Intelligent Multi-functional Nano-Micelles for Cancer Therapy

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Abstract:

Multifunctional micelles for cancer cell targeting, distribution imaging, and anticancer drug delivery were prepared from poly(N-isopropyl acrylamide-co-methacryl acid)-g-poly(*D,L*-lactide) (P(NIPAAm-co-MAAc)-g-PLA), methoxy poly(ethylene glycol)-b-poly(*D,L*-lactide) (mPEG- PLA) and two functionalized diblock copolymers, galactosamine-PEG-PLA (Gal-PEG-PLA) and fluorescein isothiocyanate-PEG-PLA (FITC-PEG-PLA). Multifunctional micelles target specific tumors by an asialoglycoprotein-Gal receptor-mediated tumor targeting mechanism. This mechanism then causes intracellular pH changes which induce structural deformation of the graft copolymer inner core of multifunctional micelles and thereby increases HepG2 cell cytotoxicity by releasing doxorubicin (Dox). Confocal laser scanning microscopy (CLSM) reveals a clear distribution of multifunctional micelles.

Introduction:

This study presents a multifunctional micelle encapsulating Dox, an anticancer drug. The micelle was prepared by dialysis from a graft copolymer[1], a diblock copolymer and two functionalized diblock copolymers, as shown in Figure 1. This type of multifunctional micelle has several important advantages over other particulate drug delivery systems. These advantageous characteristics include the ability to overcome some limitations in ionic charge materials in drug delivery[2,3], easy dialysis-method preparation[2], better control of particle size and particle distribution for suitable physiological conditions, easy modification for displaying functionalities (e.g., specific tumor targeting or fluorescent imaging for micellar distribution)[4,5], stimulus-response behavior for drug release control, and the ability to use a single polymeric micelle for multiple purposes.

Experiments:

A graft copolymer poly(N-isopropyl acrylamide-co-methacryl acid)-g-poly(D,L-lactide) (P(NIPAAm-co-MAAc)9970-g-PLA6150, [NIPAAm]:[MAAc]:[PLA] = 91:6.3:2.7, with a polydispersity index (PDI) = 1.24, and a critical micelle concentration (cmc) = 1.3 mg/L), was synthesized by traditional free-radical copolymerization from the NIPAAm monomer, the MAAc monomer and the PLA-EMA (PLA with end-capped of ethyl methacrylated group, Mn 2030) macromonomer (Mn 2000) using 2,2'-azobisisobutyronitrile (AIBN) as an initiator.[2,3] Block I (mPEG5000-PLA530, PDI = 1.05, cmc = 84 mg/L), Block II (mPEG5000-PLA1088, PDI = 1.15, cmc = 16 mg/L) and Block III (mPEG5000-PLA1750, PDI = 1.20, cmc = 5.4 mg/L) copolymers were synthesized by ring-opening polymerization from methoxy poly(ethylene glycol) (mPEG, Mn 5000) and D,L-lactide using stannous octoate as a catalyst. These diblock copolymers have the same chemical nature, but differ in composition ratio. Two functional end-capped diblock copolymer galactosamine (Gal)-PEG3400-PLA830 (Gal-PEG-PLA, [Gal]:[PEG]:[LA] = 8.4.:7.6:84) and fluorescein isothiocyanate (FITC)- PEG3400-PLA830 (FITC-PEG-PLA, [FITC]:[PEG]:[LA] = 4:8:88) were synthesized by thiol-maleimide coupling reaction.

Results and Discussion:

Before preparing multifunctional micelles, two-component mixed micelles composed of a graft copolymer and a diblock copolymer (Block I, Block II or Block III) were employed to investigate the influence of chain length and cmc of the diblock copolymers on the morphology and structure of mixed micelles. First, a graft copolymer and a diblock copolymer were dissolved together. Mixed micelles were then prepared by dialysis against Milli-Q water using a cellulose membrane bag. The Milli-Q water was replaced every 3 h. The core-shell structure and particle size of three mixed micelles from a graft copolymer and a diblock copolymer were observed by TEM. The TEM process involves staining the methacrylic acid groups of the graft copolymers using uranyl acetate (2wt%). The transmission electron micrograph was taken on a Hitachi H-600 microscope at an accelerating voltage of 100 kV. TEM observation produced three results (Fig. 2). (1) For all mixed micelles, the dark region of the graft copolymer is the inner core. (2) The radius of the core region decreased as the chain length of PLA of diblock copolymer increased. (3) Mixed micelle particle size increased as the chain length of PLA of diblock copolymer increased. A short PLA length produces smaller mixed micelles.

The multifunctional micelle incorporated with Dox was also prepared using the dialysis method. Fifty mol% of graft copolymer, 20 mol% of BlockⅢ, 15 mol% of Gal-PEG-PLA, and 15 mol% of FITC-PEG-PLA were then dissolved in the drug solution. The mixture was dialyzed against Milli-Q water for 72 h using a membrane with a molecular-weight cut-off of 6000-8000 at room temperature. The Milli-Q water was replaced every 3 h. Multifunctional micelles were obtained by a freeze-drying process. The DOX loading level was about 31 wt% in weight. The multifunctional micelle particle size was approximately 160 nm. To evaluate the effects of stimulus-response behavior on controlled drug delivery, the in vitro drug release behaviors of multifunctional micelles were studied in two different buffered solutions (pH 7.4 and 5.0). Figure 3 shows results. In neutral surroundings (pH 7.4), multifunctional micelles exhibited initial burst effects, losing about 15 wt% at 37 ℃. Release behavior remained constant after 140 h. In acidic surroundings (pH 5.0), release behavior was obviously divided into two periods. A rapid release in the first period was followed by a sustained and slow release over a prolonged time, up to a hundred hours for physically-encapsulated intelligence drug carriers. The initial rapid release (35 wt%) was observed in the initial 2 h and followed by a sustained release for 140 h until reaching a 70 wt% release profile.

To evaluate the functionality of multifunctional micelles in biomarker applications, CLSM was used to observe the fluorescence images of multifunctional micelles and

released Dox after HeLa cells uptake. Figure 4 shows fluorescence images of multifunctional micelles incubated with HeLa cells for 6 h. HeLa cells showed green fluorescence in the cytoplasm, indicating that the multifunctional micelles were located there..

To evaluate the functionality of multifunctional micelles in specific tumor targeting, multifunctional micelles were incubated with HepG2 cells to confirm drug cytotoxicities. Because of their specific ligand-receptor binding, the internalization of multifunctional micelles into cancer cells can be performed by the receptor-mediated endocytosis process and delivered to the lysosomes. The viability (percentage of surviving cells) of HepG2 cells after 24 h and 48 h incubation was compared with multifunctional micelles. Figure 5(a) shows the effects of specific tumor targeting and nonspecific tumor targeting of Dox-loaded micelles on receptor-mediated endocytosis. Cell viability was assessed using a direct count of 0.2% trypan blue dye exclusion assay to observe the rate of growth inhibition caused by the released Dox. The multifunctional micelles had lower cell viabilities than those without Gal in either positive control or negative control. This is because the multifunctional micelles bound with asialoglycoprotein and then internalized into cancer cells to release Dox by intracellular pH changes. Additionally, at 37 ℃ the cell viability of all cells incubated with multifunctional micelles was lower than that of the cells incubated at 4 ℃, suggesting an endocytosis process and a large accumulation. The specific asialoglycoprotein-multifunctional micelle interactions were verified by an inhibition assay. The incubation of cells with 150 mM galactose completely abolished micelle cell binding and indicated sugar specificity of the process involved (Fig. 5(b)).

Conclusion:

Mixed micelles encapsulating Dox were successfully prepared by dialysis, producing multifunctionalities which can be used as cancer diagnosis agents and cancer drug delivery carriers. TEM images revealed that the multifunctional micelles are spherical in shape and about 160 nm in size, which is suitable for intravenous injection and close to the typically required size under physiological conditions. Tumor targeting assay and CLSM measurements revealed that multifunctional micelles exhibited a high cytotoxicity by receptor-mediated endocytosis and showed clear fluorescence imaging of their distribution. Our goal here was to show a proof-of-concept: that is, producing an ideal micelle with a long circulation time, tumor recognition, and combined cancer diagnosis and controlled drug delivery for cancer therapy.

References

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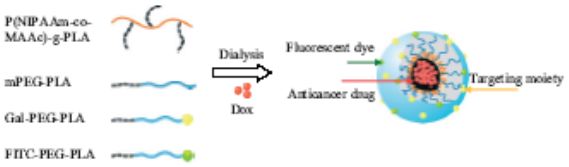

Figure 1. Schematic representation of multifunctional micelle structure made of a graft copolymer, a diblock copolymer and two functionalized diblock copolymers.

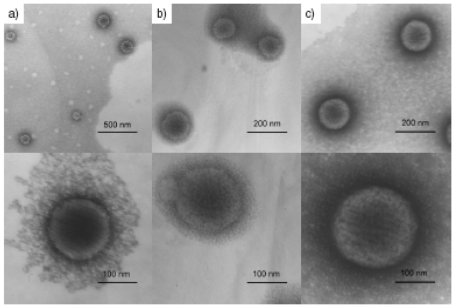

Figure 2. TEM images of mixed micelles formed from (a) 25 mol% of graft copolymer and 75 mol% of Block I, (b) 25 mol% of graft copolymer and 75 mol% of Block II and (c) 25 mol% of graft copolymer and 75 mol% of Block III.

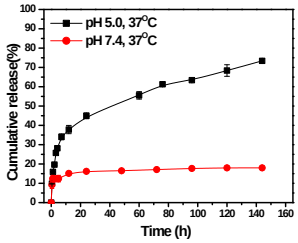

Figure 3. Release of Dox from multifunctional micelles under acidic (pH 5.0) and neutral (pH 7.4) conditions at 37 ℃. Mean ± sd (n=3).

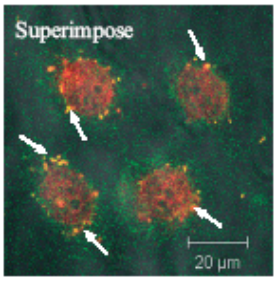

Figure 4. Confocal images of HeLa cells incubated with multifunctional micelles (50μg/mL) showing the particulate distribution and localization of released Dox.

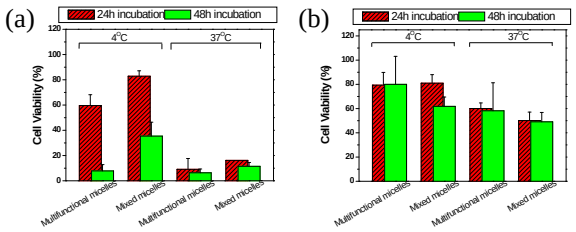

Figure 5. Multifunctional micelle cytotoxicity and mixed micelle cytotoxicity after incubation with (a) HepG2 cells and (b) HepG2 cells in the presence of 150 mM galactose for 24 h and 48 h.