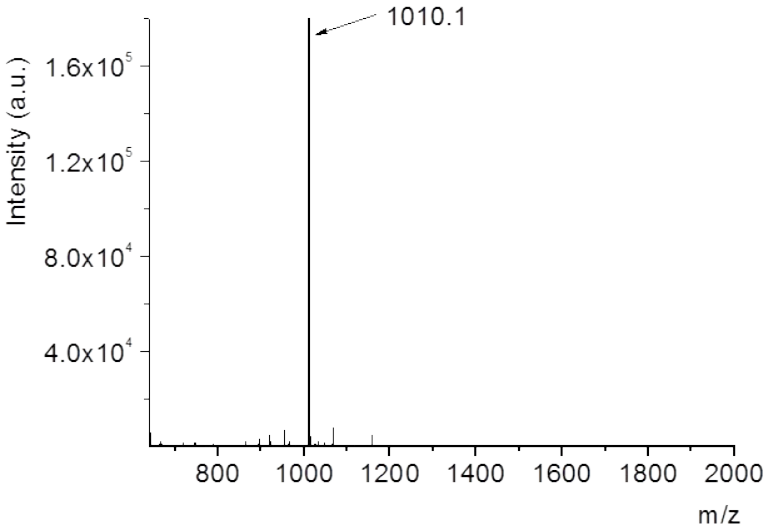

Fig. S4 SELDI-TOF MS of Fmoc-FFFRGD-OH.

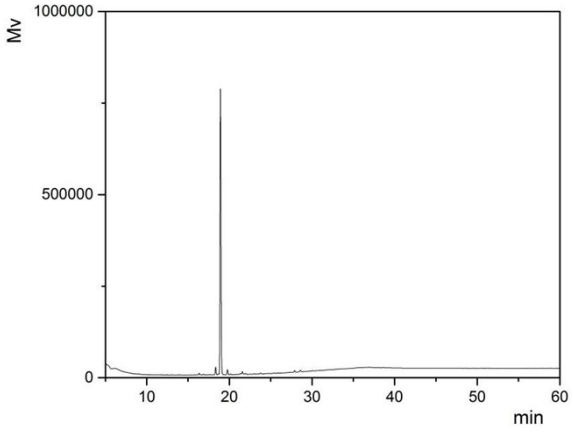

Fig. S5 HPLC profile of Fmoc-FFFRGD-OH.

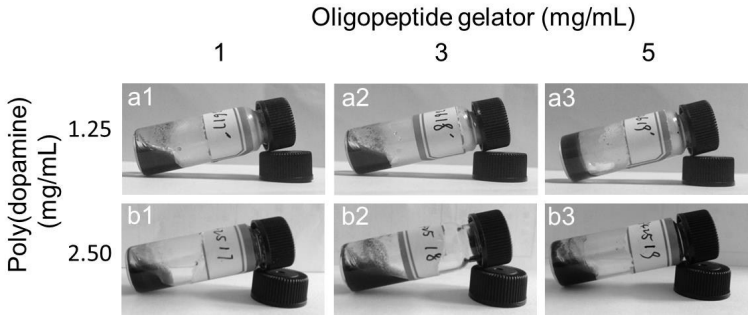

Fig. S6 The pictures of hybrid hydrosols and hydrogels consisting of different concentrations of oligopeptide gelator and PDA.

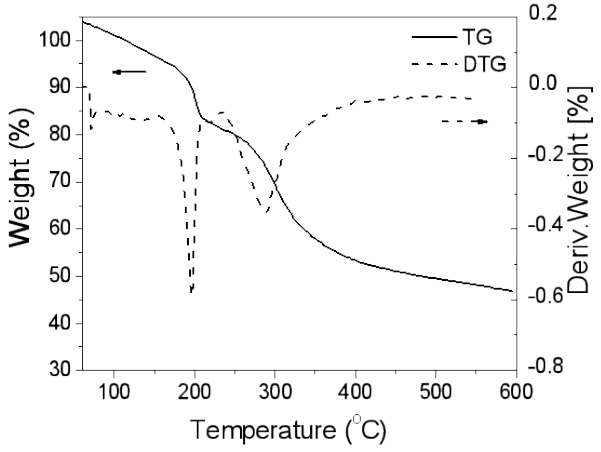

Fig. S7. TGA curve of the dry peptide gel.

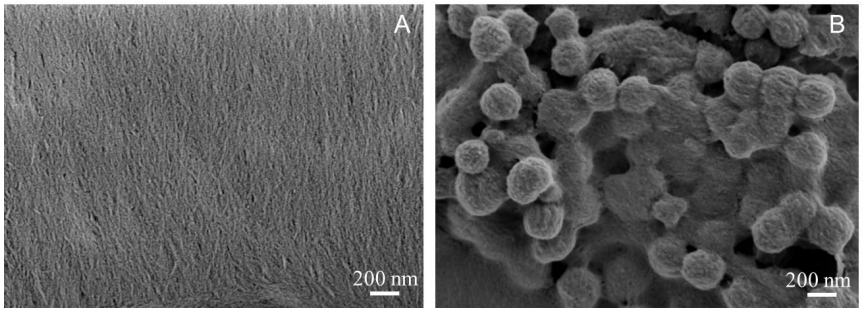

Fig. S8 (A) and (B) SEM images of native oligopeptide hydrogel and hybrid hydrogel, respectively.

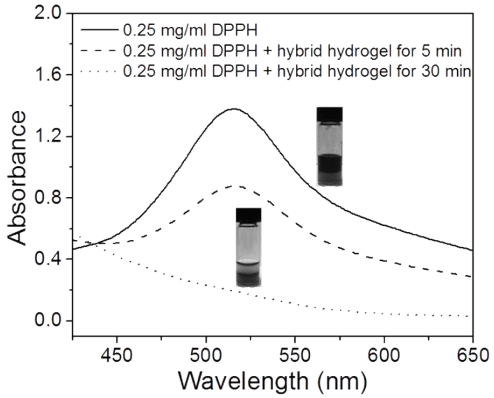

Fig. S9 DPPH radical scavenging activity of PDA based hybrid hydrogel. The concentration of DPPH is 0.25 mg/mL.

[S1] K. Y. Ju, Y. Lee, S. Lee, S. B. Park and J. K. Lee, *Biomacromolecules* **2011**, *12*, 625.