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Hydrazone cross-linked micelles based on redox degradable block copolymer for enhanced stability and controlled drug release

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ABSTRACT

In this work, an amphiphilic copolymer, PCL-SS-P(PEGMA-*co*-MAEBA), which contained a disulfide joint in backbone was designed and synthesized. The subsequent micelles that self-assembled from the copolymers were cross-linked by hydrazone, resulting in novel stimuli-responsive degradable micelles with a reversible cross-linked shell. By way of the hydrazone cross-linking of the micellar shell, SCMs owned a good stability against the extensive dilution by water or organic solvent. Doxorubicin (DOX) was used as the model drug for studying the *in vitro* release profiles of the SCMs. In normal physiological conditions at pH 7.4, a quite slow speed was observed with DOX release (only 23% after 72 h); when conditions were changed to pH 5.0, the SCMs successfully de-crosslinked, DOX release was accelerated (62%). Moreover, drug release was further promoted and reached 87% when 10 mM GSH was present, which was primarily due to the breakage of the disulfide joint. The intracellular uptake assay proved that DOX from DOX-loaded SCMs could be efficiently delivered into HepG2 cells after 12 h incubation. MTT assays confirmed that DOX-loaded SCMs owned a high cytotoxicity against HepG2 cells. These redox-responsive, degradable SCMs could be a potential candidate for efficient insoluble anticancer drug delivery and therapy.

1. Introduction

Recently, polymeric architectures showed several advantages as chemotherapeutics delivery platforms for cancer therapy [1]. Polymeric vehicles, such as nanoparticles [2], nanogels [3], polymersomes [4] and micelles [5], have been widely used. Furthermore, polymeric micelles have drawn substantial attention because of their several key features, the solubilization for hydrophobic drugs, longer blood circulation, reduction of protein adsorption [6,7] and selectively accumulation in targeted tumor site by mean of the enhanced permeation and retention (EPR) effect [8,9]. All these advantages above make it an excellent chemotherapeutic carrier for cancer therapy in recent years. As an ideal drug delivery system, high drug delivery efficiency to tumor cells is one of the most important properties and requirement. However, the delivery efficiency of traditional micellar systems was usually low. Two of the major reasons were the leakage of drug molecules in bloodstream triggered by micelle disassembly and the slow drug release within cancer cells. How to conquer the two weaknesses and promote the drug delivery efficiency was a challenge for successful chemotherapy.

Micelle solution is extensively diluted when injected into the bloodstream, causing the architectures to dissociate or disintegrate, resulting in a premature drug leakage [5,10,11]. Chemical crosslinking of the outer shell (or inner core) structure of micelle has been shown to be an efficient strategy for maintaining the stability of micelles and minimizing the drug leakage in circulation [12–15]. Wooley et al. [16,17] and Armes et al. [18–20] have conducted pioneering studies related to the crosslinking process and effects respectively. However, every coin has two sides. Irreversible chemical crosslinking is also a barrier for drug molecules that induces limited and incomplete intracellular drug release at targeted sites [21,22].

To overcome the drawback of irreversibly cross-linked micelles, the so-called “stimuli-responsive reversible cross-linked micelles” (SCMs) concept was introduced [23]. The cross-linked structures containing dynamic covalent bonds in SCMs can be cleverly broken with specific stimulus, such as acid pH and a high-concentration reductant in tumor cells, or the stimulus (*e.g.*, light) may be imposed from outside the body [24–28]. SCMs have excellent stability during circulation in the bloodstream, while presenting fast de-crosslinking when the cross-linked structures break due to the specific stimulus. Hennink's group crosslinked an amphiphilic triblock polymer containing a ketone group, poly(*N*-(2-hydroxy propyl)methacrylamide)-*b*-poly(1-(acetonylamino)-2-methyl-2-propen-1-one)-*b*-poly(*N*-(2-benzoyloxypropyl)

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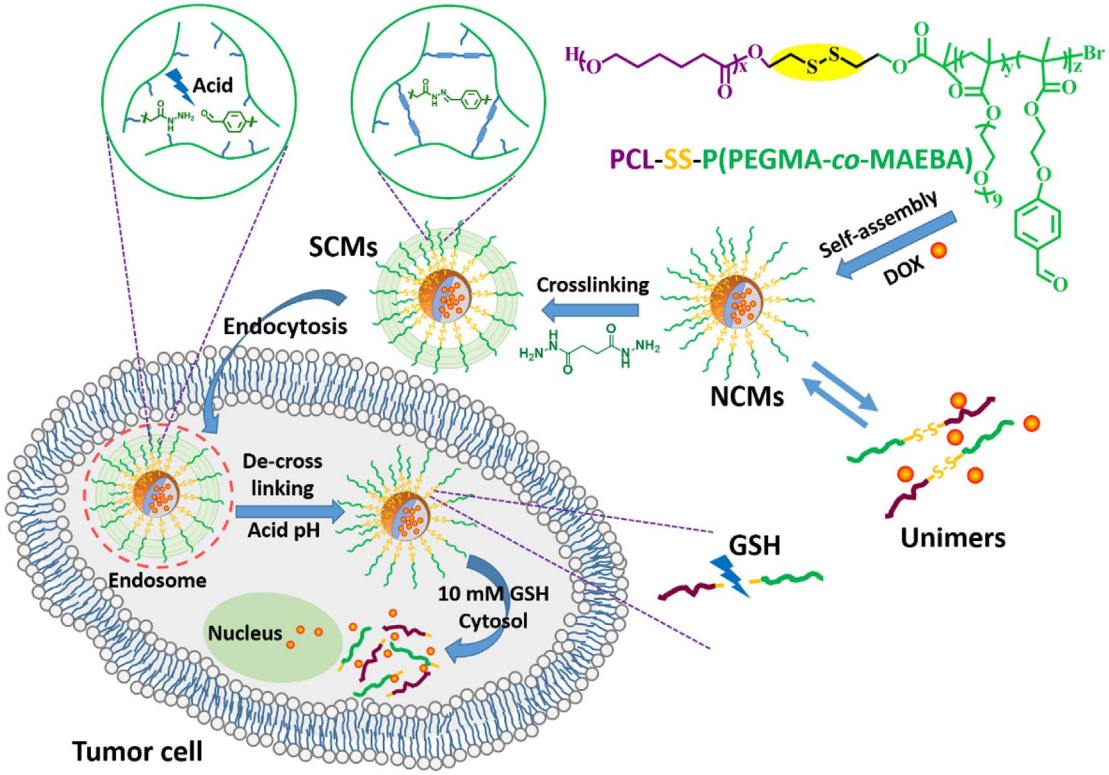

Scheme 1. Illustration for self-assembly of copolymer, drug entrapment and pH/redox-responsive release of SCMs.

methacrylamide-*co*-*N*-(2-hydroxypropyl) methacrylamide monolactate), p(HPMAm)-*b*-p(AMPO)-*b*-p(HPMAm-Bz-*co*-HPMAm-Lac), followed by self-assembly, and obtained SCMs with hydrazone bond-based cross-linked structures [15]. In the condition with pH 7.4, these SCMs showed good stability and an enhanced retention effect for release of the anticancer drug paclitaxel, while fast de-crosslinking occurred when it comes to pH 5.0, and the release of paclitaxel was efficiently accelerated. Liu's group prepared SCMs with a disulfide containing crosslinkage at the corona between the micellar core and shell [22]. This group found that the release of doxorubicin was slow with no reductant, while efficiently accelerated release occurred after adding 10 mM reductant GSH, which was primarily for breaking the disulfide bonds in the cross-linked structure.

Apart from good stability during blood circulation and fast de-crosslinking at targeted sites, the success of cancer chemotherapy for micellar carriers has also relied on fast release and high level accumulation of drug molecules within tumor cells, breaking through the multidrug resistance (MDR) barrier [29–31]. Unfortunately, the intracellular drug release from the traditional micellar core usually involves an uncontrolled and slow drug release process that needs to overcome the interaction between micellar core components and hydrophobic drug [32,33]. Micelle disassembly (degradation) within tumor cells in a short time is a powerful strategy to solve above problem. The smart degradable micellar system is the so-called stimuli-responsive degradable micelles (SRDMs), which composed of one or several cleavable linkage located in the micelle structure. And the cleavable linkage can intelligently respond to external stimulus, such as acidic pH or reducing condition in tumor cells [29,34–36]. SRDMs have good structure stability and morphology in the normal physiological environment but undergo quick destabilization or degradation in intracellular conditions at tumor sites, leading to enhanced drug release. Oh's group has produced a series of detailed studies on the redox responsive degradation of micellar architecture [32,37]. In their work, disulfide bonds were inserted as cleavable joints between a hydrophobic block and hydrophilic block. The micelles were stable at normal physiological conditions (low or no GSH present), while the architecture disassembled on account of the disulfide breakage triggered by GSH, and then the hydrophilic shells were shed from the hydrophobic cores, resulting in an obvious acceleration of the release of encapsulated drug. Han's group developed a dual stimulus-responsive degradable micellar system based on the copolymer methoxypolyethylene glycols-*b*-poly (6-*O*-methacryloyl-D-galactopyranose)-disulfide bond-doxorubicin, mPEG-*b*-PMAGP-SS-DOX [38]. By means of acid and redox cleavable linkages (disulfide and hydrazone), the hydrophobic drug DOX was conjugated on the side chain of the copolymer mPEG-*b*-PMAGP. When at the acidic and highly reductive condition within tumor cells, the disulfide and hydrazone broke up, followed by a fast drug release.

Aiming at conquering the drug leakage during blood circulation and slow intracellular drug release, the combination of the two strategies SCMs and SRDMs were taken into consideration [39,40]. For example, Zhong's group obtained a degradable micelle with reversible core from the copolymer poly(ethylene glycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate-*co*-pyridyl disulfide carbonate), PEG-P(TMBPEC-*co*-PDSC) [40]. When exposed to acidic and high reductive intracellular environment in tumor cells, the de-crosslinking occurred with the breakage of the disulfide bonds, and the cleavage of acetal resulted in the hydrophilicity change of core structure. In a short time, the micelles degraded (disassembled), leading to a fast release rate and almost complete drug release.

By taking advantages of SCMs and SRDMs, we designed a novel redox-triggered degradable SCM system with acid responsive de-crosslinking site and redox triggered degradation site to improve the drug delivery efficiency. For this goal, an amphiphilic copolymer poly(ε-caprolactone)-*b*-poly(poly(ethylene glycol) methylether methacrylate-*co*-p-(2-methacryloxyethoxy)benzaldehyde) (PCL-SS-P(PEGMA-*co*-MAEBA) was synthesized by the ring-opening polymerization (ROP) of ε-CL, and the subsequent copolymerization of PEGMA and MAEBA, using activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP) method. The hydrophobic block PCL is a matrix material