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Specific light-up pullulan-based nanoparticles with reduction-triggered emission and activatable photoactivity for the imaging and photodynamic killing of cancer cells

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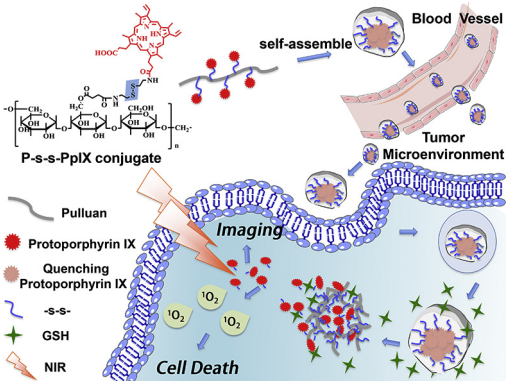
GRAPHICAL ABSTRACT



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ABSTRACT

Activatable photosensitizers that can be activated by cancer-associated stimuli have drawn increasing attention for simultaneous fluorescence imaging and photodynamic ablation of cancer cells. Here, we developed a cancer-cell specific photosensitizer nano-delivery system by synthesizing protoporphyrin IX (PpIX)-conjugated pullulan (P) with reducible disulfide bonds. The amphiphilic P-s-s-PpIX conjugate self-assembled in aqueous condition to form core-shell structured nanoparticles (P-s-s-PpIX NPs) with average size of 166 nm, showing reduction-controllable stability. In *in vitro*, the photoactivity of P-s-s-PpIX NPs in an aqueous environment was significantly suppressed by the self-quenching effect, which kept P-s-s-PpIX NPs in a photo-inactive and quenched state. But in the presence of GSH, P-s-s-PpIX NPs quickly dissociated by reductive breakage of disulfide linkers, followed by the significant recovery of fluorescent emission and singlet oxygen generation. In MCF-7 cells, compared to non-reducible P-PpIX NPs with stable amide linkages, P-s-s-PpIX NPs displayed higher cytotoxicity and induced higher apoptosis rate of tumor cells with light irradiation treatment. As a result, the P-s-s-PpIX NPs may serve as an effective smart nanomedicine platform for specific light-up and reduction-triggered cancer imaging and photodynamic therapy with the prominently reduced damage to normal tissues and cells.

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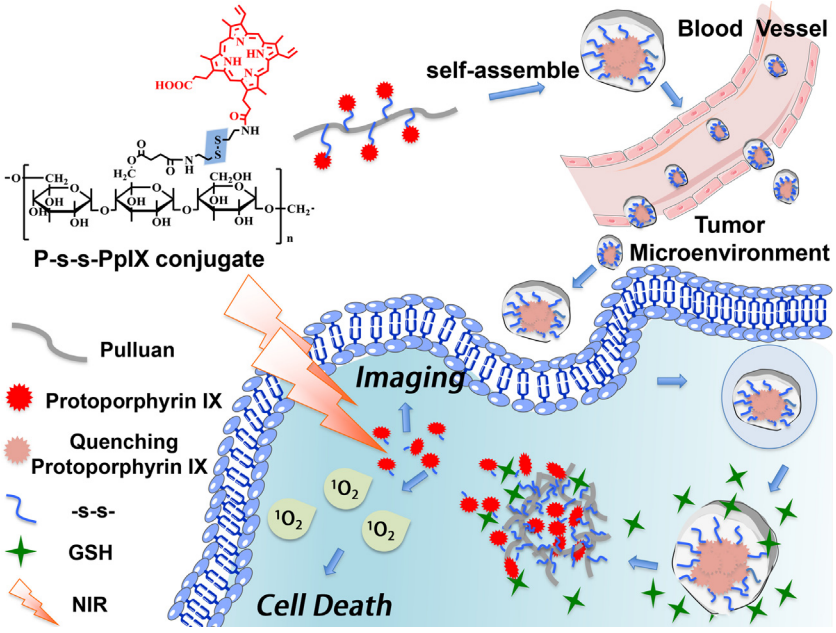
1. Introduction

Photodynamic therapy (PDT) has been increasingly recognized as an attractive approach for cancer treatment because of its precise controllability, minimal invasive nature and high spatiotemporal accuracy [1,2]. PDT has been proven to be effective in treatment of various types of cancers [3,4]. In a typical PDT process, upon appropriate wavelength irradiation, the photosensitizers (PSs) are able to generate toxic reactive oxygen species (ROS), especially singlet oxygen to induce necrosis or apoptosis of targeted tumor cells and activation of an immune response against targeted cancer cells [5,6]. Additionally, the fluorescence emitted by PSs could be exploited for the imaging of cancer cells, facilitating the imaging-guided theranostic process [4,7–10]. However, the extensive clinical application of this therapeutic approach has been hampered by several limitations associated specifically to the PS agents such as non-specific skin phototoxicity, poor water solubility (~1 μg/mL) and inefficient delivery to tumor tissues [3,11–13]. To overcome these drawbacks, the passive delivery of PS to the tumor site using nanostructured drug carriers based on the enhanced permeability and retention (EPR) effect [14–19] and active targeting approaches [20–22], such as targeting-ligands conjugation, have drawn much more attention in PDT. Unfortunately, during circulation in the blood, the continuous release of PSs from nano-delivery system at non-tumor sites would damage the normal tissues and organs of patients. Recently, activatable PS delivery systems that can be activated selectively by tumor-associated stimuli have drawn increasing attention [23,24]. Commonly, in normal tissues, the fluorescence and singlet oxygen generation of activatable PSs are silent or weak with light irradiation, but show photodynamically active and enhanced fluorescence at tumor sites through kinds of activation mechanisms, including enzymatic activation [8], nucleic acid-activation [25] and tumor-specifically environmental activation [26,27] such as the remarkable decrease in pH value, significantly high level GSH and hypoxia. Thus, activatable PSs can achieve both fluorescent imaging and selective killing of targeted cancer cells without threatening normal tissues. Therefore, the development of new activatable PSs for future PDT clinical application will be of great significance.

In the present study, a novel pullulan-based nanoparticles, self-assembled from amphiphilic conjugate (called as P-s-s-PpIX) with reduction-activated fluorescence emission and enhanced photoactivity, was described. The P-s-s-PpIX was synthesized by grafting hydrophobic protoporphyrin IX (PpIX) with hydrophilic pullulan polysaccharide (P) via disulfide bond (-s-s-) (Scheme 1). PpIX, one of the porphyrin-based photosensitizers, has been widely selected for PDT because of its strong singlet oxygen quantum yields, well-known chemistries and naturally occurring context [4,28]. Pullulan, produced by *Aureobasidium pullulans*, is a homopolysaccharide consisting of maltotriose units. Due to its excellent biocompatibility and modifiability, pullulan has been widely employed as drug/gene and PS delivery building materials [29–36]. Moreover, due to the significantly higher concentration of GSH in most tumor cells than normal cells [37], the reduction-sensitive disulfide bond has potential to trigger the rapid dissociation of nanoparticles (NPs), inducing efficient releasing and activation process of photosensitizer in target tumor cells [38–40]. As shown in Scheme 1, P-s-s-PpIX NPs may display controlled photoactivity. During circulation in the blood, the fluorescent emission and photoactivity of PpIX molecules in P-s-s-PpIX NPs may be turned off by self-quenching effect between PpIX molecules grafted to the pullulan backbone similar to the fluorescence resonance energy transfer (FRET) effect [4]. Once P-s-s-PpIX NPs penetrates tumor tissue and internalizes in cancer cells, FRET effect is destroyed by the cleavage of disulfide bond via the higher concentration of GSH in cancer cells. As a result, the released PpIX can recover their fluorescence for cancer cells imaging and generate singlet oxygen upon laser irradiation focused on tumor site, leading to the improved targeted antitumor effect of PDT and reduced damage to normal tissue and cells. P-PpIX NPs without disulfide bond were also prepared as a reduction-insensitive control. The physicochemical and photoactivity of both nanoparticles were investigated in the terms of particle size, zeta potential, fluorescence emission intensity, and the production of singlet oxygen. Especially, to assay the potential of P-s-s-PpIX NPs as a reduction-triggered activatable photosensitizing system, changes of size, PpIX release, fluorescent intensity, singlet oxygen yield in the presence and absence of GSH imitating reduction condition in cancer cells were measured. Additionally, different behavior between the P-s-s-PpIX NPs and free PpIX *in vivo* was visualized by monitoring the fluorescent intensity over time in Kun Ming mice. Finally, the cellular internalization of both nanoparticles



Scheme 1. Illustration of the construction of reduction-sensitive P-s-s-PpIX NPs applied in reduction-triggered cancer imaging and photodynamic therapy.