

Electronic Supplementary Information (ESI)

Enhanced transcellular penetration and drug delivery by crosslinked polymeric micelles into pancreatic multicellular tumor spheroids

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Supplementary methods

Cytotoxicity tests for polymeric micelles. AsPC-1 cells were seed in a 96-well plate (4000 cells/well) and culture for 1 d under 5% CO₂ at 37°C before load the micelles. Micelles were firstly sterilized by UV radiation for 20 min in a biosafety cabinet and then serially diluted with sterile water, halving in concentration for each dilution. 100 μL of each solution was added to the wells along with 100 μL of 2 × (double concentrate) cell culture media. MilliQ water was also used as a control. The cells were then incubated for 48 hours. A sulforhodamine B (SRB) assay was applied to determine the cell number.⁴⁵ The incubation with micelles was finished by addition of cold trichloroacetic acid for 30 min. After a complete washing with distilled water, 100 μL of 0.4% sulforhodamine B (SRB) solution in 1% acetic acid (w/v) was added to each well. After staining, unbound dye was removed by washing with 1% acetic acid and plates are air dried. Finally, the SRB was solubilized with 200 μL 10mM Tris buffer and the optical density was read on a Bio-Rad BenchMark microplate reader at 490 nm.

Cytotoxicity tests for inhibitors. A WST-1 based assay was used to test the cytotoxicity of various inhibitors.⁴⁶ AsPC1-1 cells were seed in a 96-well plate (4000 cells/well) and

culture for 1 d under 5% CO₂ at 37°C before add the inhibitors. Chlorpromazine, Filipin, Amiloride, NaN₃ plus deoxyglucose and Exo1 were added to the plates with their respective concentrations (Table S1). These plates were treated with inhibitors for 90 minutes. Old media was discarded and cells were washed with PBS. 100 µL of serum plus medium with WST-1 (5 µg/mL) was added to each well. Plates were incubated for 3 hours at 37 °C before reading absorbance on a microplate reader at 450 nm with a reference at 650 nm. As opposed to the SRB Assay which measures long term impacts of cell viability, the WST-1 assay is able to evaluate cell viability shortly after inhibitor treatment. This would better reflect the cell activities following this treatment during the inhibition assays.

Table S1 Inhibitors used in the endocytosis and exocytosis studies

Name	Conc.	Pathway
Chlopromazine	10 µg/mL	Clatherin-mediated endocytosis
Filipin	10 µg/mL	Caveolae-mediated endocytosis
Amiloride	50 µM	Macropinocytosis
NaN ₃ +Deoxyglucose	5 mM + 5 mM	Energy dependent endocytosis
Exo1	100 µM	Traffic emanating from the endoplasmic reticulum

References

45. Vichai, V.; Kirtikara, K. Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening. *Nat. Protoc.* 2006, 1, 1112-1116.
46. Lu, H.; Guo, L.; Kawazoe, N.; Tateishi, T.; Chen, G. Effects of Poly(L-lysine), Poly(acrylic acid) and Poly(ethylene glycol) on the Adhesion, Proliferation and Chondrogenic Differentiation of Human Mesenchymal Stem Cells. *J. Biomater. Sci. Polym. Ed.* 2009, 20, 577-589.

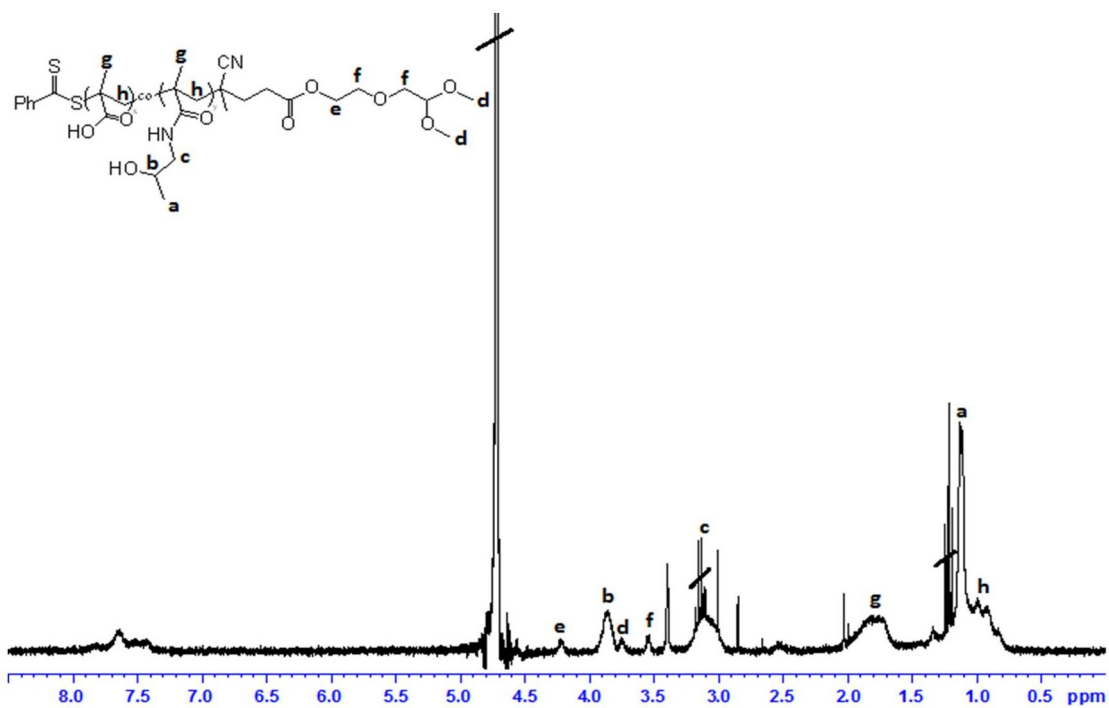


Figure S1. ¹H NMR (in D₂O) of P(HPMA-co-MAA)

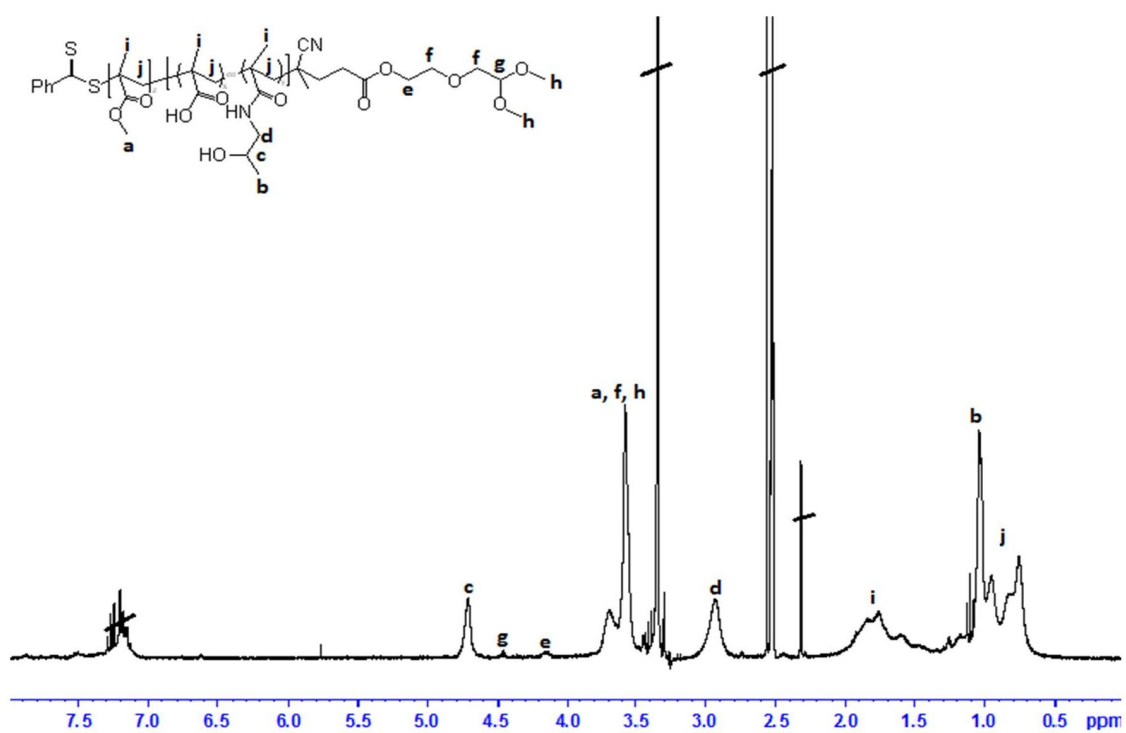


Figure S2. ¹H NMR (in D₂O) of P(HPMA-co-MAA)-b-PMMA

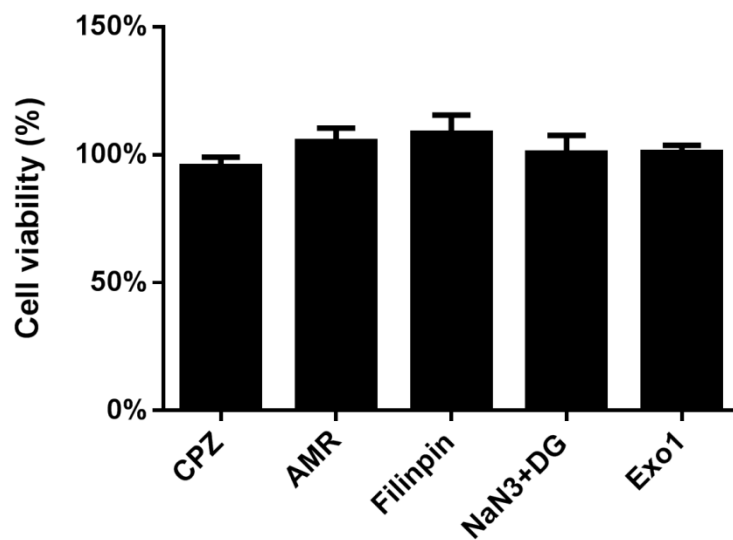


Figure S3. The inhibitors showed no toxicity to AsPC-1 cells in a WST-1 based cytotoxicity assay. Data represent mean \pm SD, n=6.

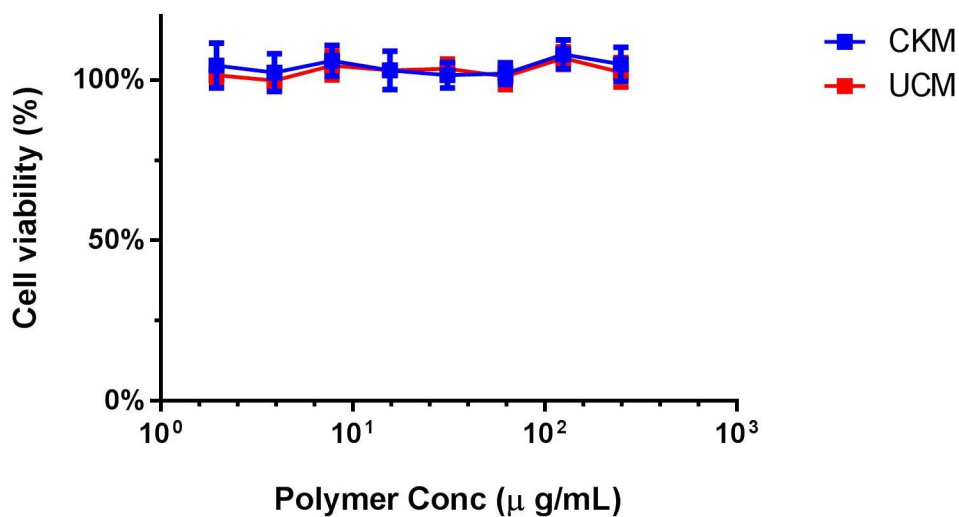


Figure S4. CKM and UCM showed no toxicity to AsPC-1 cells in a SRB-based cytotoxicity assay. Data represent mean \pm SD, n=6.

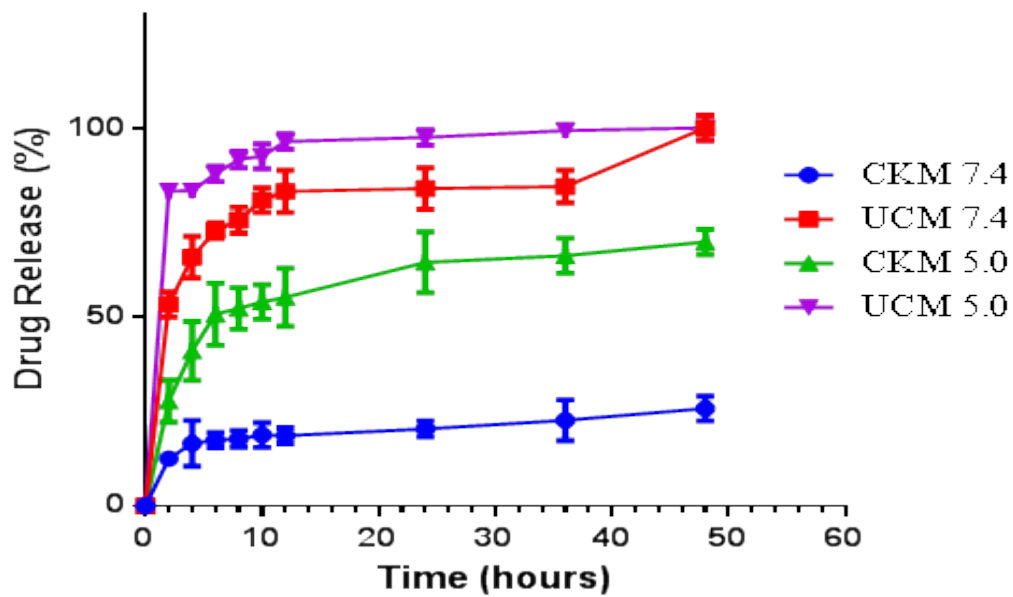


Figure S5. DOX release profile from CKM and UCM.

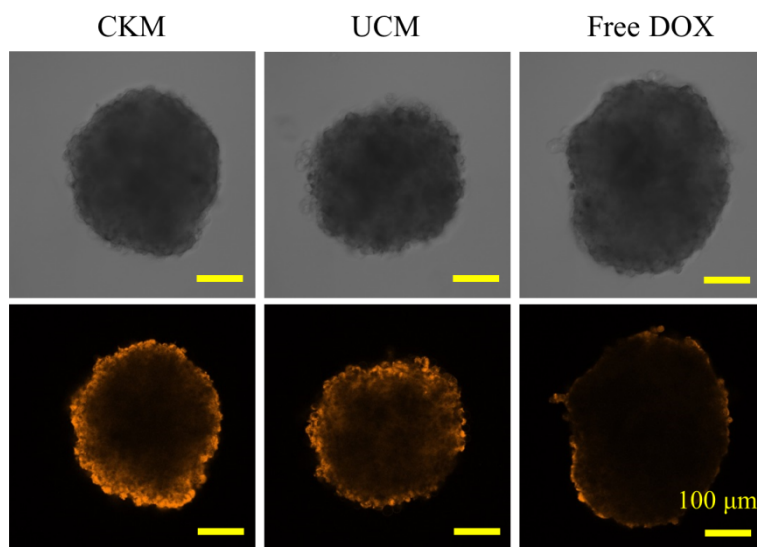


Figure S6. DOX delivery into MCTS by CKM and UCM into MCTS formed by A549 lung cancer cells.

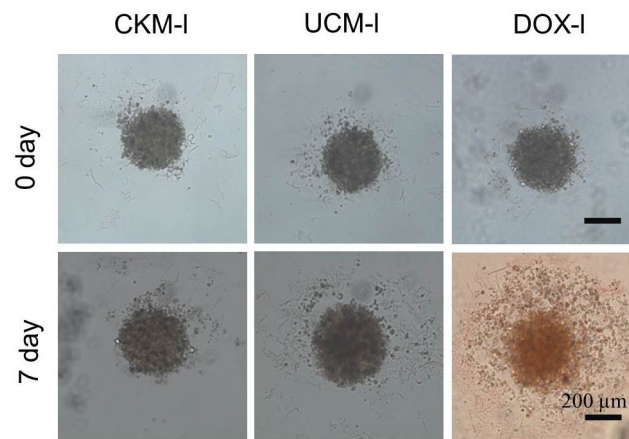


Figure S7. Microphotographs of pancreatic spheroids before and after DOX-loaded micelles treatment. Bar = 200 μm .