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# Antioxidant-photosensitizer dual-loaded polymeric micelles with controllable production of reactive oxygen species

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ABSTRACT

Poly(ethylene glycol)-b-poly(caprolactone) (PEG-b-PCL) micelles dually loaded with both pheophorbide a (PhA) as a photosensitizer and β-carotene (CAR) as a singlet oxygen ($^1O_2$) scavenger were designed to control photodynamic therapy (PDT) activity in cancer treatment. The CAR in the PhA/CAR micelles significantly diminished PhA-generated $^1O_2$ through direct $^1O_2$ scavenging, whereas the CAR molecules lost their $^1O_2$ scavenging activity when the PhA and CAR were spatially isolated by the disintegration of the PEG-b-PCL micelles. In cell-culture systems, light irradiation at a post-treatment time that corresponded to the presence of the micelles in the blood environment induced negligible phototoxicity, whereas light irradiation at a post-treatment time that corresponded to the presence of the micelles in the intracellular environment induced remarkable phototoxicity. In addition, a longer post-treatment time induced greater internalization of PhA/CAR micelles, which resulted in higher phototoxicity, suggesting an increase in photo killing activity against the tumor cells of interest. Thus, the co-loading of a $^1O_2$ generator and a $^1O_2$ scavenger into a single micelle is a potential strategy that may be useful in facilitating more accurate and reliable PDT with site-specific controllable production of singlet oxygen species for cancer treatment.



## 1. Introduction

Photodynamic therapy (PDT) is a minimally invasive method that produces localized tissue damage to treat various cancerous and other nonmalignant diseases (Ibbotson, 2010; Juarranz et al., 2008; MacDonald and Dougherty, 2001; Mitton and Ackroyd, 2008). PDT involves either the systemic or topical administration of photosensitizers (PSs), such as porphyrin and phthalocyanine derivatives, followed by photoexcitation of the PSs at the disease site using light of a specific wavelength. Upon light irradiation, an excited PS reacts in situ with molecular oxygen to generate various reactive oxygen species (ROSs), such as singlet oxygen ($^1O_2$) and free radicals, to damage cells via apoptosis and necrosis (Kohl and Karrer, 2011; Ortner, 2009). However, in clinical PDT, it is difficult to achieve a high level of selectivity for the cells of interest via intravenous administration of PSs, primarily because of the hydrophobic nature and unfavorable biodistribution of the PSs. Unwanted PS accumulation at non-target sites often results in uncomfortable adverse effects, such as phototoxic and photoallergic reactions in the skin and accidental photodamage to non-target healthy cells and tissues, such as blood cells, endothelial cells, and neighboring blood-vessel cells (Detty et al., 2004; Vrouenraets et al., 2003).

Polymeric micelles formed via the self-assembly of amphiphilic block copolymers in aqueous solutions have been extensively explored for the delivery of hydrophobic drugs and present several advantages, such as simple preparation, efficient drug loading without chemical modification, and controlled release (Kedar et al., 2010). Such micelles also exhibit long blood-circulation times and passive targeting based on the enhanced permeability and retention (EPR) effect (Deng et al., 2012; Gong et al., 2012), thus decreasing the unfavorable biodistribution of hydrophobic drugs. Indeed, PS-loaded polymeric micelles have been observed to remain in the blood for an extended period of time, thus allowing for sufficient EPR-mediated accumulation of PS in the pathological area (van Nostrum, 2004). However, PS-loaded micelles in blood circulation have been found to present a risk of skin photosensitivity, which could lead to damage of endothelial cells or neighboring blood-vessel cells (Bugaj, 2011). Therefore, there has been a continuous effort to reduce unwanted photoactivity at

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non-target sites. For example, the generation of $^1O_2$ has been controlled using self-quenchable PS nanoparticles or activatable PS molecules. In the first method, the PS is chemically linked with hydrophilic polymers or gold nanoparticles to form PS-conjugated, quenchable nanoparticles, such as folate/heparin/pheophorbide a (PhA) nanoparticles (Li et al., 2011), bioreducible glycol chitosan/PhA nanoparticles (Oh et al., 2013), and hybrid PhA/heparin/gold nanoparticles (Li et al., 2013). In these nanoparticles, the PS molecules are kept self-quenched during blood circulation, thus reducing the potential side effects. However, self-quenchable PS nanoparticles often require complicated synthetic procedures and must release or dissociate PS to generate light-induced photoactivity at target sites. For the second method, activatable PS systems have often been constructed by chemically linking PS with its quenchers (i.e., activation controllers) through stimuli-sensitive, cleavable linkers. The linkers can maintain a close distance between the PS and its quencher, thus resulting in inactive PS at non-target sites, whereas the linkers can be specifically degraded by a variety of physical or chemical stimuli that exist solely or at very high levels in target sites (e.g., a solid tumor) (Lovell et al., 2010). For example, PS-carotenoid conjugates with cancer-specific or self-folding peptide linkers, known as photodynamic molecular beacons (PMBs), have been developed that are able to specifically recognize tumor sites that differ from normal tissues (Chen et al., 2008; Zheng et al., 2007). However, current activatable PS systems are constructed with a low-molecular-weight system, which may suffer rapid elimination from the body and may possess multiple drug resistances, resulting in a short plasma half-life and low bioavailability, thereby limiting their clinical applications (Verhille et al., 2010).

In this study, we designed polymeric micelles co-loaded with PS and an antioxidant to achieve targeted and activatable PDT for cancer treatment. As illustrated in Fig. 1, PhA and β-carotene (CAR), which served as the $^1O_2$ generator and $^1O_2$ scavenger, respectively, were encapsulated in the hydrophobic cores of poly(ethylene glycol)-b-poly(caprolactone) (PEG-b-PCL) micelles. Our design concept was based on the expectation that the PhA-and-CAR dual-loaded PEG-b-PCL micelles (PhA/CAR micelles) might not exhibit phototoxicity during blood circulation because the co-loaded CAR could directly scavenge PhA-generated $^1O_2$ under light exposure. However, after the micelles were delivered to the tumor sites of interest via EPR-mediated passive targeting, they would be internalized into the tumor cells and could then release both PhA and CAR into intracellular compartments via diffusion or PCL-biodegraded micelle disassembly. The resulting spatial separation between PhA and CAR in the cells could avoid the CAR-mediated

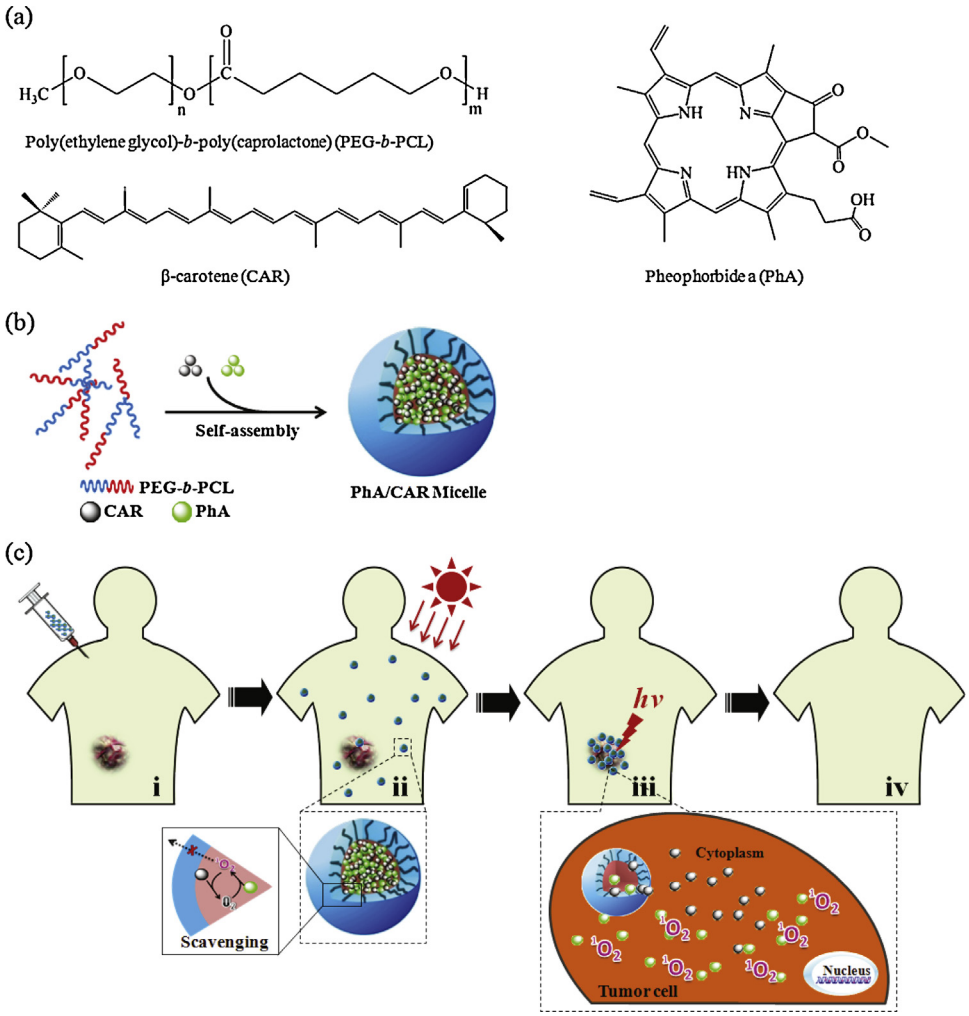


**Fig. 1.** (a) Molecular structures of PEG-b-PCL, CAR, and PhA. (b) Self-assembly of PEG-b-PCL amphiphilic copolymers accompanied by the physical co-incorporation of PhA and CAR. (c) A schematic diagram of the proposed photodynamic treatment using PhA/CAR micelles: (i) intravenous administration; (ii) CAR-mediated $^1O_2$ scavenging in PhA/CAR micelles is expected to be safe, with minimal photodamage to skin, blood cells, and non-target tissues during blood circulation; (iii) in the intracellular environment, PhA and CAR are released from the PhA/CAR micelles and then become spatially isolated, resulting in PhA-induced $^1O_2$ production and photokilling activity; (iv) tumor site restored to health after PDT.