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Photoresponsive Cross-linked Polymeric Particles for Phototriggered Burst Release

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ABSTRACT

We synthesized a series of cross-linked photoresponsive polymeric particles with photolabile monomers and cross-linkers through miniemulsion polymerization. These particles are quite stable in dark, while light irradiation caused the breakage of particles and the efficient release of encapsulated contents up to 95% based on Nile red fluorescence. Photoswitches of particle systems were confirmed by fluorescence spectroscopy, SEM and colorimetry. Particle uptake and triggered release in RAW264.7 cells were confirmed by fluorescein diacetate loaded particles.

INTRODUCTION

Lipids, carbohydrates, hydroxyl acid polymers, dendrimers, diblock copolymers etc. have been applied to form micro- and nanoparticles and have been widely used in biological applications, such as biomolecular imaging and drug delivery(1,2). Although great improvements for delivering targets into cells using micro- and nanoparticles have been achieved, it is highly desirable to be able to control the release of encapsulated targets from capsules and reach high enough target concentrations to mediate the effective responses via an external stimulus. The design and syntheses of intelligent drug delivery systems which respond to the environmental stimuli are promising research areas(3,4).

Currently, intelligent drug delivery systems are mostly based on stimuli-responsive polymers which sense a change of specific variables, such as temperature, pH, redox-potentials, the level of enzymes, ultrasound, light and specific donor molecules(4–14). Among these intelligent stimuli, light stands out as a clean and noninvasive one and has been widely used as an external trigger in biological systems(7,8,10,14–19), especially with the development of Litx^{TM} technology for internal irradiation of tissues(20). Unlike the pH and oxidation responsive systems, light does not require any chemical environmental change(21). For photoresponsive particles, the release of entrapped drugs can be accelerated at a specific location and time upon light activation, which promotes maximum efficacy. Inorganic nanoparticles, such as Au and Fe_2O_3 nanoparticles, have been used as carriers with surface modification through photolabile linkages. These nanoparticles can assist to deliver target molecules which can be released from nanoparticles upon light triggering(22,23). For nanoparticles formed by organic substances, most researches are currently limited to photolabile diblock copolymers which self-assemble into photoresponsive micellar particles. Azobenzene moieties (change of dipole moment) (24,25), spiropyran moieties (formation of zwitterionic species) (26–28), stilbene(29), pyrenyl(30), coumarinyl(31) and nitrobenzyl esters (change hydrophobic to hydrophilic block)(32–35) are the typical chromophores incorporated into amphiphilic copolymers to render them susceptible to light triggering. All these copolymers contain PEG as hydrophilic block and poly-methacrylate (PMA) derivatives as hydrophobic block. After UV irradiation, the PMA block turned into water-soluble polymethacrylic acid and the encapsulated targets were released. Even though great improvements have been achieved with photolabile copolymers for micelle formation, more photoresponsive particle systems are in need. Miniemulsion polymerization has been widely used to synthesize polymeric particles which target molecules can be encapsulated during sonication-assisted miniemusification(36). We are interested in synthesizing cross-linked photoresponsive polymeric particles for target incorporation through mimiemulsion polymerization and phototriggered target releasing.

Here, we report the syntheses of photoresponsive polymeric particles, their photoswitches from hydrophobic to hydrophilic internal environments, and the controllable release of model dyes from these particles. Different from previous works by other labs and ours which focused on self-assembly of photolabile linear polymers or photolabile PEG/PMMA block copolymers using photocleavable cross-linkers, this study illustrated cross-linked particles formed by miniemulsion polymerization of photolabile monomers with nonphotolabile cross-linkers. With cross-linkers inside emulsions, more rigid solid particles will be formed. Internal hydrophobic cores of particles are filled with hydrophobic molecules in their initial state. Upon light irradiation, the transformation from a hydrophobic environment to hydrophilic one allowed the penetration of water and the release of encapsulated molecules, as shown in Fig. 1. To develop such photoresponsive particles, we prepared hydrophobic methacrylate monomers in which the hydrophilic acid groups were temporally protected with photolabile groups.

MATERIALS AND METHODS

All commercial solvents and reagents were used without further purification except as noted below. Tetrahydrofuran (THF), dichloromethane

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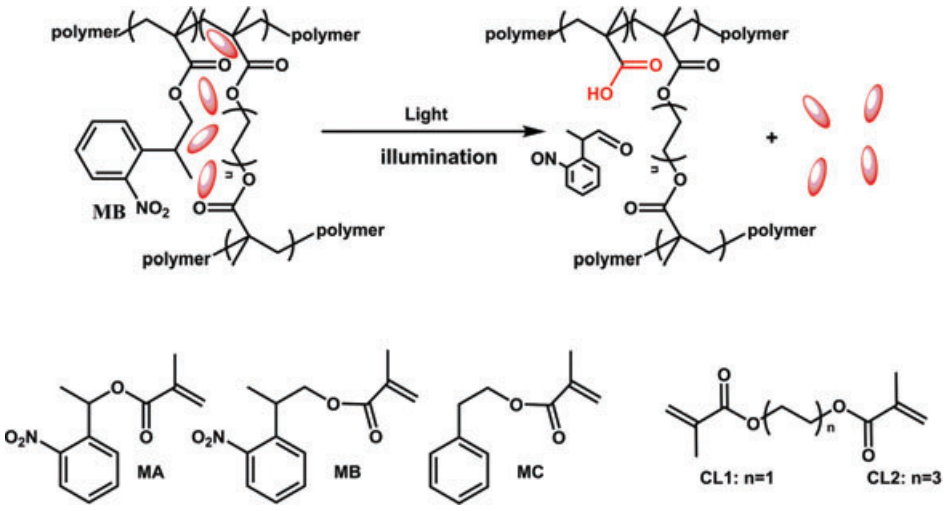

Figure 1. Strategy of controllable release from photoresponsive particles, and the structures of photolabile (MA and MB), nonphotolabile (MC) monomers and cross-linkers (CL1 and CL2) for particles synthesized through miniemulsion polymerization.

(DCM) and triethylamine (TEA) were distilled over CaH_2. Nile Red (NR) was purchased from J&K Chemicals. Fluorescein diacetate (FDA), Triton B (40% in methanol) and cross-linkers (Ethylene glycol dimethacrylate [CL1] and hexamethylene glycol dimethacrylate [CL2]) were purchased from Alfa Aesar. Methacryloyl chloride was purchased from TCI Shanghai. Hexadecane was purchased from Haltermann in Germany. sodium dodecyl sulfate (SDS) was purchased from Beijing Dingguo Biotechnology Co., Ltd. Azobisisobutyronitrile (AIBN) was purchased from Shanghai Trial of Four Hervey Chemical Co., Ltd.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Reported chemical shifts (ppm) are relative to $CDCl_3$ and coupling constants are reported in Hz. The size of nanoparticles in diameter was determined through dynamic light scattering (DLS) (Cumulant method) using the Zetasizer Nana-ZS from Malvern Instruments at 25°C. UV/Vis spectra were performed on DU800 UV/Vis Spectrophotometer (Beckman Coulter, Inc.). Fluorescence spectra were obtained using Cary Eclipse spectrofluorometer (Varian). An excitation wavelength of 550 nm was used for NR and the emission spectra were recorded from 570 and 750 nm. Scanning electron microscope (SEM, Hitachi S-4300) and transmission electron microscope (TEM) (JEM-2010; JEOL Ltd) were used to observe the particles. Typically, one drop of diluted nanoparticle solution was cast on either silicon wafer (for SEM) or carbon-coated copper grid (for TEM), and dried at 40°C. Nile red-loaded particles were visualized with Zeiss Upright Fluorescence Microscope (Axio Imager A2). And toxicity of particles was evaluated by MTT assay after incubation with blank particles for 48 h. FDA release in Raw 264.7 was detected with FlexStation 3 (Molecular Devices). Particles uptake and fluorescence images of cells were obtained using Invert Fluorescence Microscope (OLYMPUS, IX81).

*Synthesis of 1-(2-nitrophenyl)ethanol (**2**) (Scheme S1).* Compound **2** was synthesized according to a literature procedure (37) with minor modification. Aqueous solution of sodium borohydride ($NaBH_4$) (34.4 mL 10%) was added dropwise to the solution of 1-(2-nitrophenyl) ethanone (**1**) (5 g, 30 mmol) in 30 mL 1, 4-dioxane at 0°C. The mixture was stirred in an ice bath for 1 h and at room temperature for an additional 30 min. Then, excess $NaBH_4$ was quenched with 20 mL acetone. After the removal of the solvents, the residue was dissolved in water and extracted with ethyl acetate, and dried over anhydrous sodium sulfate. After the removal of solvents, the obtained light yellow oil was used in next step without further purification. Compound **2**. Yield 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 5.44 (q, J = 6.3 Hz, 1H), 1.59 (d, J = 6.4 Hz, 3H). ^{13}C NMR δ (100 MHz, $CDCl_3$): 147.9 ($C(NO_2)$), 140.9 (Ar, C), 133.6 (Ar, C), 128.1 (Ar, C), 127.6 (Ar, C), 124.3 (Ar, C), 65.5 (CH), 24.2 (CH_3).

*Synthesis of (2 -nitrophenyl)propanol (**4**) (Scheme S1).* Compound **4** was synthesized according to a literature procedure with minor modification. (38) Triton B (40% in methanol, 27 g, 66 mmol) was added to nitroethyl benzene **3** (10 g, 66 mmol). Paraformaldehyde (2 g, 67 mmol) was added to above mixture and purged with nitrogen. The solution was then heated to 60°C for 6 h. The solution was concentrated in vacuo and neutralized with 5% aqueous HCl followed by the extraction with ethyl acetate, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 2:8) to afford 4 (10 g, 84.4%). Compound 4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.1 Hz, 1H), 7.57 (t, J = 7.5 Hz, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.50 (dd, J = 13.5, 6.8 Hz, 1H), 1.88 (s, 1H), 1.32 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ150.7 ($C(NO_2)$), 138.1 (Ar, C), 132.6 (Ar, C), 128.2 (Ar, C), 127.2 (Ar, C), 124.1 (Ar, C), 67.8 (CH_2), 36.4 (CH), 17.5 (CH_3).

Synthesis of 1-(2-nitrophenyl)ethyl methacrylate (MA), 2-(2-nitrophenyl)propyl methacrylate (MB), phenethyl methacrylate (MC) (Scheme S1). Compounds **2, 4, 5** (phenethyl alcohol) (60 mmol) was dissolved in dry DCM (30 ml), dry triethylamine (10 mL, 71.8 mmol) was added, then purged with nitrogen. The mixture was allowed to stir in an ice bath for 5 min. Methacryloyl chloride (7.2 mL, 73.7 mmol) was slowly added over a period of 10 min. The reaction mixture was then warmed to room temperature while stirring overnight. The reaction mixture was then washed three times with saturated $NaHCO_3$ and once with brine. The resulting organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 15:1) to afford MA, MB, MC as light yellow oil.

Compound MA. Yield: 71.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.3 Hz, 1H), 7.65-7.59 (m, 2H), 7.45-7.39 (m, 1H), 6.38 (q, J = 6.4 Hz, 1H), 6.17 (s, 1H), 5.60 (s, 1H), 1.94 (s, 3H), 1.69 (d, J = 6.5 Hz, 3H). ^{13}C NMR δ (100 MHz, $CDCl_3$): δ=166.2 (C=O), 147.7 ($C(NO_2)$), 136.1 (C=), 126.0 (CH_2=), 138.1 (Ar, C), 133.6 (Ar, C), 128.3 (Ar, C), 127.1 (Ar, C), 124.5 (Ar, C), 68.4 (CH), 22.0 (CH_3), 18.2(CH_3). MS [M + Na]: calculated: 258.08, found: 258.10.

Compound MB. Yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.1 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.40-7.33 (m, 1H), 6.00 (s, 1H), 5.51 (s, 1H), 4.31 (d, J = 6.8 Hz, 2H), 3.75 (dd, J = 13.7, 6.9 Hz, 1H), 1.87 (s, 3H), 1.39 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1 (C=O), 150.2 ($C(NO_2)$), 135.9 (C=), 125.8 (CH_2=), 137.4 (Ar, C), 132.6 (Ar, C), 128.2 (Ar, C), 127.4 (Ar, C), 124.1 (Ar, C), 68.5 (CH_2), 33.1 (CH), 18.1 (CH_3), 17.9 (CH_3). MS [M + Na]: calculated: 272.10, found: 272.15.

Compound MC. Yield: 93.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 6.6 Hz, 2H), 7.22 (d, J = 7.2 Hz, 3H), 6.07 (s, 1H), 5.52 (s, 1H), 4.35 (s, 2H), 2.97 (t, J = 7.0 Hz, 2H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3 (C=O), 136.4 (C=), 126.5 (CH_2=), 138.0 (Ar, C), 128.9 (Ar, 2C), 128.5 (Ar, 2C), 125.4 (Ar, C), 65.2 (CH_2), 35.2 (CH_2), 18.3 (CH_3).

Preparation of Nile red/FDA-loaded particles and blank particles. Particles were prepared through miniemulsion polymerization method.

In a typical run, 50 mg of monomers, varied amounts of cross-linkers (1%, 5%, 10%, 15%, 25% of the molecular of the monomers), 0.5 mg Nile red or 4 mg FDA, 10 μL 5% (w/w) AIBN in CH_2Cl_2 and 3.1 mg costabilizer Hexadecane (for some cases) were dissolved in 500 μL CH_2Cl_2. A quantity of 5 mL distilled water (for FDA loading, sodium acetate buffer, pH = 4, was used due to the stability of FDA at 80°C) containing 5 mg SDS was purged with bubbling nitrogen for 10 min, and was then added to oil phase. This mixture was then sonicated in an ice bath for 5 min (3 s pulses with 1 s delay) with 80 W of the power. Following sonication, the mixture was transferred to 5 mL sealed container having been purged with nitrogen. The polymerization was performed at 80°C oil bath for 18 h. TLC confirmed the disappearance of monomers. After polymerization, the suspension was stirred overnight, which allowing CH_2Cl_2 to evaporate. The aqueous solutions were then dialyzed with a dialysis membrane (8000–14000 MWCO) against dialysis buffer (water with 1% DMSO) for 2 days with the exchange dialysis buffer every 4 h to remove excess surfactants and other residues, then were further dialyzed against pure water for 1 day with the exchange of water every 4 h to remove DMSO.

Nanoparticle Characterization and Degradation Study. Nanoparticles were characterized by SEM using Nanoscope IIIa. The particles were dispersed in water and then 5 μL solution was dripped onto a silicon wafer. After drying 2 h at 35°C, particles were sputter coated with aurum. Size distribution of particles was determined by DLS using Zetasizer Nano ZS.

Cell culture. Macrophage cell line, RAW 264.7 was grown in Dulbecco's modified Eagle cell culture medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 100 IU/mL Penicillin-Streptomycin in an atmosphere of 5% CO_2 and 95% relative humidity. The cells were routinely at 80–95% coverage.

FDA-loaded particle uptake and release in cell study. FDA is a nonfluorescent hydrophobic fluorescein derivative that can pass through cell membrane, where intracellular esterases hydrolyze ester groups, producing the highly fluorescent product, fluorescein. In our study, to investigate particle uptake by macrophages and triggered release in cells, FDA was mixed with monomers during microemulsion polymerization. These particles were then dialyzed for 2 days with 1% DMSO water solution and then with dialyzed with water.

Raw264.7 cells were incubated with 31.3 μg/mL FDA-loaded particles of MA, MB, MC (MA-CL110%-FDA, MB-CL110%-FDA, MC-CL110%-FDA) for 5 h. After incubation, cells were thoroughly washed with PBS buffer and were irradiated with 365 nm UV light for 10 min. Fluorescence images were taken using inverted fluorescence microscope after 2 h incubation of irradiated or nonirradiated cells. Fluorescence images of the cells incubated with FDA-loaded particles either with or without light irradiation were then taken with reverted fluorescence microscope.

Cellular toxicity of particles. Blank nanoparticles synthesized from MA, MB and MC were thoroughly dried under vacuum to remove methylene chloride inside the core of particles and resuspended in PBS buffer. Tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used for the assess of cell viability. Briefly, 5×10^4 RAW 264.7 cells were seeded in 96-well flat bottom plates (Costar, Corning, NY) and allowed to grow for 8 h. The cells were incubated with increasing concentrations of MA and MB particles or irradiated residues for 48 h. Then, 10 μL MTT (5 mg/ml in PBS) was added and incubated for another 4 h. MTT-formazan crystals formed were then dissolved in 150 μL three linked lysis solution (10% SDS/5% isobutyl alcohol/0.01 M HCl) overnight. The absorbance was measured on microplate reader at 570 nm. The cell viability (%) was determined by comparing the absorbance at 570 nm with control wells containing only cell culture media.

RESULTS AND DISCUSSION

Synthesis and Characterization of Photolabile Polymeric Nanoparticles

Photolabile monomers MA, MB and MC were synthesized by methacrylation of the corresponding 2-nitrobenzyl alcohol derivative moieties using methacryloyl chloride in dry THF and TEA, as shown in Figure S1. The emulsification were carried out with the cross-linkers (CL1 or CL2) and monomers MA, MB and MC according to reported methods.(39,40) The particles were synthesized with the initiation of AIBN and were further purified by dialysis against pure water for 2 days with alternative water replacement to remove excess surfactants. The precipitates were collected after high speed centrifuge and stored for further uses. The synthesized polymeric particles were characterized by IR as showed in Fig. 2. Strong peaks for the N-C bond at 1529 or 1527 cm^{-1} were observed in particles synthesized from MA and MB but not from MC. These specific peaks are observed in their corresponding monomers. Particle size and shape were then determined by DLS, SEM and TEM techniques. Typical results are shown in Fig. 3a and b. DLS results show that the sizes of the particles formed from monomer A and B are approximately 150–200 nm in diameter for all compositions with the cross-linker CL1 (Table S1 and Figure S1). The size of particles with the nonphotolabile monomer C and 10% cross-linker CL1 was 168 nm in diameter which is also similar to above photoresponsive particles. With the addition of hexadecane in miniemulsion, the more compact particles were formed with smaller sizes around 135–150 nm in diameter by DLS (Table S1). SEM images also show the spherical shape with particle diameter around 100–250 nm in diameter for particles synthesized from monomer A, monomer B and monomer C with different compositions (Figure S2). To further confirm the formation of particles instead of vesicles, results from TEM show that particles instead of micelles were formed from both monomer A and monomer B. (Figure S3) Nile red, a model hydrophobic guest molecule, was encapsulated inside hydrophobic particles during miniemulsion and following polymerization. The dye has higher solubility and displays stronger fluorescence in the hydrophobic environment, while its fluorescence is much weaker when it is released into water.(32) Since Nile red was encapsulated into the particles, we also characterized Nile red encapsulation by imaging particles (MA-CL1-10%-NR) with fluorescence microscope. As shown in Fig. 3c, red fluorescence spots from the particles encapsulated Nile red were clearly visible.

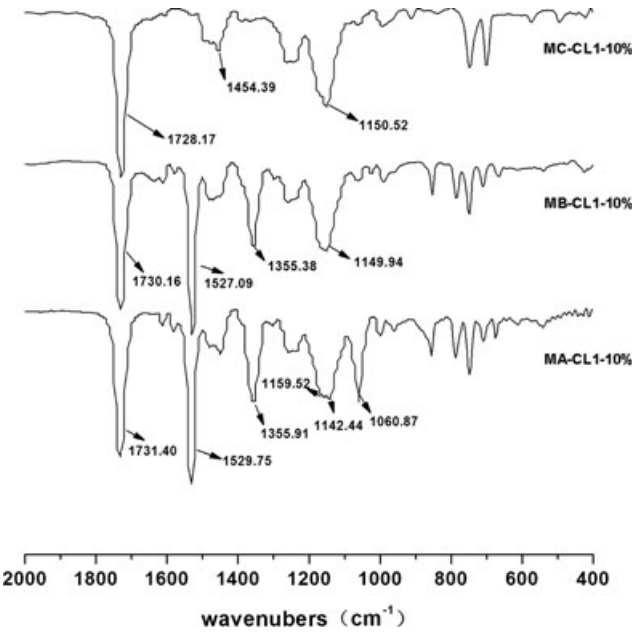

Figure 2. IR spectra of nanoparticle MA-CL1-10%, MB-CL1-10% and MC-CL1-10%. Strong peaks for C-N at 1529 cm^{-1} or 1527 cm^{-1} were observed in particles MA-CL1-10% and MB-CL1-10%, but not MC-CL1-10%.

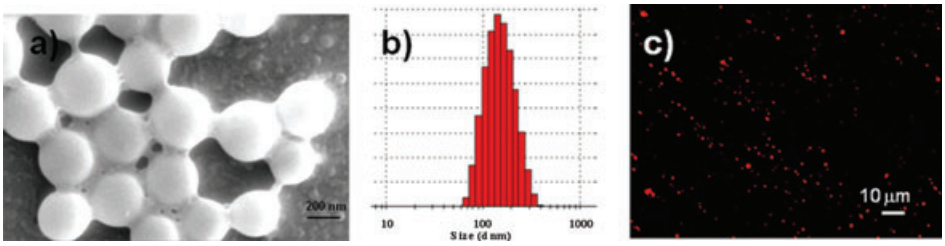

Figure 3. Characterization of photoresponsive particle MB-CL1-10%. (a) Image of scanning electron micrograph (SEM); (b) Particle size distribution by dynamic light scattering (DLS); (c) Fluorescence microscopy of Nile red-encapsulated particles MB-CL1-10%-NR.

The stability of synthetic particles in water was verified by fluorescence intensity of Nile red in particle aqueous solutions. We monitored fluorescence intensity at 609 nm of Nile red encapsulated particle solutions (0.5 mg/mL) with MB and 1%, 4% and 25% of a cross-linker (CL1) at 0, 2 and 4 days. Variations in fluorescence intensity were below 15% of initial intensities. With gentle vortexing, fluorescence intensity of the particle solutions was almost recovered. For particles (MB-CL1-25%-NR) with high percentages of the cross-linker (25% CL1), fluorescence intensity of the particle solution almost did not change in 4 days (Figure S4). These results showed that no obvious leak of the encapsulated Nile red happened.

Photoresponsive Behaviors of Nanoparticles

An essential feature of these particle delivery vehicles is the triggered release in response to external light. We tested the possibility of uncaging these photoresponsive particles under our photolysis conditions (365 nm, 11 mW cm^{-2}), as shown in Fig. 4 and Figure S5. Figure 4 demonstrated the absorption spectrum changes in particles (MA-CL1-10% and MB–CL1-10%) formed with MA or MB monomers with 10% CL1 after the irradiation of these particle aqueous solutions (0.5 mg/mL). The presence of isosbestic points at 332 and 275 nm indicates a clean conversion from esters to carbonyl moieties for MA and MB. The process of the photolysis was followed by noting the appearance of 1-(2-nitrosophenyl) ethanone and 2-(2-nitrosophenyl)propanal, as indicated by absorption peaks at 290 and 310 nm for particles MA-CL1-10% and 290 and 318 nm for particles MB–CL1-10%. The spectral changes in particle solutions were consistent to the changes of their monomers, respectively, and the literature report of similar photolabile moieties,(41) which verified the uncaging efficacy of particles as monomers under our photolysis conditions. IR spectra also indicated that light irradiation caused disappearance of C-NO_2 vibration signal at 1529.75 cm^{-1} for particle MA-CL1-10% (Figure S6). These observations confirmed the transformation of hydrophobic esters to hydrophilic acids inside the polymers, as indicated in the uncaging mechanism in previous report.

To further evaluate the photocontrolled release, light-triggered transformation of hydrophobic to hydrophilic environment inside particles was investigated by Nile red fluorescence upon exposure to light (365 nm). Fluorescence emission is used to characterize the efficiency of the triggered release due to its dramatic change in fluorescence intensity during hydrophobic and hydrophilic transformation. Figure 5 shows light triggered the changes in Nile red fluorescence spectra of aqueous particle (MB-CL1-10%-NR and MC-CL1-10%-NR) solutions (0.5 mg/mL) synthesized with 10% cross-linker CL1 and the photolabile MB or

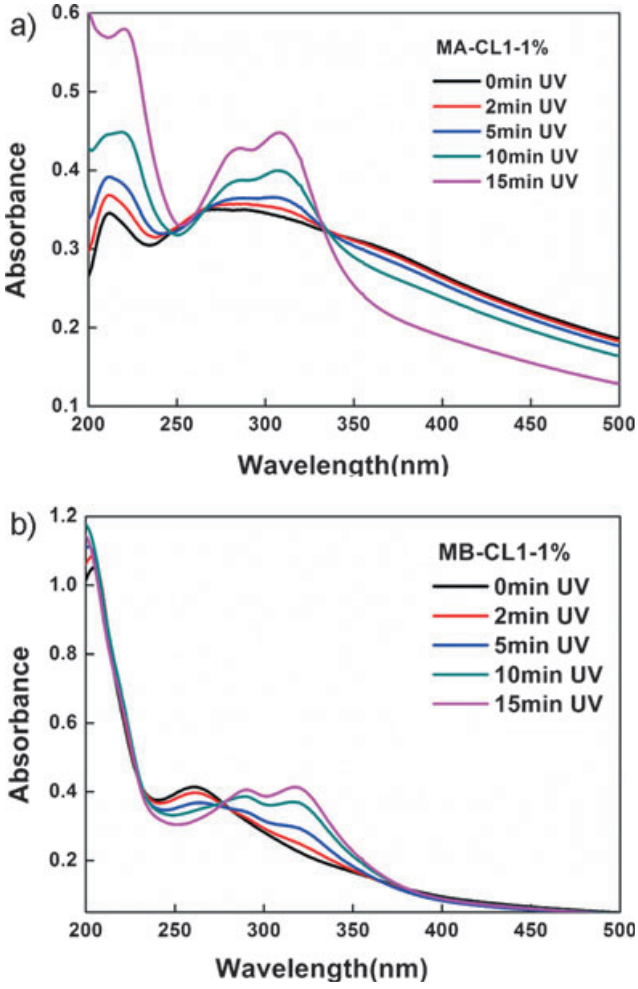

Figure 4. Absorbance spectra variation in blank polymeric particles MA-CL1-10% and MB-CL1-10% upon photolysis at 365 nm (11 mW/cm^2). The concentrations of particle solutions were 0.5 mg/mL.

nonphotolabile MC. As we can see, there was only slightly fluorescence intensity variation in 15 min light irradiation for particles MC-CL1-10%-NR (Figure S7). However, dramatic decrease in fluorescence intensity was observed for photoresponsive particles MB-CL1-10%-NR, and the remaining fluorescence intensity of the solution reached as low as 5% of the initial fluorescence intensity. The fluorescence intensity was not recovered in couple of hours due to the precipitation of Nile red. These results demonstrated that the efficient transformation of hydrophobic to hydrophilic structure of photoresponsive particles under mild UV irradiation. Particles synthesized with different percentages of the

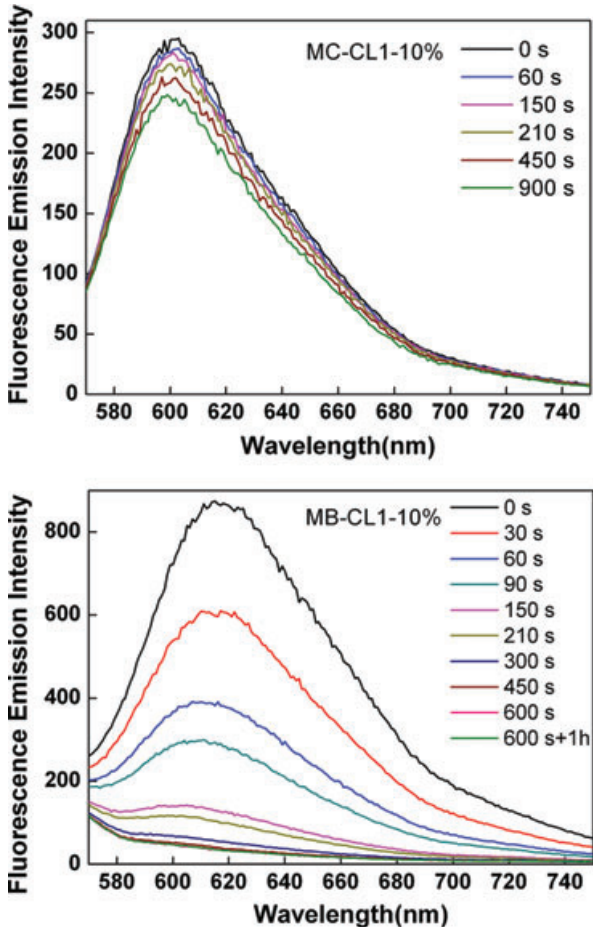

Figure 5. Fluorescence spectra of particles encapsulated with Nile red upon light irradiation (365 nm, 11 mW/cm^2) with the excitation wavelength at 550 nm. (a) particle MC-CL1-10%; (b) particle MB-CL1-10%. The concentrations of particle solutions were 0.5 mg/mL.

cross-linker CL1 (1%, 5%, 10%, 15% and 25%) to MA and MB, and their effects on photocontrolled release were also investigated. As shown in Fig. 6, normalized fluorescence intensity variation in particles (MB-CL1-1/5/10/15/25%) was recorded as a function of different irradiation time in aqueous solutions (0.5 mg/mL). For all the compositions of particles MB-CL1, the phototriggered fluorescence decrease was clearly observed and the release efficiency was around 85–95% in less than 8 min. Similar observations also happened for the photoresponsive particles MA-CL1 (Figure S8). When 10% cross-linker CL2 was used instead of CL1 to miniemulsion polymerization of MB, Nile red fluorescence intensity of aqueous particle (MB-CL2-10%) solution also greatly decreased and release efficiencies could also reach up to 95% in 15 min UV irradiation (Figure S9). The initial rate of decrease in Nile red fluorescence for particle MB-CL1-10% solution was large than that of particle MB-CL2-10% solution. This difference may be due to some small size of particle MB-CL1-10% or longer hydrophibic linker of CL2. If hexadecane was added during miniemulsion, it did not influence the sensitivity of photolabile particles, and the encapsulated Nile red could also be efficiently released upon light irradiation. In 10 min, release efficiencies were also around 90–95% for nanoparticle formed with MB and CL1. These experimental data demonstrated that the photocleavage of photolabile moieties

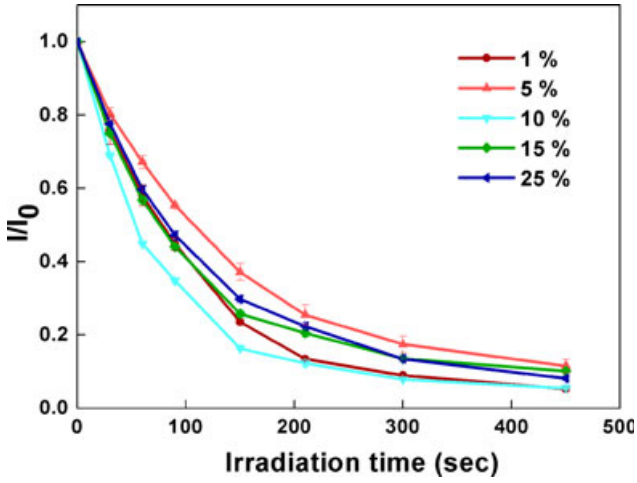

Figure 6. Plots of normalized fluorescence intensity of Nile red at 609 nm *vs* irradiation time (365 nm, 11 mW/cm^2) for aqueous solutions of photoresponsive particles MB-CL1 with different percentages of the cross-linker (1%, 5%, 10%, 15% and 25%). The concentrations of particle solutions were 0.5 mg/mL.

was efficient and the encapsulated contents were efficiently released from these photoresponsive polymeric particle systems under our photolysis conditions.

The effect of hydrophobic cores of MA-CL1-10%-NR and MB-CL1-10%-NR on kinetics of triggered release of Nile red was also studied. Under the same photolysis conditions, NR fluorescence intensity was recorded with the increase of UV irradiation time. Figure S10 shows that the normalized fluorescence intensity for particle solutions of MA-CL1-10%-NR and MB-CL1-10%-NR was an exponential change with irradiation time. To illuminate the difference, the characteristics time (τ) was introduced by fitting the data through the following equation(32):

$$I/I_0 = (I/I_0)_m + [1 - (I/I_0)_m]\exp(-t/\tau)$$

Here, $(I/I_0)_m$ is the achievable minimum of the normalized fluorescence, and τ is the characteristic time at which the fluorescence intensity decreases to 36.8% (1/e). The curve fitting results displayed τ for polymeric particle MB-CL1-10%-NR ($\tau_{MB\text{-}Nile\ red}$ = 72 s) were much shorter than that for particle MA-CL1-10%-NR ($\tau_{MA\text{-}Nile\ red}$ = 273 s), which shows that the photocleavage rate of MB was faster than that of MA. These results are consistent to the releasing efficiencies of Nile red and the previous report for photo-uncaging of these two photolabile moieties.(42)

The uncaging and crash of photolabile particles were also clearly observed by SEM, fluorescence microscopy and colorimetry. Figure 7a and b show typical SEM images of nanoparticle MB-CL1-1%. Before light irradiation, particles were in spherical shapes in SEM images. However, most spherical particles crashed or melted due to light irradiation. Similar observations also happened for the other photoresponsive particles synthesized from MA and MB, but not from MC. The color variation in aqueous particle (MA-CL1-10%) solutions was also observed before and after light irradiation, as shown in Fig. 7c–e. These aqueous particle (MB-CL1-1/4/25%) solutions remained as a red opaque suspension under ambient conditions even after 4-day standing if no light irradiation was applied (Fig. 7c). In comparison, the red

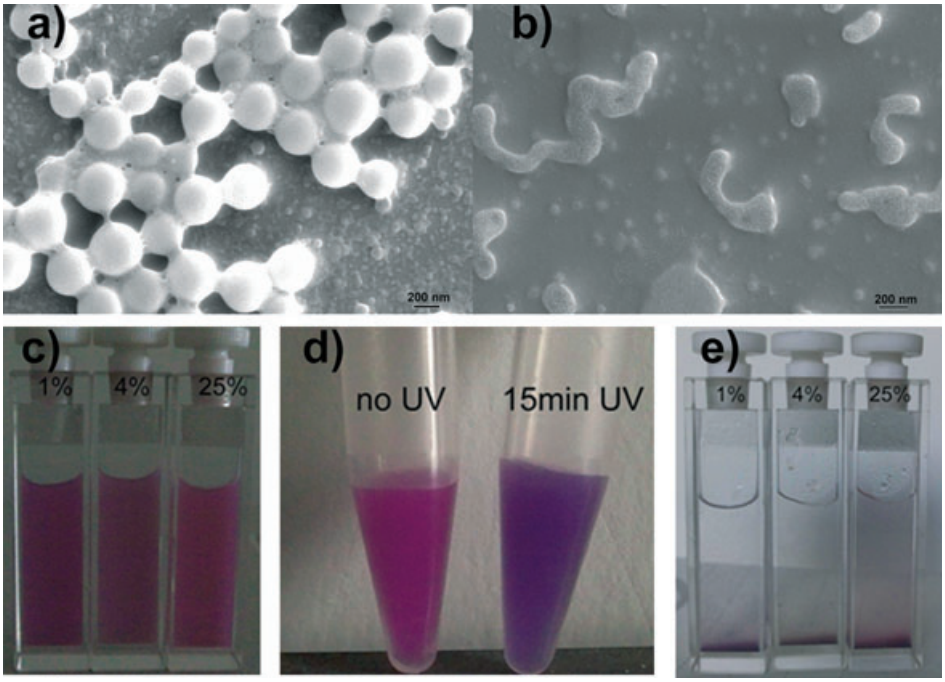

Figure 7. SEM images of photoresponsive particles MB-CL1-1% before light irradiation (a) and 15 min light irradiation with 365 nm (b); (c) a typical photo image of photoresponsive particle (MB-CL1) aqueous solutions with different percentages of cross-linker CL1 stored in the dark for 4 days; (d) photo images of photoresponsive particle (MA-CL1-10%) aqueous solution before and after 15 min irradiation with 365 nm; (e) a typical photo image of irradiated photoresponsive particle (MB-CL1) aqueous solutions with different percentages of cross-linker CL1 after 4 days. The concentrations of particle solutions were 0.5 mg/mL.

opaque suspension turned to purple red suspension with 15 min light irradiation (Fig. 7d), and purple Nile red solid gradually precipitated at the bottom of cuvettes. Figure 7e clearly shows that most Nile red solid was at the bottom and aqueous solution became transparent and almost colorless. Similar phenomena were also observed using fluorescence microscopy (Figure S11). Light irradiation could greatly reduce the fluorescence spots in the images for particles MA-CL1-10%-NR and MB-CL1-10%-NR, while lots of Nile red fluorescence spots still exist for particles MC-CL1-10%-NR after light irradiation. These results further confirmed the release of the encapsulated contents from internal particle cores upon light activation due to the breakage of these photoresponsive particles.

Nanoparticle Uptake and Photocontrolled Release In Cells

We further studied cell uptake and cell viability of photoresponsive polymeric particles synthesized from MA, MB and MC using RAW 264.7 macrophages. We used FDA-encapsulated particles (MA-CL1-10%-FDA and MB-CL1-10%-FDA) to evaluate particle cell uptake and phototriggered release in cells. FDA is nonfluorescent, upon uptake by the cells, intracellular esterases hydrolyze diacetate groups, producing the highly fluorescent product, fluorescein. Uptake and release of FDA from above particles were confirmed by fluorescence of the hydrolyzed fluorescein (Fig. 8 and Figure S12). Figure 8a–b and c–d are the images of cells incubated with MC-CL1-10%-FDA before and after light activation. No obvious fluorescence was observed which indicated FDA could not be released from the nonphotolabile nanoparticles even with light activation. Figure 8e and f are images of cells with the uptake of MA-CL1-10%-FDA without light activation. As we expected, these nanoparticles were stable in cells and there are almost no fluorescence emission. However, upon light activation, the cells clearly lighted up with the hydrolyzed FDA, as shown in Fig. 8g and h. In comparison to MC-CL1-10%-FDA, MA-CL1-10%-FDA must have been photodegraded and the breakage of polymers triggers the release of FDA, followed by the hydrolysis of FDA with intracellular esterase.

To evaluate the toxicity of these particles themselves, macrophages were incubated with varying concentrations (0–1000 μg/mL) of blank particles prepared from monomer A, B and C with 10% CL1 for 48 h. As seen in Fig. 9, the results of cell viabilities showed low toxicity at the concentration as high as 1000 μg/mL of aqueous particle (MA-CL1-10%, MB-CL1-10% and MC-CL1-10%) solutions. Upon irradiation, cell viabilities did not decrease at low concentration of particle solutions. However, cell toxicity increased greatly at high concentration for particle MA-CL1-10% and MB-CL1-10%, but not for MC-CL1-10%. We then tested cell viability of a micelle self-assembled with a diblock copolymer (PEG5000-$(MA)_{33}$). We observed low toxicity of the above micelle before light irradiation, while their toxicity increased at high concentration (1000 μg/mL) upon light irradiation, which is similar to the observation for nanoparticle MA-CL1-10% and MB-CL1-10%. (Figure S13) We postulated that the cleavaged nitrosobenzyl byproducts lead to the high toxicity. We then evaluated cell viability of these small organic compounds to RAW246.7 according to the same experimental procedure as particle solutions. High level of toxicity of these small molecules was observed before or after light irradiation even at relative low concentrations, as shown in Figure S13.

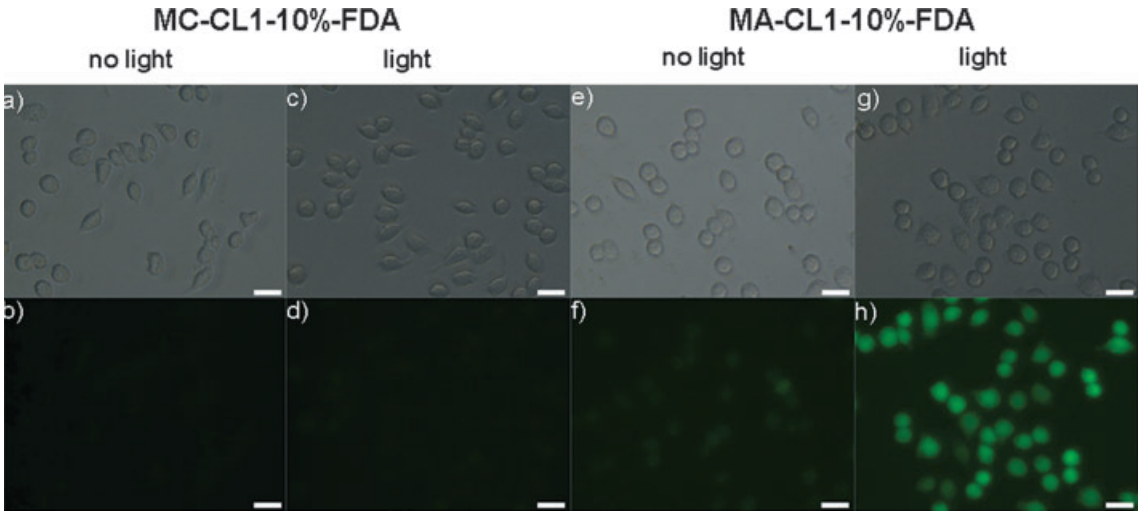

Figure 8. Fluorescence microscopy image of RAW264.7 cells incubated with MA-CL1-10% and MC-CL1-10% before or after 10 min light irradiation. (a,b) Particle MC-CL1-10%-FDA without light irradiation; (c,d) Particle MC-CL1-10%-FDA with 10 min light irradiation; (e,f) Particle MA-CL1-10%-FDA without light irradiation; (g,h) Particle MA-CL1-10%-FDA with 10 min light irradiation. The concentration of nanoparticles was 31.3 μg/mL. After particle incubation for 5 h at 37°C, the cells were thoroughly washed with PBS to remove the free nanoparticles. The cells were irradiated with UV lamp (365 nm, 11 mW/cm^2) for 10 min, then observed by inverted fluorescence microscope. All fluorescence images were taken under the same conditions.

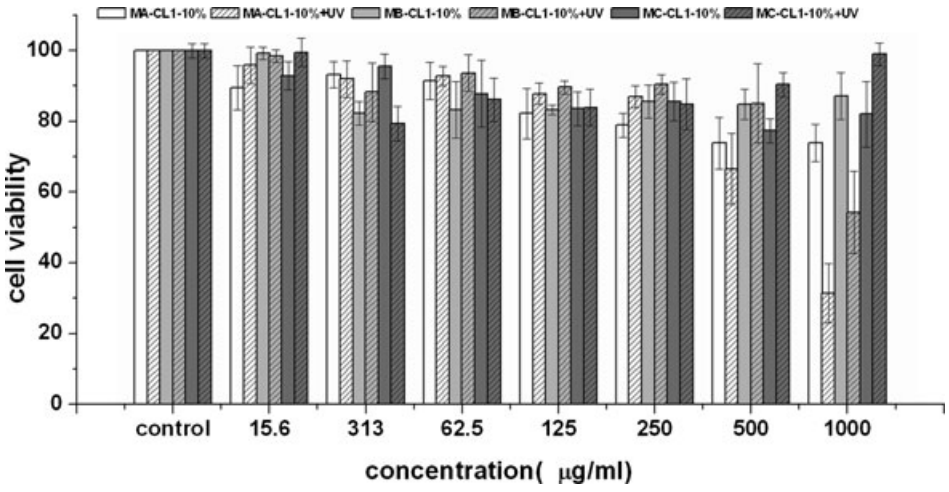

Figure 9. Toxicity of particles (MA-CL1-10%, MB-CL1-10% and MC-CL1-10% and irradiated residues) measured by incubation with macrophages for 48 h. The viability of cells incubated with particles was normalized to cells cultured without particles.

CONCLUSIONS

In summary, a series of cross-linked photoresponsive polymeric particles with different photolabile monomers and cross-linkers have been synthesized through miniemulsion polymerization and have been characterized through DLS, SEM, TEM and fluorescence microscopy. The synthesized particles were quite stable in aqueous solutions, while light irradiation caused the crash of these particles and the efficient release of encapsulated contents. Releasing efficiency could reach up to 95% based on fluorescence intensity of Nile red. Photoswitches of photoresponsive particle systems were confirmed by fluorescence spectroscopy, SEM and colorimetry. Particle cell uptake and triggered release in RAW264.7 cells were confirmed by Nile red-loaded particles and FDA-loaded particles. The cell viability of particles shows low toxicity of blank particle and particles MA-CL1-10%, MB-CL1-10% and MC-CL1-10% for macrophages at high particle concentration after 48 h incubation. Increased toxicity was observed at high concentration of irradiated particles MA-CL1-10% and MB-CL1-10%, but not for MC-CL1-10%, which is due to the toxicity of the cleaved by-products. Further studies will focus on using less toxic photolabile monomer, such as coumarin derivatives, or using copolymers with methyl methacrylate monomer for syntheses of photoresponsive particles and their photocontrolled release.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Particle characterization, NMR, DLS, TEM, SEM, photolysis, particle stability, FDA release in cells and toxicity were all included in supplementary materials.

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