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Visible-light degradable polymer coated hollow mesoporous silica nanoparticles for controlled drug release and cell imaging†

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A core–shell nanocomposite based on photo-degradable polymer coated hollow mesoporous silica nanoparticles (HMS) was successfully prepared for targeted drug delivery and visible-light triggered release, as well as fluorescence cell imaging. The HMS nanoparticles were first modified by the long-chain hydrocarbon octadecyltrimethoxysilane (C18) and fluorescent agent Rhodamine B isothiocyanate (RITC), and then encapsulated by a photodegradable amphiphilic copolymer *via* a self-assembly process. The obtained nanocarrier showed a high drug loading content due to the hollow core and mesopores of the HMS and could target folic acid receptor over-expressed tumor cells efficiently for conjugating folic acid (FA) in the amphiphilic polymer. The drug release could be triggered by the irradiation of green light (500–540 nm) due to the photodegradation of amphiphilic copolymer coated on the HMS. Furthermore, the targeted drug delivery and controlled release processes could be tracked by fluorescence imaging for the doping of RITC on the HMS. The *In vitro* results suggested that a smart visible light responsive drug delivery system was successfully prepared for the potential applications of cancer diagnosis and therapy.

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Introduction

Nanostructured materials have been developed for anti-cancer drug delivery due to their improved biodistribution and facile controllable morphology. In order to conquer the insufficient intracellular drug release and side effects of loaded anti-cancer drugs, an ideal nanocarrier should provide an excellent drug loading content, efficient tumor targeting and controlled drug release. Modifying these nanostructured materials with functional groups *via* special chemical or physical processes is continually being under way as one of the dominant development strategies.

Recently, many efforts have been made to develop multi-functional stimuli-responsive polymers for drug delivery, and most of these nanocarriers are purposely designed to exhibit tunable degradation properties induced by an external stimulus, which is usually of a chemical or physical nature such as temperature, pH, redox and light.[1–9] Compared to other stimuli-responsive polymers, light-triggered amphiphilic polymers have attracted much attention because they do not rely on changes in the specific chemical properties of the physiological environment in cancer cells, and the possibilities of remote activation as well as light-enabled spatial and temporal control are attractive features.

The known majority of photo-responsive species, including azobenzene,[10,11] *o*-nitrobenzyl[12] and spiropyran,[13,14] were proved to be responsive to ultraviolet light (UV, 250–380 nm). However, UV radiation has a relatively high energy (~6 eV),[15] which is harmful to humans, and this always hampers the biological applications of photo-responsive polymers containing these kinds of species. Hence, developing a new kind of polymer containing photochromic moieties with a low energy excitation wavelength, such as visible light (400–700 nm, ~2 eV), is quite necessary. In earlier work, the photochromic moiety containing the 9,10-dialkoxyanthracene (DN) group showed favorable visible light responsivity with synergy with the green light absorbing sensitizer (PS), eosin.[16] Eosin could transform triplet oxygen ($^3O_2$) into singlet oxygen ($^1O_2$) in solution when exposed to green light (500–540 nm), which can change 9,10-dialkoxyanthracene (DN) into 9,10-anthraquinone (AQ), and the eosin molecules have strong tendency to be bound with the DN species by π–π stacking.[17] Therefore, it is a appropriate photo-degradable moiety with which to construct a light responsive amphiphilic polymer.

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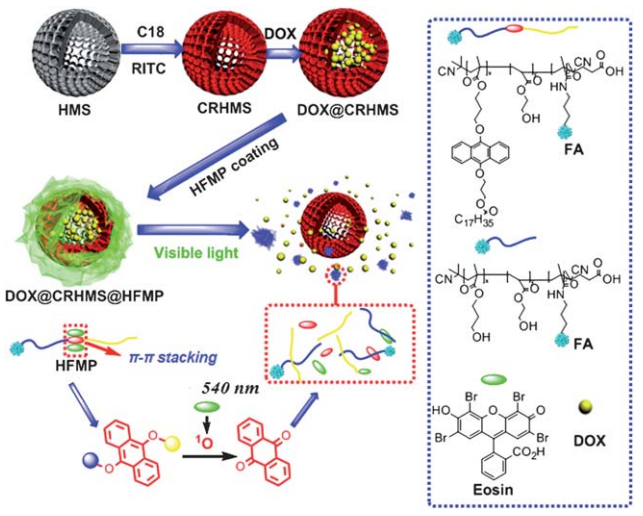
Besides, inorganic nanoparticles, such as gold nanoparticles,[18] mesoporous silica nanoparticles and Fe_3O_4 nanoparticles,[19] have attracted intense interest when acting as drug carriers, due to their excellent unique biocompatibility and chemical stability. Among these inorganic nanocarriers, hollow mesoporous silica nanoparticles (HMS) with well defined morphologies have attracted considerable interest. The hollow core of the HMS can act as storage reservoir to enhance the drug loading capacity, whereas the mesopores provide controlled and slow release pathways for the encapsulated drug molecules, and the surface of the HMS is full with Si–OH which can be easily modified with functional groups for imaging, targeting and hydrophobic properties.[20–22] However, pristine HMS cannot achieve stimulus-triggered drug delivery and thus limits their application as a smart drug carrier. Modifying the outer surface of HMS with a stimulus-responsive amphiphilic copolymer looks rather promising.

In this paper, we prepared a visible light-triggered drug delivery system for controlled drug release and cell imaging. After modification by the long-chain hydrocarbon octadecyltrimethoxysilane (C18) and the fluorescent agent Rhodamine B isothiocyanate (RITC) successively, HMS loaded the drug molecules and then was encapsulated by a photo-degradable amphiphilic copolymer through a simple self-assembly process (Scheme 1). The DN-containing copolymer was successfully synthesized *via* a reversible addition-fragmentation chain transfer (RAFT) polymerization and then conjugated with the folic acid (FA) moiety. When recognized by the FA receptor over-expressed tumor cells, the nanocarriers could be internalized efficiently and followed by a controlled drug release process under the irradiation of green light at 540 nm after degradation of the DN groups. A high drug loading content could be achieved due to the hollow core and mesopores of the HMS, and a long-term drug release could be achieved due to the hydrophobic interaction between the loaded drug and C18. In addition, the drug delivery process can be tracked by fluorescence imaging for modification of the HMS with RITC.



Scheme 1 Schematic depiction of the structure of DOX@CRHMS@HFMP and controlled release by degradation of HFMP under visible light.

Experimental section

Materials

(2-(Acryloyloxy)ethyl)trimethylammonium chloride (AETAC, 80 wt% in water), Rhodamine B isothiocyanate, 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (V-50, >97.0%), hexa-decyltrimethylammonium bromide (CTAB, >99.0%), octadecyltrimethoxysilane (C18) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Aldrich. Tetraethoxysilane (TEOS) was purchased from Alfa without any purification. Styrene (St, >99.0%) was washed through an inhibitor remover column for the removal of *tert*-butyl catechol and then distilled under reduced pressure prior to use. The analytical regents were used without further purification. 4,4-Azobis(4-cyanovaleric acid) (V-501) and azobisisobutyronitrile (AIBN) were purchased from Aldrich. *N*-Hydroxysuccinimide (NHS, 99%), *N*-(3-aminopropyl)-methacrylamide hydrochloride (APMA), dicyclohexacarbodiimide (DCC, 99%) and 4-dimethylaminopyridine (DMAP, 99%) were purchased from Alfa Aesar. Methacryloyl chloride and stearyl chloride were purchased from the Haimen Best Fine Chemical Industry Co. Ltd. and used after distillation. Hydroxyethylacrylate (HEA) was distilled under vacuum and stored at 15 °C under an inert gas atmosphere. Other reagents were commercially available and used as received.

Modification of HMS with C18 (CHMS)

The HMS were synthesized according to our previous works.[22] To prepare the PS latex templates, 0.5 g of AETAC (80 wt% in H_2O) was dissolved in 180 g of water in a 500 mL round-bottom flask. Then, 20.0 g of styrene was added slowly and kept stirring for 30 min. The mixture was purged with nitrogen for 20 min and then heated to 90 °C. Afterwards, 5 mL of an aqueous solution containing 0.5 g V-50 was added. The emulsion was kept at 90 °C for 24 h under nitrogen to complete the polymerization. The polystyrene latex was collected by centrifugation and washed with ethanol several times. To prepare the HMS, 0.8 g of CTAB was dissolved in a mixture of 80.0 g water, 60.0 g of ethanol and 1.5 mL of ammonium hydroxide solution. 0.93 g of PS powders was dispersed in 10.0 g water by sonication and then added dropwise to the above CTAB solution at room temperature under vigorous stirring, followed by sonication for 10 min. The derived milky mixture was then stirred for 30 min before adding 4.0 g of TEOS. The mixture was kept stirring at room temperature for 48 h. The precipitate was harvested by centrifugation and washed with ethanol, then dried at room temperature. Finally, the material was calcined in air at 600 °C for 8 h to remove the organic matter.

100 mg of HMS was first dispersed in 20 mL anhydrous acetonitrile, then 5 mL of C18 was added. The obtained suspension was stirred for 24 h and collected by centrifugation, washed with acetonitrile and ethanol several times and dried under vacuum to give CHMS.

Modification of CHMS with fluorescent dye RITC (CRHMS)

Modifying the external surface of CHMS with fluorescent dye Rhodamine B isothiocyanate (RITC) was done according to the literature with little modification.[24] The RITC (2.14 mg) was dissolved in 2 mL of ethanol, and APTES (1 mg) was then added. The mixture was agitated for 12 h in the dark, then 100 mg CHMS was added and then stirred 24 h. The product (CRHMS) was collected by centrifugation, washed with acetonitrile and ethanol several times, and dried under vacuum.

Synthesis of hydrophobic monomer 3-((10-(3-(methacryloyloxy)propoxy) anthracen-9-yl)oxy)propyl stearate (MAPS)

3-Iodo-1-propanol was synthesized according to the literature with some modifications.[25] 3-Chloro-1-propanol (10 g, 0.1 mol) and sodium iodide (60 g, 0.4 mol) were added to dry acetone (100 mL) and stirred at 60 °C under N_2 for 24 h. The mixture was filtered and the solvent evaporated. Then, a 1 : 1 mixture of diethyl ether–hexane was added and stirred for 10 min and then filtered. The solution was washed with a dilute sodium thiosulfate solution followed by water and then brine. The organic layer was dried with $MgSO_4$, filtered and the solvent evaporated in vacuum giving a pale yellow liquid (12.4 g, 67%). ^{1}HNMR (400 MHz, $CDCl_3$), δ (ppm): 3.74 (t, 2H, CH_2OH), 3.29 (t, 2H, CH_2I), 2.09 (m, 2H, $CH_2CH_2CH_2$).

9, 10-Bis(3-hydroxypropyloxy)anthracene: nitrogen-saturated water (150 mL) and CH_2Cl_2 (150 mL) was added to a mixture of 9,10-anthraquinone (2.57 g, 12.36 mmol), $Na_2S_2O_4$ (4.3 g, 24.7 mmol) and Adogen 464 (4.64 g, 10 mmol). The mixture was stirred for 5 min and then NaOH (4.94 g, 123.5 mmol) was added. Stirring was continued for 10 min and 3-iodo-1-propanol was added dropwise. The mixture was stirred overnight at 25 °C. Then the phases were separated and the water phase was washed with CH_2Cl_2. The combined organic phases were washed with water and dried over $MgSO_4$. The solution volume was reduced to 40 mL and the product was precipitated overnight at −20 °C. The solid was purified by column chromatography with a mixture of ethyl acetate–CH_2Cl_2 (1/4, v/v). Yield 1.13 g, 28%. ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm): 8.24 (dd, J = 6.8, 3.2 Hz, 4H, anthracene), 7.52 (dd, J = 6.8, 3.2 Hz, 4H, anthracene), 4.63 (t, J = 5.2 Hz, 2H, CH(O)), 4.16 (t, J = 6.5 Hz, 4H, OCH_2CH_2), 3.76 (m, CH_2CH_2OH, 4H), 2.1 (m, $CH_2CH_2CH_2$, 4H).

MAPS was synthesized according to the literature with little modification.[26] 9,10-Bis(3-hydroxypropyloxy)anthracene (0.4 g, 1.22 mmol) and pyridine (0.1 g, 1.22 mmol) were dissolved in dry CH_2Cl_2 (100 mL), then stearyl chloride (0.185 g, 0.61 mmol) was added dropwise to the solution under a nitrogen atmosphere. The mixture was stirred at room temperature for 2 hours. The product was purified by column chromatography with a mixture of ethyl acetate–CH_2Cl_2 (1/20, v/v) and dried in a vacuum to give 3-((10-(3-hydroxypropoxy) anthracen-9-yl)oxy)-propyl stearate. Yield: 0.25 g (0.42 mmol, 36%). Then the product (0.25 g, 0.42 mol) and triethylamine (0.101 g, 1 mmol) were dissolved in dry CH_2Cl_2 (50 mL), and methacryloyl chloride (0.104 g, 1 mmol) was added dropwise to the solution under a nitrogen atmosphere. The mixture was stirred at room temperature for 12 hours. The product was purified by column chromatography with a mixture of ethyl acetate–CH_2Cl_2 (1/20, v/v) and dried in a vacuum to give 3-((10-(3-(methacryloyloxy) propoxy)anthracen-9-yl)oxy)propyl stearate (MAPS). Yield: yellow solid, (0.145 g, 0.22 mmol, 52%). ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 8.24 (d, 4H, anthracene), 7.48 (d, 4H, anthracene), 6.18 (s, 1H, CCH_2), 5.60 (s, 1H, CCH_2), 4.64 (t, J = 6.0, 2H, CH_2OCO), 4.53 (t, J = 6.4, 2H, CH_2OCO), 4.29 (m, 4H, CH_2CH_2O), 2.45–2.35 (m, 6H, CH_2CH_2OCO, $COCH_2CH_2$), 2.01 (s, 3H, CH_3), 1.69–1,65 (m, 2H, $CH_2CH_2CH_2$), 1.29–1.25 (m, 30H, CH_2),0.88 (t, J = 13.2, 3H, CH_2CH_3).

Synthesis of RAFT agent 4-cyanopentanoic acid dithiobenzoate (CAD)

CAD was synthesized according to the literature with some modifications.[27] Briefly, 12.8 g of benzyl chloride was added dropwise to a sodium methoxide methanol solution (172 g, 12.6 wt%) containing 12.8 g of elemental sulfur. Subsequently, the obtained mixture was refluxed for 10 h under an inert atmosphere. The crude sodium dithiobenzoate solution was extracted by diethyl ether, 1.0 M hydrochloric acid and sodium hydroxide aqueous solutions, respectively and finally yielded a solution of sodium dithiobenzoate. Potassium ferricyanide solution (500 mL, 6.5 wt%) was added dropwise to the sodium dithiobenzoate over a period of 1 h with vigorous stirring. The obtained red precipitate was filtered, washed with deionized water, and dried in vacuum at room temperature overnight. Dithiobenzoyl disulfide (8.50 g, 0.28 mol) was added slowly to the distilled ethyl acetate (150.0 mL) solution containing 11.68 g of V-501 (0.42 mol). After refluxing for 18 h, the reaction solution was concentrated in vacuum and purified by column chromatography (ethyl acetate–hexane, 2/3). Yield 8.27 g, 35.5%. ^{1}HNMR (400 MHz, $CDCl_3$), δ (ppm): 7.91 (d, J = 7.59 Hz, 2H, C_6H_4), 7.58 (t, J = 7.47 Hz, 1H, C_6H_4), 7.41 (t, J = 7.79 Hz, 2H, C_6H_4), 2.75 (m, 2H, CCH_2), 2.45 (m, 2H, CH_2COOH), 1.95 (s, 3H, CH_3).

Synthesis of HAMA-*b*-MAPS

The RAFT polymerization was done according to the literature with some modifications.[28,29] HAMA (Poly(HEA-*b*-APMA)) was prepared by the RAFT polymerization of HEA and APMA using CAD as a transfer agent according to the literature with some modification.[23] Typically, 5.3 mg (0.02 mmol) CAD was added to the aqueous solution containing 2.5 mg of V-501, 1.22 g of HEA (10.5 mmol) and 268 mg of APMA (1.5 mmol). Then, the tube was sealed after three cycles between vacuum and nitrogen. After 5 h reaction in oil bath at 70 °C, the mixture was concentrated and washed with a large amount of acetone. The obtained copolymer was dried under vacuum and stored in desiccators for further polymerization.

The amphiphilic diblock polymer HAMA-*b*-MAPS was synthesized using the HAMA macro chain transfer agent. In a typical polymerization procedure, 1.2 g of HAMA was added to a dimethylsulfoxide solution containing 3 mg AIBN and 200 mg (0.31 mmol) MAPS and then placed in an oil bath at 70 °C for 5 h. The mixture was concentrated and washed with a large

amount of ethyl ether. After washing, the obtained diblock polymer was dried under vacuum overnight and stored in desiccators.

Synthesis of folate-conjugated diblock polymer HAMAFA-*b*-MAPS

A conjugation procedure was carried out according to the literature reported elsewhere.[30–32] Firstly, the thiocarbonylthio end group of the HAMA-*b*-MAPS was removed according to the literature with some modifications. Briefly, 500 mg of HAMA-*b*-DBAM was dissolved in 10 mL *N*,*N*-dimethylformamide (DMF) containing 30 mg of AIBN. The DMF solution was sealed after cycling between vacuum and nitrogen three times. After stirring at 70 °C for 5 h, the mixture was precipitated in anhydrous ether. The obtained diblock polymer was dried under vacuum and stored in desiccators for further use. Secondly, folate NHS ester was prepared by the following procedure: 0.62 g of DCC and 0.51 g NHS were added to a dry dimethylformamide (50 mL) solution containing 2.0 g of folic acid for 12 h at room temperature in the dark. The solution was filtered off and precipitated in diethyl ether. The obtained yellow powder was washed several times with anhydrous ether and used immediately for the next step. Then, FA–NHS (30 mg) was added to dry pyridine containing 100 mg of the above polymer. The solution was shaken for 12 h at room temperature and the mixture was precipitated in 40% acetone in diethyl ether. The filtrate was concentrated and stored in the dark at 4 °C. ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm): 8.63–6.62 (folic acid, CH_2NH_2 and anthracene), 4.21–3.76 ($COOCH_2CH_2$ and CH_2OH), 2.78 ($NHCH_2$ and CH_2NH_2), 1.21 (CH_3).

Preparation of CRHMS@HFMP

HAMAFA-*b*-MAPS (20 mg) were dissolved in 1 mL tetrahydrofuran, and then 0.6 mg eosin in 100 μL DMSO was added. After a continuous stirring for 1 h at RT, eosin and HAMAFA-*b*-MAPS would form into HFMP through a π–π stacking process, and then CRHMS (10 mg) was dispersed in this mixture by sonication for 5 min. Then, 5 mL of distilled water was added to the above mixture with vigorous shaking and the resulting colloid was stirred continually vigorously for 24 h at room temperature to evaporate the tetrahydrofuran. The prepared nanoparticles were isolated by centrifugation, washed with water, dried under vacuum overnight and stored in a desiccator for further use.

Photocleavage of HAMAFA-*b*-MAPS

To study the photocleavage of HAMAFA-*b*-MAPS, 1% eosin was added to DMSO-d_6 containing a certain amount of HAMAFA-*b*-MAPS, the ^{1}HNMR spectrum was evaluated after the irradiation of visible light for 2 h.

To further confirm the photocleavage of the HAMAFA-*b*-MAPS, gel permeation chromatography (GPC) of HAMAFA-*b*-MAPS containing 3% eosin in DMF was determined with irradiation of visible light at certain times.

In vitro experiments

***In vitro* drug-release.** To evaluate the drug loading capacity and release properties, doxorubicin (DOX) was used as a model drug according to the literature with some modifications.[21,33] DOX was extracted from doxorubicin hydrochloride (DOX·HCl) according to the procedure reported previously.[34] The CRHMS (4, 2 and 0.4 mg ml^{-1}) were dispersed in a 1 mL simulated body fluid (SBF) buffer solution, then the DOX solution (5 mg mL^{-1}, 40 μL) was added. After dispersion and stirring under light-sealed conditions for 24 h, the DOX@CRHMS were obtained by centrifugation and washed with 20 mL of PBS (pH 7.4), then dried at 60 °C in vacuum.

The DOX@CRHMS@HFMP and DOX@CRHMS@HFMP without eosin were prepared *via* self-assembly, as discussed before. A 5 mg sample was immersed in simulated body fluid (SBF) at 37 °C to maintain a constant temperature, and then different stimuli were introduced to determine the release of the loaded DOX.

Here, the DOX concentration was determined using a fluorescence spectrophotometer at $\lambda_{ex} = 480$ nm and $\lambda_{em} = 627$ nm. A standard plot was prepared under identical conditions to confirm the amount of drug loaded by CRHMS. The drug loading content and drug loading efficiency can be calculated as follows:

$$\text{Drug loading content (wt\%)} = (\text{weight of loaded drug/weight of nanocomposites}) \times 100\%;$$

$$\text{Drug loading efficiency (\%)} = (\text{weight of loaded drug/weight of drug in feed}) \times 100\%.$$

Cell culture and preparation

A549 human alveolar adenocarcinoma (FR−) and human KB (FR+) cell lines (purchased from Shanghai Cell Institute Country Cell Bank, China) were cultured as monolayers in a RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a humidified incubator (5% CO_2 in air, v/v).

In vitro cytotoxicity

The aminoxanthene dye, sulforhodamine B (SRB), was used in an assay for assessing the effects of drug carriers in various concentrations.[35] In brief, well-growing KB cells were placed in 96-well plates (1.3×10^4 cells per well) and four duplicate wells were set up in the sample. The culture medium was replaced with the medium containing different concentrations of drug carriers and cultured at 37 °C in a humidified incubator (5% CO_2 in air, v/v) with the cells anchored to the plates. After being cultured for 24 h, the medium was poured away and 10% (w/v) trichloroacetic acid in Hank's balanced salt solution (100 mL) was added and stored at 4 °C for 1 h. Then, the stationary liquid was discarded, the cells were washed with deionized water five times before air drying and stained with 0.4% (w/v) SRB solution (100 mL per well) for 30 min at room temperature. After removing the SRB, the cells were washed with 0.1% acetic acid solution five times. The bound SRB dye was solubilized with 10 mmol L^{-1} of a Tris-base solution (150 mL, pH = 10.5). The

optical density (OD) value of each individual well was calculated using a spectrophotometer at 531 nm absorbance.

Cellular uptake of DOX@CRHMS@HFMP

KB cells were seeded in 96-well plates at 1.3×10^4 cells per well and incubated overnight at 3 °C in a humidified incubator. The sample of the DOX@CRHMS@HFMP was dispersed in a RPMI-1640 medium. The cells were cultured with the DOX@CRHMS@HFMP solution for a certain time and observed using confocal laser fluorescence microscopy (Olympus, FV 1000) after washing three times with PBS.

The controlled therapeutic experiment was based on KB cells. The KB cells were seeded into 96-well cell culture plates at 1×10^4 per well until adherent and then incubated with the DOX@CRHMS@HFMP at a concentration of 100 ug mL^{-1} for certain times. After removal of the nanoparticles, the cells were transferred into fresh media and irradiated at 540 nm. Each irradiation time lasted for 10 min at 30 min intervals, and the cells were then incubated at 37 °C for an additional 48 h before the standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay was used to determine their viabilities, relative to the control cells.

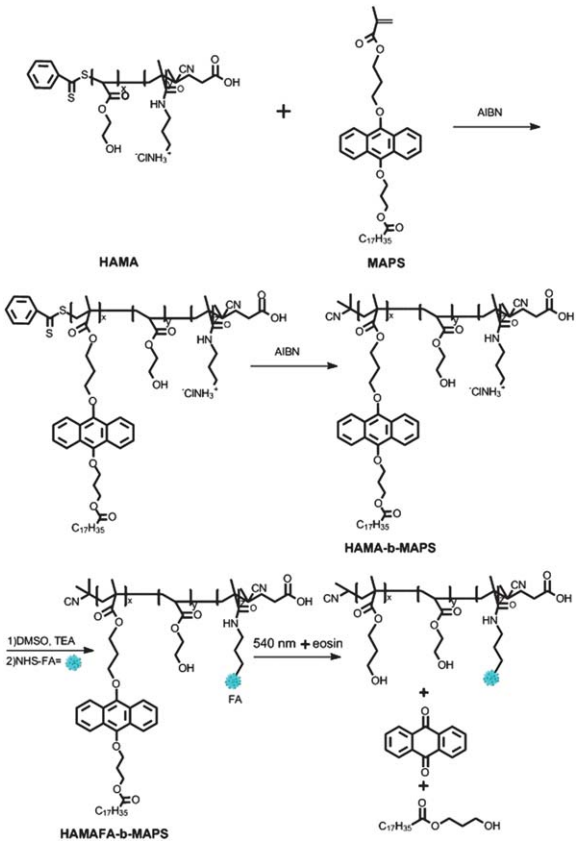
Characterization

The FT-IR measurements were performed as KBr pellets on a Nicolet 4700 spectrometer (Thermo Fisher Scientific) in the range 400–4000 cm^{-1}. The ^{1}HNMR spectra were measured by an INOVA 400 MHz NMR instrument. The TEM images were obtained using a Tecnai G220 electron microscope at an acceleration voltage of 200 kV. The number average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) were measured by gel permeation chromatography (GPC) utilizing a Waters 1515 pump. The Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) analyses were used to determine the surface area, pore size and pore volume and were obtained with a Quantachrome Autosorb 1C apparatus at −196 °C under continuous adsorption conditions. The emissions (λ_{ex} = 540 nm) were obtained using an Edinburgh-920 fluorescence spectrophotometer. The UV-vis absorption spectra were recorded by a Perkin-Elmer Lambda-17 Spectrophotometer. Confocal laser scanning microscopy (CLSM) images were observed by a confocal laser scanning microscope (Olympus, FV 1000).

Results and discussion

Synthesis of HAMAFA-*b*-MAPS and CRHMS

The amphiphilic copolymer HAMAFA-*b*-MAPS used here was synthesized following the procedure shown in Scheme 2. The successful conjugation of the FA was confirmed *via* the ^{1}HNMR spectrum of HAMAFA-*b*-MAPS (Fig. S1†).^{1}H NMR spectroscopy was next performed to prove the photocleavage of the DN groups. The ^{1}H NMR spectrum of the HAMA-*b*-MAPS in different cases is shown in Fig. S2,† the proton signals at δ = 8.25 and 7.52 ppm could be ascribed to the DN species. Then, the eosin sensitizer (3% of copolymer concentration) was added



Scheme 2 Schematic illustration of the synthesis of HAMAFA-*b*-MAPS.

to the solution of the HAMA-*b*-MAPS. When we applied a green visible light (mercury lamp of 120 W combined with a green light filter at 500–550 nm) to this solution for 1 h, two new aromatic signals at δ = 9.69 and 8.08 ppm appeared in the ^{1}HNMR spectrum (inset Fig. S2†) and were proved to be from anthraquinone.

To further confirm the photocleavage of the HAMAFA-*b*-MAPS, GPC measurement was done before and after the visible light irradiation. The decrease of M_n with the irradiation time is shown in Fig. S3.† Without visible light, the M_n of the HAMAFA-*b*-MAPS showed almost no change, but after the visible light irradiation, a sharp decrease in M_n indicated the $^1O_2$-mediated reaction had occurred and the polymer was cleaved. This result suggested the conversion of HAMA-*b*-MAPS hydrophobic segments to hydrophilic ones, with the appearance of eosin when exposed to the green light.

The surface of the HMS was enriched with Si–OH,[36,37] which could be simply modified with C18 and RITC. The FT-IR spectra of the HMS, CHMS and CRHMS are shown in Fig. 1. The Si–OH bond (950 cm^{-1}) in the spectrum of the HMS suggested that a large number of silanol groups existed on the surface of the HMS for coupling alkyl chains and functional molecules. As shown in the spectrum of CHMS, the strong bond of C–H_x (2890 cm^{-1}) revealed the successful growth of alkyl chains on the silica. Similarly, the absorption peaks around 1700 cm^{-1} in the spectra correspond to the –COOH groups of RITC. The fluorescence emission spectrum (λ_{ex} = 540 nm) of CRHMS is

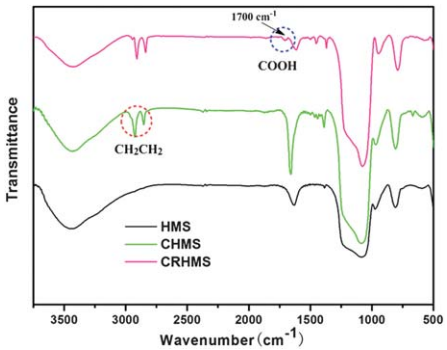


Fig. 1 FT-IR spectra of HMS, CHMS and CRHMS.

shown in Fig. S4,† which further confirms the successful conjugation between RITC and surface of HMS.

Assembly of CRHMS@HFMP

In our approach, CRHMS@HFMP was synthesized *via* a self-assembly process. HAMAFA-*b*-MAPS (20 mg) were dissolved in 1 mL tetrahydrofuran, and then 0.6 mg eosin in 100 uL DMSO was added. After continuous stirring for 1 h, eosin had strong tendency to bind with the DN species to give HFMP because of π–π stacking reactions. Then CRHMS (10 mg) was dispersed in this solution followed by the addition of 5 mL of distilled water. After evaporation of the THF at room temperature, the prepared amphiphilic HFMP would form micelles in water and hydrophobic CRHMS would be encapsulated to form core–shell nanocomposites, and long alkyl chains of hydrophobic groups of polymer would strongly associate with the hydrophobic C18 alkyl chains on the HMS surface through hydrophobic van der Waals interactions.[38,39]

The detailed morphological features of the prepared nanoparticles were examined by TEM. As shown in Fig. 2, after being grafted with C18 and RITC (Fig. 2b), no significant difference appeared in the diameter and structure between the HMS and CRHMS. Both the HMS and CRHMS maintained a good monodispersity with an average diameter of 200 nm and a very clear profile. In contrast, the profile of the CRHMS@HFMP became blurred and a thin layer of polymer shell with a thickness around 10 nm was clearly observed after the encapsulation of the HFMP (Fig. 2c). Thus, the controlled release pathways for the encapsulated drug molecules could be well blocked to prevent premature drug release from the hollow core of the HMS in normal physical environments, which is very important to clinical cancer therapy. A boundary line between the polymer film and the HMS shell could be clearly observed in the enlarged TEM images of the nanocomposites (Fig. S5†), which further confirmed the encapsulation of the polymer film. Furthermore, the prepared CRHMS@HFMP still retained a good monodispersity with an average diameter of about 220 nm, which was in accordance with the findings from the dynamic light scattering (DLS) measurements (Fig. 2d).

To ensure the appearance of eosin in the nanocomposites, UV-vis was used. The UV-vis spectra of the nanocomposites with and without eosin is shown in Fig. S6;† the enhanced absorbance at 525 nm strongly demonstrates the appearance of this complex.

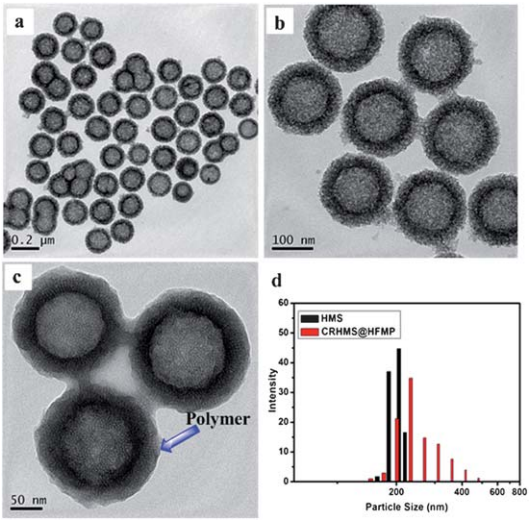


Fig. 2 TEM images of HMS (a), CRHMS (b), CRHMS@HFMP (c), and DLS of HMS and CRHMS@HFMP (d).

Drug loading and release

DOX was used here, as an model anti-cancer drug, to examine the drug loading and release behaviour of the CRHMS@HFMP. DOX was first loaded into the hollow core of the CRHMS, and the N_2 adsorption–desorption isotherms (Fig. S7†) of the HMS, CRHMS and DOX@CRHMS revealed that the modification of C18 and RITC and loading of the DOX molecules have not altered the pore structure of the HMS, which was necessary for the drug loading and release. The drug loading content was examined by measuring the DOX concentration before and after being loaded by the CRHMS. The theoretical loading content was set at 5%, 10% and 50% and the DOX loading efficiency was measured at 78, 75 and 70%, respectively (Table 1). As can be seen, the drug loading content and efficiency showed that the HMS was much more suitable for drug delivery than common mesoporous silica, owing to its hollow core. Furthermore, the HMS modified with the hydrophobic long alkyl chains surface was more suitable than the pristine HMS for the water-insoluble drug molecule loading.

Then, DOX@CRHMS@HFMP was prepared through a self-assembly process at a low drug loading content (*ca.* 5 wt%),[40] and by evaluating the DOX concentration after the centrifugation of the nanocomposites, 5.6% of the DOX loaded in the HMS was lost in this process. Then, different stimuli were introduced to determine the release of the loaded DOX. The concentration of DOX was evaluated at certain times after the centrifugation of the nanocomposites. The release quantity *versus* time in different cases is shown in Fig. 3. Without any stimuli, only less than 5% of DOX was released from HMS after 100 h. When we exerted green light and PS, a burst release (>50% in 10 h) was observed due to the uncovered pore caused by the degradation of HAMAFA-*b*-MAPS and the different

Table 1 Drug loading content and drug loading efficiency of CRHMS

Theoretical drug loading content (wt%)	Drug loading content[a] (wt%)	Drug loading efficiency (%)
5	4.1	78
10	8.1	75
50	38.5	70

[a] Drug loading content for DOX was determined by fluorescence measurement.

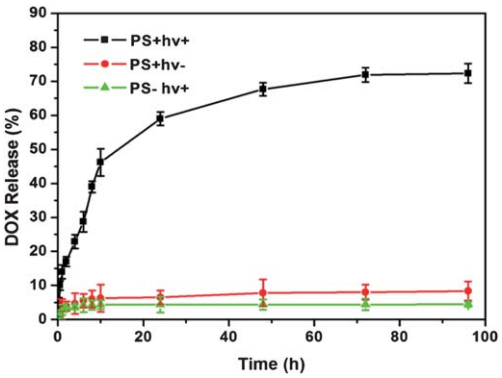


Fig. 3 Release of DOX *in vitro* from the drug-loaded DOX@CRHMS@HFMP with different stimuli at 37 °C.

concentration of DOX inside and outside of CRHMS. After 20 h, a lower concentration difference and the hydrophobic interaction between the drug and the long alkyl chains could lead to a long-term drug release (70% after 100 h), which is important for further cancer treatment.[41] This phenomenon strongly demonstrated that the amphiphilic copolymer provides a favourable sensitivity to visible light and the CRHMS@HFMP is capable of serving as a controlled drug release nanocarrier.

Evaluation of cytotoxicity and drug uptake

The sulforhodamine B (SRB) assay was used to assess the cytotoxicity of the nanocomposite. After incubation in the nanocomposite for 72 h, the cells displayed a high cell viability (>80%) as shown in Fig. 4. Even at a high concentration, the cytotoxicity still remained at a low level. The results above proved the polymer shell to be biocompatible and non-toxic. These characteristics are necessary for further biomedical applications.

The RITC was grafted onto the surface of the HMS, and then the CRHMS was encapsulated by the HFMP. For the grafted RITC on the surface of the HMS, the distribution of the nanocomposites could be observed by fluorescence imaging. Herein, *in vitro* experiments based on folate receptor positive (KB) and negative (A549) cancer cells were used to demonstrate the selective release and targeting properties of the nanocomposites. The uptake of the KB and A549 cells and the intracellular distribution of the nanocomposites with FA groups were studied by CLSM. After being incubated with the nanocomposites for 0.5 h and 2 h, the nanocomposite distributions are shown in Fig. 5.

To quantify the cellular uptake of the nanocarriers for the two different cell lines, the mean fluorescence intensity of the FR+ and FR− cells with CRHMS@HFMP and FA-free nanocomposites (CRHMS@HMP) in solution at different incubation times are shown in Fig. 6. No significant differences could be observed in the fluorescence intensity between the two nanocomposites when incubated with A549 and KB cells for 0.5 h. But after 2 h, a significant increase in the fluorescence intensity could be seen in the KB cells incubated with the FA conjugated nanocomposites when compared to other incubations. This result demonstrated that both of the two nanocomposites were internalized in a short time by the KB and A549 cells through the same endocytosis process. 2 h later, the remarkable changes in the fluorescence intensity demonstrated that the targeting moiety offered by folic acid is efficient at enhancing the tumor cell targeting *in vitro*, because the FA-conjugated nanocomposites was taken up by the KB cells through a folate receptor-mediated endocytosis.

For a further application of the nanocarriers, the DOX was loaded (drug loading content set as 38.5%) to investigate whether the therapeutic could be controlled by using the current nanocarrier. As a control, the photothermal effects and apoptosis induced by irradiation were first investigated. The results shown in Fig. 7 indicated that no obvious decrease in the cell viability was observed after irradiation with visible light, which suggested that the visible light exerted little damage on the living cells, as expected. After incubation of the nanocarriers, almost no decrease in the living cells was observed without visible light irradiation, which further confirmed that the nanocarriers showed almost no cytotoxicity in living cells, and could prevent the DOX leakage well.

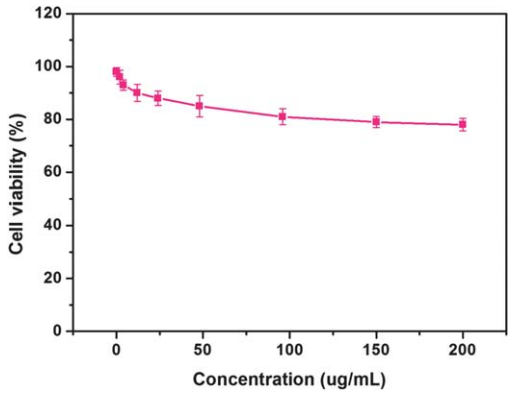


Fig. 4 *In vitro* cell viability of the CRHMS@HFMP at different concentrations.

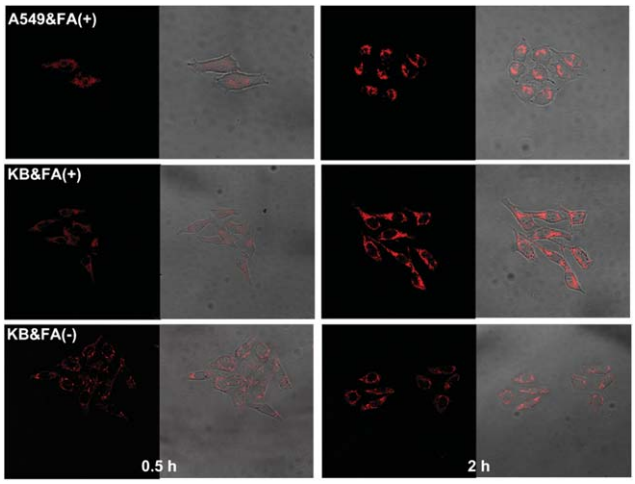


Fig. 5 CLSM of FR− and FR+ incubated with CRHMS@HFMP and CRHMS@HMP for 0.5 h and 2 h.

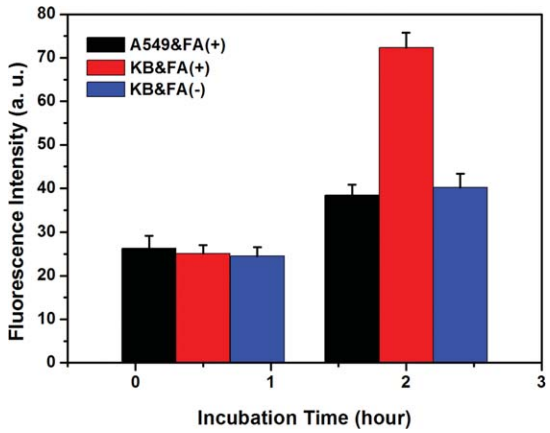


Fig. 6 Mean fluorescence intensity on FR+ and FR− cells with CRHMS@HFMP and CRHMS@HMP for 0.5 h and 2 h.

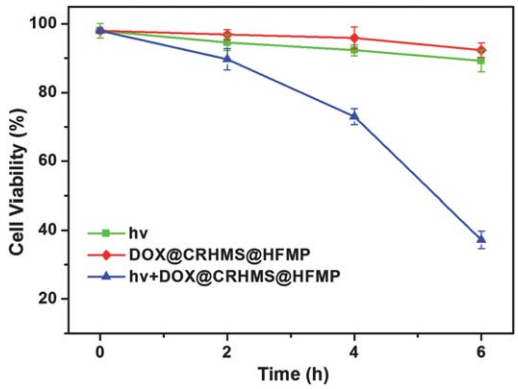


Fig. 7 Controlled therapeutic of CRHMS@HFMP under visible light.

Then the KB cells incubated with nanocarriers were exposed to visible light and the cell viability decreasing with irradiation time is shown in Fig. 7. The result showed that only 40% KB cells survived after 6 h irradiation, which confirmed that the therapeutic could be controlled by using the current nanocarrier.

Conclusion

In summary, a visible light triggered drug delivery system was successfully prepared for controlled drug release and cell imaging. HMS with a hollow core was used as the drug container and a high drug loading content could be achieved. Then, the HMS was easily encapsulated by a FA-conjugated visible light-sensitive amphiphilic polymer *via* a self-assembly process, due to modification of C18 on the HMS. This nanocarrier allowed drug release from the HMS when exposed to green light, because of the degradation of the amphiphilic polymer. Since the HMS was modified with RITC, the cancer targeting process could be easily tracked by fluorescence microscopy.

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