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Actively targeted delivery of anticancer drug to tumor cells by redox-responsive star-shaped micelles

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ABSTRACT

In cancer therapy nanocargos based on star-shaped polymer exhibit unique features such as better stability, smaller size distribution and higher drug capacity in comparison to linear polymeric micelles. In this study, we developed a multifunctional star-shaped micellar system by combination of active targeting ability and redox-responsive behavior. The star-shaped micelles with good stability were self-assembled from four-arm poly(ε-caprolactone)-poly(ethylene glycol) copolymer. The redox-responsive behaviors of these micelles triggered by glutathione were evaluated from the changes of micellar size, morphology and molecular weight. *In vitro* drug release profiles exhibited that in a stimulated normal physiological environment, the redox-responsive star-shaped micelles could maintain good stability, whereas in a reducing and acid environment similar with that of tumor cells, the encapsulated agent was promptly released. *In vitro* cellular uptake and subcellular localization of these micelles were further studied with confocal laser scanning microscopy and flow cytometry against the human cervical cancer cell line HeLa. *In vivo* and *ex vivo* DOX fluorescence imaging displayed that these FA-functionalized star-shaped micelles possessed much better specificity to target solid tumor. Both the qualitative and quantitative results of the antitumor effect in 4T1 tumor-bearing BALB/c mice demonstrated that these redox-responsive star-shaped micelles have a high therapeutic efficiency to artificial solid tumor. Therefore, the multifunctional star-shaped micelles are a potential platform for targeted anticancer drug delivery.

1. Introduction

Polymeric micelles are emerging as a promising nanocarrier for cancer therapy in improving solubility and bioavailability of hydrophobic anticancer drugs and prolonging circulation half-life in blood due to their well-defined core–shell architecture [1–4]. The micelles with a small size usually less than 100 nm can accumulate passively in solid tumors via well-known enhanced permeability and retention (EPR) effect, which is an unique anatomical-pathophysiological nature of tumor blood vessels [5,6]. Although the accumulation of these micelles in tumor tissues can be enhanced by the EPR effect, they are facing tremendous challenges, including low stability in blood circulation, poor cellular internalization and inefficient intracellular drug release [7].

The low stability of the micellar system can cause a premature release of the payloads, leading to therapeutic agent loss in blood circulation. Therefore, the stability of micelles is an important prerequisite, which can ensure the encapsulated drug be delivered accurately to target site. In recent years, covalent cross-linking of the core or shell of polymeric micelles is a general strategy to prevent de-micellization-associated drug loss [8–10]. However, a complex chemical reaction is required to perform this strategy. The generation of star-shaped micelles from amphiphilic polymers with star-shaped architectures is another viable strategy to avoid the premature drug release. Compared to traditional linear copolymers, multiple-arm star-shaped block copolymer can reduce the critical micelle concentration (CMC) without compromising its loading and delivery efficiency [11–15]. Furthermore, the star-shaped polymeric micelles have been reported to have more advantages such as

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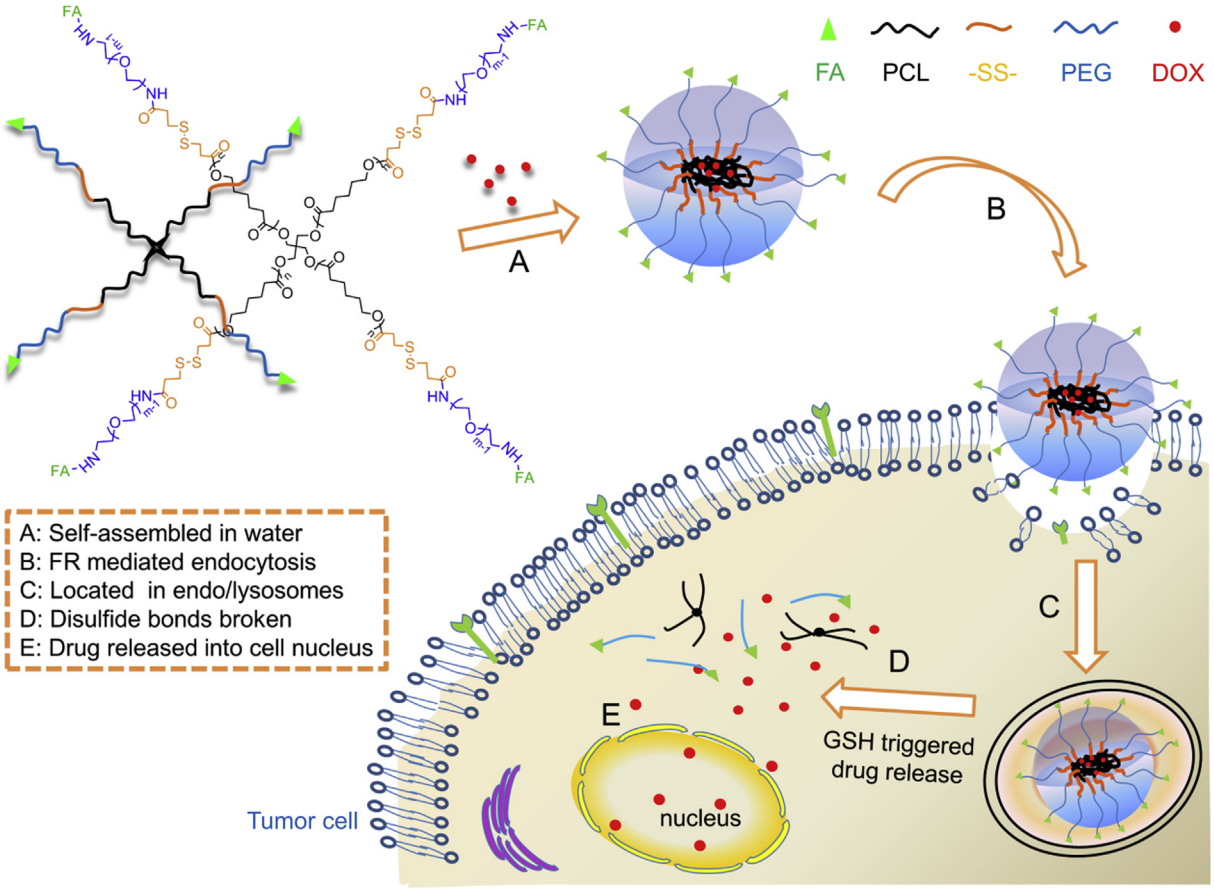
better stability, narrower size distribution, higher drug capacity, as well as more end groups for graft modifications in contrast to traditional ones [15–17].

To enhance the cellular internalization, grafting the hydrophilic shell of micelles with various targeting ligands whose receptors are overexpressed on cancer cells is an effective approach. The exposed targeting ligands on the outside surface of the micelles can lead to receptor-mediated endocytosis and subsequently promote their cellular uptake [18].The targeting agents can be broadly classified as antibodies, aptamers and small molecules [2,19]. Among these ligands, folic acid (FA), which has a high affinity for FA-binding proteins that are selectively over-expressed on the surface of many human tumor cells [20,21], is often employed to functionalize the micelles for an active tumor targeting [22–24]. After these nanocarriers enter the tumor cells through folate receptor (FR)-mediated endocytosis, the encapsulated cargos still need to be rapidly released in the cellular compartments such as the cytoplasm or nucleus to effectively kill tumor cells.

The intracellular drug release can be realized by application of external stimuli that cause micelle de-stabilization in a specially controlled manner [25]. The main intracellular signals contain glutathione [26–29], pH [22,30,31] and specific enzymes [32]. Of these stimuli, the utilization of disulfide-containing redox potential is of particular importance, owing to the fact that there exists a great difference in glutathione (GSH) concentration between the reducing intracellular space (approximately 2–10 mM) and mildly oxidizing in extracellular space (approximately 2–20 μM). The disulfide bonds of the micellar system intend to be cleaved at the high level of GSH, leading to the micelles disassembly and subsequently the anticancer drug being immediately released to cellular compartments.

In this study, we develop a multifunctional star-shaped micellar system by combination of the actively targeting ability and the redox-responsive behavior to achieve both a high therapeutic efficiency to solid tumor and low toxicity to normal tissues. The star-shaped micelles are self-assembled from four-arm poly(ε-caprolactone) (PCL)-poly(ethylene glycol) (PEG) copolymer. PEG is used as hydrophilic segment to avoid the aggregation and clearance from the reticular-endothelial system [33]. PCL is utilized as hydrophobic segment because of its good biodegradability and non-cytotoxicity. The redox-responsive behavior can be realized by connecting the hydrophilic and hydrophobic segments with disulfide bonds. The end groups of the hydrophilic segment were decorated with FA ligands to endow the active targeting. The anticancer drug, doxorubicin (DOX), can be trapped into the micelles during the self-assembly of the star-shaped PCL-PEG copolymer (Scheme 1). These micelles are specifically internalized into tumor cell through FR-mediated endocytosis, and the disulfide bonds are immediately cleaved in response to the high level of GSH and in turn DOX is released (Scheme 1). By comparison with the redox-insensitive PCL-PEG micelles, *in vitro* cellular uptake and subcellular localization of these redox-responsive micelles were studied with confocal laser scanning microscopy (CLSM) and flow cytometry (FCM) against the human cervical cancer cell line HeLa. *In vivo* antitumor effect was also evaluated in detail after being applied to the BALB/c mice bearing 4T1 tumor model.



Scheme 1. Schematic illustration of DOX-loaded star-shaped micelles based on folate functionalized and redox-responsive star copolymer (star-PECL$_{ss}$-FA) for FR-mediated endocytosis and GSH triggered intracellular drug release.