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# Visible light-induced crosslinking and physiological stabilization of diselenide-rich nanoparticles for redox-responsive drug release and combination chemotherapy

Shaodong Zhai, Xianglong Hu*, Yongjun Hu, Baoyan Wu, Da Xing**

*MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou, 510631, China*

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ABSTRACT

Undesired physiological instability of nanocarriers and premature drug leakage during blood circulation result in compromised therapeutic efficacy and severe side effects, which have significantly impeded the development of nanomedicine. Facile crosslinking of drug-loaded nanocarriers while keeping the potency of site-specific degradation and drug release has emerged as a viable strategy to overcome these drawbacks. Additionally, combination therapy has already shown advantages in inhibiting advanced tumors and life extension than single drug therapy. Herein, three kinds of diselenide-rich polymers were fabricated with distinct hydrophobic side chains. The component effect was interrogated to screen out PEG-*b*-PBSe diblock copolymer due to its favorable self-assembly controllability and high drug loading of camptothecin (CPT) and doxorubicin (DOX) that had synergistic antitumor property. Facile visible light-induced diselenide metathesis and regeneration was employed to crosslink nanocarriers for the first time. The dual drug-loaded crosslinked micelles (CPT/DOX-CCM) were stable in physiological conditions with minimal drug leakage, possessing extended blood circulation, whereas hand-in-hand dual drug release was significantly accelerated in tumor's redox microenvironments. *In vitro* cytotoxicity evaluation and *in vivo* tumor suppression with low dosage drugs further demonstrated the favorable potency of the redox-responsive nanoplatform in tumor combination chemotherapy.



## 1. Introduction

Biocompatible materials with size range from nanometers to micrometers have been frequently employed in biomedical applications (*e.g.* therapeutics, sensing and imaging), which are generally fabricated from lipids, proteins, carbohydrates as well as natural and synthetic polymers [1–5]. Among these, synthetic polymeric nanostructures tend to be more stable than low molecular weight surfactants, possessing tailorable components and on-demand functions to meet practical needs [6]. The morphological feature of polymeric nanoparticles containing hydrophobic core and hydrophilic shell makes them suitable for nanomedicine [7,8]. In addition, it is well recognized that tumors have underdeveloped blood microvessels with many pores, which are permeable to nanoparticles, including polymeric nanoparticles, and that is so-called enhanced permeation and retention (EPR) effect [9]. In this context, polymeric nanocarriers with long blood circulation and enough physiological stability would be favorable to accumulate in tumors by passive targeting, and thus it is quite attractive to develop nanocarriers with these properties [10].

Whereas some issues of polymeric nanocarriers are still unresolved, which significantly impede their advanced applications [11]. Particularly, the structural integrity and limited drug loading stability in physiological conditions have been extensively focused. Upon intravenous injection, drug-loaded polymeric nanoparticles subjected with high dilution and severe shear force are inclined to dissociate into polymer unimers, which is accompanied with undesirable drug leakage prior to accessing the targeted lesions. Moreover, when polymer unimers are frequently captured by other constituents in blood circulation (*e.g.* blood proteins, membranes and surfaces), the dissociation will accelerate due to the equilibrium shifting towards the unimer state. Thus, the nature of physical encapsulation of drugs within polymeric nanocarriers will

* Corresponding author.
** Corresponding author.
*E-mail addresses:* xlhu@scnu.edu.cn (X. Hu), xingda@scnu.edu.cn (D. Xing).

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unavoidably exhibit premature drug leakage and shorten the blood circulation life. To address the plight for drug-loaded nanocarriers, many covalently drug-modified delivery systems have been developed to alleviate these problems [12,13], but the advantages of covalently linked drug systems have been compromised by the sophisticated synthesis, limited scale-up production potency, high cost, unspecific drug release, and changed therapeutic activities [14].

Hence, various innovative strategies have been developed to improve the stability of non-covalent drug-loaded polymeric nanocarriers. Thereinto, shell cross-linking and core cross-linking strategies were frequently employed to enhance the stability of drug-loaded polymeric nanoparticles, achieving efficient drug delivery even at low micellar concentrations [15–19]. Many cross-linking methods were presented, including addition of bifunctional reagents [20–24], direct reaction of pendent functionalities (*eg.* thiol groups, silxane groups, alkoxyamine groups and boronic ester) [25–31], or polymerization of double carbon bonds within nanoparticles [32]. However, some limitations may prevent the further application, such as unreacted reagents, possible side reactions and byproducts as well as damage to the payloads [7]. In view of these issues, the ideal crosslinking profile is supposed to be no extra reagents, and the process is mild and suitable for scale-up formulation [33]. In this respect, light undoubtedly possesses distinctive advantages such as facile operation, spatiotemporal and remote control, no external additives, and wavelength selection. Many conceptually advanced systems have been reported using UV light to crosslink and stabilize polymeric nanoparticles [17,34–36]. But UV light was restricted for its low penetration depth and phototoxicity [37]. Recently, Xu and coworkers pioneered that mild visible light could induce dynamic cleavage and exchange of diselenide bonds (Se-Se) [38,39]. Park et al. reported in situ diselenide-crosslinked micelles for ROS-responsive drug delivery, which were spontaneously derived from selenol-bearing triblock copolymers to form the crosslinking shell, exhibiting excellent physiological stability [28]. Hence we envisage that visible light-sensitive dynamic diselenide bonds may be applicable in crosslinking chemistry for biomedicine using visible light as a mild and convenient method.

On the other hand, various tumors exhibit heterogeneity of cellular environments. Thus plenty of stimuli-responsive polymeric nanoparticles have been extensively developed to accomplish controlled drug release at the target sites [40–44]. Notably, glutathione (GSH) tripeptide content in the cytosol and cell nucleus ranges from 2 to 10 mM, which is about ~1000-fold higher than that in body fluids and extracellular matrices (2–10 μM) [40,45], arousing the booming development of reduction-responsive drug delivery systems [46–50]. In addition, the overproduced reactive oxygen species (ROS) within tumor cells in disease state may disrupt cellular homeostasis, giving rise to pathological conditions, which is also a characteristic of tumors [51–53]. The ROS content in tumor cells is evaluated up to 100 μM, whereas the concentration of the most abundant and stable nonradical ROS, $H_2O_2$, is just around 20 nM in normal tissues [54]. Thus ROS has been also utilized as a trigger to mediate intracellular ROS-responsive controlled drug release [55–58]. In addition, diselenide bonds were confirmed to be sensitive to both GSH and ROS, which can be both employed to mediate drug release within tumors, possessing remarkable advantages [59,60]. Therefore, diselenide bonds have gained much attention in nanomedicine during the past few years [61,62]. Nevertheless, the reported redox-sensitive polymeric nanocarriers seldom resolved the matter of physiological instability and premature drug release.

Moreover, because of the drug resistance in tumor therapy [63–65], especially in single drug therapy, combination therapy was proved to be quite effective when using antitumor drugs with different working mechanisms, thereby decreasing the developing likelihood of resistant cancer cells [66–69]. Among these, many efforts have been focused to demonstrate potent anticancer combinations with topoisomerase (top) I and II inhibitors as one of the most widely explored categories. Top I inhibitors hinder DNA replication and enhance the activity of top II, further sensitizing the cancer cells to top II inhibitors. Camptothecin (CPT, top I inhibitor) and doxorubicin (DOX, top II inhibitor) were demonstrated to inhibit cancer cells synergistically [70]. Additionally, each drug can be employed at its optimal dose in combination chemotherapy to minimize intolerable side effects [71]. Hence, polymeric nanocarriers could transport multiple drugs into tumor in an appropriate ratio to overcome limitations of single drug therapy [72–74]. Multiple drug-loaded polymeric nanoparticles render an ideal platform for the co-delivery of different drugs for tumor combination chemotherapy.

In this work, diselenide-rich amphiphilic diblock copolymers were fabricated to encapsulate two antitumor drugs with synergistic therapeutic effects, CPT and DOX, then visible light-induced dynamic exchange of diselenide bonds in the hydrophobic core promoted the formation of core crosslinking micelles, CPT/DOX-CCM, in which the effect of polymer hydrophobic components was discussed in the self-assembly and drug formulation. This mild crosslinking strategy made the platform robust and stable with minimal drug leakage in physiological conditions, prolonging blood circulation of nanocarriers. Upon intravenous administration, hand-in-hand release of CPT and DOX was accelerated significantly in tumor's redox microenvironments. *In vivo* analysis demonstrated the substantial tumor suppressing potency of the nanocarriers using low dosage of combined drugs.

## 2. Materials and methods

### 2.1. Materials

Sodium borohydride, selenium powder, 30% $H_2O_2$, dimethylformamide (DMF), dimethylsulfoxide (DMSO) and all other organic solvents used in this study were available from the Guangzhou Chemical Reagent Company. Camptothecin (CPT) was obtained from Pengzhou Maoyuan Biochemical Technology Co., Ltd and used as received. Indocyanine green (ICG), doxorubicin hydrochloride (DOX·HCl), 11-bromo-1-undecanol, 2-bromoethanol, and 4-bromobenzyl alcohol were available from Aladdin Industrial Corporation. Fetal bovine serum (FBS), penicillin, streptomycin and RPMI 1640 were purchased from GIBCO and used as received. Lysotracker Green was obtained from Thermo Fisher Scientific Inc. Water used in this study was deionized with a Milli-QSP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ cm. $PEG_{45}$-based RAFT agent [75] and three kinds of diselenide diols precursors, including di-(1-hydroxyethylene) diselenide [76], di-(1-hydroxyundecene) diselenide [59], and di-(4,1-hydroxybenzylene) diselenide [77] were synthesized according to reported literatures.

### 2.2. Synthesis of diselenide-rich amphiphilic diblock copolymers

Synthetic routes employed for the preparation of diselenide-rich samples were shown in Scheme S1. Reversible addition-fragmentation chain transfer (RAFT) polymerization technique and facile post-modification was employed. Typically, PEG-based RAFT agent (100 mg, 0.043 mmol), methacrylic acid (185 mg, 2.15 mmol), and AIBN (1.4 mg, 0.008 mmol) were charged into a glass ampoule containing 2 mL isopropanol as solvents. The ampoule was then degassed via three freeze-pump-thaw cycles and flame-sealed under vacuum. The ampoule was then immersed into an oil bath thermostated at 70 °C to start the polymerization.