Tumor-targeting peptide functionalized PEG-PLA micelles for efficient drug delivery

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Synthesis of Maleimide-PEG2000-PLA

Synthesis of HOOC-PEG2000-OH.

15.0 g PEG (7.5 mmol) was dissolved in 150 mL dichloromethane (DCM) in a three necked flask. Then 1.2 mL anhydrous pyridine (15 mmol, 2 eq), 0.092 g DMAP (0.75 mmol, 0.1 eq), and 0.75 g succinic anhydride (7.5 mmol, 1.5 eq) were added into the PEG solution and stirred under the protection of nitrogen.[1] After 12 h of reaction, another 0.375 g of succinic anhydride (3.75 mmol, 0.5 eq) was added into the reaction solution and stirred for another 12 h. The mixture was then washed twice using a separatory funnel with 0.1 M HCl and brine. The DCM phase was dried over Na_2SO_4, evaporated and finally added dropwise into 250 mL 4 °C diethyl ether. White powders were collected by filtration and dried under vacuum.

Preparation of HOOC-PEG-PLA.

2 g of the mixed HOOC-PEG-OH and HOOC-PEG-COOH product, 1.6 g D, L-lactide (11.1 mmol) were dissolved in 30 mL toluene and dried by distilling off 20 mL toluene. Stannous octoate (0.05 wt %) was used as catalyst to conduct the polymerization for 5 h at 125 °C under nitrogen atmosphere. Then the cooled product was diluted using 10 mL DCM, precipitated into 200 mL 4 °C ether and collected by filtration. The purified copolymers were dried in a vacuum for 24 h and then dissolved in 20 mL 80 °C deionized water with stirring until thick oil precipitated at the bottom.[2] Then the supernatant which contained HOOC-PEG-COOH was removed, and another 10 mL 80 °C deionized water was added again with stirring. The whole procedure was repeated three times to completely remove water-soluble HOOC-PEG-COOH. Finally, 1.3 g of white powder was obtained as a target block polymer after lyophilization.

Preparation of Maleimide-PEG-PLA.

1 g of obtained polymer (0.24 mmol) and 137 mg HEMI (0.96 mmol, 4 eq) were dissolved in 5 mL dried dichloromethane (DCM) in a round-bottom flask. Then 184 mg EDC·HCL (0.96 mmol, 4 eq) and 2.9 mg DMAP (0.021 mmol, 0.1 eq) were further dissolved in the mixture, stirred under nitrogen atmosphere at room temperature. After 72 h, the reaction mixture was washed by 0.1 M HCl (10 mL × 2) and saturated brine (10 mL ×

2). The DCM phase was dried over Na_2SO_4, concentrated and added dropwise into 100 mL 4 °C ether, followed by filtration. Finally, 0.89 g of polymer powders was obtained and dried under vacuum.

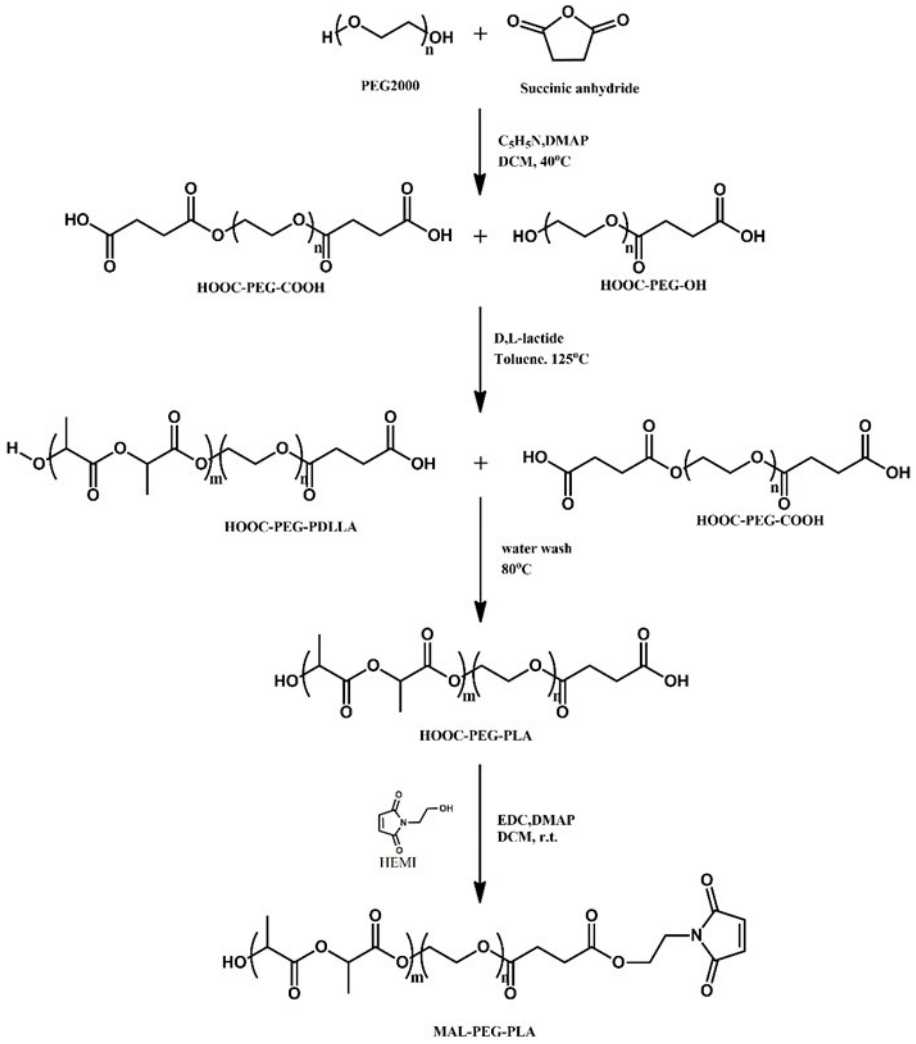

Fig.S1. Synthesis route of maleimide-Polyethylene Glycol-Polylactic Acid (mal-PEG-PLA)

Preparation of Nanoparticles

F3 peptide (30 mg) and TCEP (5 mg) were added into 5 mL 0.01 M HEPES (pH =7.0) buffer and stirred for 0.5 h. Then mal-PEG-PLA (330 mg) was added and reacted for another 8 h. The final product of F3-PEG-PLA was washed twice by ultrafiltration (Mw = 3500) and collected after lyophilization.

F3 conjugated PTX-loaded nanoparticles (F3-NP-PTX) were prepared by dialysis method. Briefly, 1 mL DMSO containing 2 mg PTX and 50 mg F3-PEG-PLA. was slowly pipetted into 10 mL deionized water with stirring. After stirring at room temperature for 2 h, the solution was loaded into a dialysis bag (Mw = 3500) to completely remove DMSO via dialysis against pure water (4 L × 3) for 24 h. After dialysis, the remained free PTX was removed by a 220 nm filter. Meanwhile, the drug-loaded nanoparticles without F3 peptide (NP-PTX) were also

prepared as the same method described above using mal-PEG-PLA. Coumarin-6 was used instead of PTX to prepare fluorescent nanoparticles. Finally, the micelles were obtained by lyophilization and stored at -20 °C under the protection of argon until used.

Coumarin-6 loaded nanoparticles (NP-C6) and F3 conjugated Coumarin-6-loaded nanoparticles (F3-NP-C6) were prepared in the same way. Briefly, 50 mg F3-PEG-PLA or PEG-PLA was dissolved in 1 mL DMSO which containing 0.1 mg Coumarin-6. Then the mixture was slowly pipetted into 10 mL deionized water with stirring. After stirring at room temperature for 2 h, the solution was loaded into a dialysis bag (Mw = 3500) to completely remove DMSO via dialysis against pure water (4 L × 3) for 24 h. After dialysis, the remained free Coumarin-6 was removed by a 220 nm filter and the micelles were obtained by lyophilization.

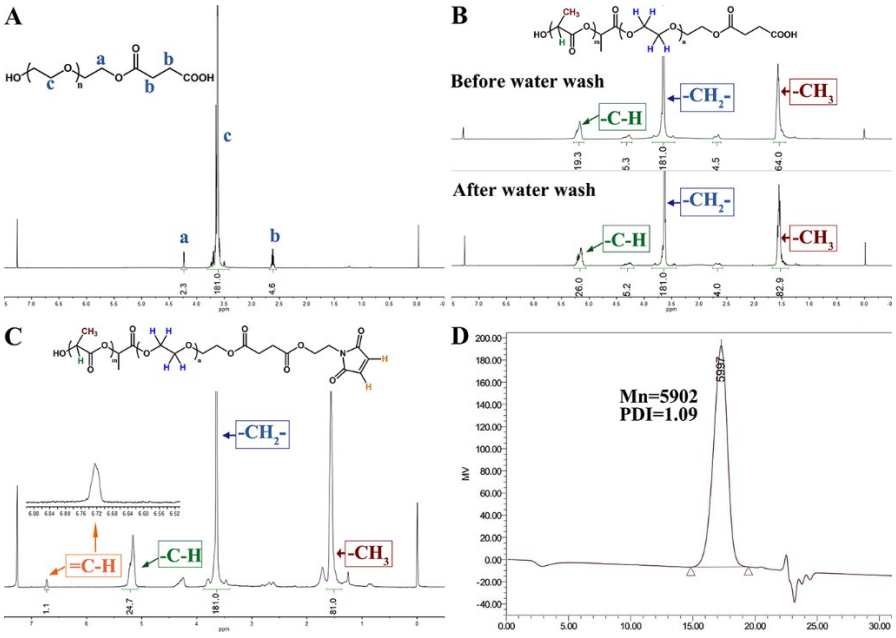

Fig. S2. 1H NMR and GPC Characterization of all synthesized polymers. (A) 1H NMR spectra of the mixture of HOOC-PEG-COOH and HOOC-PEG-OH; (B) 1H NMR spectrum of the mixture of HOOC-PEG-PLA and HOOC-PEG-COOH before and after water wash. The molecular weight of the PLA block in the HOOC-PEG-PLA increased form 1460 Da to 1930 Da after water wash, showing that the HOOC-PEG-COOH has been removed; (C) 1H NMR spectrum of mal-PEG-PLA; (D) Characteristic GPC trace of the purified mal-PEG-PLA.

Reference

1. X. Feng, Y. J. Yuan and J. C. Wu, *Bioorg Med Chem Lett*, 2002, **12**, 3301-3303.
2. J. Yang, J. Yan, Z. H. Zhou and B. G. Amsden, *Biomacromolecules*, 2014, **15**, 1346-1354.