Electronic Supplementary Material (ESI) for Chemical Communications

Materials and methods

Materials: Poly 3-caprolactone (PCL) pellets (*M*n ~ 80,000) were purchased from Solvay Interox. Fmoc-protected amino acids were obtained from GL Biochem. (Shanghai, China). Solvents and other chemicals were purchased from *Alfa* (Beijing, China). 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and Fluorescein diacetate (FDA) were obtained from Molecular Probes (Invitrogen). 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Invitrogen (Grand Island, NY). NIH3T3 fibroblast was purchased from (ATCC, Manassas, VA, USA).

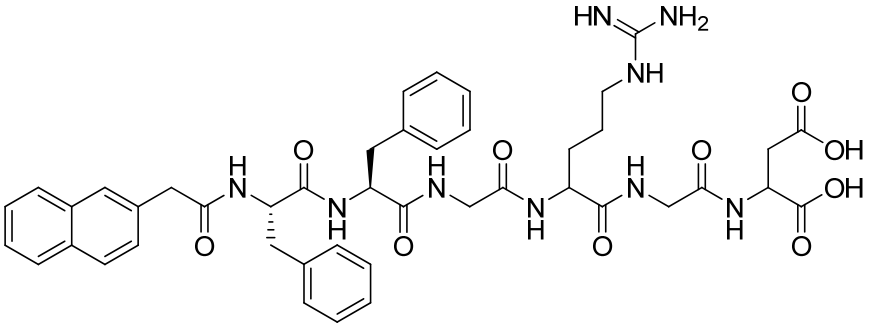
General methods: ^{1}H NMR (Bruker ARX 400) was used to characterize the synthesized compounds. ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. AFM was done on a Veeco multimode V system with the tip of Veeco RTESP. Emission spectra were recorded on a Perkin-Elmer LS-55 luminance spectrometer. Data between the wavelengths of 300 nm and 500 nm were collected with an excitation wavelength of 272 nm and bandwidth of 2.5 nm. Fourier transform infrared (FTIR) spectra were obtained on a Bio-Rad FTS6000 FT-IR spectrophotometer. Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500m. Bio-RAD iMark^{TM} Microplate Reader (Bio-Rad, America) was used in the MTT assay.

Synthesis of Nap-FFGRGD

Nap-FFGRGD prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids. The first amino acid (Fmoc-Aspartic acid) was loaded on the resin at the C-terminal with the loading efficiency about 0.85 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide

(DMF) was used during the deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, the N-terminus of the peptides was coupled with 2-naphthalene acetic acid to attach the aromatic group on the hexapeptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 ml per gram of resin), followed by five steps of washing using dichloromethane (DCM) for 2 min (5 ml per gram of resin). The peptide derivatives were cleaved from the resin by ice-cold reagent B and the mixture was stirred at room temperature, concentrated by the rotary evaporator, and poured into ice-cold diethylether. The resulting precipitate was centrifuged for 10 min at 20°C at 10,000 rpm. Afterward the supernatant was decanted and the solid was dissolved in DMSO. The final product was obtained by HPLC and lyophilization.

Characterizations of Nap-FFGRGD (1)



Scheme S-1. Chemical structure of compound **1**

^{1}H NMR (600 MHz, DMSO-d6) δ 8.20-8.33 (m, 4H), 8.06-8.08 (m, 1H), 7.84-7.87 (m, 1H), 7.73-7.79 (m, 2H), 7.581 (s, 1H), 7.44-7.50 (m, 2H), 7.22-7.23 (m, 4H), 7.16-7.19 (m, 8H), 4.48-4.56 (m, 3H), 4.30-4.35 (m, 1H), 3.67-3.82 (m, 5H), 3.47-3.59 (m, 3H), 2.95-3.10 (m, 5H), 2.68-2.85 (m, 3H), 2.60-2.63 (m, 2H), 1.74-1.77 (m, 1H) , 1.49-1.57 (m, 3H). MS: calc. M^+ = 865.4, obsvd. $(M+H)^+$ =866.9, HR-MS obsvd. $(M+H)^+$ =866.3840.

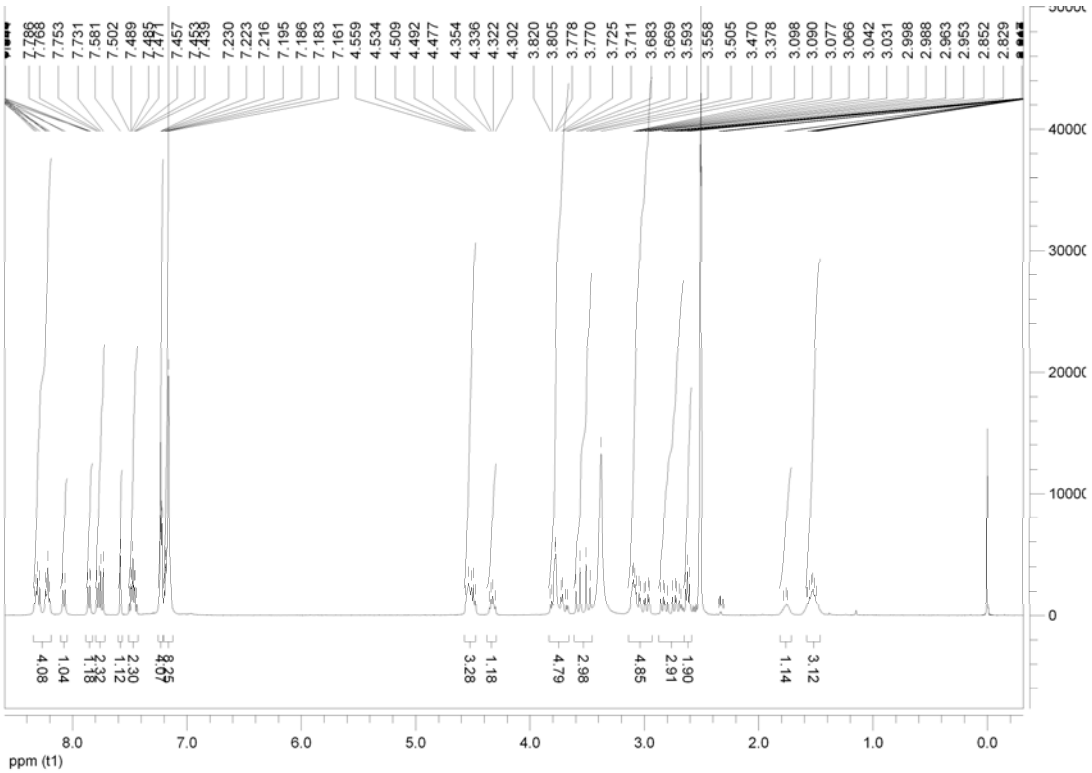


Fig. S-1. ^{1}H NMR of compound **1**

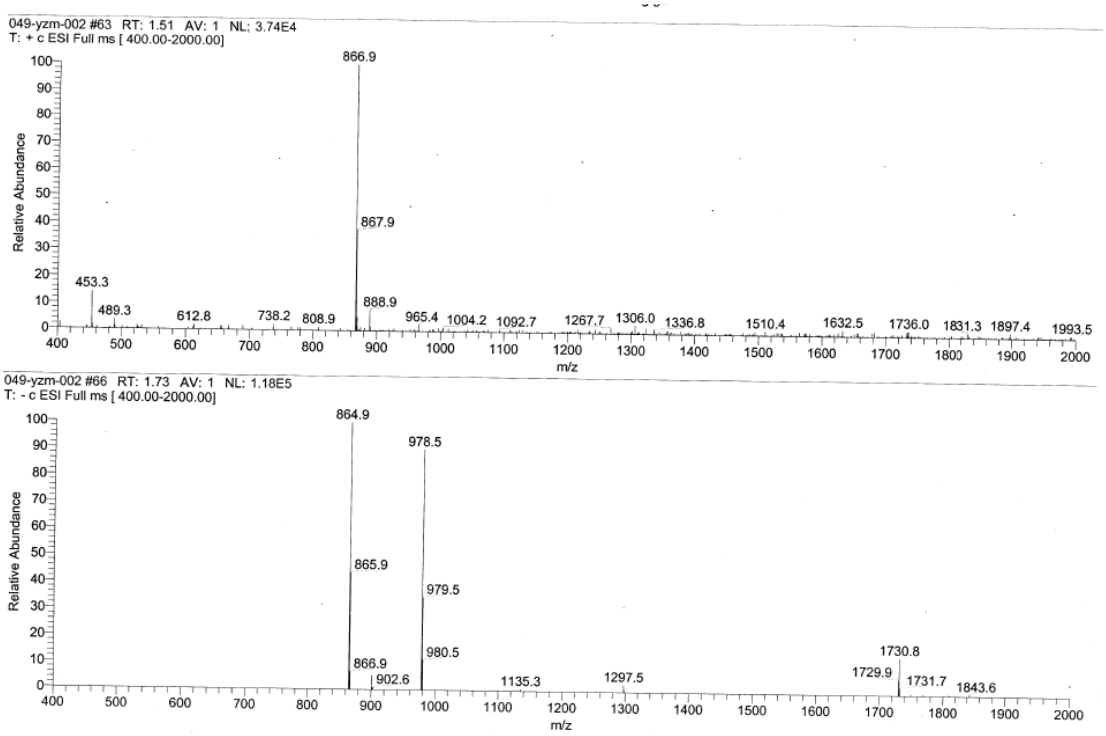


Fig. S-2. MS of compound **1**

Fig. S-3. HR-MS of compound **1**

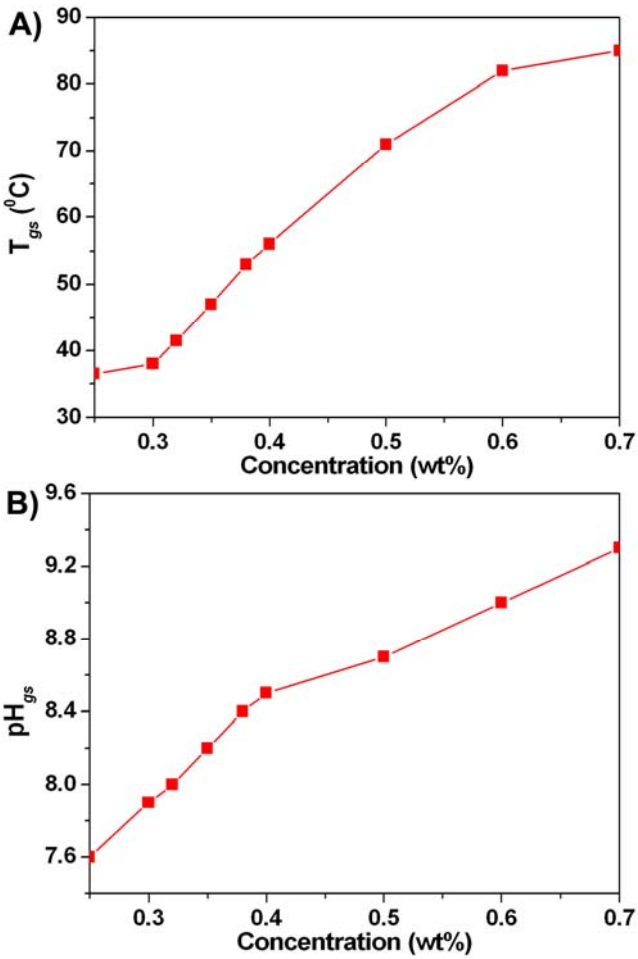


Fig. S-4. Gel-sol phase transition A) temperature (T_{gs}) and B) pH value (pH_{gs}) of gels containing different concentration of **1**

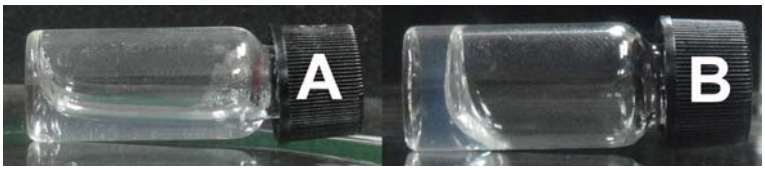


Fig. S-5. Optical images of A) a PBS solution containing 0.5 wt% of **1** formed by heating and B) a gel formed from the solution in A) by cooling to room temperature

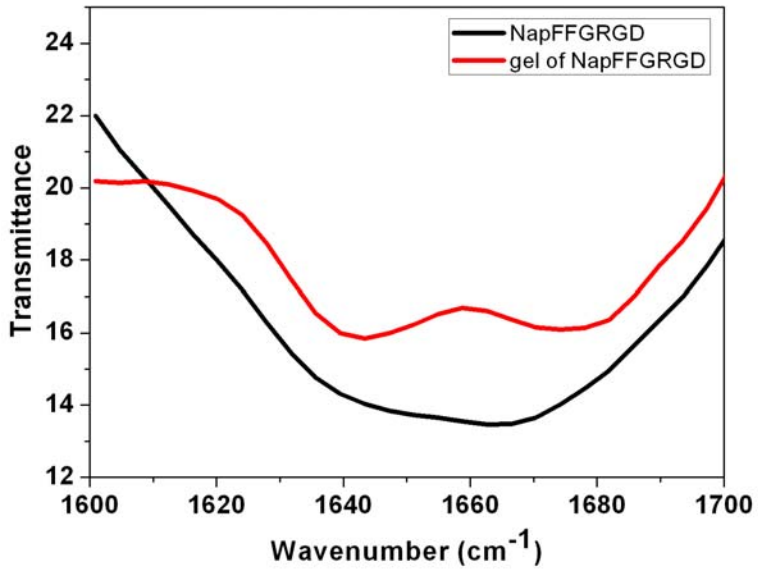


Fig. S-6. FTIR spectra of NapFFGRGD (**1**) in powder form (black) and in dried gel (red): the red shift of the peaks suggested the formation of extensive hydrogen bonds and supramolecular structures in gel stage

Fabrication of PCL films by electro spinning

PCL films were fabricated by the electro spinning method as previously described in detail [1]. Briefly, PCL was dissolved in 5:1 mixture of chloroform / methanol by stirring at room temperature over night. The electro spinning set-up includes a syringe pump (New Era Pump Systems, Inc.), a high-voltage supply (Gamma High Voltage Research, Inc.), and a rotating mandrel to collect the fibers. A positive voltage (18 kV) was applied to the polymer solution by the power supply. PCL solution (10%w/v) was delivered through a 22-gauge blunt tip syringe needle at a constant flow rate of 3 ml/h using the syringe pump. The collecting mandrel was a rotating stainless steel rod (5 cm in diameter, 30 cm in length). The distance between the syringe tip and the

mandrel surface was 12 cm. The rotation rate was 100 rpm. The films were vacuum-dried in a desiccator at room temperature for 48 h, and sterilized in 70% alcohol for 30min.

Surface coating of PCL films by Nap-FFGRGD

The Nap-FFGRGD was prepared at a concentration of 0.2 mg/ml in distilled water with 0.0245 mg/ml Na_2CO_3. 300 μL of the peptide solution was added onto the surface of PCL films in 48 wells cell culture plate and then incubated overnight at 37 °C. The samples were then washed three times with PBS (pH 7.4) and dried under vacuum at room temperature.

Surface and mechanical properties characterization

For chemical composition analysis, films before and after coating were characterized using an X-ray photoelectron spectroscopy (XPS, Axia Ultra DLD, Kratos). All spectra were collected at an electron take-off angle of 90 from the surface. For the morphology analysis, the films were scanned by scanning electron microscopy (SEM, Quanta 200, Czech) after sputtering the surface with gold. The SEM images were analyzed with ImageJ (1.42, NIH). For the hydrophilicity analysis, water contact angles were measured at room temperature with Contact Angle Meter (Harke-SPCA, Beijing, China), relying on the sessile drop technique. Each measurement was taken 60s after dispensing. Five samples were tested in each group and the resulting values were averaged. The mechanical tests were performed using an Instron mechanical system, equipped with a 1000 Newton load cell. Specimens (n=4) were cut into strips of 1cm×4cm and tested with a crosshead speed of 10 mm/min. Peak stress at rupture was calculated and plotted.

Cytocompatibility

To investigate cell attachment, PCL films with or without surface coating were placed in 48-well cell culture plates. NIH3T3 fibroblasts (ATCC, Manassas, VA, USA) or

bone marrow mesenchymal stem cells (MSC) prepared according to previous report [2] were labeled with DiI or FDA seeded at a density of 5×10^4 cells per well and cultured in DMEM medium with 10% FBS, respectively. After 1 hour, the non-adhered cells were eliminated by PBS washing and the adhered cells were visualized by fluorescent microscopy. Fluorescent images were acquired by a microscope (Olympus BX-41, Tokyo, Japan). And further MTT assay was performed to quantitatively assess the cell attachment on films before and after coating. Cell proliferation on the films was examined by MTT assay. NIH3T3 fibroblasts were seeded at a concentration of 2×10^4 on PCL films in a 48-well tissue culture plate. Non-adherent cells were washed at 1 day and 3 days after seeding. Then, 50 μL MTT solution (5 mg/ml in PBS) was added to each sample and the plates were incubated at 37°C for 4 h. The supernatant was removed and the formazan salt was dissolved in 300 μL DMSO. The absorbance was measured at 492nm.

[1] Zhang M,Wang Z,Feng S,Xu H,Zhao Q,Wang S, et al. Immobilization of anti-CD31 antibody on electrospun poly(varepsilon-caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells. *Colloids Surf B: Biointerfaces* **2010** PMID: 21123036.

[2] Cheng Z, Liu X, Ou L, Zhou X, Liu Y, Jia X, et al. Mobilization of mesenchymal stem cells by granulocyte colony-stimulating factor in rats with acute myocardial infarction. *Cardiovasc Drugs Ther.* **2008** Oct;22(5):363-71