Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Core/shell stabilized polysaccharide-based nanoparticle with intracellular environment-sensitive drug delivery for breast cancer therapy

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Materials

ε-Caprolactone (ε-CL), monohydroxyl terminated poly(ethylene glycol) (Polyethylene glycol monomethyl ethers, MPEG, M_n = 1900), stannous(II)octoate ($Sn(Oct)_2$), and calcium hydride (CaH_2), were purchased from Aladdin (China). All other reagents and solvents used were analytical grade.

Synthesis of MPEG-PCL

According to Shuai's work,[1,2] ε-CL was purified by vacuum distillation in the presence of CaH_2. Toluene was dried by sodium via refluxing, and then distilled under dry argon. MPEG was purified by precipitation in n-hexane from tetrahydrofuran. After drying the precipitates at vaccum, the product was further dehydrated by azeotropic distillation with dry toluene. The diblock copolymer (MPEG-PCL) was synthesized by ring-opening polymerization of ε-CL using MPEG as a macro-initiator and $Sn(Oct)_2$ as a catalyst. Briefly, MPEG and ε-CL (molar ratio = 1:17) reacted in an oil bath at 115 °C under dry argon for 24 h, using $Sn(Oct)_2$ as a catalyst (ca. 0.1% of ε-CL in molar amount). The product was dissolved in dichloromethane and then precipitated in cold methanol. After two washes with cold methanol, the product was vacuum-dried at 40 °C (yield > 90%).

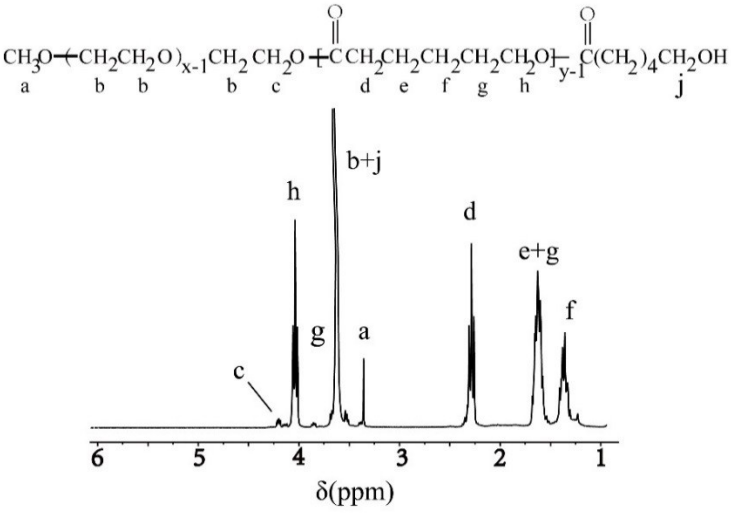
Proton Nuclear Magnetic Resonance (^{1}H-NMR)

The ^{1}H-NMR spectrum of MPEG-PCL was recorded in a 500 MHz Bruker AVANCE III (Bruker, Germany) spectrometer at room temperature. MPEG-PCL was dissolved in $CDCl_3$ (5 mg/mL). The polymerization degree of the PCL block was calculated by comparing the integrals of characteristic peaks at 2.25 ppm (triplet, -$COCH_2$-) from the PCL block and 3.35 ppm (mono, -OCH_3) from the MPEG block, as shown in the following equation (1):

$$\frac{3}{2y} = \frac{S_a}{S_d} \tag{1}$$

Where *y* is the polymerization degree of the PCL block, S_a is the integral intensity of proton a, and S_d is the integral intensity of proton d.

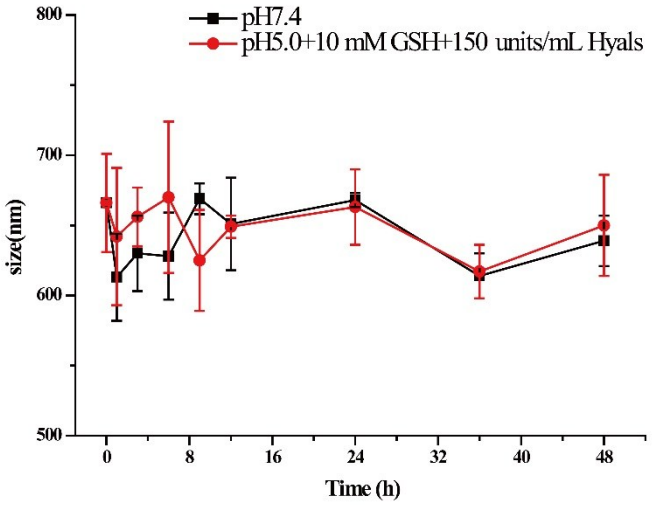
Based on the polymerization degree of the PCL block, the molecular weight of MPEG-PCL diblock copolymer was calculated to be 3326.



S1 ^{1}H-NMR spectrum of MPEG-PCL diblock copolymer in $CDCl_3$.

Stability of MPEG-PCL nanoparticles

The stability of MPEG-PCL nanoparticles by GSH/Hyals/pH multi-stimuli was evaluated. Briefly, 10 mg of MPEG-PCL nanoparticles were dispersed in 10 mL of PBS solution (pH 5.0) containing 10 mM GSH and 150 unit/mL Hyals, and incubated in an incubator (100 rpm) at 37 °C. At desired intervals, 1 mL of nanoparticle suspension was taken out for particle size test in a Zetasizer (Nano-ZS, Malvern Instruments Ltd., U.K.). As compared, the size change of MPEG-PCL nanoparticles in PBS (pH 7.4) was investigated using the same process as above.



S2 Size change of MPEG-PCL nanoparticles with time under different conditions. Data are represented as mean ± SD (n=3).

Preparation of SNX-loaded MPEG-PCL nanoparticles

The MPEG-PCL diblock copolymer and SNX (m:m, 5:1) was dissolved in a mixed solvent of DMSO and CH_2Cl_2 (v:v,1:10) at a concentration of 2 mg/mL with stirring at room temperature for 4 h. After the solvents were evaporated at 45 °C under vaccum in a rotary evaporator, 20 mL deionized water was added followed by stirring for 2 h. The unloaded drugs were thoroughly removed by centrifugation (5000 rpm) and washing with deionized water for five times. The SNX-loaded MPEG-PCL nanoparticles, named as SNX@MPEG-PCL, were obtained by lyophilization.

Drug loading capacity and delivery of SNX@MPEG-PCL nanoparticles

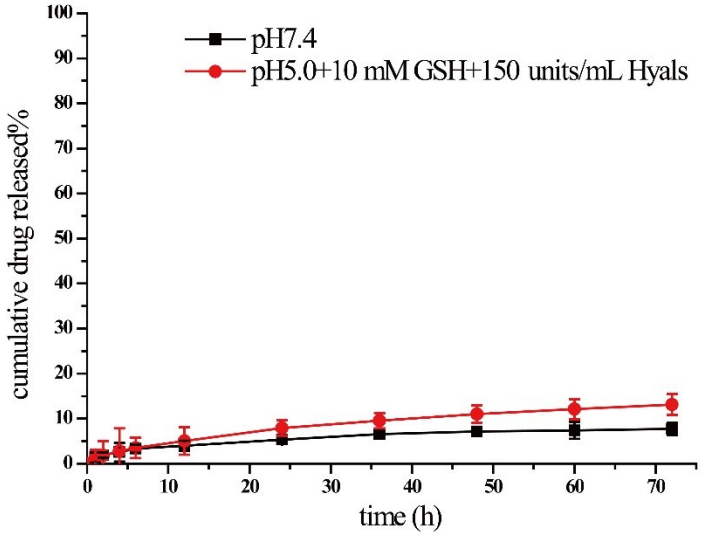
A certain amount of SNX@MPEG-PCL nanoparticles were dissolved in DMSO/CH_2Cl_2 (v/v, 1:10), and then the absorbance at 361 nm is determined in an UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan). The encapsulated SNX in SNX@MPEG-PCL nanoparticles was calculated according to the standard curve of free SNX in the DMSO/CH_2Cl_2 mixed solvent at concentrations of 0.2, 0.5, 1.0, 5.0, 20, and 50 μg/mL. The drug loading capacity (DLC) of SNX@MPEG-PCL nanoparticles was calculated to be 101 μg/mg according to the following equation (2):

$$DLC = \frac{m_e}{m} \times 100\% \quad (2)$$

Where, m_e is the mass of encapsulated SNX, and m is the total mass of dry SNX@MPEG-PCL

nanoparticles respectively.

For the drug delivery by GSH/Hyals/pH multi-stimuli, 2 mL of SNX@MPEG-PCL nanoparticles suspension (2.0 mg/mL) was placed in a dialysis bag (MWCO: 8000-14000 Da). Then, the dialysis bag was immersed in 15 mL of PBS release medium (pH 5.0) containing 0.5% (v/v) Tween 80, 10 mM GSH and 150 unit/mL Hyals, and incubated in an incubator (100 rpm) at 37 °C for drug delivery. At desired intervals, the release medium was taken out and replaced with the same volume of fresh release medium. The absorbance of SNX in the release medium was measured at 361nm in an UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan). The percentage release of SNX was calculated by the ratio of SNX delivered to the medium and the total SNX loaded in SNX@MPEG-PCL nanoparticles, and the percentage cumulative release of SNX was the sum of SNX delivery at each interval during the whole delivery period. As compared, the drug delivery of SNX@MPEG-PCL nanoparticles in PBS (pH 7.4) was performed using the same process as above.



S3 Drug delivery profiles of SNX@MPEG-PCL nanoparticles under different environments at 37 °C. Data are represented as mean ± SD (n=3).

References

1. X. Shuai, H. Ai, N. Nasongkla, S. Kim, J. Gao. J. Control. Release 2004, **98**, 415-426.

2. X. Shuai, T. Merdan, F. Unger, A. Matthias Wittmar, T. Kissel. Macromolecules 2003, **36**, 5751-5759.