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Redox-sensitive nanoparticles from amphiphilic cholesterol-based block copolymers for enhanced tumor intracellular release of doxorubicin

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Abstract

A novel amphiphilic cholesterol-based block copolymer comprised of a polymethacrylate bearing cholesterol block and a polyethylene glycol block with reducible disulfide bonds (PC5MA-SS-PEO) was synthesized and evaluated as a redox-sensitive nanoparticulate delivery system. The self-assembled PC5MA-SS-PEO nanoparticles (SS-NPs) encapsulated the anticancer drug doxorubicin (DOX) with high drug loading (18.2% w/w) and high encapsulation efficiency (94.9%). DOX-encapsulated PC5MA-SS-PEO self-assembled nanoparticles (DOX-encapsulated SS-NPs) showed excellent stability and exhibited a rapid DOX release in response to dithiothreitol (DTT) reductive condition. Importantly, following internalization by lung cancer cells, the reducible DOX-encapsulated SS-NPs achieved higher cytotoxicity than the non-reducible thioester NPs whereas blank nanoparticles were non-cytotoxic. Furthermore, *in vivo* imaging studies in tumor-bearing severe combined immunodeficiency (SCID) mice showed that the nanoparticles preferentially accumulated in tumor tissue with remarkably reduced accumulation in the healthy non-target organs. The results indicated that the SS-NPs may be a promising platform for cancer-cell specific delivery of hydrophobic anticancer drugs.

Key words: Redox-sensitive nanoparticles; Block copolymer; Cholesterol; Intracellular release; Cancer therapy

In recent years, self-assembled nanoparticles from amphiphilic block copolymer have emerged as one of the most promising platforms for targeted cancer therapy due to their ability to encapsulate and stabilize hydrophobic anticancer drugs, and accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect.[1-4] Furthermore, the polymeric nanoparticles allowed conjugation with different targeting ligands to achieve active tumor targeting.[5,6] Unfortunately, translational potential of current polymeric nanoparticle delivery systems is limited due to low drug loading levels, poor *in vivo* stability, and slow drug release in tumor tissue and/or inside the tumor cells. Many anticancer drugs such as DOX and camptothecin can only induce therapeutic effects when they are in the cell nuclei.[7] The slow and incomplete release of the drugs from the nanoparticles may retard the drug influx into the nuclei along with the increased risk of drug deactivation in the endosome/lysosomes where the nanoparticles are retained after endocytosis.

To increase drug release in the tumor tissue or tumor cells, stimuli-responsive nanocarriers have been developed that release a therapeutic payload in response to a microenvironment trigger such as pH, redox, and enzyme.[8-15] Redox-sensitive nanocarriers containing disulfide bonds have received much attention for intracellular drug delivery due to the existence of a high glutathione (GSH) concentration in the tumor microenvironment and cancer cells.[16-18] For instance, several groups have reported redox-sensitive polymer/DNA complexes,[19] polyion complex

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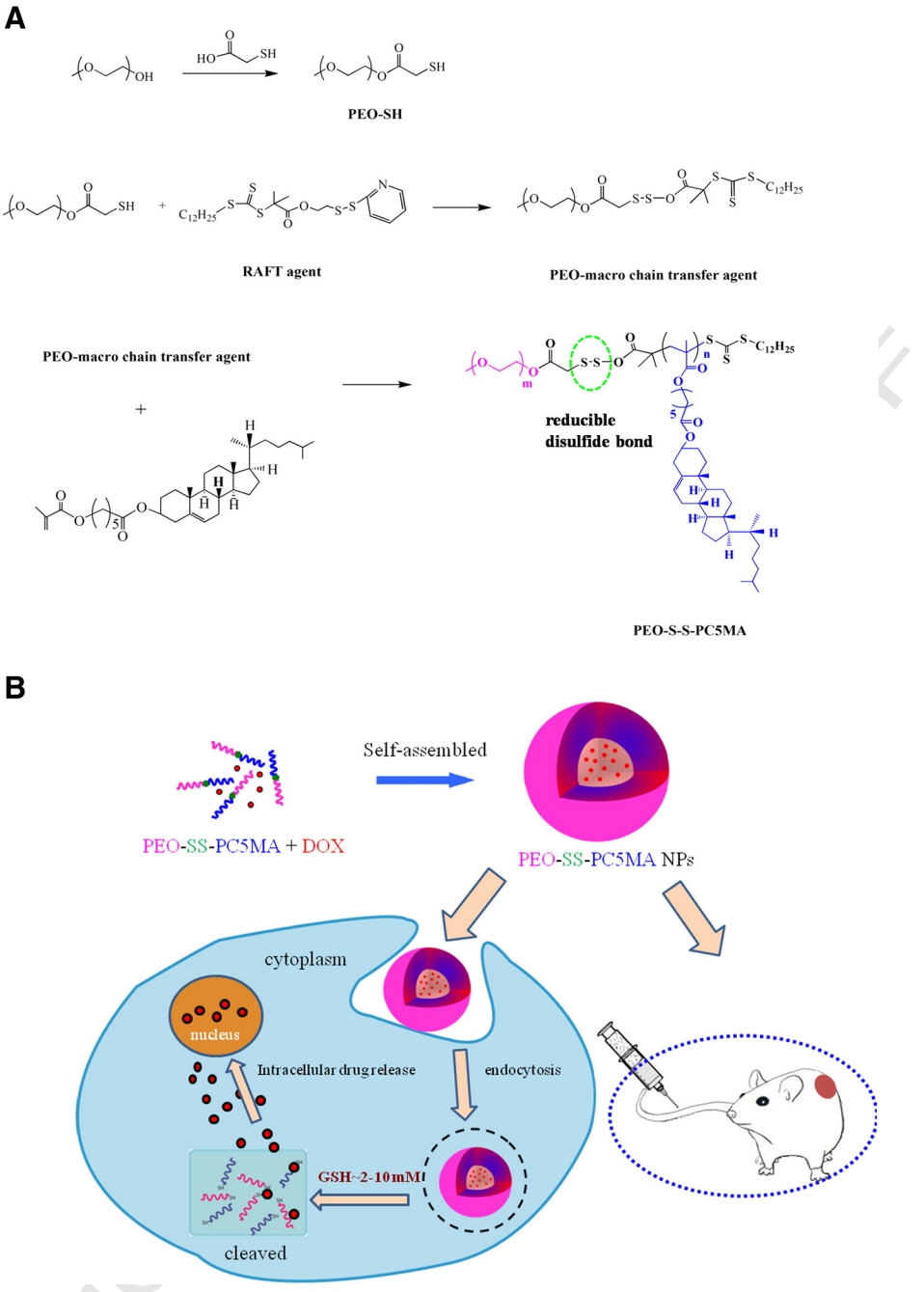

Figure 1. **(A)** Synthetic route of cholesterol-based block copolymer with disulfide linkage PC5MA-SS-PEO and **(B)** reduction-sensitive in aqueous media of PC5MA-SS-PEO NPs for intracellular drug release.

micelles for siRNA delivery,[20,21] cross-linked micelles,[22] and degradable nanogels[23] with good stability under the physiological conditions that rapidly released encapsulated drugs in the intracellular reductive environment. However, many synthetic copolymers upon erosion *in vivo* yield non-endogenous oligomers and monomers that might adversely interact with the surrounding tissue.[24] Therefore, reducible block copolymers based on biologically-compatible components may be promising nanocarriers for intracellular drug delivery with minimal toxicity of the polymer carrier and its degradation products.

Cholesterol is an important component of cell membranes involved in lipid organization, signal transduction, cell adhesion, and cell migration.[25] Therefore, cholesterol-based block copolymers are interesting materials for cell attachment and proliferation, forming the basis of polymeric scaffolds, and drug delivery.[26-30] Our previous studies have shown that amphiphilic brush block copolymers containing cholesterol blocks formed long circulating self-assembled nanoparticles for improved DOX delivery to tumor.[31,32] However, these nanoparticles showed a very slow drug release pattern that reduced their anti-tumor effect.[32]