Novel Fluorinated Polymer-Mediated Upconversion Nanoclusters for pH/Redox Triggered Anticancer Drug Release and Intracellular Imaging

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This paper designs and synthesizes a novel amphiphilic fluorinated block copolymer, composed of oligo ethylene glycol methyl ether methacrylate, 2-((N,N-dimethylamino)ethyl methacrylate, and developing 2-((2-methacyloxy ethyl) disulfanyl) ethyl 3,5-bis(trifluoromethyl) benzoate, by reversible addition–fragmentation chain transfer polymerization. This amphiphilic block copolymer can self-assemble with hydrophobic lanthanide-based upconversion nanoparticles to form hybrid colloidal nanoclusters. The obtained nanoclusters exhibit not only good water-dispersibility, high biocompatibility, and strong stability in biological milieu, but excellent pH/redox-dual-responsive release in vitro behavior for doxorubicin (DOX). And the DOX-loaded nanoclusters have much better therapeutic effect than free DOX and can be uptaken by MCF-7 cells with evident upconversion luminescence signal for intracellular imaging.

1. Introduction

Lanthanide-based upconversion nanoparticles (UCNPs) have been widely investigated for biological applications, including bioimaging, biomolecular sense, and photodynamic therapy,[1–3] because they can provide visible or near-infrared emission under excitation at 980 nm wavelength and exhibit excellent chemical, biological, and optical properties, such as low cytotoxicity, narrow emission lines, large anti-Stokes shifts, high penetration depth in tissues, and high resistance to photobleaching.[4–7] The most efficient host matrix of UCNPs is NaYF₄ codoped with Yb³⁺ as a sensitizer, and Er³⁺ or Tm³⁺ as emitter. However, these UCNPs are usually hydrophobic as they are capped by oleic acids, which compromise their water solubility and their compatibility with the biological milieu.[8–11] Accordingly, surface hydrophilization of UCNPs has become essential for their potential biomedical applications.

So far, several strategies have been developed to transfer the hydrophobic nanocrystals into an aqueous phase, including addition of silica, or replacement of the hydrophobic ligand with other ones, or self-assembly with amphiphilic polymers or molecules.[12–14] Among these methods, amphiphilic polymers are especially promising owing to their unique chain architecture and self-assembling properties.[15,16] Although some polymers have been used for surface modification, most of them...
just endow these nanoparticles with water solubility or single stimuli for drug delivery, which cannot be used for effective diagnosis and treatment.\textsuperscript{[17–20]} To work out this issue, intelligent drug delivery systems have recently gained great attention because of their controllable on demand release of anticancer drugs in response to intracellular microenvironmental stimuli, including pH, light, redox, and enzyme,\textsuperscript{[21–24]} producing stronger anticancer activity with fewer side effects. Particularly, pH-/redox-responses have drawn much attention for drug delivery because tumors usually have the lower pH value and higher glutathione (GSH) than normal tissue.\textsuperscript{[25–28]}

In addition, in vivo circulation time of the nanocarriers is also very important since it can enhance this passive targeting of the tumor cells in chemotherapy. Unfortunately, the current responsive nanocarriers based on non-covalent self-assembles often show an easy dissociation in blood due to their interaction with hydrophobic blood components.\textsuperscript{[29]} By contrast, fluorinated nanocarriers are both hydrophobic and lipophobic, which not only improve the stability of blood proteins without altering their structures and functions but also extend circulation time by preventing hydrophobic interactions with blood components.\textsuperscript{[30,31]} Moreover, fluorination has a high phase-separation tendency in both polar and non-polar environments, and thus can improve the affinity of polymers to cell membrane and cross the lipid bilayer of the cell membrane, as well as the endosome/lysosome membrane, facilitating nanocarriers’ transport.\textsuperscript{[32]} Therefore, fluorinated polymer have been widely used for biological application.\textsuperscript{[33–36]} Nonetheless, few responsive fluorinated polymers have been used for the surface hydrophilization of UCNPs for drug delivery and fluorescent imaging.\textsuperscript{[37,38]}

Motivated by the above considerations, in this study, we design and synthesize a novel pH-/redox-responsive (poly)oligo ethylene glycol methyl ether methacrylate (OEGMA)-co-2-(N,N-dimethylamino)ethyl methacrylate (DMAEMA))-b-2-((2-methacryloyloxy ethyl) disulfanyl)ethyl 3,5-bis(trifluoromethyl) benzoate (MBSEF)) block copolymer through reversible addition–fragmentation chain transfer polymerization (RAFT) polymerization of OEGMA, DMAEMA, and our developing MBSEF. This new amphiphilic block copolymer can self-assemble with UCNPs and anticancer drug (DOX), showing very good pH-/redox-dual-responsive controllable in vitro release behavior with obvious toxicity in tumor cells, via both the cleavage of disulfide by glutathione and the protonation of amino groups by acidic environment (Scheme 1). At the same time, the UCNPs fluorescent imaging can efficiently monitor the drug distribution, delivery, and release in MCF-7 cells. All these results suggest that synthesized fluorinated amphiphilic polymers we present here may be used to endow hydrophobic nanocrystals with good water-dispersibility, high biocompatibility, and strong biological stability, pH/redox-dual-responsive capability which are similar to the tumor microenvironment. And the assembled nanoclusters may be considered a very promising candidate for novel intelligent delivery system and cell imaging.

2. Experimental Section

2.1. Materials

OEGMA ($M_n = 500$) was received from Sigma-Aldrich and passed through basic alumina column to remove inhibitors prior to use. DMAEMA (99%), 3,5-bis(trifluoromethyl) benzoyl chloride (BTBFC), methacryloyl chloride, 2,2′-azobis(2-methylpropionitrile) (AIBN), 6-diamidino-2-phenylindole (DAPI), and anhydrous 1,4-dioxane were obtained from J&K Chemical Ltd. AIBN was recrystallized three times from ethanol. Oleic acid (90%), NH$_4$F (98%), sodium hydroxide (NaOH, 98%), 1-octadecene (90%), ethanol, cyclohexane, triethylamine (TEA), MgSO$_4$, NaHCO$_3$, ethyl acetate, petroleum ether, dichloromethane (DCM) (anhydrous grade), and tetrahydrofuran (THF, 99.5%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). THF was refluxed and distilled over sodium (Na) before use. 4-(Dimethylamino)pyridine (DMAP) (99%), GdCl$_3$·6H$_2$O (99.99%), YCl$_3$·6H$_2$O (99.99%), YbCl$_3$·6H$_2$O (99.9%), ErCl$_3$·6H$_2$O (99.9%), 2-hydroxyethyl disulfide (technical grade), doxorubicin hydrochloride (DOX), and 2-cyano-2-propyl dodecyl triiodocarbonate (CTA, 97%) were purchased from Sigma-Aldrich. All these chemicals were used as received unless otherwise stated.

2.2. Synthesis of 2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate (HSEMA)

HSEMA was synthesized by monoesterification of 2-hydroxyethyl disulfide with methacryloyl chloride as follows: TEA (6.07 g,
60 mmol) and 2-hydroxyethyl disulfide (6.17 g, 40 mmol) were dissolved into anhydrous THF (300 mL) in an ice-water bath, and then methacryloyl chloride (4.18 g, 40 mmol) in 50 mL anhydrous THF was added dropwise using a syringe pump within 1 h under vigorous magnetic stirring. The reaction mixture was stirred at room temperature overnight. After filtration and evaporation of all solvents, the residues were diluted with ethyl acetate and washed twice with water and brine, respectively. The organic layer was dried over anhydrous MgSO4, filtered, and finally purified by silica gel column chromatography using ethyl acetate/petroleum ether (1/4 v/v) as the eluent, affording HSEMA as a yellowish liquid. $^1$H NMR (TMS, CDCl3, ppm as shown in Figure S1, Supporting Information): 6.1 (s, 1 H), 5.56 (s, 1H), 4.38 (t, J = 6.7 Hz, 2H), 3.84 (t, J = 5.6 Hz, 2H), 2.93 (t, J = 6.7 Hz, 2H), 2.82 (t, J = 5.8 Hz, 2H), 1.9 (s, 3H). $^{13}$C NMR (CDCl3, ppm, TMS):167.27, 135.94, 126.15, 62.6, 60.2, 41.5, 36.8, 18.2.

2.3. Synthesis of Reduction-Responsive Monomer, MESEF

Typically, HSEMA (2.24 g, 10 mmol), DMAP (18 mg, 0.15 mmol), and TEA (1.5 g, 15 mmol) were dissolved in dry DCM (30 mL) under argon atmosphere in ice water. BF3·OEt2 (4.14 g, 15 mmol, in 10 mL dry THF) was added dropwise via a syringe pump. The reaction mixture was stirred overnight at room temperature. After filtration and evaporation of all the solvents, the residues were diluted with DCM and washed twice with 1 m HCl solution (100 mL x 3), twice with saturated NaHCO3 aqueous solution (100 mL x 2), respectively. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated on a rotary evaporator. The crude product was purified by column chromatography using ethyl acetate and petroleum ether (1/8 v/v) as eluent, affording MESEF as a yellowish liquid. $^1$H NMR (TMS, CDCl3, ppm as shown in Figure S2, Supporting Information): 8.49 (m, 1H, -CF3CHCF3–), 8.06 (m, CF3CC–), 6.12 (m, 1H, -CHH=C(CH3)–), 5.56 (m, 1H, CHH=C(CH3)–), 4.67 (t, 2H, -COOCH2CH2SS–), 4.42 (t, 2H, -COOCH2CH2SS–), 3.1 (t, 2H, -CH2SSCH2–), 3.0 (t, 2H, -SSCH2CH2–), 1.92 (s, 3H, CH2=C(CH3)–). Yield: 85%. $^{13}$C NMR (CDCl3, ppm, TMS):167.05, 163.64, 135.96, 131.69, 129.77, 125.96, 124.15, 121.45, 63.89, 62.39, 37.26, 36.86, 18.16.

2.4. Synthesis of Poly(OEGMA-co-DMAEMA) Copolymer

In a typical polymerization, OEGMA (0.4 g, 0.8 mmol), DMAEMA (1.57 g, 10 mmol), CTA (34.5 mg, 0.1 mmol), and AIBN (3.28 mg, 0.02 mmol) were dissolved in 1,4-dioxane (4 mL) under stirring within a 25 mL flask equipped with a magnetic stirring bar. After three freeze-pump-thaw degassing cycles, the solution was subjected to 70 °C for 7 h for the RAFT polymerization, and then quenched in ice water and exposed to air for termination. The mixture was precipitated in hexane and redissolved in THF. The dissolution and precipitation were carried out for three times. After dried in a vacuum oven overnight at 40 °C, the poly(OEGMA-co-DMAEMA) copolymers were obtained as yellow viscous solid (1.38 g, yield: 92%). Because terminal CTA agent lacks characteristic $^1$H NMR peaks, the actual DP (degree of polymerization) of poly(OEGMA-co-DMAEMA) was estimated to be about =6 and 75 based on the conversion of OEGMA (80%) and DMAEMA (75%).

2.5. Synthesis of Poly(OEGMA-co-DMAEMA)-b-MESEF Block Copolymer

This synthetic procedure is the same as above. Typically, poly(OEGMA-co-DMAEMA) (0.4 g, 0.023 × 10^3 mol), MESEF (0.4 g, 0.85 × 10^3 mol), AIBN (0.9 mg, 0.0054 × 10^3 mol), and 1,4-dioxane (3 mL) were charged into a 25 mL flask equipped with a magnetic stirring bar. The flask was carefully degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After thermostated at 70 °C in an oil bath and stirred for 24 h, the reaction was quenched in ice water and exposed to air for termination, the mixture was then precipitated into an excess of hexane and redissolved in THF. After dried in a vacuum oven overnight at 35 °C, poly(OEGMA-co-DMAEMA)-b-MESEF was obtained as light yellowish viscous solid (0.7 g, yield: 87%). DP of the MESEF was determined to be about =30 by $^1$H NMR analysis in CDCl3.

2.6. Preparation of Micellar Solution and Colloidal Nanoclusters

The synthetic procedure of NaYF4:Yb/Er@NaGdF4 nanocrystals was described in the Supporting Information. The preparation of polymeric micelles and colloidal nanoclusters were as follows: Poly(OEGMA-co-DMAEMA)-b-MESEF copolymer (10 mg) was dissolved into THF (1 mL) at 30 °C under stirring, and 10 mL water was slowly added to the solution using a syringe pump within 1 h. The solution was then dialyzed (MWCO: 3.5 KDa) against water at 30 °C for 8 h to remove THF. Besides, colloidal nanoclusters were obtained by the solvent evaporation induced self-assembly. The poly(OEGMA-co-DMAEMA)-b-MESEF (20 mg) was well dispersed in the mixture of THF (1 mL) and distilled water (10 mL) under sonication for 5 min, then the oleic acid-capped UCNP nanocrystals (0.3 mmol) in 3 mL DCM was added and the milk-like mixture was sonicated for another 10 min. After that, the suspension was vigorously sonicated at 70 °C for 30 min to evaporate the DCM and THF for the self-assembly of amphiphilic block copolymers and nanocrystals. The obtained assemblies were centrifuged to remove the agglomerations and further dialyzed against distilled water to produce hydrophilic UCNP nanoclusters.

2.7. Loading of DOX in Colloidal Nanoclusters

DOX (1.5 mg) and TEA (20 μL) were dissolved in 0.5 mL THF at 25 °C under stirring for 10 h. Then the above 5 mL colloidal UCNP nanoclusters were added into the solution for sonication for 5 min. This mixture was dialyzed against 7.4 phosphate buffer saline (PBS) buffer at 25 °C for 5 h to remove THF, TEA, and unloaded DOX. The loaded DOX content was quantified by fluorescent spectrum. The encapsulation efficiency (EE %) was calculated as follows: EE% = (Ntotal - N unloaded) / Ntotal × 100%. The loading content (LC %) = Nloaded / (Nnanoclusters + Nloaded) × 100%, where Ntotal, N unloaded, and N loaded refer to the masses of drug used, unloaded drug, drug loaded in nanoclusters, and nanoclusters, respectively.
2.8. In Vitro Release of DOX

The DOX-loaded colloidal nanoclusters were dispersed in 0.1 mol L\(^{-1}\) phosphate buffer (pH 7.4) and transferred to a dialysis bag (MWCO: 3.5 kDa) immersed in PBS solutions at different pH (7.4 and 5.0), without or with GSH (10 \(\times\) 10\(^{-3}\) mol L\(^{-1}\)), at 37 °C. At certain time intervals, samples of the external medium were withdrawn and replaced with the same volume of fresh buffer solution. The concentration of DOX was calculated based on the fluorescence intensity of DOX at 590 nm. The cumulative amount of released drug was calculated and plotted against time.

2.9. Cell Culture and Cytotoxicity Assay

MCF-7 cells cultured with Dulbecco minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% antibiotics penicillin, and streptomycin were incubated under a fully humidified atmosphere at 37 °C with 5% CO\(_2\). The cytotoxicity of sample (nanoclusters, free DOX, and the DOX-loaded nanoclusters) was evaluated using a CCK-8 assay. 100 \(\mu\)L of culture medium was removed and the cells were incubated for another 4 h, and the absorbance at 450 nm was measured using a microplate reader (Thermo SCIENTIFIC MULTISKAN MK3). Cell viability was calculated by:

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\text{Cell viability} (\%) = \frac{A_{\text{treated}} - A_0}{A_{\text{control}} - A_0} \times 100\%
\]

The assays were conducted in triplicate.

2.10. Confocal Laser Scanning Microscopy (CLSM)

The MCF-7 cells were plated on microscope slides of a 24-well plate at 1.2 \(\times\) 10\(^5\) cells per well in DMEM medium containing 10% FBS and incubated with free DOX or DOX-loaded nanoclusters at 37 °C under 5% CO\(_2\) atmosphere. After incubation for 24 h with sample, the culture medium was removed and the cells were washed three times with PBS. Then the cells were fixed with 4% formaldehyde for 15 min, and rinsed three times with PBS. Then the cell nucleus was counterstained with DAPI for 15 min and washed with PBS again. The CLSM microimages of cells were observed using a confocal laser scanning microscope (Nikon, Japan), with an excitation wavelength of 408 nm for DAPI and 488 nm for DOX.

2.11. Characterizations

Chemical structures were characterized by \(^1\)H NMR spectra (Bruker 400 MHz NMR instrument). Molecular weight and molecular weight distribution were determined on a gel permeation chromatograph (GPC) with THF as the eluent, polystyrene as the standard at 30 °C, and a flow rate of 1.0 mL min\(^{-1}\). Luminescence spectra of samples were recorded on an Edinburgh LFS-920 spectrometer. X-ray diffraction (XRD) patterns were obtained on a Bruker D8 powder X-ray diffractometer using Cu K\(\alpha\) radiation (40 kV, 40 mA). The images of the polymeric micelles, nanocrystals, and nanoclusters were scanned at a JEOL JEM-2010 transmission electron microscope at an accelerating voltage of 200 kV. Energy-dispersive X-ray analysis of the samples was performed on high resolution transmission electron microscope. Dynamic light scattering (DLS) measurements were carried out in a Malvern Instrument Nano ZS90. Fluorescent images were observed under a confocal laser scanning microscope (CLSM, Nikon, Japan).

3. Results and Discussion

3.1. Synthesis and Characterization of Poly(OEGMA-co-DMAEMA)-b-MESEF

The amphiphilic poly(OEGMA-co-DMAEMA)-b-MESEF block copolymers were successfully prepared via RAFT polymerization as shown in Scheme 2, and confirmed by \(^1\)H NMR spectroscopy and GPC in Figure 1. The broad peak at 4.03 ppm (f and c) is assigned to \(-\text{CH}_2\text{CH}_2\text{O}\) of OEGMA and \(-\text{COOH}_2\) of DMAEMA, the peak at 3.63 ppm (e) belongs to the repeating units of the OEGMA segment, and the singlet 3.36 ppm (d) is ascribed to \(-\text{OCH}_3\). The characteristic signals at 2.82 and 2.55 ppm (a and b) are assigned to the resonances of \(-\text{CH}_3\text{N(CH}_3\text{CH}_3\)_. Compared to the \(^1\)H NMR of poly(OEGMA-co-DMAEMA), the characteristic signals at 8.45 and 8.02 ppm (i and h) are assigned to benzene, signals at 4.63 and 4.21 ppm (j and n) are ascribed to \(-\text{COOH}_2\), and signals at 3.07 and 2.92 ppm (k and l) are assigned to \(-\text{SSCH}_2\). Other signals are attributed to the block copolymer backbone and the RAFT agent. Furthermore, the molecular weight and molecular weight distribution of amphiphilic block copolymers demonstrate the relatively narrow and symmetric peaks indicating the relatively ideal macromolecular chain structures in these copolymers. And the molecular weight of block copolymers increases with the addition of MESEF monomer. These results clearly indicate that the novel poly(OEGMA-co-DMAEMA)-b-MESEF block copolymer has been successfully synthesized via RAFT polymerization.

The self-assembly of poly(OEGMA-co-DMAEMA)-b-MESEF block copolymer was triggered by adding water into the THF solution of polymer (1 mg mL\(^{-1}\)), followed by the dialysis of the solution against water to remove the THF, then they were able to form the stable micelles, the critical micellar concentration of the block copolymer was determined to be ~0.17 mg mL\(^{-1}\) in water using Nile Red (NR) as fluorescence probe (Figure S3, Supporting Information). During the self-assembly process, because water is the poor solvent for fluorocarbon chains and further collapses MESEF segments to minimize the overall free energy of the system, thus the micelles with hydrophobic core by the noncovalent bonding force and the water soluble OEGMA and DMAEMA segments as the outer hydrophilic shell are formed.
3.2. Self-Assembled Colloidal Nanoclusters

As shown in Figure 2a, the size of the oleic acid capped NaYF₄:Yb/Er nanocrystals is about 28 nm with almost uniform spherical morphology. The increase of average nanoparticles size from 28 to 35 nm indicates the successful shell NaGdF₄ growth of crystals onto the surface of NaYF₄:Yb/Er (Figure 2b). The high crystalline phase of the core–shell nanocrystals were confirmed by powder XRD spectrum (Figure S4, Supporting Information). When the hydrophobic nanocrystals were added into the amphiphilic block copolymer solution under vigorous ultrasonication, the oleic acid chains of nanocrystals can interact with the hydrophobic fluorinated benzene segments of amphiphilic block copolymers through hydrophobic interaction, because fluorinated benzene segments of polymers show good compatibility with the oleic acid of the nanocrystals, while the hydrophilic OEGMA and DMAEMA segments stretch toward the aqueous phase. After assembled with poly(OEGMA-co-DMAEMA)-b-MESEF block copolymers, most of the nanocrystals have been embedded into polymeric micelles (Figure 2c), which are finely dispersed in aqueous phase with an average size of about 248 nm (Figure 2d,e), and the elemental distribution can be further confirmed by elemental mapping images (Figure 2f–k).

Figure 3a presents the upconversion luminescence spectrum change before and after the assembly with this block copolymer. Under excitation at 980 nm, there are three emission peaks at 520, 541, and 653 nm, which are...
ascribed to the $^{4}H_{11/2}$$-^{4}I_{15/2}$, $^{4}S_{3/2}$$-^{4}I_{15/2}$, and $^{4}F_{9/2}$$-^{4}I_{15/2}$ transitions of erbium ions, respectively. The upconversion emission of the core–shell nanocrystals is significantly enhanced by seven times as much as that of the NaYF$_4$:Yb/Er core alone in cyclohexane. However, after being transferred into hydrophilic ones, the luminescence intensity of the colloidal nanoclusters decreases by about 30% compared with that of oleic acid capped core–shell nanocrystals, probably due to the quenching arising from solvent or surface ligands,[16] in addition, we also investigate the luminescence spectrum of colloidal nanocluster after GSH/pH treatment, it can be seen that the luminescence intensity at 500–600 nm remained unchanged at different pH (Figure S5, Supporting Information), by contrast, the luminescence intensity increases with the addition of GSH, it may be attributed to the biodegradation of block polymer.

The hydrodynamic size and stability of colloidal nanoclusters were characterized by the DLS upon different conditions. As shown in Figure 3b, the average size of colloidal nanoclusters is recorded as about 248 nm at pH 7.4, which is consistent with TEM size, and there is little change in PBS and DMEM solution for up to 5 d (Figure S6, Supporting Information). The high stability of the colloidal nanoclusters may be attributed to the presence of the fluorinated segments,[38] which facilitate their cellular application. At pH 5, average size of nanoclusters is about 260 nm which is slightly bigger than that at pH 7.4, because PDMAEMA segments are extending into aqueous well owing to the protonation of $N,N$-dimethylaminoethyl groups. After addition of GSH into the colloidal nanocluster solution, the average size of the nanoclusters further decreases to about 140 nm with some small polymer chains and aggregates (Figure 3b), this phenomenon could be attributed to hydrophilic segments detached and hydrophobic carriers may further aggregated, and some disordered aggregates and nanocrystals can be found in the TEM image due to the reductive cleavage of the disulfide bonds with GSH (Figure 3c).

### 3.3. In Vitro Drug Loading and Triggered Release

To investigate whether colloidal nanoclusters possess pH-/redox-dual-responsive drug release, DOX was loaded and further released from the colloidal nanoclusters in PBS buffer solution containing reducing agent GSH at various pH values by dialysis method. The calculated drug-loading efficiency and drug-loading content are 83% and 11%, respectively. As shown in Figure 3d, under physiological conditions (pH 7.4), the DOX is released slowly than that under triggering conditions. At pH 5, much higher release rate is observed because the PDMAEMA chains are stretched by electrical repulsion after protonation of the $N,N$-dimethylaminoethyl groups. This profile indicates that colloidal nanoclusters exhibit pH-triggered release performance. When the DOX-loaded colloidal nanoclusters were placed at high GSH concentration ($10 \times 10^{-3}$ M), much faster release rates are observed than their corresponding pH conditions, which is should be ascribed to the cleavage of the disulfide bonds. Especially, the drug-loaded colloidal nanoclusters can release as high as 83% of DOX after 12 h at pH 5 and GSH. All these results indicate that the release rate of DOX from colloidal nanoclusters can be highly enhanced under pH-/redox-dual-responsive conditions. Since tumors usually have the lower pH value (e.g., $\approx 5.0$–5.5) and higher glutathione (e.g.,

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**Figure 1.** a) $^{1}$H NMR spectra of the poly(OEGMA-co-DMAEMA) copolymer and poly(OEGMA-co-DMAEMA)-b-MESEF block copolymer. b) GPC curves of poly(OEGMA-co-DMAEMA) copolymer and poly(OEGMA-co-DMAEMA)-b-MESEF block copolymer.
≈10 × 10^{-3} \text{M}} than normal tissue, the above experimental results strongly suggest that the colloidal nanoclusters assembled with this novel fluorinated pH-/redox-dual-responsive block copolymer we develop here is a very promising intelligent drug load and delivery system.

3.4. Cytotoxicity Assay and Cellular Uptake

To further investigate the anticancer effect of the DOX-loaded colloidal nanoclusters, the cytotoxicity of the blank colloidal nanoclusters, free DOX, and the DOX-loaded colloidal nanoclusters against MCF-7 cells was evaluated by the CCK-8 assay, respectively. As shown in Figure 4a, the relative viability of the cells incubated with the blank nanoclusters for 24 h is over 90% at all of the measured concentration even up to 300 µg mL^{-1}, indicating the colloidal nanoclusters have a good biocompatibility. However, the MCF-7 cells treated with free DOX show the dose dependent cytotoxicity, with the cell viability decreasing to 37% for the 30 µg mL^{-1} concentration. By contrast, the DOX-loaded colloidal nanoclusters exhibit high cytotoxicity against MCF-7 cells (Figure 4b), indicating much better therapeutic effect than the free DOX, which could be attributed to high hydrophilicity of colloidal nanoclusters and good affinity of fluorinated polymer for cell uptake.

The cellular uptake behavior of the colloidal nanoclusters was also investigated by CLSM. As shown in Figure 5, after incubation for 8 h, the DOX fluorescence can be seen in the cytoplasm and nuclei for cells treated with the DOX-loaded colloidal nanoclusters (Figure 5b), indicating the DOX-loaded nanoclusters have been taken up efficiently. Meanwhile, some of the DOX could be further released from colloidal nanoclusters due to intracellular reductive and acidic environment. Besides, the evident green upconversion luminescence signal can be seen from the MCF-7 cells under the excitation of 980 nm laser (Figure 5c). Eventually, it can be seen from the merged images that UNCP intracellular imaging can be useful to monitor or guide the drug delivery (Figure 5d).

4. Conclusions

In summary, we have successfully synthesized a novel pH/redox-dual-responsive fluorinated block amphiphilic copolymer. This polymer can self-assemble with hydrophobic UCNPs to form colloidal nanoclusters, endowing the latter with good biocompatibility and...
stability for biomedical applications. More importantly, because of the unique structure of the obtained fluorinated block amphiphilic copolymer, the colloidal nanoclusters exhibit excellent pH- and redox-dual responses for controllable drug release manner, e.g., at pH 5 and GSH \(10 \times 10^{-3}\) M which are similar to the tumor micro-environment, as high as 83% of DOX being released. Moreover, intracellular uptake and cytotoxicity confirm that the colloidal nanoclusters still have remarkably enhanced cellular uptake and antitumor efficacy. And the evident upconversion luminescence imaging on MCF-7 cells could be used to monitor or guide the drug delivery. Thus, the fluorinated polymer-mediated colloidal nanoclusters we present here may have potential applications in cellular imaging and intelligent drug delivery.

Figure 3. a) Upconversion emission spectra of samples under excitation at 980 nm. b) Hydrodynamic diameter distribution of colloidal nanoclusters. c) TEM image of colloidal nanoclusters treated with \(10 \times 10^{-3}\) M GSH. d) In vitro drug release of the drug-loaded colloidal nanoclusters at pH 7.4, pH 7.4 with \(10 \times 10^{-3}\) M GSH, pH 5, and pH 5 with \(10 \times 10^{-3}\) M GSH.

Figure 4. a) Cytotoxicity of blank colloidal nanoclusters with various concentrations. b) Cytotoxicity of free DOX and the DOX-loaded colloidal nanoclusters with different DOX concentrations treated MCF-7 cells.
**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Figure 5.** Confocal microscopic images of the DOX-loaded colloidal nanoclusters treated MCF-7 cells. a) Nucleus was stained by DAPI (blue), b) DOX was imaged as red, c) UCNPs were imaged as green, and d) the merge images, scale bars are 20 μm.


