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Supplementary Information

Co-delivery of dual chemo-drugs with precisely controlled, high drug loading polymeric micelles for synergistic anti-cancer therapy

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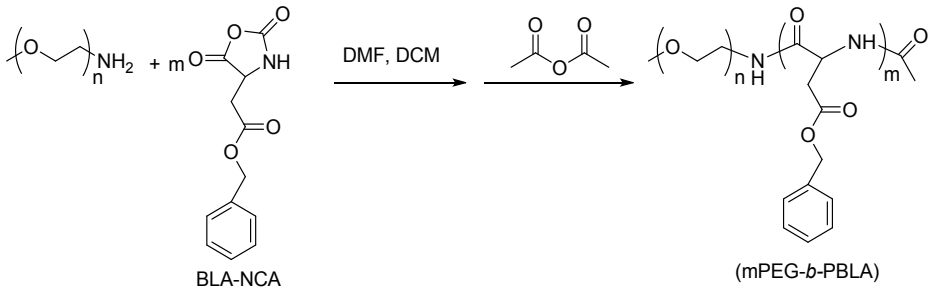
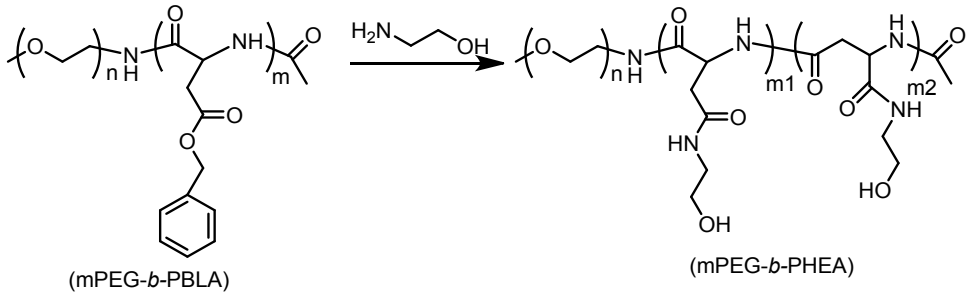
Synthesis of mPEG-*b*-PBLA copolymer



The mPEG-*b*-PBLA di-block copolymer was synthesized via ring-opening polymerization (ROP) of the BLA-NCA monomer using mPEG-NH_2 as the macro-initiator. Briefly, BLA-NCA (5.58 g, 24.4 mmol) was dissolved in a mixture of dry DMF (5.6 mL) and DCM (50.2 mL). Subsequently, mPEG-NH_2 was dissolved in dry DCM and added into the above solution by a syringe under the protection of argon and the resulting solution was stirred gently at 35 °C for 3 days. Then, excessive acetic anhydride was added to the solution and was allowed to react for 12 h at 35 °C to block the terminal amino group. The mPEG-*b*-PBLA copolymer was obtained via precipitation by excessive ice diethyl ether after the mixture was concentrated (yield 86%). The degree of polymerization (DP) of mPEG-*b*-PBLA was 21 (determined by ^{1}H NMR in TFA-*d*).

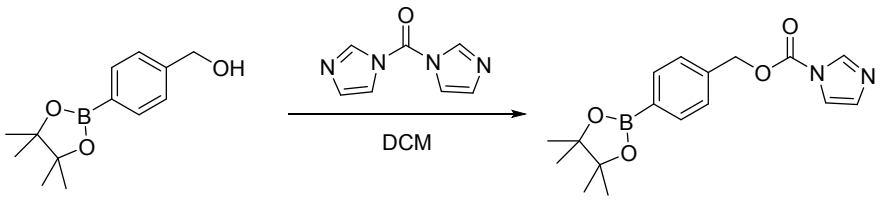
Synthesis of mPEG-*b*-PHEA



mPEG-*b*-PHEA was synthesized via the aminolysis of mPEG-*b*-PBLA with ethanolamine in DMF. For reactions, mPEG-*b*-PBLA (4.0 g) was dissolved in DMF (40 mL) and ethanolamine (3 equivalents to the BLA units) was added. After being stirred at 35 °C for 12 h, the solution was precipitated by excessive ice diethyl ether to remove the excessisve uncreated molecules and the precipitate was washed by diethyl ether for three times. The precipitate was dried under vacuum and the obtained

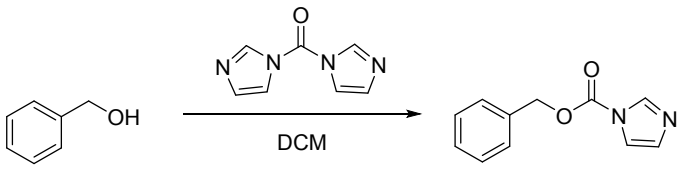
precipitate was dissolved in DMF and dialyzed against distilled water. Finally, the purified mPEG-*b*-PHEA was freeze-dried to obtain the final product as a white solid (yield 83%).

Synthesis of 4-(hydroxymethyl)phenylboronic acid pinacol carbonyl imidazole



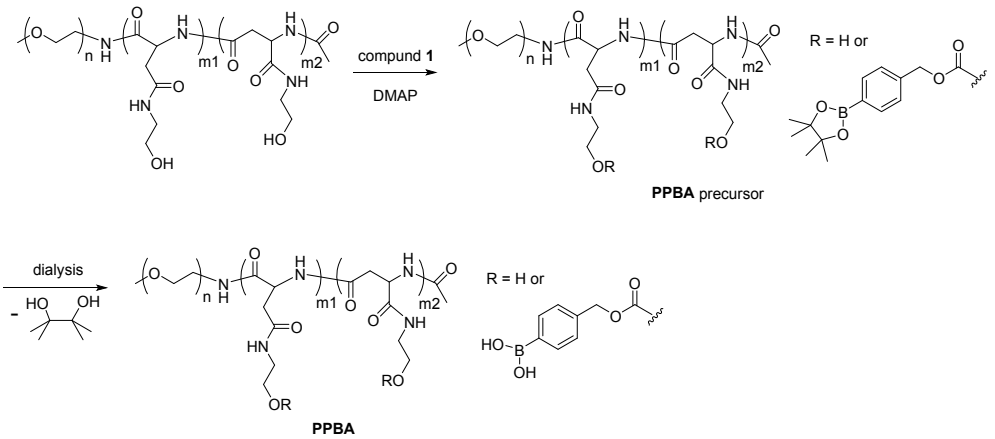
4-(Hydroxymethyl)phenylboronic acid pinacol carbonyl imidazole was synthesized according to the literature.[1] Firstly, 4-(hydroxymethyl)phenylboronic acid pinacol ester was dissolved in dry DCM (50 mL) and carbonyldiimidazole (10.2 g, 62.9 mmol) was added. The mixture was then stirred overnight at room temperature. Then, the obtained solution was diluted by ethyl acetate (200 mL) and washed with distilled water (100 mL × 3). Finally, the organic phase was washed with brine (50 mL × 3), dried with anhydrous $MgSO_4$, concentrated under vacuum to afford the final product as white powder (yield 85.7%).

Synthesis of benzyl carbonyl imidazole



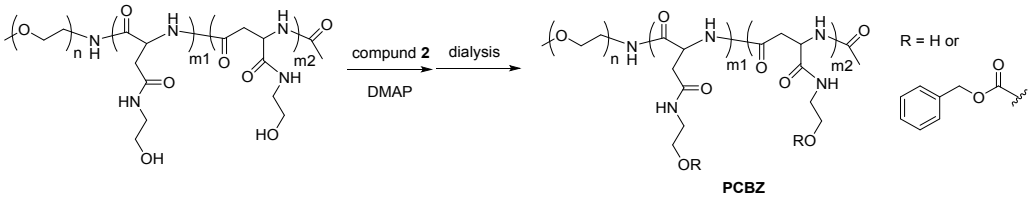
Benzyl carbonyl imidazole was synthesized according to the literature.[1] Firstly, benzyl alcohol (Bn-OH, 5.0 g, 46.2 mmol) was dissolved in dry DCM (50 mL) in a dried flask and carbonyldiimidazole (15 g, 92.5 mmol) was added to the solution and stirred overnight. Then, the obtained solution was diluted by ethyl acetate (200 mL) and washed with distilled water (100 mL × 3). Finally, the organic phase was washed with brine (50 mL × 3), dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford the product as yellow liquid (yield 82.3%).

Synthesis of mPEG-*b*-PHEA-PBA (PPBA)



Firstly, 4-(hydroxymethyl)phenylboronic acid pinacol carbonylimidazole (948 mg, 2.88 mmol), mPEG-*b*-PHEA (2.00 g, 0.24 mmol), and DMAP (412 mg, 3.38 mmol) were dissolved in anhydrous DMF (30 mL) in a dry flask, and the mixture was stirred overnight at 50 °C. Then, the solution was precipitated with excessive diethyl ether. The precipitate was dried with a vacuum pump to afford the PPBA precursor. Finally, the PPBA precursor was dissolved in DMF, dialyzed against distilled water, and the solution was freeze-dried to obtain the final PPBA as white solid (yield 81.2%).

Synthesis of mPEG-*b*-PHEA-Bn (PCBZ)



Similar to the synthesis of PPBA, benzyl carbonylimidazole (310 mg, 1.53 mmol), mPEG-*b*-PHEA (1.00 g, 0.24 mmol), and DMAP (190 mg, 1.56 mmol) were dissolved in anhydrous DMF (30 mL) in a dry flask, and the mixture was stirred overnight at 50 °C. Then, the obtained solution was precipitated with excessive diethyl ether. Finally, the precipitate was dissolved in DMF, dialyzed against distilled water, and freeze-dried to obtain PCBZ as white solid (yield 80.6%).

Characterizations

The nuclear magnetic resonance (NMR) spectra were recorded on an Agilent 400 MHz spectrometer. The size and zeta potential of micelles in the aqueous solution

were measured by dynamic light scattering (DLS) conducted on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) with 90° collecting optics. The morphology of micelles was observed by an FEI Tecnai G2 Spirit Twin transmission electron miscroscopy (TEM).

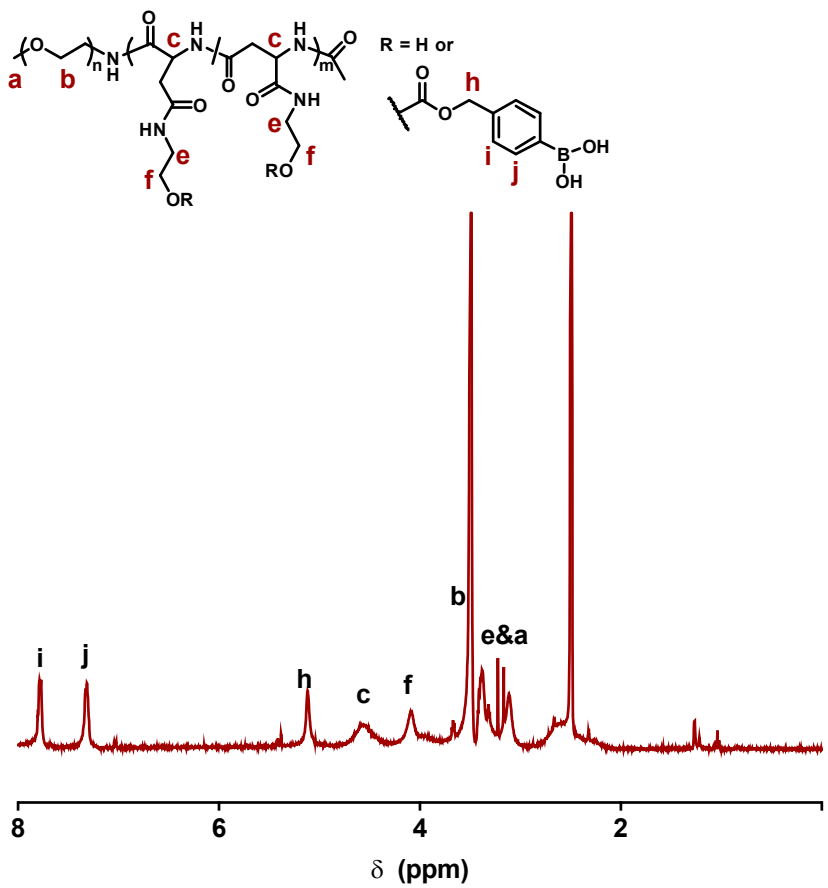


Fig. S1. ^{1}H NMR spectrum of PPBA in d_6-DMSO.

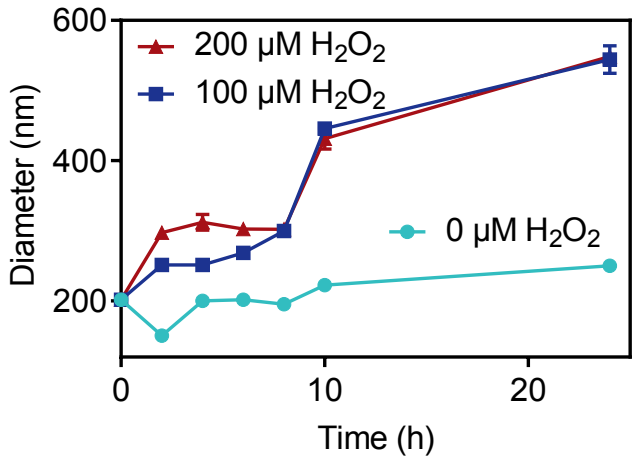


Fig. S2. Diameter alteration of PPBA micelles after treatment with PBS containing H_2O_2 at different concentrations.

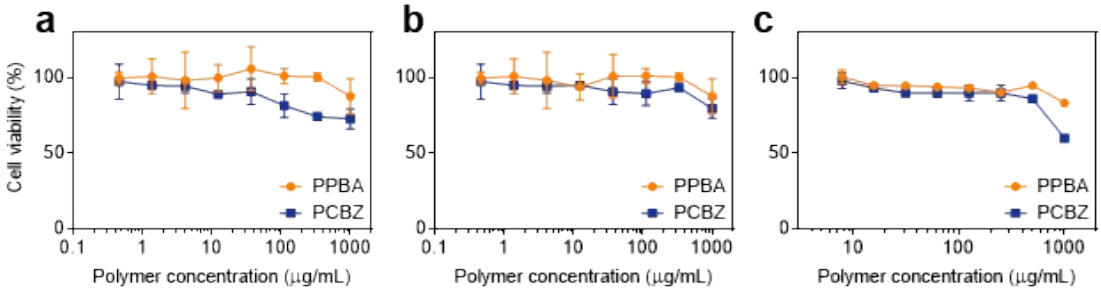


Fig. S3. Viability of (a) LLC, (b) SKOV, and (c) NIH-3T3 cells after incubation with PPBA or PCBZ micelles at different concentrations for 48 h (n = 3).

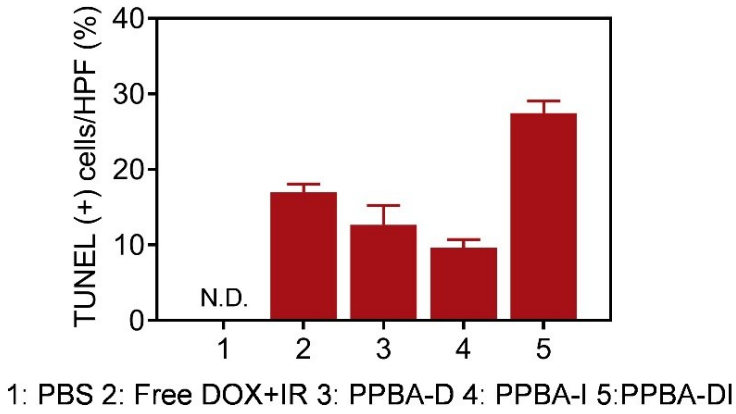


Fig. S4. Quantitative analysis of the apoptosis level of LLC tumor cells *in vivo*. N.D. stands for "not detectable".

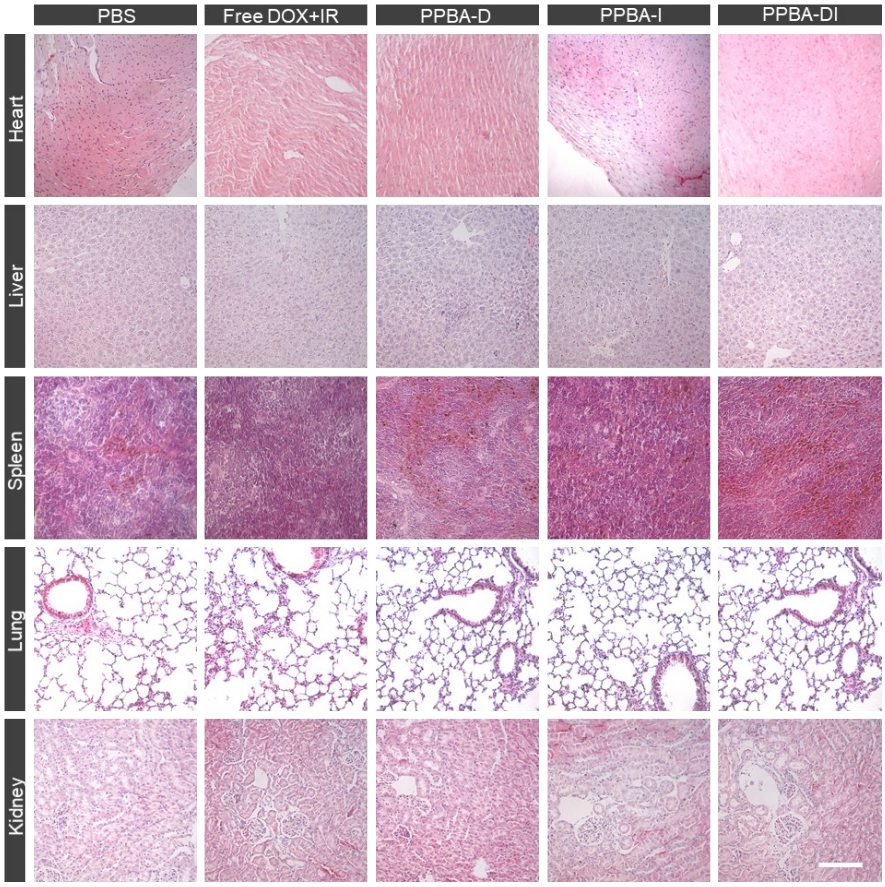


Fig. S5. H&E staining of major organs harvested from LLC xenograft tumor-bearing mice on day 16 in the efficacy study (bar = 200 μm).

Table S1. Summary of IC_{50} and CI of the free drugs against LLC and SKOV-3 cells.

Cell lines	Micelles	$IC_{50\ (DOX)}$ (μg/mL)	$IC_{50\ (IR)}$ (μg/mL)	CI
LLC	Free DOX	0.72	-	0.62
	Free IR	-	36.6	
	Free DOX+IR	0.37	3.74	
SKOV-3	Free DOX	0.14	-	0.39
	Free IR	-	14.5	
	Free DOX+IR	0.05	0.5	

Reference:

1 S. Lv, Y. Wu, K. Cai, H. He, Y. Li, M. Lan, X. Chen, J. Cheng and L. Yin, *J. Am. Chem. Soc.*, 2018, **140**, 1235–1238.