JUSTC

Article

http://justc.ustc.edu.cn

Received: October 28, 2022; Accepted: November 28, 2022

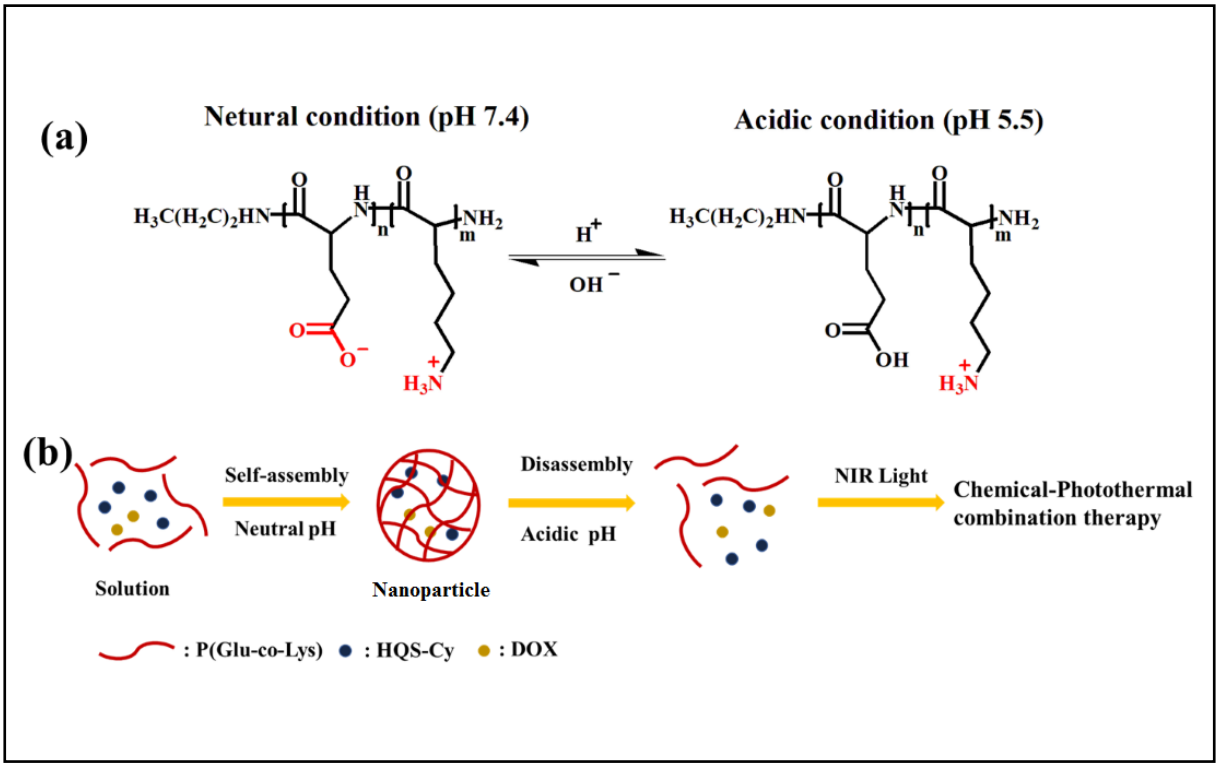
Synthesis of pH-responsive supramolecular polypeptide nanoparticles from α-amino acids for combined chemo-photothermal therapy

Hongyun Qian[1], Huiping Dang[1], Changchang Teng[1], Dalong Yin[2], and Lifeng Yan[1] ✉

[1]*Department of Chemical Physics, University of Science and Technology of China, Hefei 230026, China;*
[2]*Department of Hepatobiliary Surgery, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230001, China*

✉Correspondence: Lifeng Yan, E-mail: lfyan@ustc.edu.cn

Graphical abstract



pH-switchable polypeptide nanoparticles for chemo-photothermal therapy.

Public summary

- A new polypeptide copolymer, P(Glu-co-Lys), was synthesized by the polymerization of α-amino acids via the N-thiocarboxylic acid anhydride (NTA) method.
- Both drugs and organic dyes can be efficiently encapsulated by the self-assembly of the copolymer, and the drug delivery system shows pH-sensitive performance.
- In vitro experiments reveal that the nanoparticles show efficient smart combined chemo-photothermal therapy.

Citation: Qian H Y, Dang H P, Teng C C, et al. Synthesis of pH-responsive supramolecular polypeptide nanoparticles from α-amino acids for combined chemo-photothermal therapy. *JUSTC*, **2023**, 53(3): 0305. DOI: 10.52396/JUSTC-2022-0154

JUSTC

Article

http://justc.ustc.edu.cn

Received: October 28, 2022; Accepted: November 28, 2022

Synthesis of pH-responsive supramolecular polypeptide nanoparticles from α-amino acids for combined chemo-photothermal therapy

Hongyun Qian[1], Huiping Dang[1], Changchang Teng[1], Dalong Yin[2], and Lifeng Yan[1] ✉

[1]*Department of Chemical Physics, University of Science and Technology of China, Hefei 230026, China;*
[2]*Department of Hepatobiliary Surgery, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230001, China*

✉Correspondence: Lifeng Yan, E-mail: lfyan@ustc.edu.cn

Cite This: *JUSTC*, **2023**, 53(3): 0305 (6pp)

Read Online

Abstract: A new smart supramolecular polypeptide copolymer P(Glu-co-Lys) was synthesized by the polymerization of α-amino acids using the N-thiocarboxylic acid anhydride (NTA) method, using the pH dynamic response peptide of L-glutamic acid and L-lysine as a carrier for tumor cells. The drug delivery system activated by external acid can self-assemble (pH 7.4) and disassemble (pH 5.5) under the adjustment of pH to load the drug and control its release. Doxycycline (DOX) and the photothermal reagent hydrophilic quanternary stereo-cyanine (HQS-Cy) were loaded into the peptide copolymer to obtain HQS-Cy/DOX nanoparticles (NPs) for chemo-photothermal therapy. Gentle photothermal heating can enhance the absorption of drugs by cells and enhance the efficacy of chemotherapy. In addition, chemo-photothermal therapy can solve the defect of easy recurrence after single photothermal therapy. The ingenious nanodrug delivery system of HQS-Cy/DOX NPs provides great potential for the improvement of chemo-photothermal therapy and will achieve excellent therapeutic effects in cancer treatment.

Keywords: organic near infrared dye; nanoparticle; N-thiocarboxylic acid anhydride; chemo; photothermal therapy

CLC number: TQ460.1 **Document code:** A

1 Introduction

Chemotherapy plays a fundamental role in the treatment of metastatic cancer. However, to date, the severe side effects and multidrug resistance caused by high-dose chemotherapeutic drugs have been difficult to overcome[1, 2]. Encouragingly, some studies have shown that hyperthermia caused by near-infrared (NIR) radiation can not only directly kill cancer cells but also significantly inhibit the spread of cancer cells, enhance the sensitivity of cancer cells to chemotherapy drugs, and produce additive or synergistic anticancer effects[3, 4]. Therefore, the combination of chemotherapy and photothermal therapy (PTT) is considered to be a promising strategy. Compared with single chemotherapy, the combination of chemotherapy and photothermal therapy can significantly increase cytotoxicity at lower drug doses.

α-Amino acid N-thiocarboxylic acid anhydride (NTA) is the thio analog of N-carboxyanhydrides (NCAs). It is a more stable monomer, which has reduced sensitivity to moisture and heat and does not require the use of phosgene derivatives in the synthesis. NTA can be exposed to air for synthesis and purification and stored at room temperature for months to years. Although NTA compounds have been discovered since 1950[5], they are usually used for the stepwise synthesis of oligopeptides[6, 7]. Due to their low reactivity, research on their polymerization was very limited before 2014[8]. Only in the past five years has the controlled polymerization of NTA and NNTA initiated by primary amines and rare earth borohydrides in polar and nonpolar solvents been promoted. Because NTA is easy to synthesize and can efficiently produce PAA, researchers are paying increasing attention to it.

Synthetic peptides are one of the most important biodegradable polymers, and they have been widely used in biomedical fields such as biosensors, drug delivery, tissue engineering, and medical diagnosis[9, 10]. Poly-L-glutamic acid and poly-L-lysine are typical polypeptides with many pH-responsive groups on their side chains. It is reported that the PNiPAM (PLG-co-PLLys) copolymer responds to the pH value in aqueous solution. The unique response behavior of PNiPAM (PLG-co-PLLys) to pH is controlled by the competition between lysine and glutamic acid residues for protonation and deprotonation[11]. Lecommandoux and his colleagues reported that diblock copolymers (PGA-b-PLys) form pH-sensitive nanoparticles, and vesicles can be reversibly produced in purified water according to the pH value[12]. Amphiphilic peptides with opposite charges can undergo copolymerization reactions at neutral pH to form supramolecular hydroparticles, which are triggered by intermolecular attraction, hydrogen bonds, and π-π stacking interactions[13, 14]. The interactions will destroy the stability of the hydroparticle, thereby providing a potential pH response for the controlled release of

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305

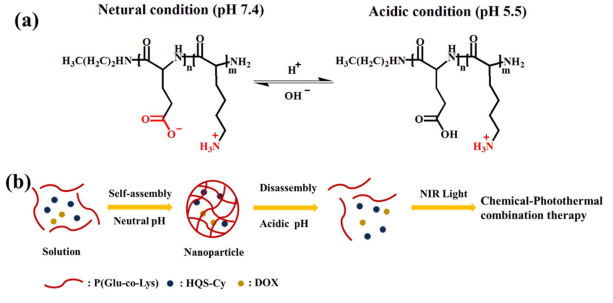
the loaded drug.

Inspired by these studies, here, we designed a drug delivery system that uses the pH dynamic response peptides of L-glutamic acid and L-lysine as carriers that are sensitive to the lyso/endosome acidic environment of tumor cells, which can be adjusted by pH to carry out self-assembly (pH 7.4) and disassembly (pH 5.5) to load the drug and control its release (Scheme 1). L-Lysine and L-glutamic acid provide opposite charges. Under neutral pH (pH 7.4), the responsive peptide is biocompatible due to hydrogen bonds and intermolecular electrostatic interactions between cations and anions. However, under acidic conditions (pH 5.5), the carboxylic acid group of glutamic acid is protonated to change the size of the nanoparticles to control the release. The study of two-component supramolecular networks based on amphiphilic peptides with opposite charges may provide reference ideas and suggestions for the development of required pH-switchable nanoparticles. DOX and the photothermal reagent HQS-Cy were loaded into the polymer to perform chemotherapy and photothermal combined therapy. Gentle photothermal heating can enhance the absorption of drugs by cells to enhance the efficacy of chemotherapy[15, 16], and chemo-photothermal therapy can solve the defect of recurrence after photothermal treatment.

2 Materials and methods

2.1 Synthesis N-(Ethoxycarbonothioyl)-N-methylglycine

17 mL ethanol, 2.19 g NaOH (54.7 mmol), and 42 mL deionized water were added to the two-necked flask under stirring at 0 °C with nitrogen protection. After the NaOH was completely dissolved, 4 mL of carbon disulfide was added to the reaction through a syringe (65.6 mmol), and the mixture was allowed to react under a N_2 atmosphere at 25 °C for 2 h after the addition was completed. 7.6 g of bromoacetic acid was added into 20 mL of deionized water, and after fully dissolving it, it was added to the light yellow reaction solution through the addition funnel. The light yellow reaction solution turned darker and darker orange. The reactants were stirred at 25 °C for 14 h, and finally, an appropriate amount of concentrated hydrochloric acid was slowly added dropwise to the resulting solution. After the reaction solution was acidified to pH=1, an appropriate amount of dichloromethane was added to it and extracted by a separatory funnel several times to obtain an orange liquid. The liquid was washed with 10 mL



Scheme 1. (a) Chemical structure of P(Glu-co-Lys) under neutral and acidic conditions. (b) Conceptual illustration of pH-switchable HQS-Cy/DOX NPs for chemo-photothermal therapy.

brine, and then anhydrous magnesium sulfate was added to remove the residual water in the product. The dichloromethane solvent was removed by a rotary evaporator to obtain a yellow solid crude product, which was passed through chloroform/n-hexane. The system was recrystallized to obtain a white needle-like crystal product.

2.2 Synthesis of Zlys-NTA

2.87 g NaOH (71.8 mmol) was dissolved in cooled 120 mL deionized water, 10.04 g H-Lys(Z)-OH (35.8 mmol) and 6.47 g XAA (35.9 mmol) were added to the above solution in order, and turbidity solution was obtained. The mixture was stirred at room temperature for 3 d. An appropriate amount of 4 mol/L HCl acidified solution was added to pH = 3, and then an appropriate amount of ethyl acetate was added to extract the reaction solution several times through a separatory funnel. The resulting extract was dehydrated with anhydrous sodium sulfate to remove a small amount of water, filtered, and then concentrated by rotary evaporation. Then, 3.7 mL of phosphorus trichloride was added to the liquid under nitrogen protection, the reaction was stirred at room temperature for 20 h, and saturated $NaHCO_3$ solution, deionized water, and saturated NaCl solution were added for washing. The upper organic solution was separated, dried over magnesium sulfate to remove water, and then filtered with a Buchner funnel. The resulting solution was concentrated by a rotary evaporator to obtain a pale yellow oily crude product, which was dissolved by adding THF and then adding excess n-hexane (poor solvent) and stirring to obtain a white solid product (8.26 g, 25.7 mmol, 72% yield).

2.3 Synthesis of BLG-NTA

4.95 g H-Glu(Obz)-OH (20.9 mmol) and 3.76 g XAA (20.9 mmol) were dissolved in a saturated $NaHCO_3$ aqueous solution, and the mixture was stirred at room temperature for 3 d. An appropriate amount of 4 mol/L HCl acidified solution was added to pH = 3, and then an appropriate amount of ethyl acetate was added to extract the reaction solution several times through a separatory funnel. The resulting extract was dehydrated with anhydrous $MgSO_4$ to remove a small amount of water, filtered, and then concentrated by rotary evaporation. Two milliliters of phosphorus trichloride was added to the liquid under nitrogen protection, the reaction was stirred at room temperature for 20 h, and then saturated $NaHCO_3$ solution, deionized water, and saturated NaCl solution were added for washing. The upper organic solution was separated, dried over magnesium sulfate to remove water, and then filtered with a Buchner funnel. The resulting solution was concentrated by a rotary evaporator to obtain a pale yellow oily crude product, which was dissolved by adding THF and then adding excess n-hexane (poor solvent) and stirring to obtain a white solid product (3.24 g, 25.7 mmol, 81% yield).

2.4 Copolymerization of ZLys-NTA and BLG-NTA initiated by n-hexylamine

N-hexylamine was ustilized as the initiator, and the molar ratio of the total NTA to the initiator was 15∶1, and BLG-NTA (1.67 g, 6.0 mmol) was added to the Schlenk bottle, which was vacuum-baked in advance to remove water and oxygen. ZLys-NTA (1.93 g, 6 mmol) and n-hexylamine (81.0 mg, 0.8 mmol) were added in n-hexylamine anhydrous THF

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305

solution (1.0 mL). After the reaction was carried out for 48 h in an oil bath at 60 °C, solid product was observed at the bottom of the bottle. Then, it was dissolve with DMF, and excess ether was added to precipitate it again. After it was centrifuged and dried in a vacuum drying oven at a constant temperature of 60 °C, a white powder product P(BLG-co-Zlys) was obtained.

2.5 Preparation of P(Glu-co-Lys)

A total of 5 mL of trifluoroacetic acid was added to polymer P (BLG-co-Zlys) (350 mg, 1.97 μmol), and HBr (33% acetic acid solution, 500 μL) was added under ice-water bath cooling after it was completely dissolved so that the sample∶fluoroacetic acid∶HBr/hydrobromic acid was 1∶10∶3 (m/v/v). After stirring for 1 h at room temperature, a large amount of ether was added to promote sedimentation, and the precipitate was collected by centrifugation, dissolved with a small amount of DMF, and dialyzed with a dialysis bag with M_w = 1000. The residual acetic acid was removed and then freeze-dried to obtain a white powder product P(Glu-co-Lys).

2.6 Measurement of the pKa of polymer P(Glu-co-Lys)

20 mg of P(Glu-co-Lys) was added into a beaker, and 20 mL of deionized water was then added to it until the polymer is completely dissolved, and then 1 mol/L HCl was added to adjust the solution to pH<3. Next, 40 μL of 0.1 mol/L NaOH solution was added dropwise to the solution. After the dropwise addition was complete, the solution was stirred evenly, and a pH meter was used to measure and record the pH of the solution.

2.7 Preparation of HQS-Cy/DOX NPs

The small-molecule NIR-II fluorophore HQS-Cy was synthesized as we previously reported[17]. 0.5 mL of doxorubicin hydrochloride (1 mg/mL, DMF) was added in the flask, and 130 μL of triethylamine (1 mg/mL, DMF, 1.5 equivalents) was added to neutralize it under stirring for 30 min under dark conditions. Next, 1 mg HQS-Cy, 50 mg polymer, and 1 mL DMF were added and stirred until all of them were dissolved under the shading condition of tin foil. Then, the solution was added dropwise to 4 mL of high-speed stirring deionized water at a constant speed and stirred for 2 h in the dark. After dialysis against water for 9 h (M_w = 3000), the dialyzed water was PBS buffer with pH = 7.4.

2.8 In vitro release test

The dialysis method was used to study the release characteristics of different formulations of drugs under neutral (pH = 7.4) and acidic (pH = 5.5) conditions in PBS buffer. The drug-loaded nanoparticle solution (500 μL) was sealed in a dialysis bag (3000 Da, Spectrum USA) and placed in a culture flask containing 50 mL PBS (containing 2% Tween 80), and the culture flask was placed at 37 °C in a constant temperature shaker at 100 r/min. At the designated time interval (0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72 h), 2 mL of solution was collected for DOX release analysis and supplemented with an equal volume of PBS buffer liquid. All collected media were analyzed with a fluorescence spectrophotometer to calculate the DOX release rate.

2.9 In vitro cytotoxicity study

To measure the cell dark toxicity of drug-loaded nanoparticles, HepG2 liver cancer cells (1×10^4) were inoculated in each well of a 96-well plate. The 96-well plate contained 150 μL of DMEM per well and was cultured at 37 °C and 5% CO_2. After 24 h, equal volumes of test samples of different concentrations were added to replace the incubation for 12 h. The cells were washed with PBS and then treated with 5 mg/mL MTT in 20 μL PBS for 4 h. Finally, the medium was aspirated, 150 μL of DMSO was added to each well to dissolve the formed formazan crystals, and then the absorbance was measured at 570 nm with a microplate reader.

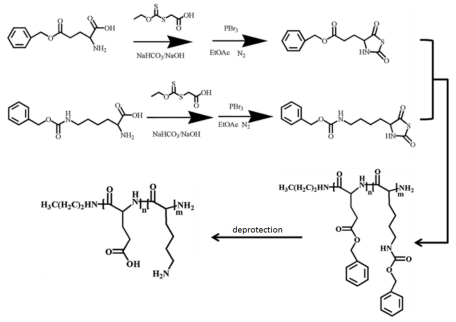
To evaluate the effect of nanoparticles on cytotoxicity under light conditions, the cytotoxicity of the drug-loaded nanoparticles under 915 nm laser light was also carried out by inoculating 1×10^4 HepG2 liver cancer cells per well in a 96-well plate, where each well of the 96-well plate contained 150 μL of DMEM at 37 °C in a 5% CO_2 atmosphere. After nurturing for 24 h, the medium was removed and incubated with test samples of various HQS-Cy concentrations (2.5, 5, 7.5, 10, 15 μg/mL) for 6 h and irradiated with a near-infrared laser (915 nm, 0.75 W/cm^2).

3 Results and discussion

3.1 Synthesis and characterization of HQS-Cy/DOX NPs

The P(Glu-co-Lys) copolymer was synthesized by the NTA method according to the method shown in Scheme 2. The ^{1}H NMR spectrum of P(Glu-co-Lys) is shown in Fig. 1. The characteristic signal of hydrogen on the benzene ring of P(BLG-co-Zlys) at δ 7.26 ppm disappeared in the ^{1}H NMR spectrum of P(Glu-co-Lys) (Fig. 1), indicating that it was completely removed. The molar ratio of glutamic acid and lysine in the random copolymer was calculated based on the signal intensity ratio of 3.69 ppm (-CH-$(CH_2)_2$COOH, b) and 4.25 ppm (-CH-$(CH_2)_3NH_2$, c) derived, and the final ratio was close to 3∶8.

As shown in Fig. 2, we used acid-base titration to determine the pKa value of the P(Glu-co-Lys) polymer to confirm whether it has a pH response, indicating that when the environmental pH value is lower than the pKa, the carboxyl group of the upper glutamic acid part of the polymer begins to protonate, and it may cause deprotonation when the pH is higher than the pKa. The change in the carboxyl group from deprotonation to protonation leads to the self-assembly and depolymerization of the P(Glu-co-Lys) polymer. This change causes



Scheme 2. Synthesis of P(Glu-co-Lys) by the NTA method.

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305

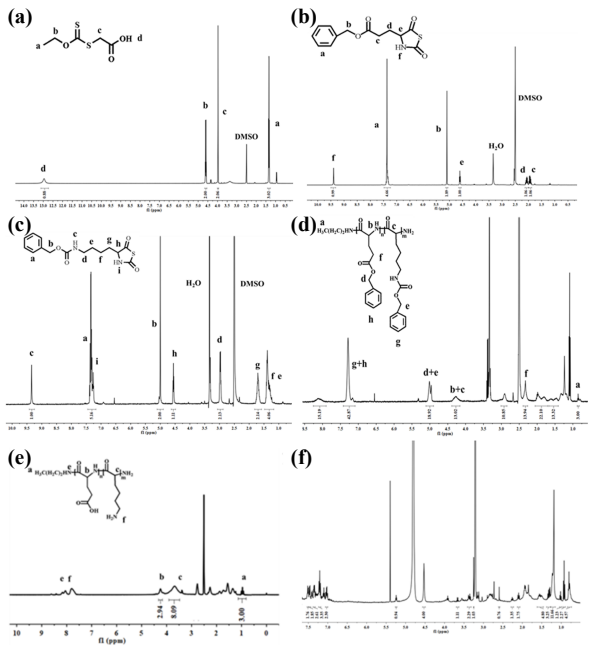


Fig. 1. ^{1}H NMR spectra of (a) XAA, (b) BLG-NTA, (c) ZLys-NTA, (d) P(BLG-co-Zlys), (e) P(Glu-co-Lys), and (f) the NIR dye HQS-Cy.

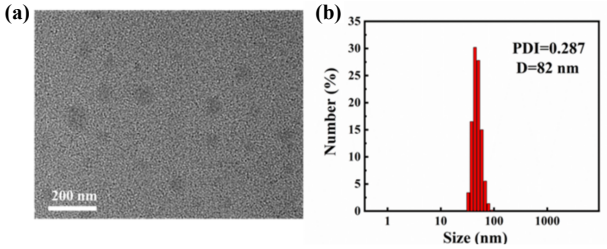


Fig. 2. (a) TEM and (b) DLS methods to determine the nanoparticle size distribution of HQS-Cy/DOX NPs.

the decomposition of the original micelles formed by the copolymer. The titration curve of the P(Glu-co-Lys) copolymer has a plateau buffer period between pH 5.73 and 7.67, showing a certain acid-base buffering effect, and the pKa value is approximately 6.7.

Then, the cyanine fluorescent small molecule HQS-Cy dye and doxorubicin were encapsulated with polypeptide P(Glu-co-Lys) to prepare HQS-Cy/DOX NPs. The resulting HQS-Cy/DOX NPs were fully characterized by TEM (Fig. 3a) and DLS (Fig. 3b). According to TEM and DLS results, the average diameter of HQS-Cy/DOX NPs is approximately 82 nm.

3.2 In vitro drug release test

To prove the pH-responsive release characteristics of HQS-Cy/DOX NPs, DOX in vitro release experiments were performed in PBS buffer at pH 7.4 and 5.5 in a constant temperature shaker at 37 °C.

The DOX release of HQS-Cy/DOX NPs nanoparticles in PBS was performed in vitro. The concentration of doxorubicin released from nanoparticles was measured with time by an external standard method, and the fluorescence absorption intensity was taken as the *Y* axis. The release curve is shown in Fig. 4. The release of DOX occurs in a controlled and continuous manner. A slightly faster drug release was observed in the pH 5.5 PBS buffer solution than in the pH 7.4 PBS buffer solution, and the final total amount of DOX released was also greater. This may be due to the protonation of the carboxyl group of the glutamic acid unit at pH 5.5, which weakens the complexation of DOX with the active groups on the polymer side chain. Nanoparticles are intermolecular through hydrogen bonds and ionic forces. Doxorubicin coated by force is easier to dissociate, the solubility is improved, and the release amount is increased.

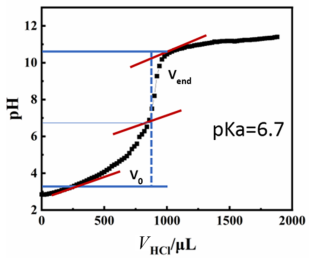


Fig. 3. Determination of pKa of peptide copolymer P(Glu-co-Lys).

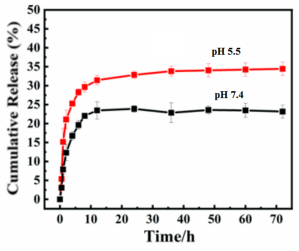


Fig. 4. DOX release from HQS-Cy/DOX NPs at pH 7.4 or 5.5 and 37 °C (triplicate).

3.3 In vitro cytotoxicity test

The MTT method was used to further evaluate the in vitro cytotoxicity and biocompatibility of HQS-Cy@P(Glu-co-Lys), DOX, and HQS-Cy/DOX NPs at the level of human liver cancer HepG2 cells. Clearly, HQS-Cy@P(Glu-co-Lys) has excellent cell viability under dark conditions when the concentration is below 15 μg/mL, indicating good biocompatibility of the nanoparticles. However, DOX showed toxicity with increasing concentration (Fig. 5). For the NPs of HQS-Cy/DOX, the temperature of the cell environment was controlled at approximately 30 °C ± 1 °C during the experiment. When the concentration of HQS-Cy/DOX nanoparticles reached 10 μg/mL, using a 915 nm laser (0.75 W/cm^2) to irradiate for 10 min, the lethality of HepG2 cancer cells reached more than 80% (Fig. 6). When the administered dose reaches

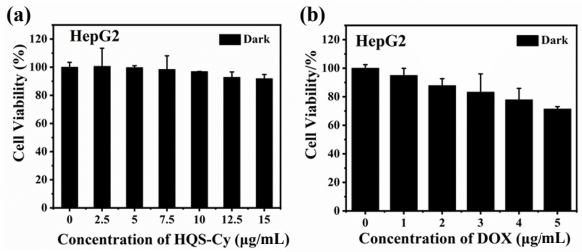


Fig. 5. Cytotoxicity assays of (a) HQS-Cy@P(Glu-co-Lys) and (b) DOX@P(Glu-co-Lys) against HepG2 cells under dark conditions.

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305

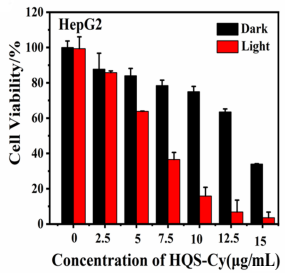


Fig. 6. MTT method to test the therapeutic effect of HQS-Cy/DOX NPs on HepG2 cells.

15 μg/mL, the temperature of the medium in the illuminated group will cause significant overheating, which will lead to rapid tumor cell death, revealing an efficient combined chemo-photothermal therapy. At the same time, under dark conditions, with only the chemotherapeutic effect of DOX, the nanoparticles showed gradient cytotoxicity. When the concentration reached 15 μg/mL, the cell death rate increased to more than 60%, indicating that HQS-Cy/DOX NPs have certain chemotherapeutic effects.

To further directly observe the therapeutic effect of HQS-Cy/DOX NPs, we also performed live/dead cell staining assays for FDA and PI by fluorescence microscopy. After irradiation with a 915 nm laser (0.75 W/cm^2) for 10 min, strong red fluorescence emission (representing dead cells stained with PI) was observed in the 6-well plate containing HepG2 cancer cells, and the morphology of the tumor cells became elliptical (Fig. 7). These results indicate that HQS-Cy/DOX NPs exhibit excellent in vitro cytotoxicity and have certain tumor treatment effects.

4 Conclusions

We have developed a simple strategy to prepare an HQS-Cy/DOX NP drug delivery system that is pH responsive. When the environmental pH value changes, it causes the protonation of the side chains of lysine and glutamic acid on the polymer, and the change of the carboxyl group from deprotonation to protonation leads to the depolymerization and depolymerization of the P(Glu-co-Lys) polymer, resulting in the pH-responsive release of the encapsulated DOX and hydrophobic NIR-II dye (HQS-Cy). In vitro experiments revealed that the nanoparticle system showed combined chemo-photothermal therapy. It provides a potential nanomaterial for NIR-II fluorescence imaging-guided combined chemo-photothermal therapy for tumors.

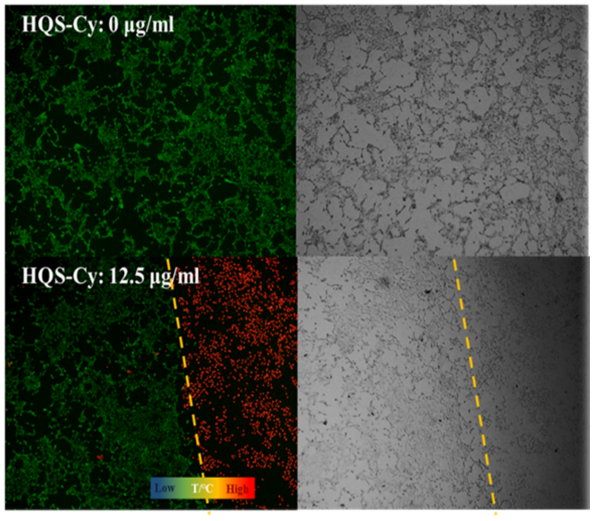


Fig. 7. Cell death staining method to test the therapeutic effect of HQS-Cy/DOX NPs on HepG2 cells.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Universities (YD2060002015), the National Natural Science Foundation of China (51873201, 82172071), and the Key Research and Development Program of Anhui Province (202104b11020025).

Conflict of interest

The authors declare that they have no conflict of interest.

Biographies

Hongyun Qian obtained her master's degree at the School of Chemistry and Materials Science, University of Science and Technology of China, under the supervision of Prof. Lifeng Yan. Her research mainly focuses on biomaterials.

Lifeng Yan received his Ph.D. degree in Chemistry from the University of Science and Technology of China. He is currently a Professor at the University of Science and Technology of China. His research interests include nanomedicine, polymeric science, and green chemistry.

References

[1] Fan L, Jin B Q, Zhang S L, et al. Stimuli-free programmable drug release for combination chemo-therapy. *Nanoscale,* **2016**, *8*: 12553–12559.
[2] Suo A L, Qian J M, Zhang Y P, et al. Comb-like amphiphilic polypeptide-based copolymer nanomicelles for co-delivery of doxorubicin and P-gp siRNA into MCF-7 cells. *Mat. Sci. Eng. C-Mater.,* **2016**, *62*: 564–573.
[3] Xu W J, Qian J M, Hou G H, et al. Hyaluronic Acid-functionalized gold nanorods with pH/NIR dual responsive drug release for synergetic targeted photothermal chemotherapy of breast cancer. *ACS Appl. Mater. Inter.,* **2017**, *9*: 36533–36547.
[4] Wang Z Z, Chen Z W, Liu Z, et al. A multi-stimuli responsive gold nanocage-hyaluronic platform for targeted photothermal and chemotherapy. *Biomaterials,* **2014**, *35*: 9678–9688.
[5] Aubert P, Knott E B. Synthesis of thiazolid-2:5-dione. *Nature,* **1950**, *166*: 1039–1040.
[6] Higashimura T, Kato H, Suzuoki K, et al. Condensation polymerization of N-dithiocarbonyl alkoxycarbonyl-amino acids. Part I. Synthesis and condensation polymerization of N-dithiocarbonyl ethoxycarbonyl-amino acids. *Makromolekul Chem.,* **1966**, *90*: 243–248.
[7] Dewey R S, Schoenewaldt E F, Joshua H, et al. Synthesis of peptides in aqueous medium. V. Preparation and use of 2, 5-thiazolidinediones (NTA' s). Use of 13C-H nuclear magnetic resonance signal as internal standard for quantitative studies. *J. Am. Chem. Soc.,* **1968**, *90*: 3254–3255.
[8] Kricheldorf H R, Bösinger K. Mechanismus der NCA-polymerisation, 3. Über die amin-katalysierte polymerisation von sarkosin-NCA und-NTA. *Makromol. Chem.,* **1976**, *177*: 1243–1258.

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305

[9] Deming T J. Synthetic polypeptides for biomedical applications. *Prog. Polym. Sci.,* **2007**, *32*: 858–875.

[10] Choe U J, Sun V Z, Tan J K Y, et al. Self-assembled polypeptide and polypeptide hybrid vesicles: From synthesis to application. In: Deming T, editor. Peptide-Based Materials. Berlin, Heidelberg: Springer, **2011**: 117–134.

[11] Li J G, Wang T, Wu D L, et al. Stimuli-responsive zwitterionic block copolypeptides: Poly(N-isopropylacrylamide)-block-poly(lysine-co-glutamic acid). *Biomacromolecules,* **2008**, *9*: 2670–2676.

[12] Rodríguez-Hernández J, Lecommandoux S. Reversible inside-out micellization of pH-responsive and water-soluble vesicles based on polypeptide diblock copolymers. *J. Am. Chem. Soc.,* **2005**, *127*: 2026–2027.

[13] Frisch H, Unsleber J P, Lüdeker D, et al. pH-switchable ampholytic supramolecular copolymers. *Angew. Chem. Int. Ed.,* **2013**, *52*: 10097–10101.

[14] Behanna H A, Donners J J J M, Gordon A C, et al. Coassembly of amphiphiles with opposite peptide polarities into nanofibers. *J. Am. Chem. Soc.,* **2005**, *127*: 1193–1200.

[15] Wang C, Xu H, Liang C, et al. Iron oxide @ polypyrrole nanoparticles as a multifunctional drug carrier for remotely controlled cancer therapy with synergistic antitumor effect. *ACS Nano,* **2013**, *7*: 6782–6795.

[16] Liu T, Wang C, Cui W, et al. Combined photothermal and photodynamic therapy delivered by PEGylated MoS_2 nanosheets. *Nanoscale,* **2014**, *6*: 11219–11225.

[17] Qian H Y, Cheng Q, Tian Y L, et al. An anti-aggregation NIR-II heptamethine-cyanine dye with a stereo-specific cyanine for imaging-guided photothermal therapy. *J. Mater. Chem. B,* **2021**, *9*: 2688–2696.

DOI: 10.52396/JUSTC-2022-0154
JUSTC, **2023**, 53(3): 0305