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Tumor microenvironment-responsive micelles for pinpointed intracellular release of doxorubicin and enhanced anti-cancer efficiency

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ABSTRACT

Internal stimuli, such as intracellular lysosomal pH, enzyme, redox and reduction, can be applied to improve biological specificity of chemotherapeutic drugs for cancer therapy. Thus, functionalized copolymers based on their response to specific microenvironment of tumor regions have been designed as smart drug vesicles for enhanced anti-cancer efficiency and reduced side effects. Herein, we reported dually pH/reduction-responsive novel micelles based on self-assembly of carboxymethyl chitosan-cysteamine-*N*-acetyl histidine (CMCH-SS-NA) and doxorubicin (DOX). The tailor-made dually responsive micelles demonstrated favorable stability in normal physiological environment and triggered rapid drug release in acidic and/or reduction environment. Additionally, the nanocarriers responded to the intracellular environment in an ultra-fast manner within several minutes, which led to the pinpointed release of DOX in tumor cells effectively and ensured higher DOX concentrations within tumor areas with the aid of targeted delivery, thereby leading to enhanced tumor ablation. Thus, this approach with sharp drug release behavior represented a versatile strategy to provide a promising paradigm for cancer therapy.

1. Introduction

Over the past decade, nanocarriers have been extensively explored for the controlled and targeted delivery of hydrophobic chemotherapy drugs owing to their abilities in improving poor solubility of chemotherapy drugs, prolonging the half-life time of payloads *in vivo* and passively accumulating in the tumor regions *via* the enhanced permeability and retention (EPR) effect (Brannon-Peppas and Blanchette, 2004; Wang et al., 2012; Maeda et al., 2013). However, issues still remain because of the limitation of pinpointed drug delivery or on-demand drug release. For instance, it is highly desired to explore more effective smart nanocarrier to maximize intracellular delivery of anticancer drugs for superior cancer therapy owing to their complex intracellular trafficking to target sites. Consequently, it is a major challenge to develop a new smart nanocarrier with multiple stimuli-responsive features for optimal anticancer efficacy (Mura et al., 2013). To date, by taking advantage of the stimuli (pH, GSH and enzyme) of tumor microenvironment, various stimuli-responsive nanoparticles (NPs) have been investigated for targeted and controlled drug delivery. Among them, pH- or reduction- responsive NPs have been researched most frequently and comprehensively, owing to significant difference in pH and GSH between extracellular and

Abbreviations: DOX, doxorubicin; CS, chitosan; EPR, enhanced permeability and retention; GSH, glutathione; CMCH, carboxymethyl chitosan; NAHis, *N*-acetyl-L-histidine; CMCH-SS-NA, carboxymethyl chitosan-cysteamine-*N*-acetyl histidine; CMCH-LA, CMCH-lauramine; DOX/CMCH-SS-NA, DOX loaded CMCH-SS-NA micelles; DOX/CMCH-LA, DOX loaded CMCH-LA micelles; DOX·HCl, doxorubicin hydrochloride; NPs, nanoparticles; EDC·HCl, 1-(3-Dimethylaminopropyl)-3-ethyl carbon carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide; DS, degree of substitution; DLS, dynamic light scattering; TEM, transmission electron microscopy; DLC%, Drug loading content; EE%, encapsulation efficiency; MTT, 5-diphenyl-2H-tetrazolium bromide; NIRF, near-infrared fluorescent; CMC, critical micelle concentration; ROI, region of interest.

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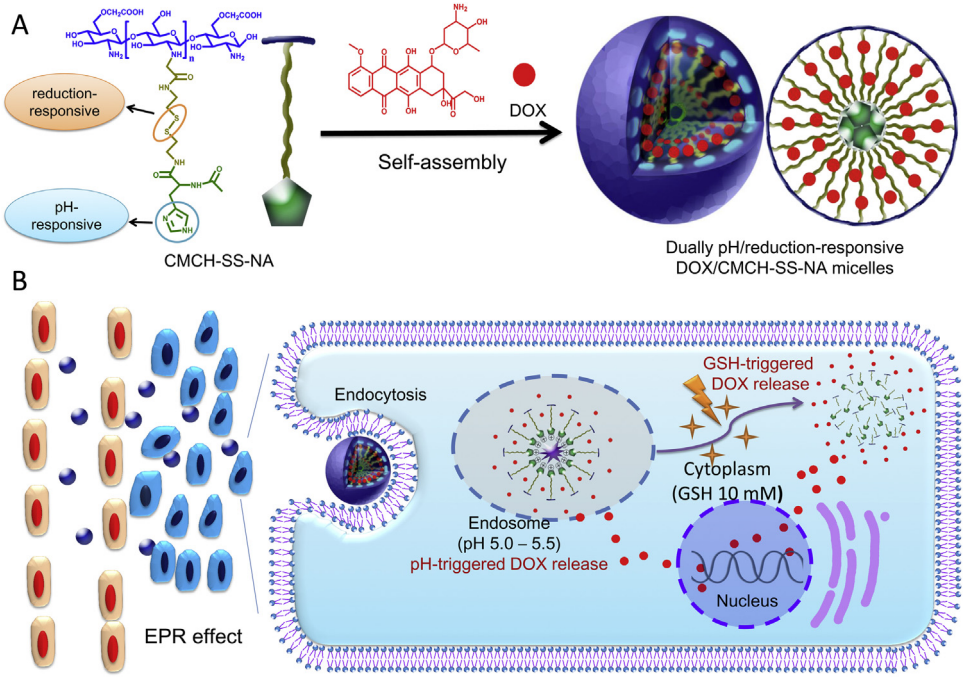
intracellular conditions (Liu et al., 2014; Deng et al., 2015a). However, the majority of pH- or reduction- responsive NPs remain limited to exhibit complete and significantly rapid drug release within tumor cells. Few types of NPs have exhibited marked or visible changes in response to low pH or GSH, which is the foundation of rapid intracellular drug release.

Compared with normal tissues, tumor regions possess specific intrinsic biological signals, such as low pH and active redox/reduction atmospheres, which play important roles in triggering stimuli-responsiveness for rapid drug release (Yu et al., 2014). Among these signals, the pH stimulus of tumor regions has been widely studied. Most tumor tissues present a mildly acidic extracellular pH value (≈6.0) as a result of rapid anaerobic respiration and they can also yield more significantly acidic endosome/lysosome pH value (5.0–5.5), which is much lower than the pH values of normal tissues and blood circulation (Hu et al., 2013; Meng et al., 2014; Hou et al., 2015). Responding to the specific acidic micro-environment of tumor regions, pH responsiveness of NPs contributes to the extracellular charge reversal of nanocarriers or the intracellular rapid release of payloads and thereby improving the cellular uptakes or enhancing the cytotoxicity of chemotherapy drugs (Du et al., 2011). Many previous studies have reported that pH responsiveness could be easily triggered because of the protonation of amino and imidazole groups; or the cleavage of acid labile ortho ester, hydrazone, *cis*-aconityl, and acetal bonds within the acid microenvironment in tumor regions (Tong et al., 2014). Among various promising pH-responsive polymers, polymeric nanoscales based on histidine have emerged as a viable platform because the imidazole group in histidine yields a pKa value of around 6.5 and can be protonated in acidic endosomes. Consequently, this type of polymer can quickly forfeit the hydrophilic/hydrophobic balance and then rapidly release the payloads in cells. Additionally, the protonation of histidine can help NPs escape from endosomes owing to the "proton sponge" effect (Jiang et al., 2012; Zeng et al., 2012). Park et al. reported novel pH-responsive NPs self-assembled by N-acetyl histidine-conjugated glycol chitosan polymer for intracytoplasmic delivery of drugs; these NPs responded to the acidic intracellular microenvironment and had an effect on escaping from endosomes and releasing the payloads into cytoplasm, thereby inhibiting cell growth (Park et al., 2006).

Different from the pH-responsiveness in response to the extracellular or endosomal pH environment, reduction-responsiveness can be triggered by the reducing environment in the cytoplasm, where the concentration of glutathione (GSH) is 100-fold to 1000- fold higher (approximately 2–10 mM) than that of extracellular fluids (approximately 2–20 μM) (Cheng et al., 2011). Reduction-responsive NPs assembled by polymers containing disulfide bonds can disassemble quickly and release the payloads under the stimuli of high-concentration GSH in the cytoplasm (Williford et al., 2014; Deng et al., 2015b). Given the structure containing a central disulfide bond, cysteamine and 3, 3′-dithiodipropionic acid have been widely adopted as a reduction-responsive cleavable bridge to contact a hydrophobic segment (*e.g.*, poly(caprolactone), stearic acid) with a hydrophilic one (*e.g.*, polyethylene glycol, hyaluronic acid) to obtain an amphiphilic polymer (Meng et al., 2009). Compared with the insensitive control, the reduction-responsive micelles exhibited enhanced cytotoxicity and much higher tumor-targeting capacity (Li et al., 2012). However, so far, few studies have been successfully conducted on the contact of a pH-responsive segment with a hydrophobic or hydrophilic segment *via* a reduction-responsive cleavage bridge to obtain a polymer with dual pH/reduction sensitivity.

It is promising that the combinational application of pH and reduction responsiveness can obviously take advantage of both stimuli environments, including endosomal pH and high-concentration GSH in the cytoplasm, leading to a more rapid and more complete release of payloads (Wu et al., 2013). Compared with single pH or reduction-responsive NPs, dually pH/reduction-responsive NPs performed more efficiently in the delivery of chemotherapy drugs (*e.g.*, doxorubicin (DOX)) because these NPs could cause stimuli-triggered release and markedly improve the uptake, thereby enhancing therapeutic efficiency. (Chen et al.,



Scheme 1. (A) Self-assembly of pH/reduction-responsive DOX/CMCH-SS-NA micelles. (B) DOX/CMCH-SS-NA for the pinpointed intracellular delivery of doxorubicin.