Reduction-triggered formation of FEK8 molecular hydrogel for 3D cell culture

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Experimental Section
Chemicals

All Fmoc-protected amino acids were obtained from GL Biochem (Shanghai). All the other Starting materials were obtained from Alfa. Commercially available reagents were used without further purification.

Analytical Methods

The synthesized compounds were characterized using 1H NMR (Bruker ARX 300) using DMSO-d6 as the solvent and ESI-MS spectrometric analyses were performed at the Finnigan TSQ7000 System. Emission spectra were recorded on a Perkin-Elmer LS-55 luminance spectrometer at excitation wavelength of 265 nm; HPLC analysis were performed on Waters 600E Multi-solvent Delivery System using a C18 RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents.

Synthesis and characterization

Fmoc-cystamine succinic acid (Fmoc-CS) for solid phase peptide was synthesized according to previous reported procedure.1 The peptide derivative 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. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.8 mmol/g. Fmoc-EEE was synthesized by standard Fmoc solid-phase peptide synthesis (SPPS) and 20% piperidine in anhydrous N,N’-dimethyl formamide (DMF) was used during the deprotection of Fmoc group. Then Fmoc-CS was coupled to the free amino group of Fmoc-EEE 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, acetic anhydride was used to attach on the peptide. 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 DCM for 1 min (5 mL per gram of resin). The peptide derivative was cleaved from the resin using 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H₂O for 1 hour. The mixture was poured into ice-cold diethyl ether (20 mL per gram of resin) and centrifuged at 10,000 rpm for 10 min at 4 °C. Then the supernatant was decanted and the resulting solid was purified using preparation reverse-phase HPLC (Lumtech). The purity of obtained Ac-FEFKFEFK-CS-EEE was determined by ¹H-NMR and HR-MS. ¹H NMR (300MHz, DMSO-d6) δ 12.16 (s, 6H), 7.99-8.14 (m, 12H), 7.87-7.88(d,3H), 7.18-7.23 (m, 22H), 4.55 (s, 5H), 4.21 (s,8H), 2.90-3.05 (m, 6H), 2.67-2.75 (d, 13H), 2.34 (s, 5H), 2.25-2.27 (d, 7H), 2.15-2.18 (d, 5H), 1.93 (s, 4H), 1.82 (s, 5H), 1.51 (s,7H), 1.24(s, 5H). HR-MS: calc. M⁺ = 1784.7507, obsvd. (M+1)⁺ = 1785.7598.
Fig. S1 $^1$H NMR spectrum of Ac-FEFKFEFK-CS-EEE.

Fig. S2 HRMS spectrum of Ac-FEFKFEFK-CS-EEE.
Fig. S3 FT-IR spectrum of Ac-FEFKEFK-CS-EEE. (BIO-RADFTS3000 spectrometer with KBr pellets): 3408.5 cm\(^{-1}\) (-OH), 3295.0 cm\(^{-1}\) (-NH\(_2\)), 3074.6 cm\(^{-1}\) (Ar-H), 2920.6 cm\(^{-1}\) (\(-\text{CH}_2\)), 1738 cm\(^{-1}\) (\(-\text{CO}\)); 1625 cm\(^{-1}\), 1530 cm\(^{-1}\) (-\(\text{NH}\)), 1402 cm\(^{-1}\) (-CN of –CO-NH-), 1223.6 cm\(^{-1}\) (-CN of –NH\(_2\)), 702.5 cm\(^{-1}\) (-S-S-).

Fig. S4 The cleavage of disulfide bond in the peptide monitored by LC-MS.
References: