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pH-responsive unimolecular micelle-gold nanoparticles-drug nanohybrid system for cancer theranostics

Wenjing Lin [a], Na Yao [a], Long Qian [c], Xiaofang Zhang [a], Quan Chen [a], Jufang Wang [b,*], Lijuan Zhang [a,*]

[a] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, PR China
[b] School of Bioscience & Bioengineering, South China University of Technology, Guangzhou 510640, PR China
[c] Department of Biology and Center for Genomics and Systems Biology, New York University, NY 10003, USA

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ABSTRACT

The development of an in situ formed pH-responsive theranostic nanocomposite for anticancer drug delivery and computed tomography (CT) imaging was reported. β-cyclodextrin-{poly(lactide)-poly(2-(dimethylamino) ethyl methacrylate)-poly[oligo(2-ethyl-2-oxazoline)methacrylate]}$_{21}$ [β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$] unimolecular micelles served as a template for the in situ formation of gold nanoparticles (GNPs) and the subsequent encapsulation of doxorubicin (DOX). The formation of unimolecular micelles, microstructures and the distributions of GNPs and DOX were investigated through the combination of experiments and dissipative particle dynamics (DPD) simulations. β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$ formed spherical unimolecular micelles in aqueous solution within a certain range of polymer concentrations. GNPs preferentially distributed in the PDMAEMA area. The maximum wavelength (λ_{max}) and the size of GNPs increased with increasing concentration of $HAuCl_4$. DOX preferentially distributed in the PDMAEMA mesosphere, but penetrated the inner PLA core with increasing DOX concentration. DOX-loaded micelles with 41–61% entrapment efficiency showed fast release (88% after 102 h) under acidic tumor conditions. Both *in vitro* and *in vivo* experiments revealed superior anticancer efficacy and effective CT imaging properties for β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$/Au/DOX. We conclude that the reported unimolecular micelles represent a class of versatile smart nanocarriers for theranostic application.

Statement of Significance

Developing polymeric nanoplatforms as integrated theranostic vehicles for improving cancer diagnostics and therapy is an emerging field of much importance. This article aims to develop an in situ formed pH-responsive theranostic nanocomposite for anticancer drug delivery and computed tomography (CT) imaging. Specific emphases is on structure-properties relationship. There is a sea of literature on polymeric drug nanocarriers, and a couple of polymer-stabilized gold nanoparticles (GNPs) systems for cancer diagnosis are also known. However, to our knowledge, there has been no report on polymeric unimolecular micelles capable of dual loading of GNPs without external reducing agents and anticancer drugs for cancer diagnosis and treatment. To this end, the target of the current work was to develop an in situ formed nanocarrier, which actively dual wrapped CT contrast agent GNPs and hydrophobic anticancer drug doxorubicin (DOX), achieving high CT imaging and antitumor efficacy under *in vitro* and *in vivo* acid tumor condition. Meanwhile, by taking advantage of dissipative particle dynamics (DPD) simulation, we further obtained the formation process and mechanism of unimolecular micelles, and detailed distributions and microstructures of GNPs and DOX on unimolecular micelles. Taken together, our results here provide insight and guidance for the design of more effective nanocarriers for cancer theranostic application.

* Corresponding authors.
E-mail addresses: jufwang@scut.edu.cn (J. Wang), celjzh@scut.edu.cn (L. Zhang).

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1. Introduction

Owing to the versatility of polymer chemistry and ease of fabrication, multi-molecular micelles self-assembled from amphiphilic block copolymers offer an attractive means by which to accommodates hydrophobic drugs tunably, and thus have played a key role in the rapid development of cancer nanomedicine [1–6]. However, these micelles are thermodynamic aggregates of amphiphilic molecules above their critical micelle (CMC) concentration. They could disassemble into unimers, when the concentration of copolymer is below the CMC (e.g., in the blood stream), furthermore, other external conditions, for example, temperature, pH, flow stress, ionic strength, etc., may also reduce the stability of micelles. The instability could result in the burst-release of loaded drugs before reaching the tumor target and cause serious toxicity problems [7].

The construction of thermodynamically stable unimolecular micelles (micelles composed of a single copolymer), formed from multi-arm star-like polymers, presents one effective way to solve the structure instability problem of multi-molecular micelles. They show high stability and a narrow nanoparticle size distribution, which is insensitive to concentration, temperature, pH, etc, and could offer high drug loading capacities for cancer treatments [8–11]. Based on a fourth-generation hyperbranched polyester (Boltorn H40) core, unimolecular micelles have been extensively exploited in anticancer drug delivery systems [12–15]. For instance, Gong's group developed unimolecular micelles formed from a series of polymers like H40-PCL-MPEG, H40-PLA-MPEG, H40-PLA-PEG-OH/FA, H40-PLA-PEG-Apt and H40-BPLP-PEG-cRGD for the enhancement of therapeutic efficacy in dilute solution [16–20]. Although H40-based systems require simple synthesis methods and can form unimolecular micelles at a wide range of concentrations, their drawbacks such as the irregularity of polymer structure, poor controllability, and broad molecular weight distributions are not conducive to the structural regulation of unimolecular micelles.

In the field of cancer diagnostics and therapy, various nanoplatforms have been developed as integrated theranostic vehicles to deliver anticancer drugs and contrast agents to the diseased sites, providing a comprehensive therapeutic approach with lower toxicity and enhanced efficacy [21–24]. Unimolecular micelles could serve as nanocarriers for the simultaneous delivery of anticancer drugs and gold nanoparticles (GNPs), which points to potential applications in theranostic systems. GNPs, a potent computed tomography (CT) contrast agent, are ideal for CT imaging due to its prolonged blood circulation time and higher CT imaging sensitivity than conventional iodine-based agents (e.g., Omnipaque) [25–27]. For example, 30-nm GNPs had a 5.7-time higher X-ray attenuation intensity than iodine [28]. Currently, the most common method for preparing GNPs from unimolecular micelles in situ is through chemical reduction, namely, by adding reducing agents such as sodium borohydride ($NaBH_4$), sodium citrate (SC), hydrazine hydrate ($N_2H_4{\cdot}H_2O$) and tetrabutyl boron-amine (TBAB) in gold-salt precursor solutions [28,29]. Lin's group reported the fabrication of unimolecular micellar templates from a series of polymers such as 21-arm β-CD-PAA-PS, β-CD-P4VP-PAA-PS and β-CD-PAA-PEO, which formed well-dispersed and small gold nanoparticles (≤20 nm) with ethanol or TBAB as the reducing agent [30]. Filali et al. synthesized five-arm block copolymer PEO-*b*-PCL unimolecular micelles and prepared 3 nm GNPs at the optimal molar ratios of $KAuCl_4/NaBH_4$ (reducing agent) and $KAuCl_4$/EO were 1/2 and 1/4, respectively [31]. However, in the above studies, the use of reducing agents such as $NaBH_4$ and TBAB not only complicated the post-treatment process, but might also induce toxic side effects in biomedical applications. Alternatively, it has been reported that GNPs was fabricated in just one step using certain polymers with amine groups as both the stabilizer and the reducing agent [32–34]. For example, diblock polymer PMPC-*b*-PDMA synthesized by Arms et al. was found that the $AuCl_4^-$ could be reduced to zero-valent gold in situ without additional reducing reagents [32]. Meanwhile, research efforts of in situ GNP preparation using unimolecular micelles as templates have mainly focused on the preparation methods and the characterization of GNP morphology. How polymer structure affects the distribution of GNPs, as well as the regulation of particle size and shape have yet to be explored.

Herein, we reported the development of an unimolecular micelle system synthesized from 21-arm star-like polymer β-cyclodextrin-{poly(lactide)-poly(2-(dimethylamino) ethyl methacrylate)-poly[oligo(2-ethyl-2-oxazoline)methacrylate]}$_{21}$ [β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$]. β-CD, a cyclic oligosaccharide consisting of 21 hydroxyl groups serves as a desirable inner core with a variety of advantages including easy modification and regulation of structures, good biocompatibility and biodegradability and non-toxicity. The polymeric unimolecular micelles were utilized as a template for both the in situ formation of gold nanoparticles and the encapsulation of doxorubicin (DOX), enabling the integration of cancer imaging and chemotherapy (Scheme 1). β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$ formed unimolecular micelles in aqueous solution with both β-CD and PLA as the hydrophobic core for DOX loading, and EtOxMA as a dense hydrophilic shell for the maintenance of micelles. Notably, the PDMAEMA mesosphere with amine groups has dual functionalities. On the one hand, it acts as a reducing agent and a stabilizer simultaneously to ensure the in situ reduction of the precursor $AuCl_4^-$. On the other hand, protonation of the pH-responsive PDMAEMA block triggers the rapid release of DOX in weakly acidic tumor environments, which could be leveraged to achieve high anticancer efficacy. By a combination of experiments and DPD simulations, the formation of unimolecular micelles, the microstructures and detailed distributions of GNPs and DOX, as well as the inherent structure and mechanism of unimolecular micelle-GNPs-drug nanohybrid system (β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$/Au/DOX) were investigated, with the goal of gaining a deeper understanding of its structure-property relationships for applications in cancer theranostics.

2. Materials and methods

2.1. Materials

21-arm star like polymer β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$ ($M_{n,NMR}$ = 334,174 g/mol, $M_{n,GPC}$ = 57,752 g/mol, PDI = 1.05) was synthesized as reported in our previous paper [35]. Doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Huafeng United Technology Co., Ltd., Beijing, China. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen, Carlsbad, CA, USA. HepG2 cells were purchased from the American Type Culture Collection (ATCC), Manassas, VA, USA, and cultured under conditions recommended by the supplier. 3-(4,5-Dimethyltlliazol-2-yl)-2,5-diphenyltetrazoxium bromide (MTT) and Hoechst 33324 were purchased from Sigma Chemical Co. $HAuCl_4{\cdot}4H_2O$ (AR, J&K Scientific Ltd., China), Omnipaque (98%, J&K Scientific Ltd., China), paraformaldehyde (99%, Aldrich), pelltobarbitalum natricum (Aldrich), and other reagents were used as received.

2.2. Fabrication of unimolecular micelles

β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$ (20 mg) was dissolved in DMSO (20 mL) for 4 h. The solution was then dialyzed against 2 L deionized water in dialysis tubing with a molecular weight cut-