2016 中技社科技創意獎學金

CTCI Science and Technology Research Scholarship

開發具主動標靶與多重酸敏感性的自組裝多胜肽奈米藥物載體應用於癌症藥物傳輸及腫瘤轉移抑制

Tailored Design of Self-assembling and Stimulus-responsive Polypeptides for Active Targeted Drug Delivery

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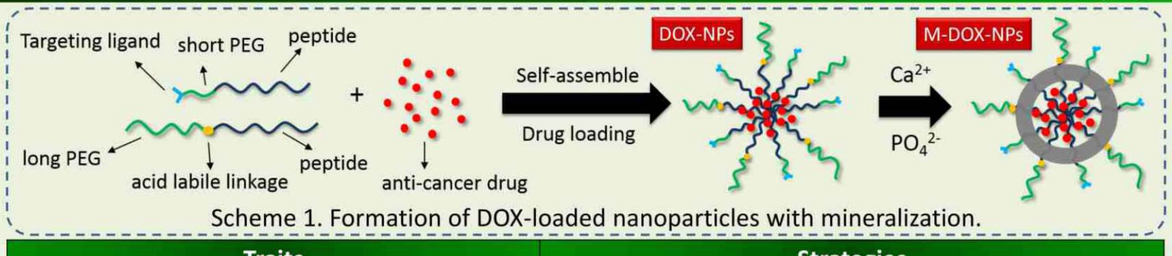
創意重點

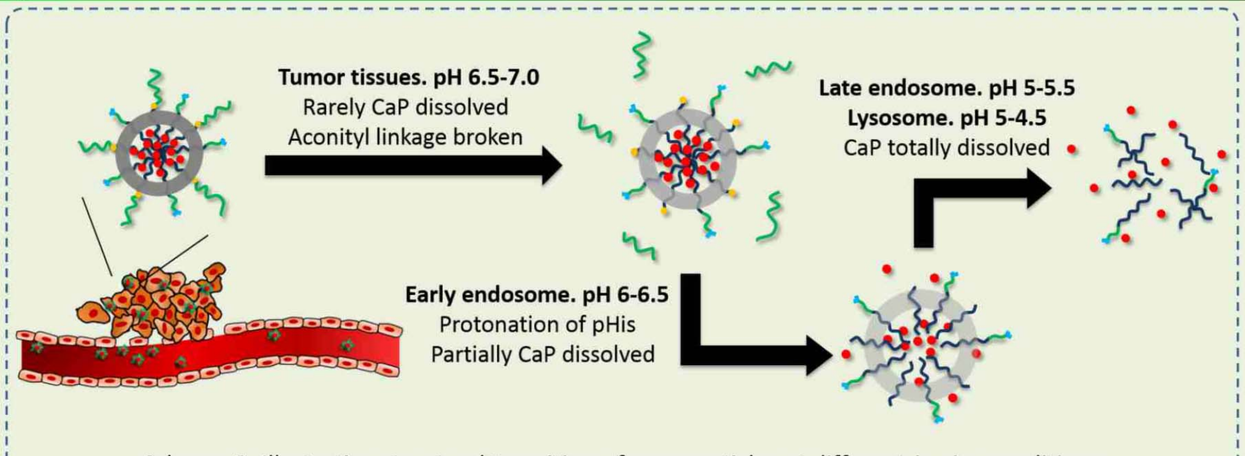
The purpose of this study is to develop biodegradable nanoparticles composed of natural polypeptides with sequential pH-responsivity to tumor microenvironments for active targeted drug delivery. Two different amphiphilic copolymers, poly(ethylene glycol)3400-aconityl linkage-poly(L-glutamic acid)x-poly(L-histidine)y-poly(L-leucine)z and LyP1-poly(ethylene glycol)1100-poly(L-glutamic acid)x-poly(L-histidine)y-poly(L-leucine)z, were exploited to self-assemble into micelles in aqueous phase. The bio-stable nanoparticles provide three distinct functional domains: the anionic PGlu shell for calcium phosphate mineralization, the protonation of Phis segment for facilitating anticancer drug release at target site, and the hydrophobic core of PLeu for encapsulation of anticancer drugs. Furthermore, the hydrated PEG outer corona is used for prolonging circulation time, while the active targeting ligand, LyP-1, is served to bind to specific cancer-related cells for inhibiting metastasis. Mineralized DOX-loaded nanoparticles (M-DOX NPs) efficiently prevent the drug leakage at physiological pH value and facilitate drug release at acidic condition. M-DOX NPs with LyP-1 targeting ligand effectively accumulated in MDA-MB-231 breast cancer cells. The inhibition effect on cell proliferation also enhances with time, illustrating the prominent anti-tumor efficacy. Moreover, the in vitro metastatic inhibition model shows the profound inhibition effect on cancer cell invasion. In brief, this self-assembling peptide-based drug delivery nanocarrier with multifunctionality and programmable pH-sensitivity is of great promise and potential for anti-cancer therapy.

創意圖示說明



Scheme 1. Formation of DOX-loaded nanoparticles with mineralization.

Traits	Strategies
Biocompatibility	Natural materials (peptide & CaP)
High stability in blood	Mineralization of CaP & conjugation of PEG
Enhanced accumulation at the target site	Breaking of aconityl amino linkage
Minimized drug loss	Mineralization of CaP
Effective uptake	Nano-size & active targeting ligand
Facilitated release of drugs within cancer cells	Protonation of histidine



Scheme 2. Illustrative structural transition of nanoparticles at different *in vivo* conditions.

創意成果

Physicochemical properties

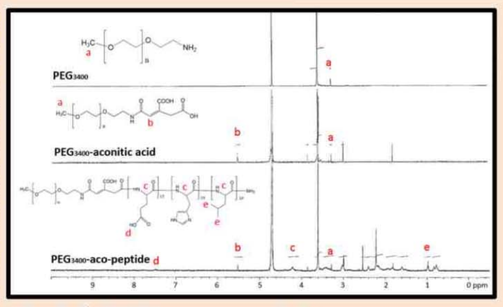


Fig. 1. ^{1}HNMR spectra of of long sequence.

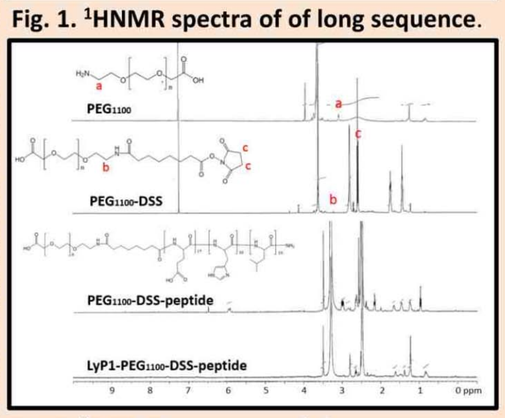


Fig. 2. ^{1}H NMR spectrum of short sequence.

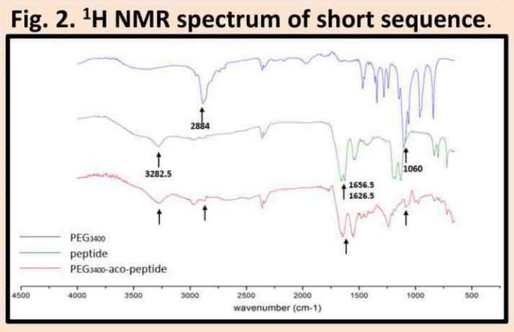


Fig. 3. FT-IR spectra of long sequence.

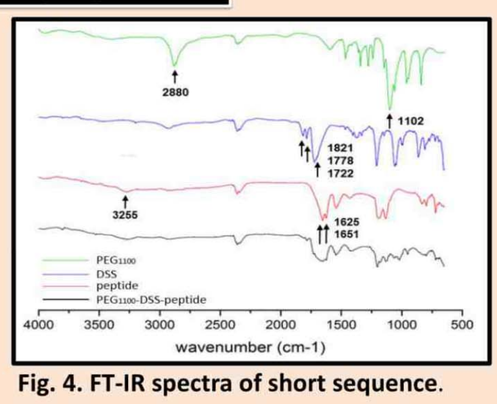


Fig. 4. FT-IR spectra of short sequence.

Table 1. Characteristics of nanoparticles

Sample	Size(nm)	ζ (mV)
DOX-NPs pH 5.0	143.7 ± 6.6	22.5 ± 1.0
DOX-NPs pH 7.4	77.5 ± 3.8	-45.5 ± 1.0
M-DOX-NPs pH 5.0	291.2 ±25.1	21.7 ± 2.1
M-DOX-NPs pH 7.4	179.4 ±33.9	-21.9 ± 1.6

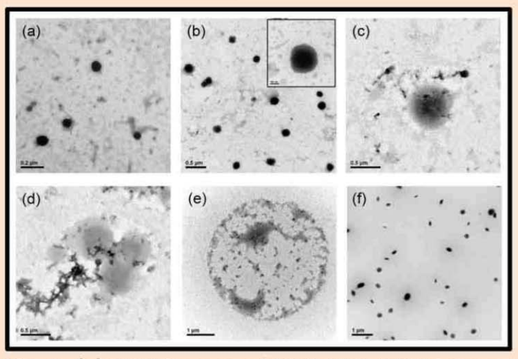


Fig. 5. (a) TEM images of DOX NPs. TEM images of M-DOX NPs at pH 7.4 (b) and pH 5 at different time points (c-f). c: 20 mins; d: <4 hours; f: > 4hrs.

In vitro study

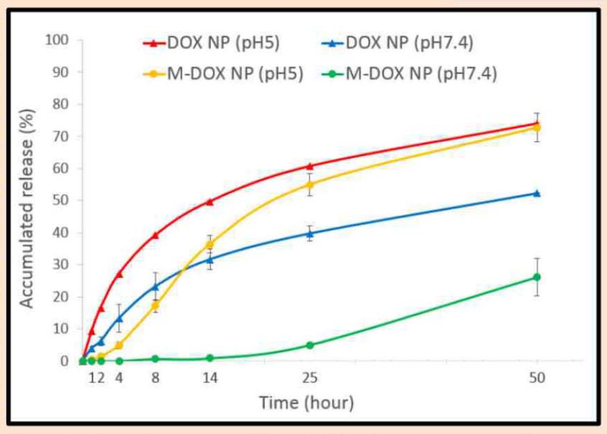


Fig. 6. The release profile of DOX from nanoparticles in different pH value.

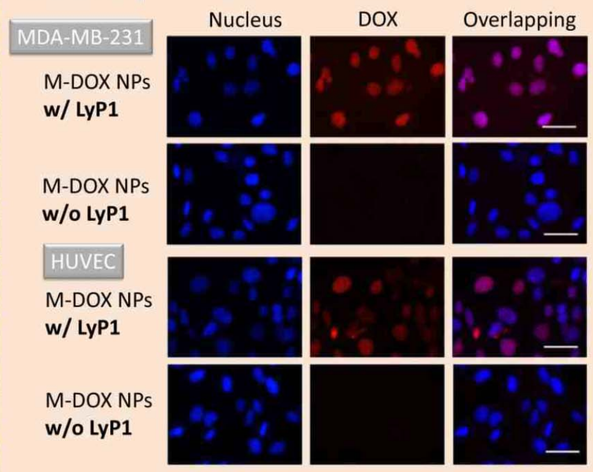


Fig. 7. Intracellular DOX release and integrated into nuclei. MDA-MB-231 breast cancer cells and HUVEC were incubated for 4 h at a DOX-equivalent dosage of 10ug/ml. Nuclei were stained with DAPI.

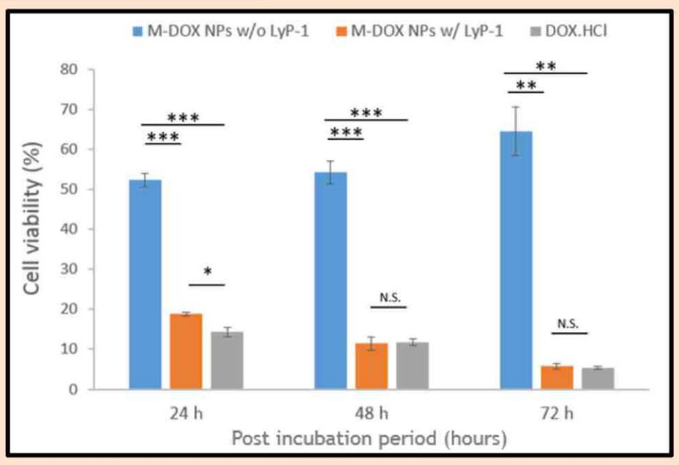


Fig. 8. The anti-tumor effect of M-DOX NPs to MDA-MB-231 cells.

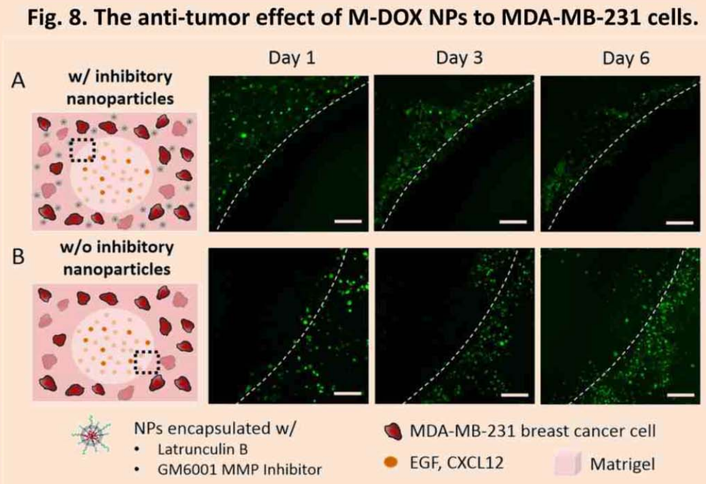


Fig. 9. The inhibition effect of MDA-MB-231 cell invasion via incubating cells with M-inhibitor NPs (A) and without M-inhibitor NPs (B). Cells were stained with CellTracker Green CMFDA. Scale bar: 200 µm.

創意心得

研究過程中有挫折、難關，很慶幸自己一路上沒有放棄，也非常榮幸自己能夠帶著多方的肯定與鼓勵一直大步向前進；很幸運有一群很好的研究夥伴，能夠相互扶持、一起成長；衷心感謝我的指導教授王子威老師，在這期間的提攜與鼓勵，並讓我了解「如果你不鼓足勇氣去嘗試， 那就永遠沒有成功的機會。」這句話的真諦！除此之外，非常感謝戴念華老師與王潔老師，在我對未來感到困惑迷惘時，總像是一盞明燈給我指引方向。我會帶著這份熱情與衝勁繼續在生醫領域持續向前邁進！最後謝謝中技社給予的肯定，讓我能帶著這筆獎學金去出國自我精進。希望未來能夠為台灣生醫產業盡一份心力，致力為「提供病人更好的生活品質」這個理念而努力。

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