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Singlet oxygen-responsive micelles for enhanced photodynamic therapy

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ABSTRACT

Photodynamic therapy (PDT) efficacy is limited by the very short half-life and limited diffusion radius of singlet oxygen ($^1O_2$). We report a $^1O_2$-responsive micellar nanoplatform subject to considerable size-expansion upon light triggering to facilitate on-demand release of photosensitizers. Imidazole, a well-known $^1O_2$ scavenger, was incorporated in the hydrophobic core of amphiphilic copolymer micelles, and was used to coordinate with biocompatible Zn^{2+} and encapsulate the photosensitizer chlorin e6 (Ce6). The micelles are highly sensitive to light irradiation: $^1O_2$ triggering induced dramatic particle size expansion due to the conversion of imidazole to hydrophilic urea, resulting in instantaneous release of Ce6 and rapid intracellular distribution. This $^1O_2$-responsive, size-expandable nanosystem delivered substantially more Ce6 to tumor sites as compared to free Ce6, and exhibited improved anti-tumor efficacy *in vivo* in 4T1 tumor-bearing mice. This work opens new avenues of particle expansion-induced PDT enhancement by controlled imidazole chemistry.

1. Introduction

Nanocarriers (NCs) have been extensively employed to improve the efficacy of photodynamic therapy (PDT) [1–3]. Key challenges of classical PDT include very short life time, rapid cellular elimination, and limited diffusion radius of singlet oxygen ($^1O_2$) [4–6]. It is pivotal to enable instant photosensitizer (PS) release upon light irradiation to exhibit sufficient PDT efficacy. In contrast to traditional pH- and redox-sensitive PS nanocarriers [7–9], reactive oxygen species (ROS)-responsive nano-PDT has gained increasing attention as a self-triggered delivery system due to the PS-generated ROS could induce either the cleavage of covalently linked cargo, or the solubilization and degradation of NCs, followed by cargo release [10–12]. Among these, $^1O_2$-responsive NCs are particularly appealing because of the highly reactive nature of $^1O_2$ and rapid onset of responsive cascade events. To date, there have only been limited reports on $^1O_2$-responsive PDT nanosystems with primary use of vinyldithioether linker as the trigger-responsive domain [13–15].

Particles that can undergo significant size expansion are of particular interest and importance in controlled release and cargo delivery [16–18]. Ideally, particles do not release encapsulated cargo in the off-state. Upon triggering, size expansion results in substantially improved release of the encapsulated cargo due to combined effects of significant enlargement of the particle pore size in aqueous phase and influx of water that changes the hydrophobicity/hydrophilicity balance within the particle. While there are numerous reports of trigger-responsive hydrogel particles with model triggers, NCs responsive to clinically relevant triggers that can undergo substantial size change and controlled burst release are rare [19–21]. Here, we report the use of $^1O_2$-induced imidazole (IM)-urea transformation for the design of the first ever $^1O_2$-responsive size-expansion NCs for controlled cargo release (Fig. 1).

The key of triggered size expansion of the designed NCs in the current work is the peculiar $^1O_2$-scavenger reaction, which results in scavenger oxidation and carrier modification. Imidazole has often been utilized as a molecular scavenger to modulate $^1O_2$ activity [22,23]. Upon oxidization, the generated $^1O_2$ will oxidize imidazole and facilitates its transformation to urea [24–27]. Imidazole is known to form stable and tight coordination complex with Zn^{2+} as the electron donor [28–30]. Such ionic crosslinking could enhance the particle

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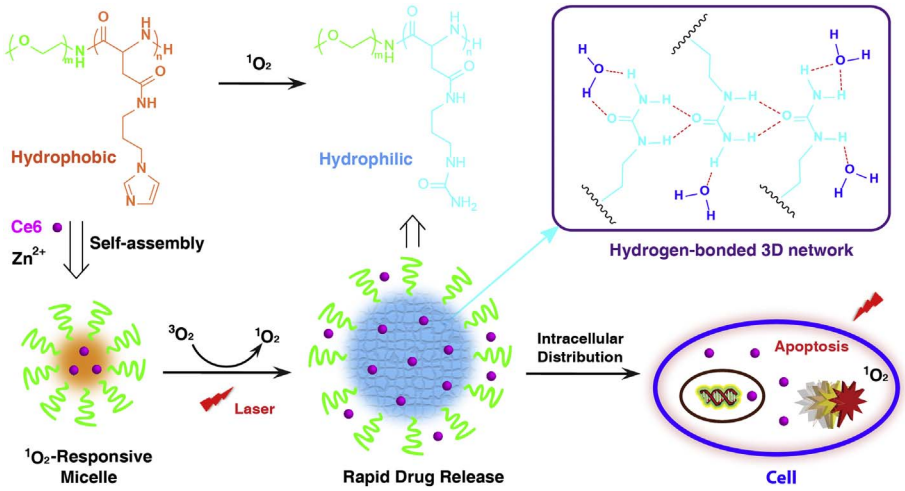


Fig. 1. Illustration of singlet-oxygen responsive micelles for enhanced photodynamic therapy. The imidazole-bearing amphiphilic copolymer can self-assemble into micelles containing Zn^{2+} crosslinkers. Chlorin e6 (Ce6) was encapsulated within micelles. Laser activation generates singlet oxygen, thereby converting imidazole to urea, and resulting in micelle expansion, rapid Ce6 release, and onset of apoptosis. Upon size increase, the urea-containing polymers form various types of hydrogen bonds and do not lose nanocarrier integrity.

systemic stability for extended circulation [31]. Upon transformation to urea, the coordination complex disappears, accompanied with significant increase of water solubility. Urea has both H-bonding donor and acceptor properties (via NH_2 and C═O), and as such has a remarkable capability of absorbing water, by forming an extensive H-bonding network with large amounts of water molecules (Fig. 1). We envisioned the opportunity of utilizing such transformation for the design of smart size-expandable NCs and postulated that the employment of imidazole in $^1O_2$-responsive NCs would provide triggered PS delivery nanosystems.

To develop a smart imidazole-based on-demand PDT nanosystem, we employed the multivalent, biocompatible methoxyl poly(ethylene glycol)-*co*-poly(aspartic acid) (mPEG-PAsp) as the backbone copolymer [32,33]. Imidazole was conjugated to the backbone, forming an amphiphilic polymeric conjugate (mPEG-PAsp-IM) that can self-assemble into micellar nanocarriers in aqueous medium (Scheme 1) [34,35]. The prototypic PS chlorin e6 (Ce6) was encapsulated within the Zn^{2+}-crosslinked NCs, and the *in vitro* and *in vivo* efficacy of the newly developed NC formulation was evaluated in the highly aggressive 4T1 breast cancer model in mice.

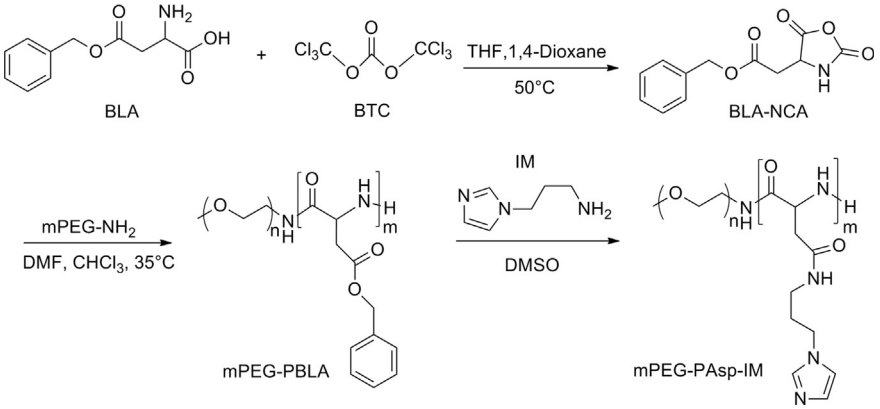
2. Materials and methods

2.1. Materials

L-Aspartic acid β-benzyl ester was purchased from Beijing HWRK Chem Co., Ltd. (Beijing, China). Methoxypolyethylene glycol amine (mPEG-NH_2, 5000 Da) was sourced from Beijing JenKem Technology Co., Ltd. (Beijing, China). Triphosgene and 1-(3-Aminopropyl)imidazole (IM), phosphate buffered saline (PBS) tablets, urea and *p*-dimethylaminobenzaldehyde were obtained from Sigma-Aldrich (Beijing, China). Tetrahydrofuran (THF), *N*,*N*′-dimethylformamide (DMF), 1,4-dioxane, ether, hexane, dimethyl sulfoxide (DMSO), trichloromethane ($CHCl_3$), hydrochloric acid (HCl), trifluoroacetic acid (TFA), nitrilotriacetic acid, disodium hydrogen phosphate, sodium hydroxide, and sulphuric acid were from Guangfu Fine Chemical Research Institute (Tianjin, China). 3-morpholinopropanesulfonic acid (MOPS) and pyrene were purchased from Jingchun Reagent Co., Ltd. (Shanghai, China). Dimethyl sulfoxide-d_6 and chloroform-d were purchased from Jinouxiang Science & Technology Co., Ltd. (Beijing, China). Acetone was sourced from Jiangtian Chemicals (Tianjin, China). Chlorin e6 was obtained from Beijing J & K Scientific Co., Ltd. (Beijing, China). Zinc chloride ($ZnCl_2$) was purchased from Tianjin Yuan Li Chemical Co., Ltd. (Tianjin, China). Acetonitrile, methanol, and absolute ethanol were obtained from Tianjin Concord Technology Co., Ltd. (Tianjin, China).

2.2. Monomer synthesis

L-Aspartic acid β-benzyl ester (5 g, 22.4 mmol) was suspended in 25 mL anhydrous 1,4-dioxane, followed by oxygen removal. Then, triphosgene (3.3 g, 11.1 mmol) dissolved in 25 mL anhydrous THF was added at ambient temperature under nitrogen atmosphere. The



Scheme 1. Synthesis of singlet oxygen-responsive polymer (mPEG-PAsp-IM) and the control polymer (mPEG-PBLA). mPEG, PBLA, PAsp, and IM represents methoxyl poly(ethyl glycol), poly(β-benzyl-L-aspartate), poly(aspartic acid), and imidazole, respectively.