Electronic Supplementary Information (ESI)

A Collagen telopeptide binding peptide shows potential in aiding collagen bundle formation and fibril orientation

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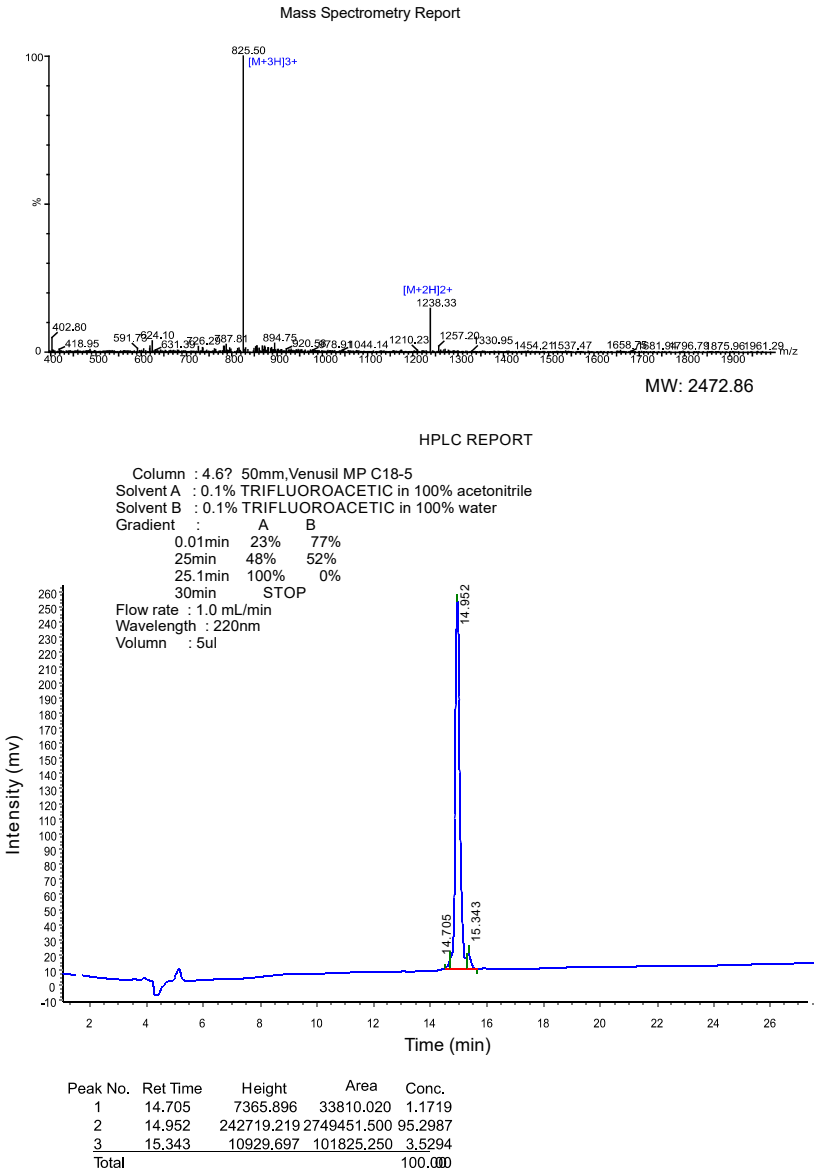

Fig.S1 Characterization of the synthesized CTBP by mass spectrometry (MS) and high efficiency liquid chromatography (HPLC).

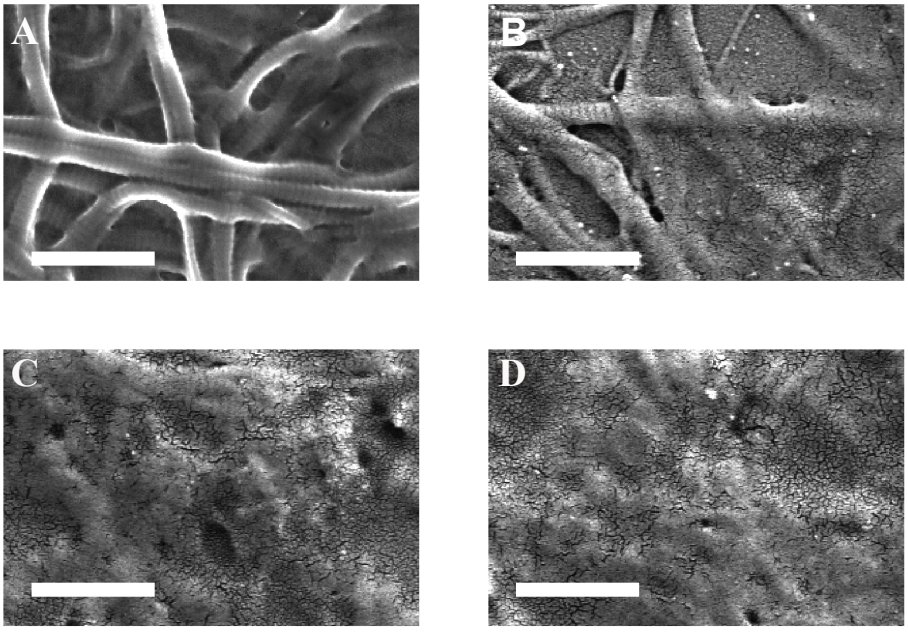

Fig.S2 The inhibitory effect of the CTBP peptide on collagen fibrils formation. (A), The SEM image of the collagen fibril formed by co-incubation of 10× PBS and collagen solution; (B) – (D) the SEM images of air-dried samples after co-incubating CTBP peptide and collagen suspension, the mole ratio of CTBP to collagen molecule was, (B) 1:1, (C) 10:1 and (D) 100: 1. Scale bar: 1μm.

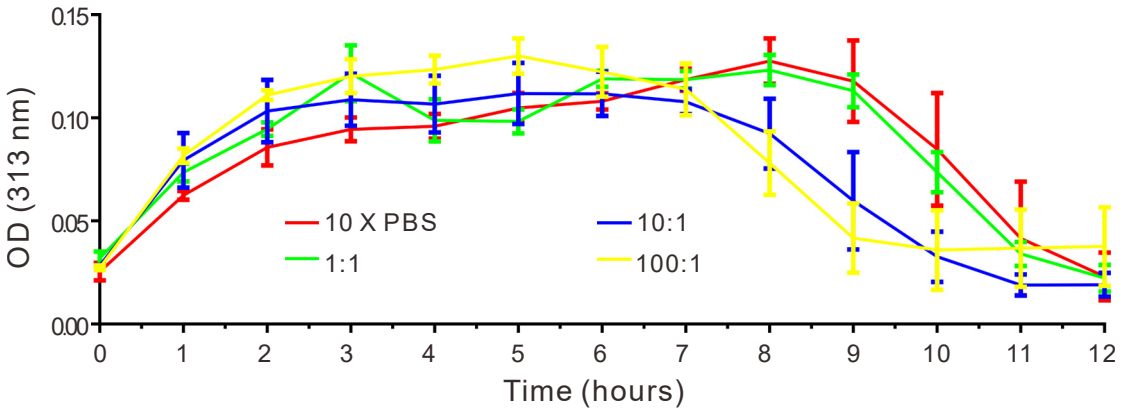

Fig.S3 The effect of CTBP on collagen fibrils formation from atelocollagen. Turbidity curves of atelocollagen with 10× PBS (red) or 10× PBS containing CTBP peptide based on the changes of optical density (OD) relative to that of pure atelocollagen suspension (initial OD value ~0.03) at 313 nm, n = 3. The OD values had no significant differences among different groups at the same time point, although the averages of the OD value of different samples were different. The mole ratios of CTBP to atelocollagen molecule were 1:1, 10:1 and 100:1, respectively.

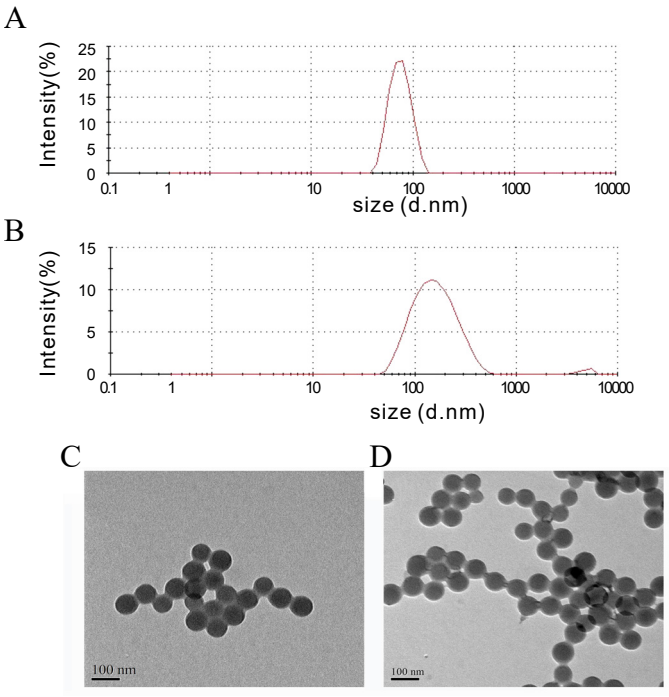

Fig.S4 (A) The typical diameter distributions of PS suspended in ultrapure water with average diameter 73.6 ± 0.67 nm, with a narrow size distribution. While (B) the typical diameter distributions of CTBP-PS particles suspended in ultrapure water with average diameter 144 ± 0.79nm that may indicate that the CTBP-PS particles can form dimer spontaneously. (C) The TEM images of PS and (D) CTBP-PS particles. Both the PS and CTBP-PS particles had a spherical shape. Scale bar, 100nm.

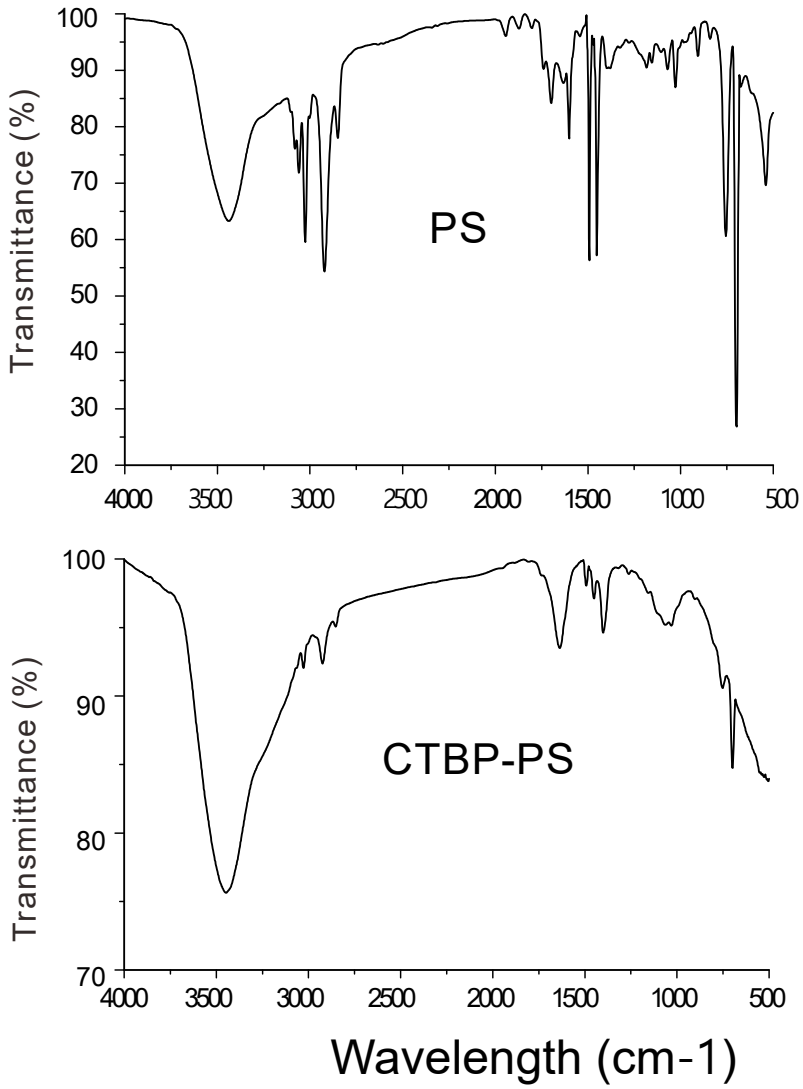

Fig.S5 IR spectra of the PS and CTBP-PS particles. (A) IR spectrum of the PS particles without peptide. (B) IR spectrum of the CTBPPS particles. After modifying by the P1 peptide, the IR spectrum of the PS particles showed the characteristic bands of PS disappeared (1601) or decreased (1493 and 698), while the characteristic bands of peptide, for example at 1635, 1267 cm^{-1}, namely amides I and amides III appeared.

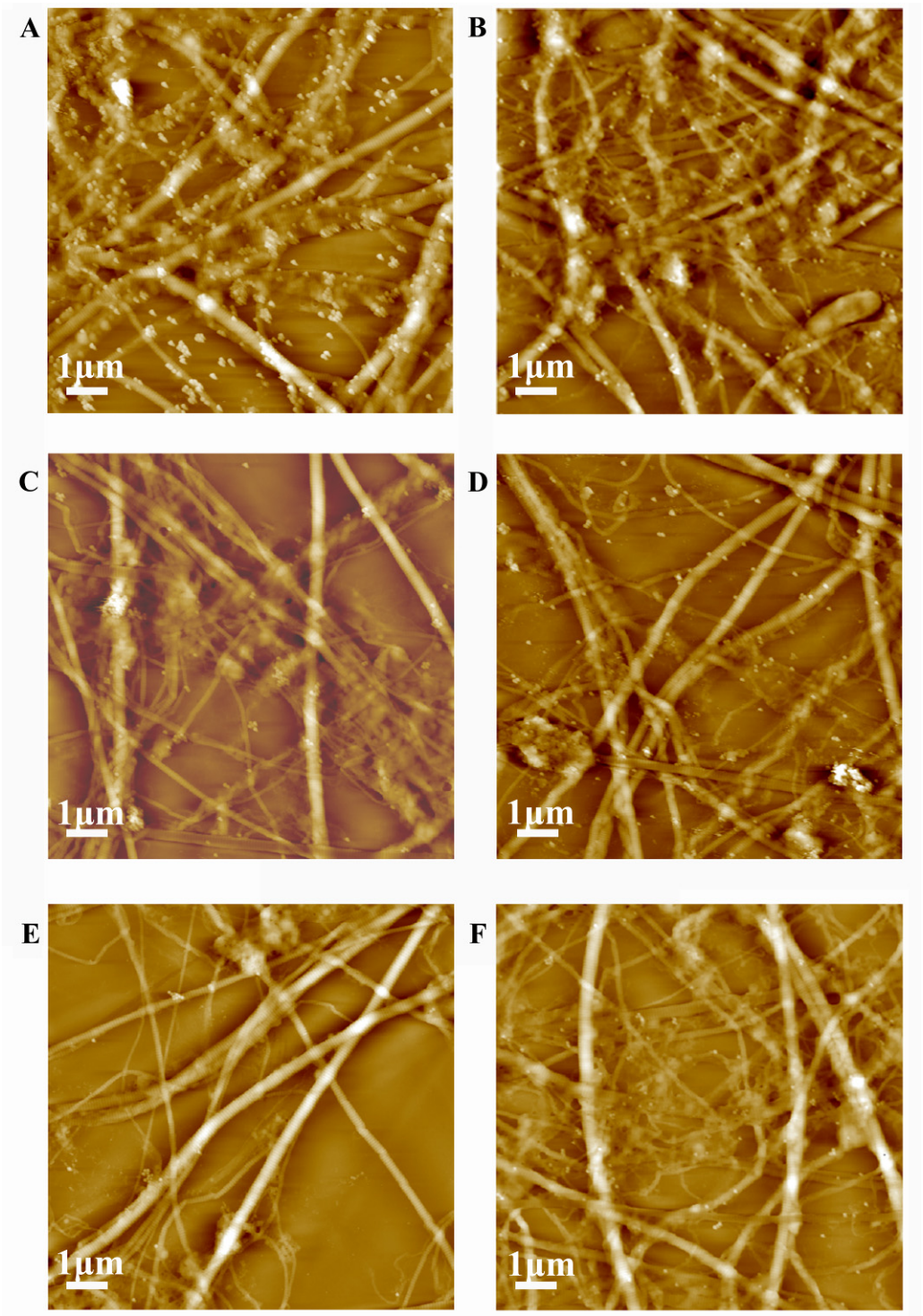

Fig.S6 The AFM images of the PS (A), (C) and (E), and CTBP-PS particles (B), (D) and (F), distributed on collagen fibrils substrate. Both particles were re-dispersed in 0.05M TBS buffer at various ratios of particles to collagen molecules; (A) and (B), 1:1800; (C) and (D), 1:3600; (E) and (F), 1:5800. Scale bar, 1μm.

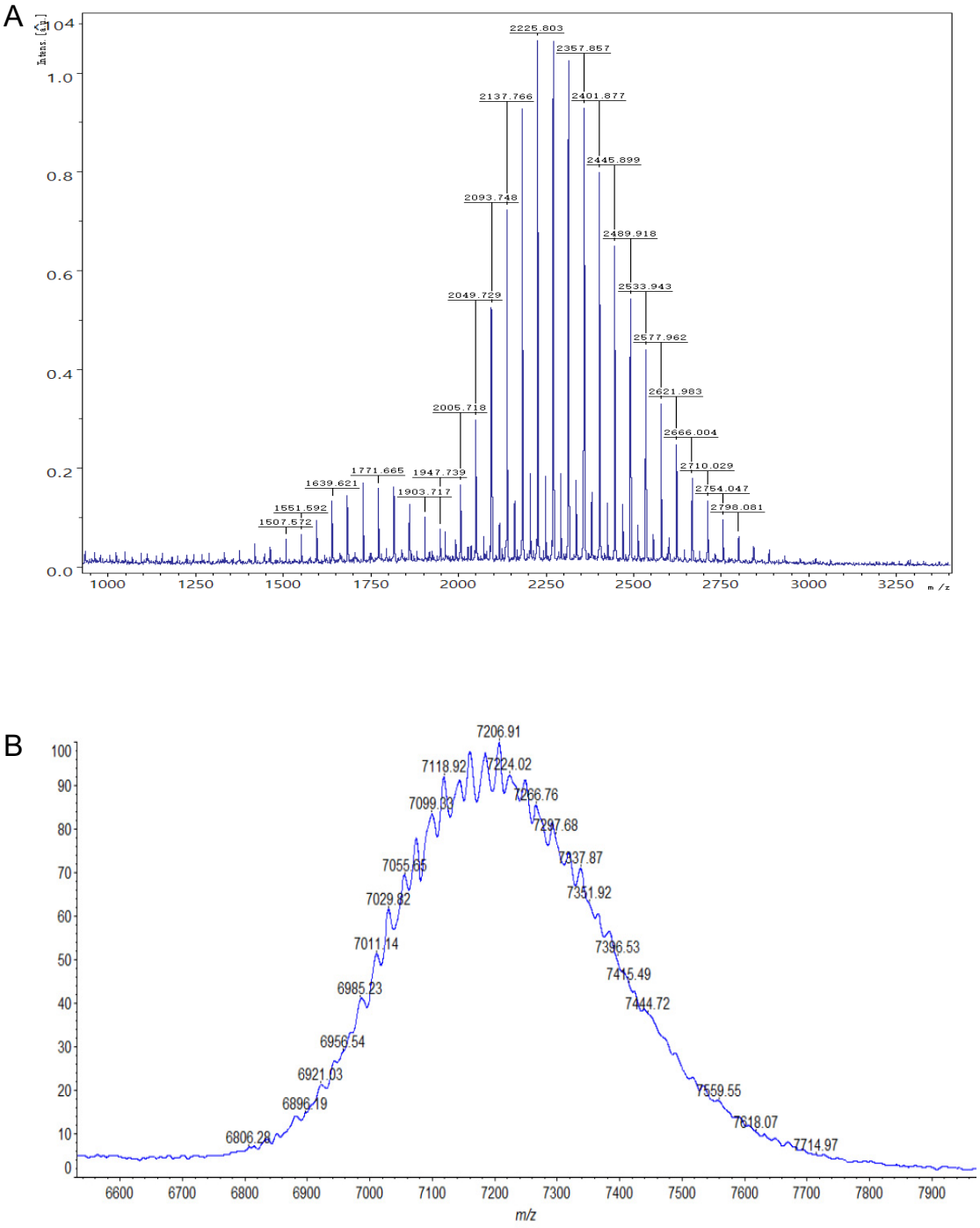

Fig.S7 The distributions of molecular weight of HOOC-PEG-COOH and CTBP-PEG-CTBP characterized by mass spectrometry (MS).

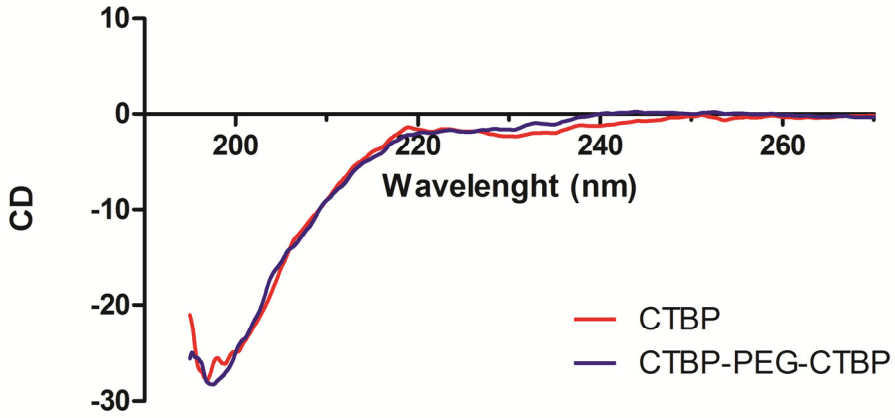

Fig.S8 CD spectra of CTBP-PEG-CTBP and CTBP at room temperatures. Either the CTBP and CTBP-PEG-CTBP display a strong negative peak at ~198 nm with nor positive peaks, indicating the conformation of the both molecules might be similar.

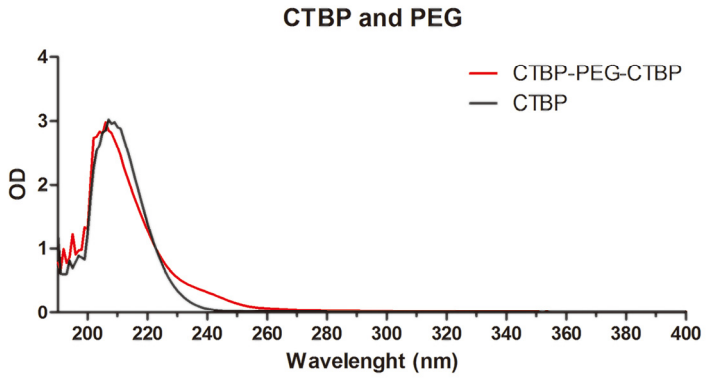

Fig.S9 The UV spectra of CTBP-PEG-CTBP and CTBP at room temperatures. And both the solution of CTBP and CTBP-PEG-CTBP showed special UV absorption (0.00625 mg/mL to 0.1 mg/mL, R2= 0.9998) at ~207 nm.

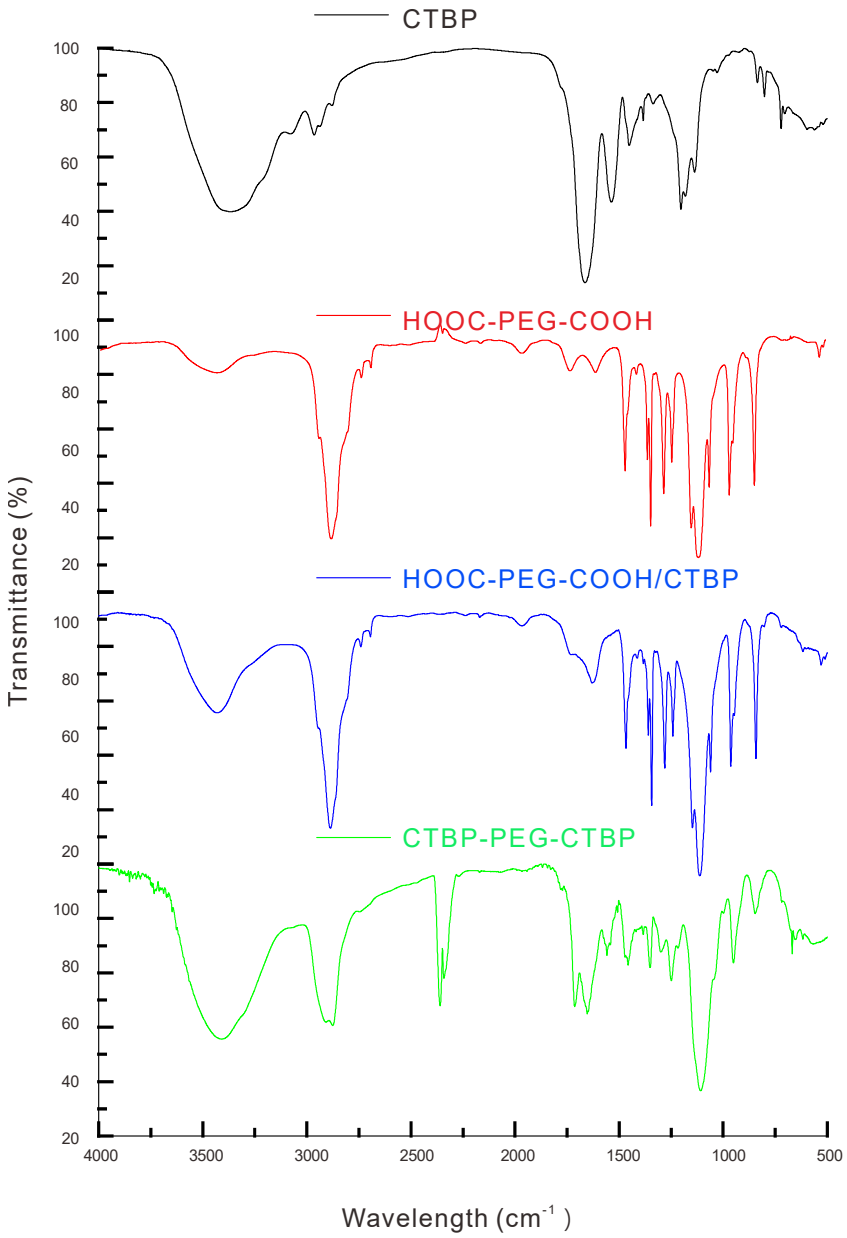

Fig.S10 IR spectra of the CTBP peptide, HOOC-PEG-COOH, CTBP-PEG-CTBP as well as physical mixture of CTBP and HOOC-PEG-COOH at ratio of 5:1. For CTBPP-PEG-CTBP, the intensity of the peak at ~3400 cm-1 increased , the peak at ~2896 cm-1 still existed but widened, the characteristic bands of CTBP that located at 1628, 1552 and 1234 cm-1 (amide I, II, and III) remained, but is widened and deepened due to the conjugation of PEG, respectively. These results suggest that CTBP-PEG-CTBP was successfully synthesized.

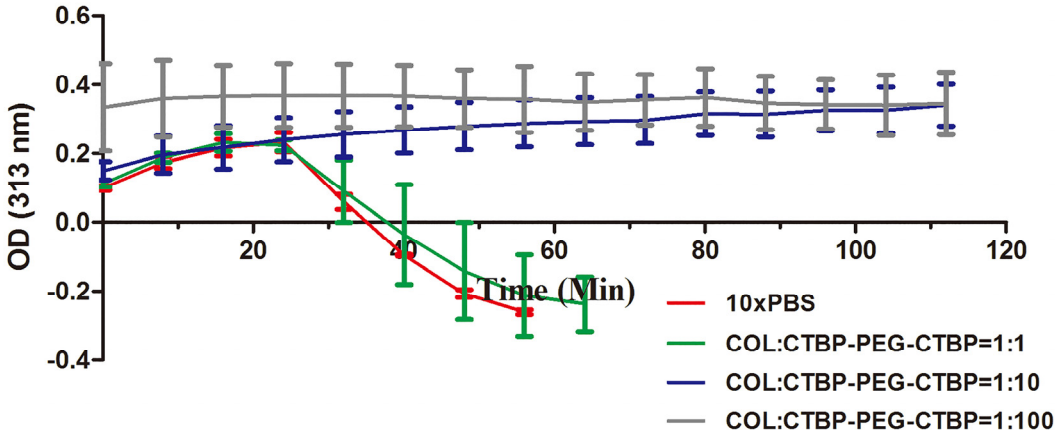

Fig.S11 Turbidity curves of collagen suspension with 10× PBS or 10× PBS containing CTBP-PEG-CTBP (mole ratio of CTBP-PEG-CTBP to collagen, 1:1, 10:1 and 100:1) based on the changes of optical density (OD) relative to that of pure collagen suspension at 313 nm, n = 5.

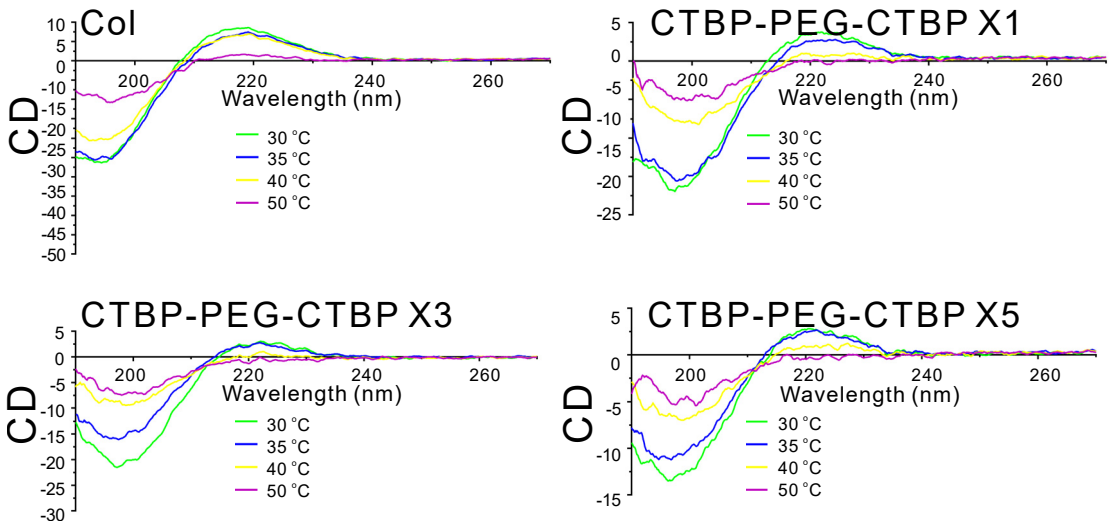

Fig.S12 The CD spectra of collagen fibrils treated by CTBP-PEG-CTBP at different ratios in suspensions for temperatures 30 °C, 35 °C, 40 °C and 50 °C during heating. Col, freeze-dried samples of collagen fibril without any additional treatment; CTBP-PEG-CTBP X1, X3 and X5, freeze-dried samples of fibrous collagen matrix treated by CTBP-PEG-CTBP at ratios (CTBP-PEG-CTBP to collagen) of 1:1, 3: 1 and 5:1, respectively.

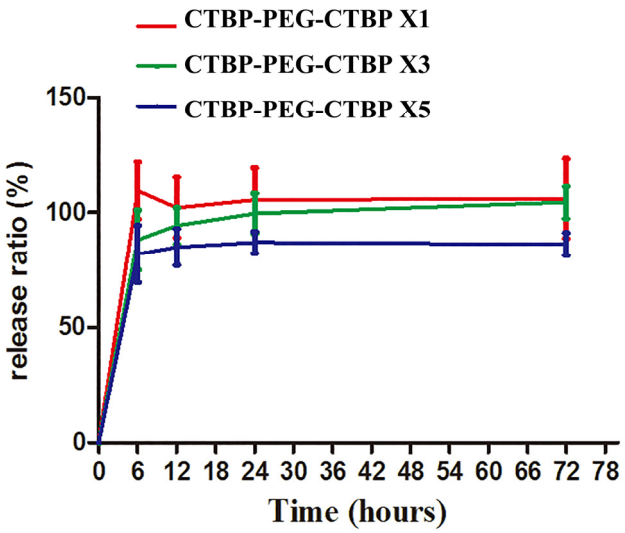

Fig.S13 Cumulative release CTBP-PEG-CTBP from the collagen-based matrixes at different ratios. The data were obtained based on the specific UV absorption of CTBP-PEG-CTBP at ~207nm, while the collected release medium from both the pure collagen and HOOC-PEG-COOH treated matrixes showed no UV absorption at ~207nm. CTBP - PEG - CTBP X1, X3 and X5, freeze - dried samples of fibrous collagen matrix treated by CTBP - PEG - CTBP at ratios (CTBP - PEG - CTBP to collagen) of 1:1, 3: 1 and 5:1, respectively. n=3, mean ± SD.