Research Article

A pH-Responsive Multifunctional Nanocarrier in the Application of Chemo-Photodynamic Therapy

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In cancer therapy, combined utilization of anticancer drug and photosensitizer attracts increasing interest due to enhanced curative effects and reduced side effects. Since the drug delivery system is an effective method to enhance curative effects, drug carriers for codelivery of the two abovementioned molecules are essentially important for chemophotodynamic therapy. Based on the foundation, a nanocarrier with pH-responsive and targeted properties was designed, prepared, and researched in the work. A pH-sensitive nanoparticle was fabricated by acetylated β-cyclodextrin (Ac-β-CD) using oil-in-water (O/W) emulsion technique. During the fabrication processing, a functional emulgator (gelation-folic acid ester (G-FA)) with a biorecognition domain was absorbed onto the surface of the nanoparticle, which endowed a nanoparticle-targeted property. The nanoparticle exhibited a coarse surface, pH-responsive property, and similar fluorescence characteristic as G-FA. The cell endocytosis profile revealed that equilibrium endocytosis could be reached after being cocultured with 1.0 mg/mL nanoparticle for 8 h. Furthermore, camptothecin (CPT) as an anticancer drug and phthalocyanine (PcZn) as a photosensitizer were encapsulated into the nanoparticle during the fabrication processing. The nanoparticle enhanced the fluorescence effects of PcZn on water solution, and CPT encapsulation proportion was slightly influenced by initial CPT concentration. The pH value influenced the PcZn fluorescence behavior and CPT release behavior of the nanoparticle. In vitro cytoviability evaluation confirmed the therapeutic effect of the nanocarrier on HEP2 cells. Finally, the results of preliminary in vivo evaluation revealed that the reported nanocarrier in the research could inhibit cancer development with little effects on the body weight of mice.

1. Introduction

Photodynamic therapy (PDT) combined with chemotherapy for cancer treatment has been intensively concerned on account of their enhanced curative effect and lesser side effects [1–5]. In PDT, a photosensitizer, which is an essential chemical, can obtain enough energy from laser and convert it to heat that induces cancer cell apoptosis and death [6]. However, most photosensitizers had slight solubility and tend to agglomerate into dimer in water, which leads to loss of photosensitive characteristic and thus hinder the curative effect of PDT. In the other aspect, some anticancer drug in chemical therapy has also some disadvantage of severe side effect due to large toxicity and reduced curative effect due to limited water solubility [6–11]. Therefore, an effective and efficient carrier for codelivery of anticancer drug and photosensitizer is a premise to realize the combined therapy [9, 10]. Recently, Zhang et al. developed a nanoscale drug delivery system (nDDS) for codelivery of cisplatin prodrug and chlorin e6 (Ce6), which exhibited potent anticancer activity against A549R cells [12]. Additionally, the Ce6- and doxorubicin- (Dox-) loaded pegylated graphene oxide (pGO) nanophysisorplexes (Ce6/Dox/pGO) were reported to have synergistic curative effects [13]. Based on these
foundations, a nanoparticle drug delivery system for code- 
delivery of anticancer drug (camptothecin (CPT)) and 
photosensitizer (phthalocyanine (PcZn)) was designed, 
fabricated, and evaluated in the research, which is aimed 
at providing an effective and efficient nanocarrier for 
chemo-photodynamic therapy.

On account of a lower pH value environment in tumor 
tissue, low pH-triggered carriers can respond to acid envi-
ronment and control drug release at the tumor site, which 
have attracted intense attention in the field of drug 
delivery [8, 14–18]. Furthermore, biocompatibility and 
biodegradation are essential prerequisites for materials in 
their biomedical application. Although a lot of synthesized 
 pH-sensitive polymers have been used to prepare 
PH-sensitive carriers, the biodegradation of these polymers is 
not easy to realize and the biocompatibility of these 
polymers including their degradation products is also a 
problem. In consideration of biocompatibility and biodegra-
dation, natural materials like polysaccharides and proteins are 
opimal choices to fabricate all kinds of carriers on 
account of natural biodegradation and excellent biocompati-
bility [19–26]. Therefore, pH-sensitive natural polysaccha-
rides have been designed and synthesized through 
reversible acetylation, which also endow material-
hydrophobic groups [27–33]. The hydrophilic-lipophilic 
characteristic of polysaccharides is simultaneously altered 
by acetylation, which benefits the O/W emulsion method 
for nanoparticle fabrication. The pH response property of 
acetylated materials is attributed to the transition of undissol-
vable hydrophobic acetal groups to dissolvable hydrophilic 
dehydroxy groups in pH 5.5 aqueous solution [27–33]. In 
our previous work, pH-sensitive β-cyclodextrin (β-CD) was 
synthesized and investigated concerning the effects of 
reaction condition on pH responsiveness and stability 
[33]. In a further step, a biocompatible and pH-responsive 
β-CD nanoparticle was obtained and studied in view of 
synthesis parameters, pH response property, and biocom-
patibility [33].

Besides fundamental biocompatibility and special 
response properties, accurate orientation to targeted cells or 
tissue for nanocarriers is a proven measure for effec-
tive and efficient drug delivery [34–37]. Therefore, an additional 
targeting design for nanoparticle is a feasible choice to opti-
mize nanocarriers for drug and photosensitizer co-delivery.

Herein, a targeted pH-responsive nanoparticle was 
designed and prepared for codelivery of anticancer drug 
and photosensitizer in the work. The active targeting based 
on biological recognition and passive targeting based on 
magnetic interaction are frequently used methods to 
design targeting properties for the nanoparticle [34–38].

It has been reported that folic acid and folic acid receptors 
are a pair of ubiquitous biological recognition in both 
human tissue and tumor [39–41]. Nevertheless, either the 
number or activity of folic acid receptors in tumor is 
higher than that in normal tissue. Moreover, folic acid 
itself has good structural stability, low immunogenicity, 
and good biocompatibility, which becomes an ideal target-
ing molecule [39–41]. Therefore, folic acid was modified 
on the surface of a nanoparticle during the fabrication 
process in the research. At the same time, camptothecin 
(CPT) as a model anticancer drug and zinc phthalocya-
nine (PcZn) as a model photosensitizer were encapsulated 
into the nanoparticle during the fabrication process. 
Finally, in vitro and preliminary in vivo investigations 
were conducted to evaluate the nanocarrier.

2. Experimental Section

2.1. Materials. Gelatin, folic acid (FA), β-cyclodextrin 
(β-CD), 2-methoxypropene, pyridinium 4-toluensulfonate, 
zinc phthalocyanine (PcZn), anhydrous dimethyl sulfoxide 
(DMSO), and dichloromethane (DCM) were purchased from 
Shanghai Medicine and Chemical Company, China. N,N-Di-
cyclohexylcarbodiimide (DCC) and N-hydroxsuccinimide 
(NHS) were obtained from Shanghai Energy Chemical Com-
pany Limited. Camptothecin (CPT), dialysis tube (Mw = 
8000–14000), 3-(4,5-dimethyl) thiazol-2-yl-2,5-dimethyl tet-
razolium bromide (MTT), trypsin, rhodamine B isothioce-
ninate (RBITC), 4',6-diamidino-2-phenylindole (DAPI), and 
Dulbecco’s minimum essential medium (DMEM) were 
obtained from Sigma. Antifade mounting medium and fetal 
bovine serum (FBS) were purchased from Beyotime Biotec.
Co., China. All other reagents and solvents were of analytical 
grade and used as received.

2.2. Synthesis and Characterization of G-FA. 0.441 g FA was 
dissolved in anhydrous DMSO, into which 0.404 g DCC 
and 0.229 g NHS were sequentially added. After the reaction 
had lasted for 12 h at 30°C, the resultant solution was 
filtered to wipe out sediment and further precipitated by mixed 
solution (acetone: ethyl ether = 3 : 7). After the obtained 
sediment was washed by ethyl ether for several times and 
then vacuum dried, FA active ester was obtained.

In the next step, 1 g gelatin was dissolved in 100 mL PBS 
to obtain 1% gelatin solution, into which 8 mL FA-active 
osti DMSO solution was added drop by drop in 1 h. After 
the reaction had lasted for 12 h at 40°C, the resultant solution 
was sealed in a dialysis bag with a cutoff molecular weight of 
10 kDa and dialyzed in a large amount of triple-distilled 
water for 3 d. Finally, gelation-folic acid ester (G-FA) was 
obtained by freeze drying and characterized by nuclear mag-
netic resonance hydrogen spectrum (1H NMR, BRUKER 
AV500). The structure of G-FA was shown in Figure 1(b). 
The degree of substitution (DS) for G-FA was calculated by 
the same measuring parameter.

2.3. Fabrication and Characterization of the FA Functional 
 pH-Responsive Nanoparticle. In order to prepare the nano-
particle, acetylated β-cyclodextrin (Ac-β-CD) was first syn-
thesized using the previous method [33]. Briefly, 1 mol 
2-methoxypropene was dropped into 500 mL 50 mM 
β-CD/anhydrous DMSO solution containing 5 mM pyridi-
nium p-toluene sulfonate in an anhydrous anaerobic envi-
ronment. After the reaction had lasted for 1 h at 30°C,
Ac-β-CD was precipitated from basic water, collected by filtration, and last lyophilized to white powder. The structure of Ac-β-CD was shown in Figure 1(a).

FA functional pH-responsive nanoparticles were prepared by a similar microemulsion method as mentioned in a previous research [33]. But differently, G-FA was used as a functional emulsifier. Briefly, 10% Ac-β-CD/DCM solution was emulsified via probe sonication (Scienz, JY92-II) into 3% G-FA aqueous solution. The obtained emulsion was immediately added into 1% concentration G-FA aqueous solution to evaporate DCM under magnetic stirring. After 10 h, nanoparticles were collected by centrifugation (14000 rpm, 10 min), washed several times with basic water, and lyophilized. The lyophilized nanoparticles were used for further characterization. G-FA solution after separation could be dialyzed for purification and lyophilized for recycling. The final nanoparticles were characterized by Fourier-transformed infrared spectroscopy (FTIR) (IS10), scanning electron microscope (SEM) (S8100), and spectrofluorometer (FS5). In characterization, nanoparticles were dispersed in alkaline water to form nanoparticle solution, which was further dropped onto an aluminum film and dried by an infrared lamp. Then the sample was coated with Pt, which was further observed under SEM. The fluorescence intensity of acid nanoparticle dissolved solution was determined to quantify the G-FA content on the nanoparticle surface, which was calculated by the above-mentioned calibration curve in the last section.

2.4. Cell Endocytosis of the Nanoparticle. The nanoparticle was marked with rhodamine isocyanate. Briefly, nanoparticles were dispersed in 0.1 mg/mL rhodamine isocyanate alkaline solution (pH 7.5). After the reaction had lasted for 24 h, nanoparticles were washed several times, last lyophilized, and sterilized before use.

The cell endocytosis profile was evaluated by coculture of the nanoparticle and adherent cells. Briefly, HEP2 cells were incubated in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. The used cells were detached by 0.25% trypsin, washed by PBS, and resuspended by culture medium with a cell density of 50-100 cells/μL. Then the resuspended cells were seeded on one 12-well culture plate and several 3.5 cm² culture dishes with embedded glass slides. After cells were fused by about 40-60% on each well (approximately 24 h), original culture medium was discarded and fresh culture medium containing the nanoparticle as a function of nanoparticle concentration was supplemented. For different intervals, cells on each well of the culture plate were detached and detected by flow cytometry (FCM, BD, C6) using cells without the nanoparticle as a gate. At the same time, cells on dishes were fixed by 2.5% glutaraldehyde, stained by 0.1 μg/mL DAPI for 20 min, washed by PBS three times, sealed by anti-fade mounting medium, and finally observed in a fluorescence microscope (IX73). Theoretically, DAPI was a fluorescent dye, which could penetrate cell membrane and react with double-chain DNA. After reaction, DAPI-DNA could produce blue fluorescence 20 times higher than DAPI itself.

2.5. In Vitro Evaluation for the Drug-Encapsulated Nanoparticle. PcZn (Figure 1(c)) and CPT (Figure 1(d)) were chosen as model drugs to be encapsulated into the above-mentioned nanoparticle during the process of nanoparticle fabrication. Briefly, 100 μL CPT/DMSO solution of different concentrations was mixed with 10% Ac-β-CD/DCM solution containing saturated PcZn, which was further emulsified via probe sonication into G-FA aqueous solution, just as mentioned above. Final drug-encapsulated nanoparticles were obtained using the same abovementioned method.

PcZn/DCM, PcZn/water solution, and nanoparticle suspension were characterized by spectrofluorometer (FS5). The fluorescence intensity of nanoparticle suspension in different media as a function of time was recorded by spectrofluorometer (FS5). Quantification of CPT was accomplished by ultraviolet spectrophotometric method using UV spectroscopy (Cary 50). In order to qualify the encapsulated CPT amount, nanoparticle suspension was dialyzed in 15 mL 1 mM HCL solution to dissolve all nanoparticles. The encapsulated CPT amount was obtained by absorbance of HCL dialysis solution and calculation according to the standard curve. For CPT release assay, nanoparticle suspension was dialyzed in 15 mL water solution with different pH values. At appropriate intervals, 3 mL released dialysis solution was withdrawn and the absorbance at 360 nm was

![Figure 1: The chemical or schematic structure of (a) Ac-β-CD, (b) G-FA, (c) PcZn, and (d) CPT.](image-url)
recorded to calculate the cumulate CPT release. Simultaneously, 3 mL fresh solution was supplemented into dialysis solution.

Besides the in vitro drug release profile, effects of drug-encapsulated nanoparticles on viability of HEP2 cells were evaluated by MTT assay. Briefly, nanoparticle DMEM solution with different nanoparticle concentrations was added into a 96-well culture plate with 80-90% cell confluence. At different intervals, after being supplemented with 20 μL MTT, the cells were continually cultured for another 4 h. 200 μL of DMSO was added to dissolve the formed formazan pigment. The absorbance of 150 μL above solution at 570 nm was recorded by a microplate reader (Tecan M200 PRO).

2.6. In Vivo Evaluation. All the animal experimental protocols were performed in compliance with the Animal Management Rules of the Ministry of Health of the People’s Republic of China (no. 55, 2001) and the guidelines for the Care and Use of Laboratory Animals of the Nanjing University of Chinese Medicine (Nanjing, China). The protocol was approved by the Jiangsu Experimental Animal Committee. The protocol used was also approved by the Ethics Committee of Nanjing University. HEP2 tumor-bearing nude mice were used to give a preliminary assessment of the drug-encapsulated nanoparticles. The mice were divided into a nanoparticle-treated group (40 mg/kg) and treated with normal saline (the control group, n = 5 per group). The sample was intravenously injected via tail vein every day. Tumor size and animal body weight were measured every day during the study, and the tumor volume was calculated by the formula width2 × length/2. After 12 days, animals were sacrificed to the experiment under the institutional guideline and the tumor tissue was fixed by glutaraldehyde, embedded in paraffin, and cut into 5 μm slices. The slice was dewaxed with xylene, washed with various levels of ethanol and water, and further stained by hematoxylin-eosin (H-E). Finally, the slide was dehydrated, sealed, and characterized by a microscope (IX73).

2.7. Statistical Analysis. Data were analyzed using the t-test for differences. Results are reported as means ± standard deviation. The significant level was set at p < 0.05.
3. Result and Discussion

3.1. Synthesis and Characterization of G-FA. In order to obtain the FA functional nanoparticle, FA was first grafted onto gelatin, an emulgator by amidation reaction. $^1$H NMR spectra provided detailed structural information of gelatin before and after modification, which was shown in Figure 2(a). The peaks are assigned as follows: a peak of 0.86 ppm belonging to the methyl residues of leucine, valine, and isoleucine; peaks of 1.16, 1.36, 1.61, and 1.72 ppm belonging to the methyl residues of threonine, alanine, lysine, and arginine, respectively; peaks of 2.64, 2.93, 3.14, and 3.57 ppm belonging to methylene residues of aspartic acid, lysine, arginine, and proline, respectively; peaks of 7.04-7.20 ppm belonging to the phenyl group of phenylalanine (b position); and peaks of 6.86 and 7.67 ppm belonging to the phenyl group of FA (a and c position). The emergence of groups belonging to FA confirmed the successful grafting of FA onto the gelatin chain. In addition, the grafting ratio of FA was calculated by integration of peaks a and b, considering that one gelatin molecule has 60 phenylalanine groups and 32 lysine groups containing NH$_2$, which was 94%.

The fluorescence spectra of FA and G-FA water solution were detected to clarify the fluorescence property for G-FA in Figure 2(b). Excited by 336 nm fluorescence, FA solution showed an emission peak with small intensity at 455 nm but equal G-FA solution showed an emission peak with high intensity nearly ten times as high as that of FA, which indicated that gelatin enhanced the fluorescence property of FA. The enhancement of protein on the fluorescence property for FA had been confirmed by previous reports, which was consistent with our results [42]. Furthermore, fluorescence intensity of G-FA at 455 nm as a function of its concentration was recorded in Figure 2(c). It was found that when G-FA concentration is at 0.2-5.0 μg/mL, fluorescence intensity of G-FA had a good linear relationship with G-FA concentration, which could be used to quantify the G-FA content in the following part.

Figure 3: (a) FTIR spectra of Ac-β-CD and G-FA functional nanoparticles. (b) SEM images of the G-FA functional nanoparticle. (c) Fluorescence spectrum of the G-FA functional nanoparticle.
3.2. FA Functional pH-Responsive Nanoparticle. Since detailed information of Ac-β-CD had been researched and discussed in our previous research, the synthesis and characteristic of Ac-β-CD were not discussed here. It was found in our previous research that the emulgator was inevitably absorbed onto the surface of the nanoparticle. Therefore, a FA functional emulgator, namely, G-FA, was expected to obtain the FA functional nanoparticle surface in nanoparticle fabrication. In order to clarify the surface characteristic, the obtained nanoparticle and its precursor of Ac-β-CD were characterized by FTIR spectra (Figure 3(a)). Compared with the spectrum of Ac-β-CD, appearance of peaks at 2834 cm$^{-1}$ and 1280 cm$^{-1}$ belonging to carboxylic acid or amino groups of gelatin or FA and obvious enhancement of peak at 1655 cm$^{-1}$ belonging to amide characteristic groups of G-FA confirmed the existence of G-FA on the surface of the nanoparticle, which is consistent with our previous report [33].

The formed G-FA functional pH-responsive nanoparticle showed a coarse surface with a diameter of about 100-300 nm from SEM images (Figure 2(b)), which was similar to a previously prepared Ac-β-CD nanoparticle [33]. Although some aggregation was witnessed in a dried state, homogeneous nanoparticle solution with a defined effective diameter of about 250 nm (PDI: 0.149) was confirmed by DLS results.

Furthermore, a fluorescence spectrum of nanoparticle solution was detected in Figure 2(c). Excited by 336 nm fluorescence, nanoparticle solution showed an emission peak with small intensity at 455 nm, which confirmed the existence of G-FA on the surface of the nanoparticle. The G-FA content on the nanoparticle surface was quantified by fluorescence intensity of acid-dissolved nanoparticle solution and calculated to be $1.04 \pm 0.29$ mg/g by referring Figure 2(c).

Additionally, it was found from transparency of nanoparticle solution that the nanoparticle could stably exist in PBS (pH 7.2-7.4) for 10 days, gradually degrade in pure water (pH 6.0-6.5) within 2 days, and rapidly dissolve in mild acid solution (pH 5.0-5.5) within 2 hours. The result confirmed the pH-responsive properties of the nanoparticle and was consistent with the previous research [33].

3.3. Cell Endocytosis of the Nanoparticle. In order to track cell endocytosis of the nanoparticle, nanoparticles were labeled with a fluorescence dye (RBITC) and further cocultured with adherent HEP2 cells. It was found from the FCM result in Figure 4 that the number fraction of fluorescent cells increased rapidly with cocultured time until 8 h (Figure 4(a)) and increased rapidly with nanoparticle concentration until it reached 1.0 mg/mL (Figure 4(b)). Intelligibly, cells would have fluorescence if they endocytosed fluorescent nanoparticles, that is, fluorescent cells were considered to the cells that endocytosed the nanoparticle. Therefore, FCM results confirmed the successful cell endocytosis for nanoparticles and the endocytosis reached equilibrium for the 1.0 mg/mL nanoparticle and after 8 h. Furthermore, cell endocytosis behavior was observed by a fluorescence microscope, in which cell nucleus was stained to blue color and the nanoparticle showed red color (Figure 5). In one aspect, after being cocultured for 24 h, without the nanoparticle, only blue nucleus could be observed in the fluorescence image (Figure 5(a)); with the nanoparticle, the red nanoparticle could be observed to distribute around the nucleus or inside the nucleus (Figures 5(b)–5(e)). But no obvious linear relationship between the red nanoparticle amount and cocultured nanoparticle concentration was found in these fluorescent images. In other aspects, with 0.5 mg/mL nanoparticles, a few nanoparticles were scattered inside the cells (either
inside the nucleus or inside the cytomembrane) after 2 h; further, the nanoparticle number seemed to increase a lot inside the cells and nearly all nucleuses were surrounded by many nanoparticles after 6 h. Surprisingly, the nanoparticle amount inside the cells decreased after 12 h and 24 h, which might be ascribed to dissolution of the nanoparticle inside the cells or exocytosis by cell. In view of no obvious exocytosis phenomenon from FCM results, degradation of the nanoparticle might be a main reason for the nanoparticle reduction after 12 h.

In brief, these results of the cell endocytosis profile indicated that the nanocarrier could realize the intracellular delivery of drugs.

3.4. In Vitro Evaluation. On account of slight solubility, PcZn in water solution was difficult to be quantified by spectroscopy. Thus, the fluorescence characteristic of PcZn solution was qualitatively tracked in Figure 6. Excited by 355 nm fluorescence, PcZn-saturated DCM solution exhibited a maximal emission peak with a fluorescence intensity of $7.5 \times 10^7$ at
However, the fluorescence intensity at 670 nm for PcZn-saturated water solution decreased to 3.0 \times 10^3 on account of insolubility and aggregation in water, which would hinder the absorbance of light energy and then reduce curative effects (Figure 6(a)). Interestingly, nanoparticle suspension showed the same emission peak with a fluorescence intensity of 1.5 \times 10^7, which indicated that the nanoparticle could enhance fluorescence effects of PcZn due to better dispersibility in water. In order to track the stability of the PcZn encapsulation nanoparticle in water, fluorescence intensity of nanoparticle water suspension in different media as a function of time was recorded in Figure 6(b). In a neutral environment (PBS, pH 7.4), fluorescence intensity of nanoparticle suspension gradually decreased along time within 2 weeks; in mild acid solution (pH 5.0), fluorescence intensity rapidly reduced to very low in a day; in water, the nanoparticle lost majority of their fluorescence in 4 days. The gradual decline of fluorescence intensity is assumed to be attributed to gradual diffusion of PcZn into solution since the nanoparticle had been verified to be stable in PBS and PcZn had very low fluorescence intensity; quick loss of the fluorescence property for the His et al. (2018) CPT encapsulation efficiency in the nanoparticle with different initial CPT concentrations. (b) In vitro cumulate CPT release behaviors in different pH value media at 37°C.
nanoparticle in a mild acid environment is ascribed to the degradation of the nanoparticle and aggregation of PcZn in solution.

Differently, quantification of CPT was accomplished by spectroscopy, which was shown in Figure 7. It was found that the CPT encapsulation rate decreased slightly along with initial CPT concentration increasing (Figure 7(a)). Since CPT did not dissolve in DCM, a higher CPT/Ac-β-CD mixture and stability of nanoparticle-prepared emulsion, which resulted in a lower encapsulated CPT rate. In the drug release profile (Figure 7(b)), 80% encapsulated CPT was gradually released to PBS (pH 7.4) within 24 h, 90% encapsulated CPT was gradually released to water (pH 6.5) within 24 h, and nearly 100% encapsulated CPT was burst released to acid medium (pH 5.0) within the first hour. In view of the degradation rate of the nanoparticle in different media, the release of CPT in PBS was mainly driven by molecular diffusion for CPT, while burst CPT was attributed to quick degradation of the nanoparticle in an acid environment.

To sum up, relative temporary stability of either drug or photosensitizer in a normal physiological environment (pH 7.4) and burst release in a mild acid environment (pH 5.0) ensured effective delivery of the drug and photosensitizer to targeted tissue and did limited harm to normal tissue.

Moreover, since CPT and ZnPc have been proven to have reduced curative effects due to slight solubility and G-FA had no cytotoxicity according to common sense and our experiment, cytoviability of only the nanocarrier had been examined. In vitro effects of the nanocarrier on HEP2 cells were investigated in Figure 8. After coculture for 6 h, cytoviability decreased with the increase of nanoparticle concentration until nanoparticle concentration reached 2 mg/mL. Many dead cells confirmed the effectiveness and efficiency of nanocarriers on HEP2 cells.

3.5. Preliminary In Vivo Evaluation. The curative effect of the nanocarrier was preliminary evaluated by tumor’s volume and body weight of nude mice in Figure 9. In the research, only a common cancer model (bearing cancer) under the skin with a short-term test was used to give a preliminary evaluation for multifunctional nanocarriers. The tumor’s volume kept its original size along with time within 12 days for the treatment group, while the tumor’s volume kept increasing along with time within 12 days for the control group (Figure 9(a)). Especially after 6 days, the tumor’s volume of the treatment group was significant smaller than that of the control group, which indicated that the nanocarrier could inhibit the growth of tumor. Although the error bar of the tumor volume was big due to mobility and uncontrollability of bearing tumor for the control group, the error bar of the tumor volume was much smaller for the treatment group. Obvious tumor development was found in the control group along with time, while no obvious tumor development was found in the treatment group along with time. It was inferred that imbalance of tumor development coming from biological individual differences was also the reason for the big error bar. In other aspects, the mouse’s weight kept constant along with time either for the treatment group or for the control group (Figure 9(b)) and no obvious difference for the mouse’s weight between the treatment group and the control group was detected, which inferred that the nanocarrier had little influence on conventional life activities of mice. After 12 days, tumor tissue was sectioned and stained by H&E in Figures 9(c) and 9(d). For the treatment group, some tissue frame without obvious cell nucleus was observed in the image (Figure 9(c)), which might be attributed to the death of cancer cells. Besides this, some relative smaller blue nucleus in the image might be a sign of cellular apoptosis. As a contrast, many normal cancer cells were observed in the image for the control group, except some blank region (Figure 9(d)). In a word, the results of preliminary in vivo evaluation showed that the nanocarrier could inhibit cancer development.

To sum up, the characteristic of intracellular delivery from in vitro cell endocytosis results indicated that the nanocarrier would induce the delivered drug to targeted tissues even cells. Simultaneously the nanocarrier could encapsulate both drug and photosensitizer to some extent and adjust their release according to the component-structure characteristic of the nanocarrier. These characteristics ensured more effective and efficient targeted molecule delivery. Preliminary in vivo evaluation further verified the cancer inhabitation effect for the nanocarrier. These results exhibited bright prospects for the nanocarrier in their potential biomedical application.

4. Conclusion

FA functional emitter, namely, G-FA, was successfully synthesized with DS of 94%. The synthesized G-FA exhibited
enhanced fluorescence property and good linear intensity-concentration correlation. The G-FA functional pH-responsive nanoparticle with a G-FA content of 1.04 ± 0.29 mg/g was successfully prepared by emulsion method. The formed nanoparticle showed a coarse surface with a diameter of about 100-300 nm, pH-responsive property, and similar fluorescence property to G-FA. The nanoparticle could be successfully endocytosed by cells. Equilibrium endocytosis could be reached after 8 h with 1.0 mg/mL nanoparticle. Moreover, degradation of the nanoparticle might be involved in cell endocytosis process. Anticancer drug (CPT) and photosensitizer (PcZn) could be encapsulated in the nanoparticle. The nanoparticle enhanced the fluorescence effects of PcZn on water solution, and the CPT encapsulation rate decreased slightly along with initial CPT concentration increasing. A gradual decline of fluorescence intensity for the PcZn encapsulation nanoparticle in PBS solution and a quick loss of fluorescence intensity in mild acid solution were witnessed by a fluorescence spectrum. Similarly, gradual CPT release in PBS within 24 h and burst release in acid medium within the first hour were detected. In vitro cytoviability evaluation showed that the nanocarrier could decrease viability of HEP2 cells to a very low value. The results of preliminary in vivo evaluation revealed that the nanocarrier could inhibit cancer development with little effects on the body weight of mice. In conclusion, these results indicated that the nanoparticle with a pH-responsive property and targeted characteristic was an effective and efficient nanocarrier for codelivery of anticancer drug and photosensitizer.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the research.

Authors’ Contributions

Xiaohong Hu and Ziyu Gao contributed equally to this work and should be considered co-first authors.
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