Supporting Information

General methods: The synthesized compounds were characterized by ^{1}H NMR (Bruker ARX 300) using DMSO-d_6 as the solvent and ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C_{18} RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents, LC-MS was conducted at the LCMS-20AD (Shimadzu) system, and rheology was performed on an AR 2000ex (TA instrument) system using a parallel plates (40 mm) at the gap of 500 μm. TEM was done on a Tecnai G2 F20 system, operating at 200 kV. Confocal microscopy images were obtained on a Leica TCS SP5 system (Germany).

Synthesis and characterization of the peptide:

The peptide was prepared by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group or Pbf group or Boc group. After the first amino acid was loaded on the resin by its C-terminal, 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used to 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 and diisopropylethylamine (DIEA) as catalysis reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. After the last amino acid was coupled, excessive reagents were removed by a single DMF wash for 5 min (5 mL per gram of resin), followed by 5 times DCM wash for 2 min (5 mL per gram of resin). The peptide was cleaved using 95 % of trifluoroacetic acid (TFA) with 2.5 % of trimethylsilane (TMS) and 2.5 % of H_2O for 30 min. TFA was removed by rotary-evaporate process, then 20 mL per gram of resin of ice-cold diethylether was added. The resulting precipitate was centrifuged for 10 min at 4 ℃ at 10000 rpm. After the supernatant was decanted, the resulting solid was dissolved in DMSO for HPLC separation with MeOH containing 0.05 % of TFA and H_2O containing 5 % MeOH as eluents.

The synthesized compound was characterized by ^{1}H NMR (Bruker ARX-300) using DMSO-d_6 as the solvent. HR-MS was conducted at the Agilent 6520 Q-TOF LC/MS using ESI-L low-concentration tuning mix (lot no. LB60116 from the Agilent Tech).

^{D}FEFK^{D}FEFKYRGD: ^{1}H NMR (400 MHz, DMSO) δ 9.19 (s, 1H), 8.55 (d, J = 7.7 Hz, 1H), 8.34 – 8.00 (m, 11H), 7.82 (dd, J = 54.9, 30.3 Hz, 7H), 7.31 – 7.00 (m, 23H), 6.64 (d, J = 8.4 Hz, 2H), 4.53 (ddd, J = 21.9, 20.5, 13.2 Hz, 5H), 4.38 – 4.16 (m, 5H), 4.11 (s, 1H), 3.75 (ddd, J = 21.9, 17.0, 5.7 Hz, 3H), 3.13 – 2.89 (m, 8H), 2.81 – 2.58 (m, 10H), 2.11 (t, J = 7.9 Hz, 2H), 1.94 (t, J = 8.0 Hz, 2H), 1.83

(d, J = 8.2 Hz, 1H), 1.73 – 1.15 (m, 17H), 1.01 (d, J = 6.9 Hz, 2H). HR-MS: calc. M^+ =1611.77, obsvd. $(M+H)^+$ = 1612.7803.

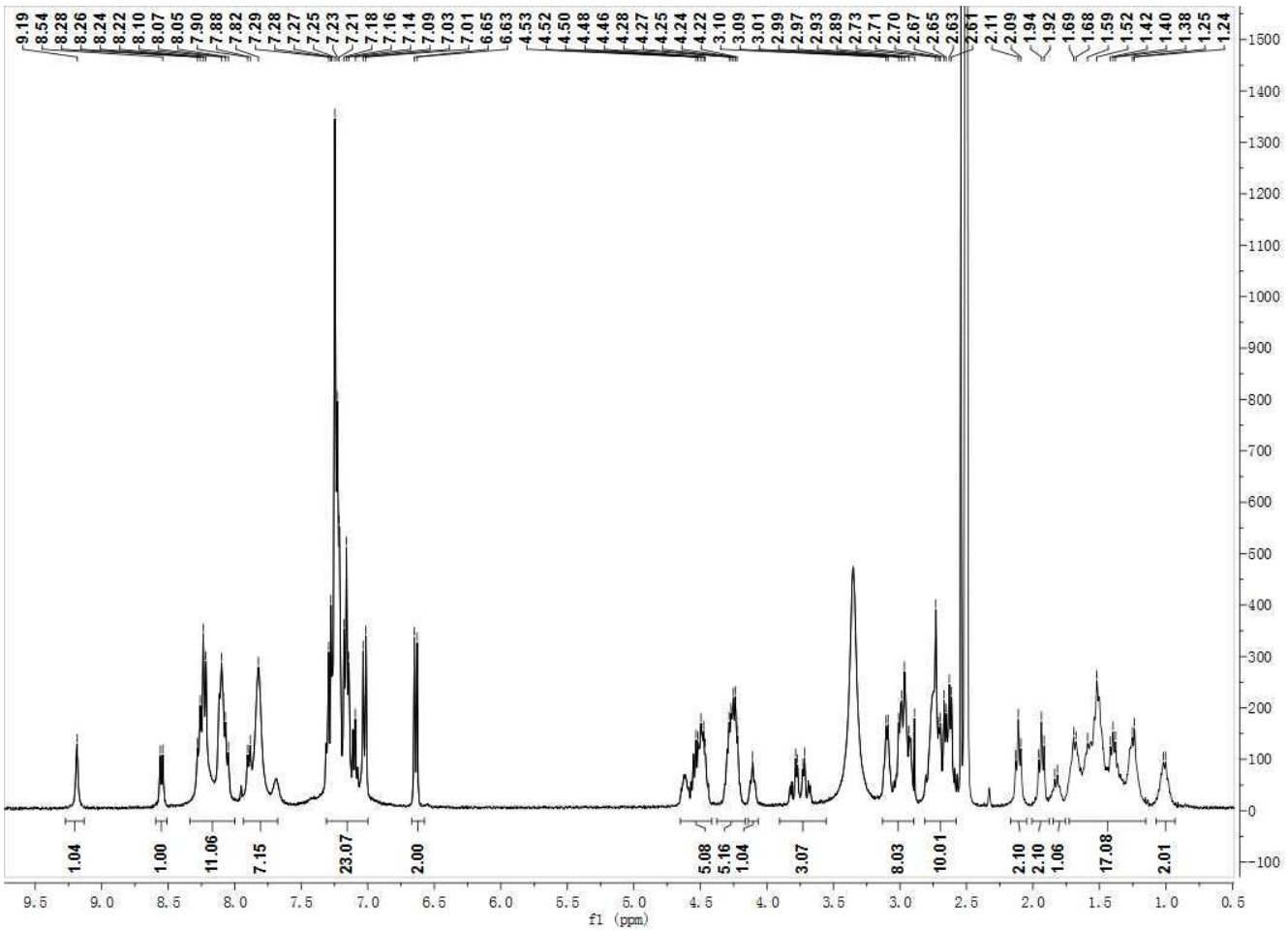

Fig. S-1. ^{1}H NMR of ^{D}FEFK^{D}FEFKYRGD

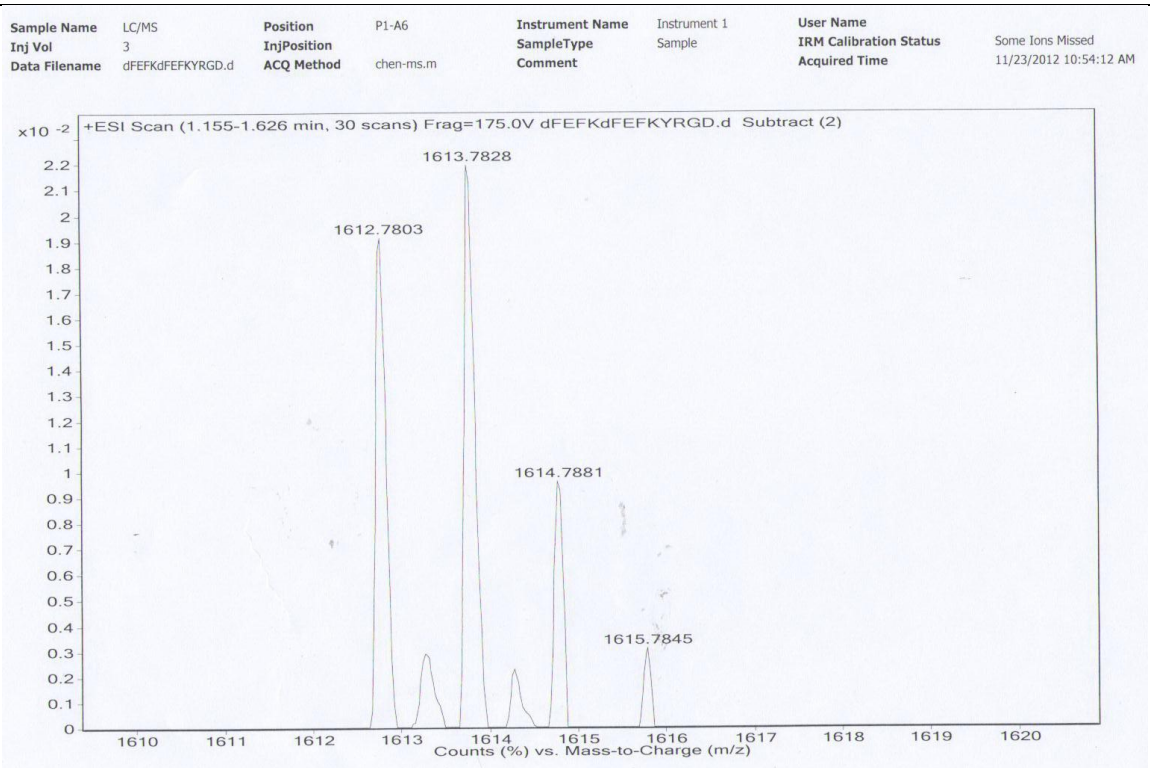

Fig. S-2. HR-MS of ^{D}FEFK^{D}FEFKYRGD

^{D}FEFKFE^{D}FKYRGD: ^{1}H NMR (400 MHz, DMSO) δ 12.28 (s, 4H), 9.17 (s, 1H), 8.53 (d, J = 7.9 Hz, 1H), 8.31 – 7.95 (m, 12H), 7.80 (s, 5H), 7.63 (s, 1H), 7.30 – 7.02 (m, 22H), 6.63 (d, J = 8.4 Hz, 2H), 4.73 – 4.19 (m, 11H), 4.10 (s, 2H), 3.76 (dd, J = 16.2, 10.7 Hz, 2H), 3.10 (d, J = 5.7 Hz, 2H), 3.03 – 2.85 (m, 6H), 2.84 – 2.60 (m, 10H), 1.99 – 1.94 (m, 2H), 1.70 (d, J = 5.7 Hz, 3H), 1.65 – 1.40 (m, 12H), 1.15 (dd, J = 29.4, 24.7 Hz, 5H). HR-MS: calc. M^{+} =1611.77, obsvd. $(M+H)^{+}$ = 1612.7781.

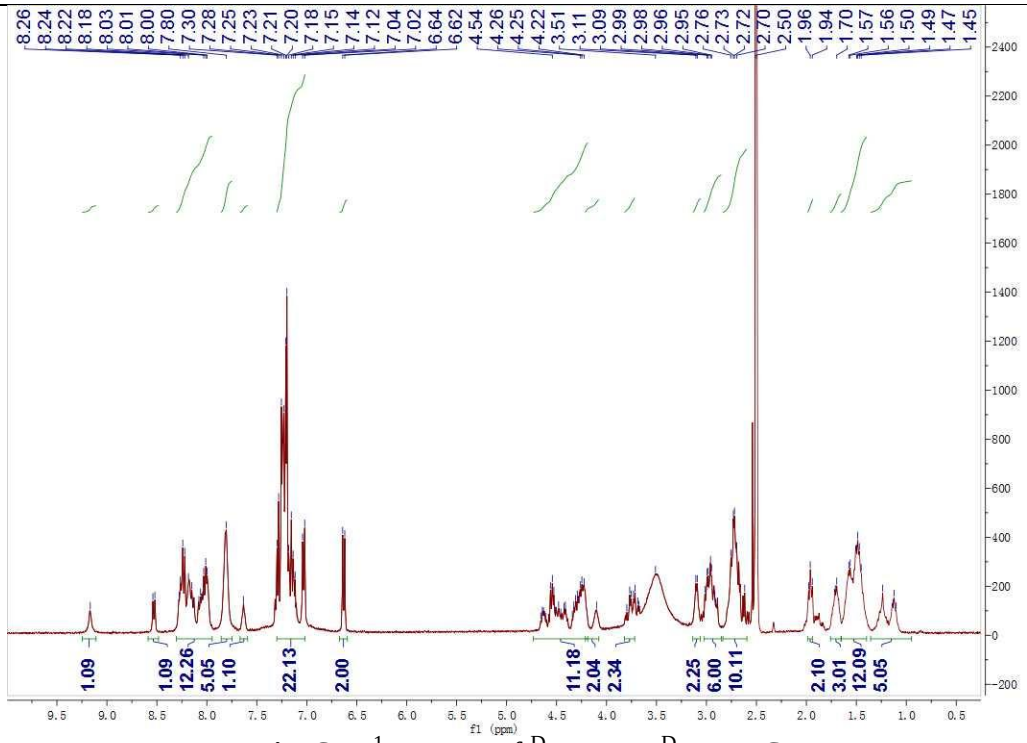

Fig. S-3. ^{1}H NMR of ^{D}FEFKFE^{D}FKYRGD

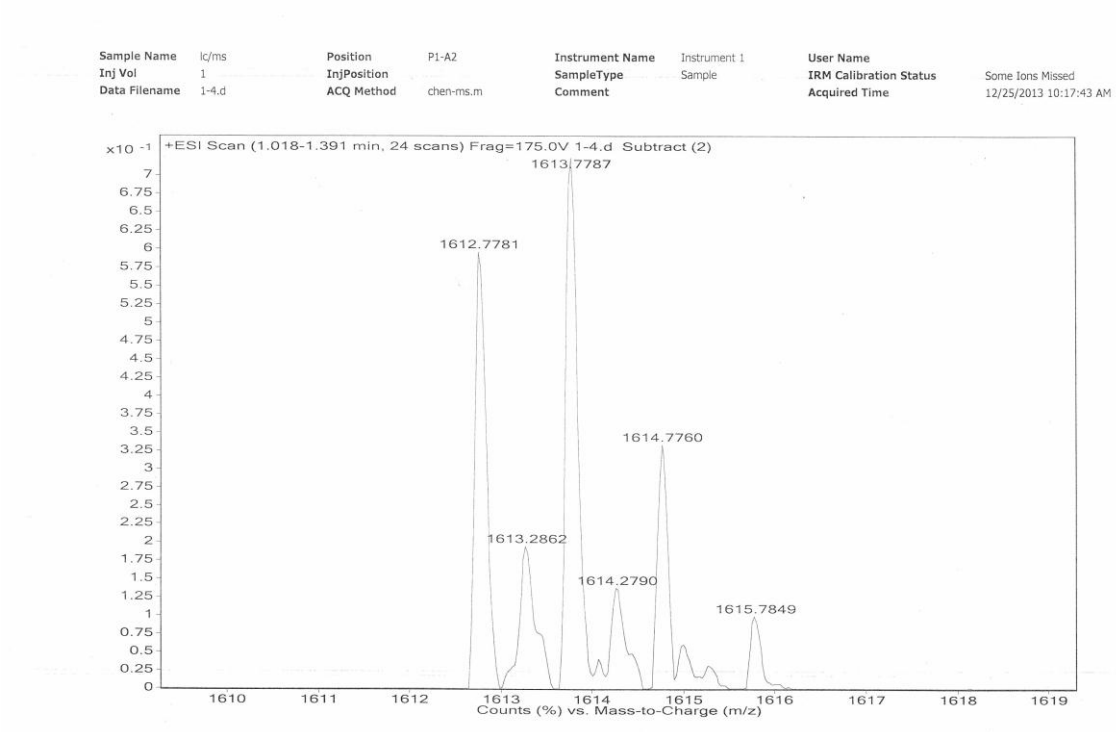

Fig. S-4. HR-MS of ^{D}FEFKFE^{D}FKYRGD

FEFK^{D}FE^{D}FKYRGD: ^{1}H NMR (400 MHz, DMSO) δ 12.29 (s, 4H), 9.17 (s, 1H), 8.54 (d, *J* = 8.2 Hz, 1H), 8.31 – 7.96 (m, 12H), 7.72 (s, 5H), 7.29 – 6.98 (m, 23H), 6.64 (d, *J* = 8.4 Hz, 2H), 4.66 – 4.15 (m, 12H), 4.08 – 4.01 (m, 1H), 3.74 (m, 3H), 3.09 (d, *J* = 10.2 Hz, 2H), 3.01 – 2.86 (m, 5H), 2.78 – 2.57 (m, 10H), 2.21 (m, 2H), 1.89 – 1.65 (m, 6H), 1.60 – 1.35 (m, 10H), 1.05 (m, 4H). HR-MS: calc. M^{+} =1611.77, obsvd. $(M+H)^{+}$ = 1612.7764.

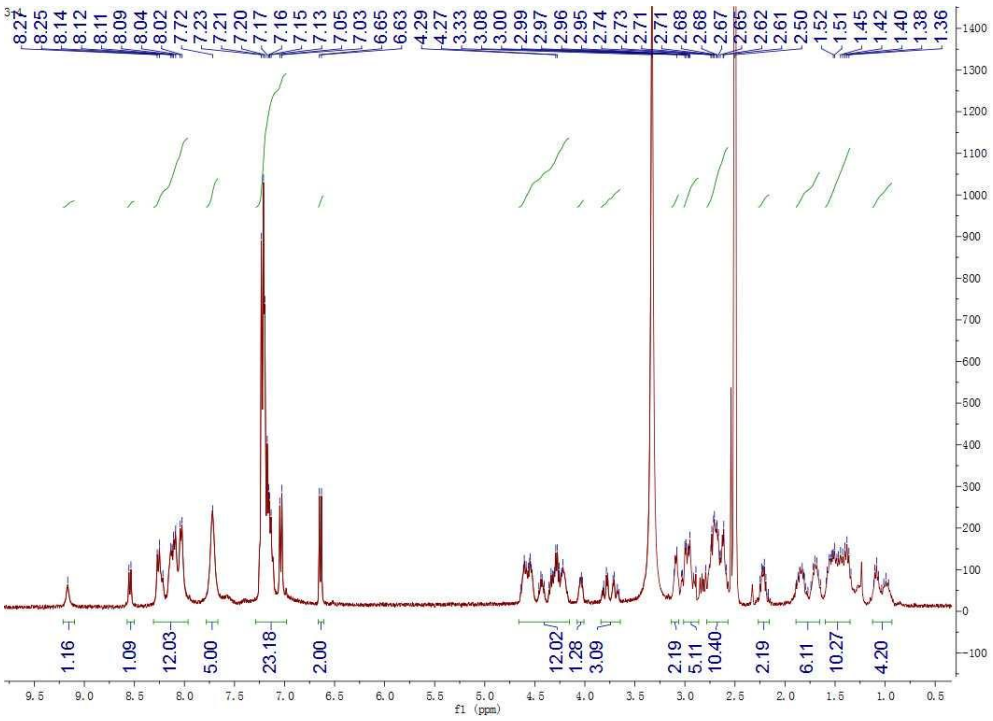

Fig. S-5. ^{1}H NMR of FEFK^{D}FE^{D}FKYRGD

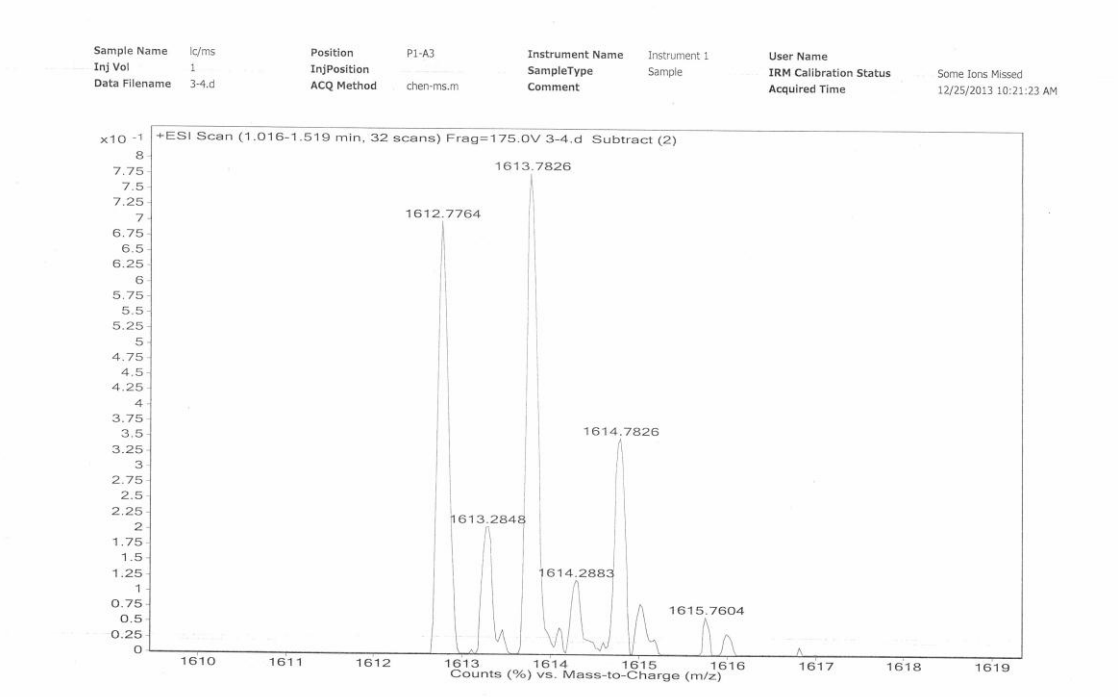

Fig. S-6. HR-MS of FEFK^{D}FE^{D}FKYRGD

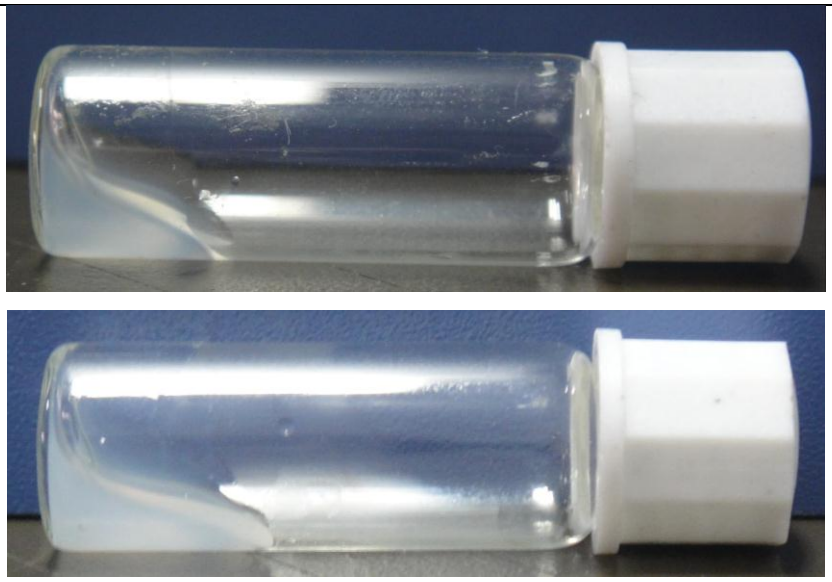

Fig. S-7. Optical images of the suspensions of top) ^{D}FEFKFE^{D}FKYRGD and down) FEFK^{D}FE^{D}FKYRGD in PBS (pH = 7.4, 1.0 wt%)

Rheology: Rheology test was carried out on an AR 2000ex (TA instrument) system, 40 mm parallel plate was used during the experiment at the gap of 500 μm. For the dynamic time sweep, the sample after mixing two solutions was directly transferred to the rheometer and it was performed at the frequency of 1 rad/s and the strain of 1 %. The gel was characterized for the dynamic frequency sweep in the frequency region of 0.1-100 rad/s at the strain of 1 %. For the dynamic strain sweep, it was characterized in the strain region of 0.1-10 % at the frequency of 1 rad/s.

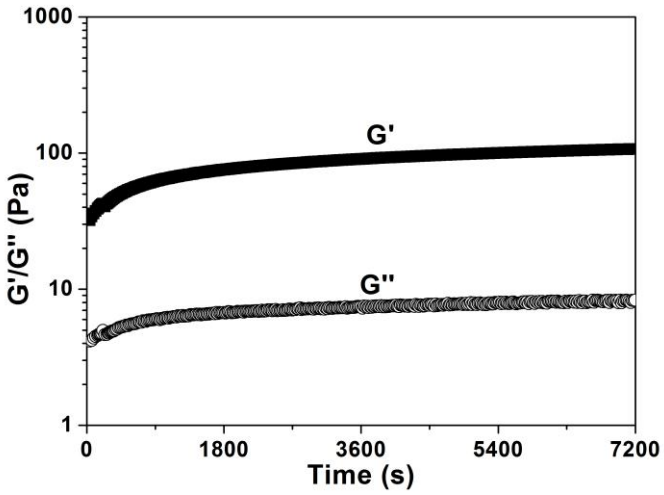

Fig. S-8. Rheological measurement with the mode of dynamic time sweep at the frequency of 1 rad/s and strain of 1% for the gel of ^{D}FEFK^{D}FEFKYRGD.

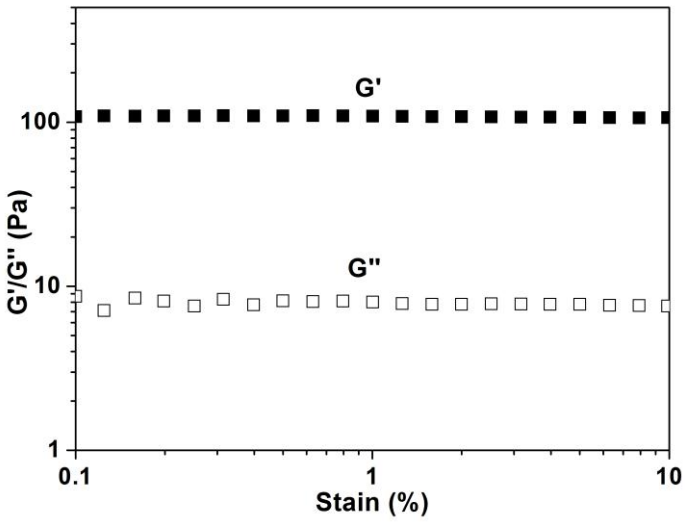

Fig. S-9. Rheological measurements with the mode of dynamic strain sweep at the frequency of 1 rad/s for the gel of ^{D}FEFK^{D}FEFKYRGD.

Fig. S-10. Optical image of the gel formed by mixing a pure water solution of peptide with equal volume of complete cell culture medium (DMEM with 10% of FBS, final peptide concentration = 1.0 wt%)

Live/dead assay: Viability of the cells cultured on the hydrogels was tested by the Live/Dead assay according to the manufacturer's instruction. Specifically, the cell-gel constructs were washed twice with PBS for 20 min each. 100 μL of staining reagent containing 2 μM calcein AM and 4 μM EthD-1 was then added onto the cell-gel constructs. After 30 min incubation in a 37◦C/5% CO_2 incubator, cells were

observed using a Nikon Eclipse TS100 inverted fluorescent micro-scope with excitation filters of 450–490 nm (green, Calcein AM) and 510–560 nm (red, EthE-1).

Determination of cell proliferation rate by CCK-8: To quantify cell proliferation inside the cell-gel constructs, a CCK-8 assay was performed at a series of time points. A 3D Culture standard was made by encapsulating cells into hydrogels following the above 3D-culture procedure. To perform the CCK-8 assay, each cell-gel construct was incubated with 100 μL of 10% (v/v) CCK-8 in serum-free DMEM. The plates were then incubated in the 5% CO2 incubator for 4 h at 37◦C. The absorbance at 450 nm was determined using the microplate reader (MultiskaniMark, Bio-Rad, USA). The experiments were conducted for five times and SD was determined.

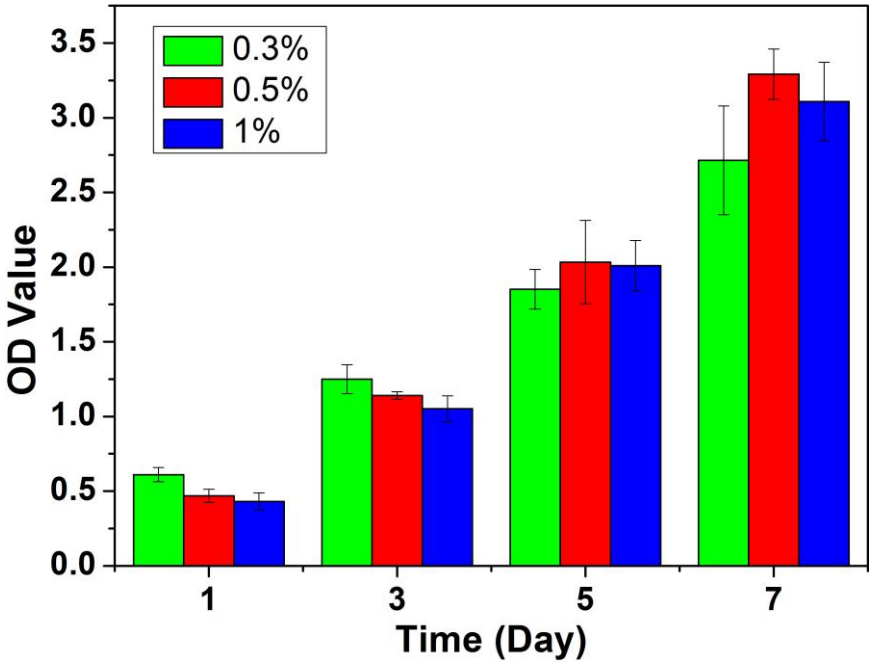

Fig. S-11. Cell proliferation rate of HeLa cells determined by CCK-8 assay cultured in the gel with different concentrations of peptides and at different time points

Distribution of cells in hydrogels by confocal image:

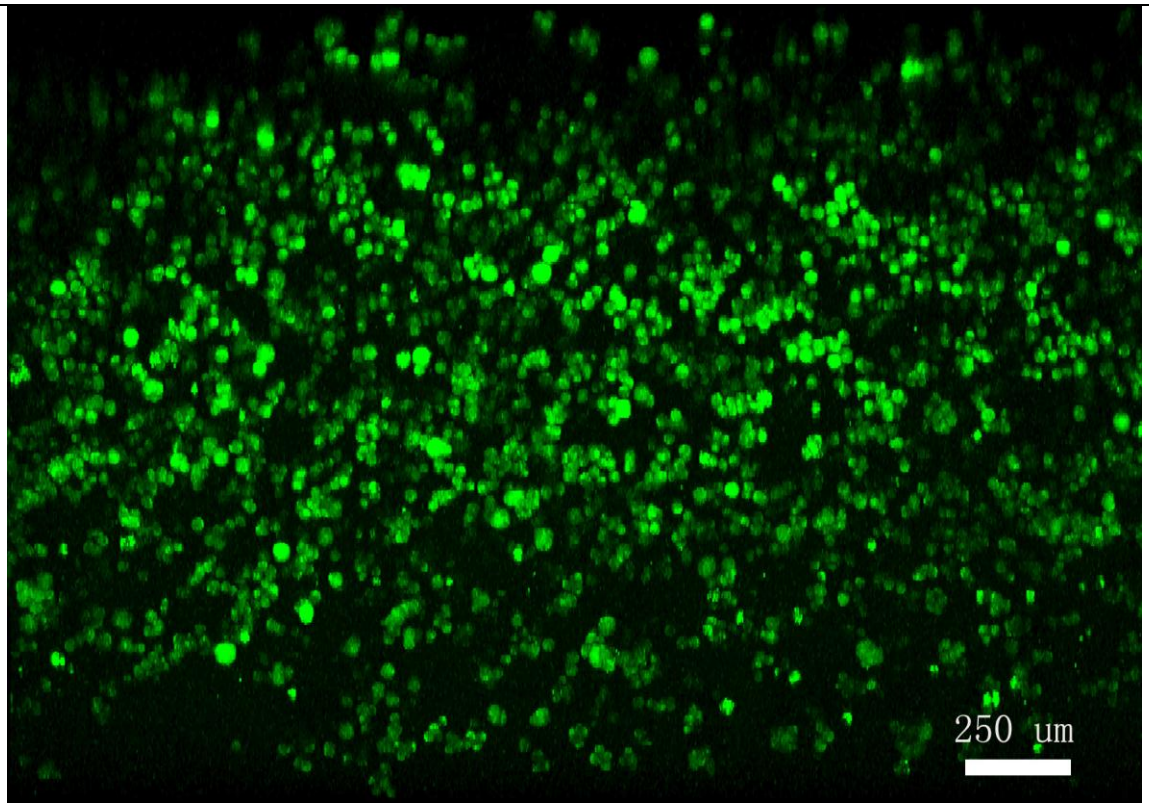

Fig. S-12. A confocal image to show the evenly distribution of cells in the hydrogels (viewed perpendicular to the Z-axis), cells were cultured in the gels for 24 h. Viable cells were stained with calcein AM (green); dead cells were labelled with EthE-1 (red).

^{125}I-Radiolabelling of ^{D}FEFK^{D}FEFKYRGD and *in vivo* imaging: 30 μL of ^{D}FEFK^{D}FEFKYRGD in pure water was placed into a polypropylene microcentrifuge tube (1.5 mL). 10 μL diluted [^{125}I] NaI (0.3 mCi) and 45 μL chloramine-T (MW 281.69, 0.160 μmol) in 0.1 M PBS (pH = 7.4) were then added. The mixture was rotated on a rotator for 20 minutes at room temperature. 45 μL sodium pyrosulfite (MW 190.09, 0.473 μmol) in 0.1 M PBS was added and the resulting mixture was rotated for another one minute. 1000mM NaCl (PH=5.0) was then added to precipitate the peptide. The mixture was then centrifuged at 5000 rpm for 20 minutes, the supernatant was decanted and pellet was re-dispersed in 30 μL of DMSO. The radiochemical purity of the products was determined by paper chromatography (95% ethyl acetate and 5% methanol as mobile phase). The 30 μL of DMSO solution of radioactive peptide was then mixed with 470 μL water solution of ^{D}FEFK^{D}FEFKYRGD, 2× PBS was then added for hydrogel formation. 50 μL of the above mixture containing different concentrations of ^{D}FEFK^{D}FEFKYRGD (0.3, 0.5 and 1.0 wt%) was injected subcutaneous of hind leg quickly. The images were recorded on a small-animal in vivo imaging instruments (KODAK IS in vivo FX, KODAK, New Haven, CT) with an analysis software of KODAK 5.1 software. The intensity of radioactive signals of injection site was used to determine the stability of the gels (3 mice in each group). Gamma counter (SN6100, Hesuo Rihuan, Shanghai, China) was used to determine the radiochemical purity and stability of radioactive

^{D}FEFK^{D}FEFKYRGD.

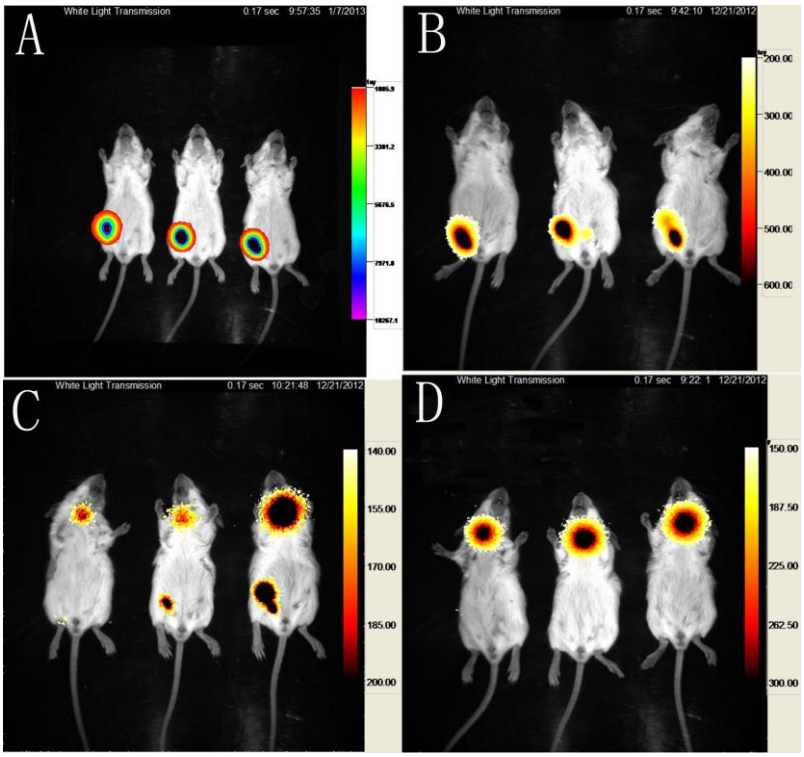

Fig.S-13. *In vivo* images of the hydrogel of 1.0 wt% A) at 0 h time point and hydrogels of B) 1.0 wt%, C) 0.5 wt%, and D) 0.3 wt% at 24 h time point (the peptide was labelled with radioactive iodine and the radioactive signal from the mouse was record at different time points. The scale bar represents the intensity of radioactive signal. Some of the radioactive signals go to thyroid grand and most of them have been detected in the excretion of the mice.)

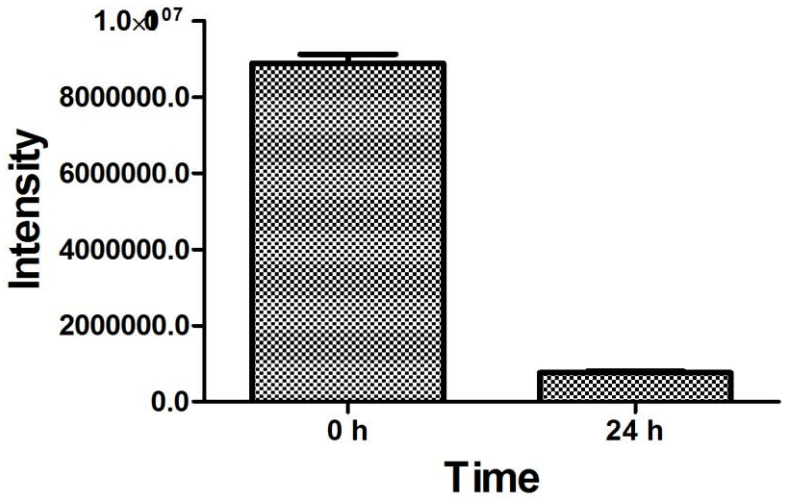

Fig. S-14. Radioactive signals intensity of the gels with 1.0 wt% of the peptide at 0 h and 24 h time points