Size Switchable Nanoplatfor for Targeting Tumor Microenvironment and Deep Tumor Penetration

Xingli Cun, Man Li, Shuying Wang, Yifei Wang, Jialing Wang, Zhengze Lu
Ruixin Yang, Xian Tang, Zhirong Zhang, Qin He*

Key Laboratory of Drug Targeting and Drug Delivery Systems, West China School of Pharmacy, Sichuan University, No. 17, Block 3, Southern Renmin Road, Chengdu 610041, China.

*Corresponding author: Fax/Tel: 86 28 85502532; Qin He: qinhe@scu.edu.cn

Table of contents

Fig. S1. 1H NMR spectra recorded in CDCl₃ for Me-PEG-PCL........................................1
Fig. S2. 1H NMR spectra recorded in CDCl₃ for COOH-PEG-PCL. ..................................1
Fig. S3. 1H NMR spectra recorded in D₂O for EGPLGVRK peptide. ..........................2
Fig. S4. 1H NMR spectra recorded in CDCl₃ for Pep-PEG-PCL. .................................2
Fig. S5. 1H NMR spectra recorded in DMSO-d₆ for DGL (A), DGL-Glu (B) and DGL-Glu-NHNH₂ (C). .............................................................................................................3
Fig. S6. Absorption spectra of the synthesized DGL, DOX and DOX-DGL. ...............4
Fig. S7. 1H NMR spectra recorded in DMSO-d₆ for DOX-DGL-PEG-PCL (A) and DOX-DGL-Pep-PEG-PCL (B).
Fig. S8. Absorption spectra of the synthesized DOX-DGL-PEG-PCL and DOX-DGL-Pep-PEG-PCL..............................................................................................................4
Fig. S9. DLS (A) and TEM (B) measurements of DGL/DOX........................................4
Fig. S10 Zeta potential of PP.
Fig. S11. XPS analysis. XPS survey spectra of PP (A), DGL/DOX-PP (B) and DGL/DOX@PP (C). Nitrogen N1s envelopes from XPS analysis of PP (D), DGL/DOX-PP (E) and DGL/DOX@PP (F).
Fig. S12. Image of red blood cells incubated with 1 mg/mL DGL/DOX, DGL/DOX-PP and DGL/DOX@PP for 12 h. ..............................................................5
Fig. S13. Time-related hemolysis rates of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP for 12 h. ..............................................................5
Fig. S14. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with PBS (pH=7.4). .................................................................5

Fig. S15. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with 10% serum. .................................................................6

Fig. S16. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with 50% serum. .................................................................6

Fig. S17. Cytotoxicity study of different blank nanoparticles in 4T1 cells.................7

Fig. S18. Cytotoxicity study of different blank nanoparticles in BxPc-3 cells. ..........7

Fig. S19. Cytotoxicity study of different blank nanoparticles in 3T3 cells. ..........8

Fig. S20. Cellular uptake of different formulations by 4T1 cells 2 h after treatment as measured by flow cytometry . .................................................................8

Fig. S21. Cytotoxicity of different formulations in 4T1 cells after 24 h of incubation ......9

Fig. S22. In vivo tumor penetration of DGL/Cy5.5, DGL/Cy5.5-PP and DGL/Cy5.5 @PP into tumor tissues of 4T1 tumor-bearing mice at a Cy5.5 dose of 0.24 mg/kg after 48 h.

Fig. S23. Ex vivo imaging of normal tissues at 24 h after treatment with different formulations. .................................................................................................................9

Fig. S24. DOX distribution of different formulations in major organs slices. .........10
Fig. S1. $^1$H NMR spectra recorded in CDCl$_3$ for Me-PEG-PCL.

Fig. S2. $^1$H NMR spectra recorded in CDCl$_3$ for COOH-PEG-PCL.
Fig. S3. $^1$H NMR spectra recorded in D$_2$O for EGPLGVRK peptide.

Fig. S4. $^1$H NMR spectra recorded in CDCl$_3$ for Pep-PEG-PCL.
Fig. S5. $^1$H NMR spectra recorded in DMSO-d6 for DGL (A), DGL-Glu (B) and DGL-Glu-NHNH$_2$ (C).
Fig. S6. Absorption spectra of the synthesized DGL, DOX and DOX-DGL.

Fig. S7. $^1$H NMR spectra recorded in DMSO-d6 for DOX-DGL-PEG-PCL (A) and DOX-DGL-Pep-PEG-PCL (B).
Fig. S8. Absorption spectra of the synthesized DOX-DGL-PEG-PCL and DOX-DGL-Pep-PEG-PCL.

Fig. S9. DLS (A) and TEM (B) measurements of DGL/DOX.

Fig. S10 Zeta potential of PP.
Fig. S11. XPS analysis. XPS survey spectra of PP (A), DGL/DOX-PP (B) and DGL/DOX@PP (C). Nitrogen N1s envelopes from XPS analysis of PP (D), DGL/DOX-PP (E) and DGL/DOX@PP (F).

Fig. S12. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with PBS (pH=7.4).
Fig. S13. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with 10% serum.

Fig. S14. Time-related absorption of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP after incubation with 50% serum.
Fig. S15. Image of red blood cells incubated with 1 mg/mL DGL/DOX, DGL/DOX-PP and DGL/DOX@PP for 12 h.

![Graph showing percentage hemolysis over time for DGL/DOX, DGL/DOX-PP, and DGL/DOX@PP.]

Fig. S16. Time-related hemolysis rates of 1 mg/mL of DGL/DOX, DGL/DOX-PP, DGL/DOX@PP for 12 h.

![Bar graph showing cell viability for different blank nanoparticles in 4T1 cells.]

Fig. S17. Cytotoxicity study of different blank nanoparticles in 4T1 cells. (n=3, mean±SD).
Fig. S18. Cytotoxicity study of different blank nanoparticles in BxPC-3 cells. (n=3, mean±SD).

Fig. S19. Cytotoxicity study of different blank nanoparticles in 3T3 cells. (n=3, mean±SD).
Fig. S20. Cellular uptake of different formulations by 4T1 cells 2 h after treatment as measured by flow cytometry (n=3, mean ± SD).

Fig. S21. Cytotoxicity of different formulations in 4T1 cells after 24 h of incubation (n=3, mean ± SD).
Fig. S22. In vivo tumor penetration of DGL/Cy5.5, DGL/Cy5.5-PP and DGL/Cy5.5 @PP into tumor tissues of 4T1 tumor-bearing mice at a Cy5.5 dose of 0.24 mg/kg after 48 h. The tumor sections were obtained at different depths from the injection site to the equatorial plane of the tumor. Red represents Cy5.5. Blue represents DAPI. Bar represents 200 μm.
Fig. S23. Ex vivo imaging of normal tissues at 24 h after treatment with different formulations.

Fig. S24. DOX distribution of different formulations in major organs slices. The scale bar represents 100 μm.