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Chemotherapeutic drug-photothermal agent co-self-assembling nanoparticles for near-infrared fluorescence and photoacoustic dual-modal imaging-guided chemo-photothermal synergistic therapy

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ABSTRACT

Multimodal imaging-guided synergistic combination therapy has shown great potential for cancer treatment. However, the nanocarrier-based theranostic systems suffer from batch-to-batch variation, complexity of multicomponent, poor drug loading, and carrier-related toxicity issues. To address these issues, herein we developed a novel carrier-free theranostic system with nanoscale characteristics for near-infrared fluorescence (NIRF) and photoacoustic (PA) dual-modal imaging-guided synergistic chemo-photothermal therapy (PTT). Indocyanine green (ICG) and epirubicin (EPI) could co-self-assemble into small molecular nanoparticles (NPs) in aqueous solution without any molecular precursor or excipient *via* collaborative interactions (electrostatic, π–π stacking, and hydrophobic interactions). The exceptionally high dual-drug loading (~ 92 wt%) ICG-EPI NPs showed good physiological stability, preferable photothermal response, excellent NIRF/PA imaging properties, pH-/photo-responsive drug release behavior, and promoted cellular endocytosis compared with free ICG or EPI. Importantly, the ICG-EPI NPs showed excellent tumor targeting ability with high spatial resolution and deep penetration *via in vivo* NIRF/PA dual-modal imaging. Moreover, in comparison with individual chemotherapy or PTT, the combinational chemo-PTT therapy of ICG-EPI NPs with NIR laser irradiation synergistically induced apoptosis and death of cancer cells *in vitro*, and showed synergistic chemo-PTT efficiency *in vivo* as evidenced by highly efficient tumor ablation. Furthermore, the ICG-EPI NPs exhibited inappreciable toxicity. This co-self-assembly of both FDA-approved agents provides a safe and "Molecular economical" strategy in the rational design of multifunctional nano-theranostic systems for real-time self-monitoring intracellular drug delivery and targeting multimodal imaging-guided synergistic combination therapy.

1. Introduction

Cancers have currently become one of the most serious diseases all over the world [1]. A number of therapies such as surgery, chemotherapy, photothermal therapy (PTT), photodynamic therapy (PDT), radiation therapy, and immunotherapy have been developed for cancer treatment. Chemotherapy is considered as a dominant therapeutic modality due to its high efficiency compared to others [2]. PTT is another promising therapeutic modality because of the advantages of noninvasiveness, harmless, and high selectivity. In PTT, a photothermal agent is exploited to absorb near-infrared (NIR) light and convert it into cytotoxic heat to kill cancer cells [3]. Each individual therapeutic modality is currently effective to some extent, but the therapeutic outcome of single modal therapy remains unsatisfied for eradication of tumor without any recurrence [3–5]. After decades of experimental and clinical studies, combination therapy has been demonstrated as an effective and preferable therapeutic modality [5,6]. The combination therapy with different therapeutic agents and anticancer mechanisms

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may cooperatively suppress tumor development by utilizing the advantages of both modalities and circumventing the drawbacks of each modality [7]. Among the selections of combination therapy, "chemotherapy + PTT" has emerged as a promising modality to augment the cytotoxicity of chemotherapeutic drug by a synergistic manner [6,8,9].

To guide the therapeutic process, track the delivery of nanomedicines, and evaluate the efficacy of cancer treatment, there has been an increasing interest focus on imaging modalities, such as near-infrared fluorescence (NIRF) imaging, magnetic resonance (MR) imaging, photoacoustic (PA) imaging, computed tomography (CT) imaging, ultrasound (US) imaging, positron emission tomography (PET) imaging, and single photon emission computed tomography (SPECT) imaging [9–14]. However, a single-modal imaging still faces enormous challenges such as limited tissue penetration depth, low contrast, low sensitivity, and poor spatial resolution [15,16]. Thus multimodal imaging is expected to compensate the intrinsic limitations of each single-modal imaging and is thus emerging as an inevitable trend in the development of new imaging techniques [15–17]. A good example is the integration of NIRF and PA, which could offer a real-time, comprehensive, and exact manner for cancer theranostics [17,18].

To date, with the aim of multimodal imaging-guided combinational chemo-PTT, a prevalent and effective strategy is to incorporate two or more different therapeutic and imaging agents into a nanoplatform [3,6,9,10,19]. Despite of the tremendous development of multimodal theranostic nanomedicine, the sophisticated design and engineering, batch-to-batch variation, poor drug loading capacity, and potential toxicity issues caused by degradation, metabolism, and excretion of nanocarriers are still major obstacles in translational medicine [20–22]. Therefore, it is highly desirable to develop a simple, safe, and efficient nano-agent that could intrinsically integrate multimodal theranostic capabilities while ensuring eradication of tumor without recurrence. Recently, nanomedicines constructed directly from small molecules without using any drug carrier have attracted great attention [20,23–27]. Compared with traditional carrier-based nanomedicines, self-delivered nanomedicines without carriers as a new generation of drug delivery systems could not only avoid the significant safety concerns from nanocarriers, but also achieve both nanoscale advantages and high drug payload simultaneously [28,29]. Moreover, it is relatively easy for large scale production and acceleration of laboratory-to-clinic-to-industry translation by applying the novel self-delivered nanomedicine [29–33]. Recently, Yan group reported that amphiphilic drug-drug conjugate could be self-assembled into nanodrug self-delivery systems for combination cancer chemotherapy [25]. Some well-known research groups also reported high drug loading nanodrugs from the self-assembly of two or more chemotherapeutic drugs (like paclitaxel, camptothecin, and doxorubicin) [34–36]. To our knowledge, no example has been reported by applying small molecular photothermal agent and chemotherapeutic drug to construct carrier-free nanomedicines for multimodal imaging-guided combination therapy.

Epirubicin (EPI) is one of the most clinically used chemotherapeutic drugs against a wide range of tumors, while indocyanine green (ICG) is widely used in NIRF/PA imaging and PTT as a clinical used diagnostic agent. It should be noted that EPI has the hydrophobic anthraquinone rings and the hydrophilic amino sugar and adjacent hydroxyl groups [37]. Moreover, ICG has two polycyclic indole skeletons imparting hydrophobic character, and two sulfate groups imparting hydrophilic character (Fig. S1) [38,39]. In consideration of the highly conjugated electron configuration and amphiphilic nature, we hypothesized that versatile ICG and EPI can be used to realize the collaborative assembly of NPs based on electrostatic, π–π stacking, and hydrophobic interactions, singly or in combination. Herein we develop a new concept of theranostic NPs that is co-self-assembled by both the two small molecular FDA-approved agents for NIRF/PA bimodal imaging-guided combinational chemo-PTT (Scheme 1). Trace amounts of PEG are used for surface functionalization to ensure long circulation time of ICG-EPI NPs [40]. Both EPI and ICG could be self-delivered themselves into tumor sites without the help of drug carriers, possibly due to enhanced permeability and retention (EPR) effect. As expected, the robust and multifunctional ICG-EPI NPs could achieve synchronous diagnostics and eradication of tumors without recurrence by a synergistic therapeutic effect in one treatment cycle. Thus, the ICG-EPI NPs system is a promising candidate as multimodal theranostic nanoplatforms for imaging-guided tumor ablation.

2. Results and discussions

2.1. Preparation and characterization of ICG-EPI NPs (without PEGylation)

Supramolecular self-assembly has attracted much interest in developing new drug delivery platforms for both biomedicine and nanotechnology [41]. In this study, the preparation of co-self-assembled ICG-EPI NPs based on two structural motifs was illustrated in Scheme 1. Hydrophilic EPI·HCl was initially transformed to hydrophobic EPI molecules by removal of hydrochloric acid in an alkaline environment. Then, carrier-free ICG-EPI NPs (without PEGylation) were prepared by injecting an organic solvent of both ICG and EPI dropwise into aqueous solution with stirring and ultrasonication.

Interestingly, it should be noted that the size of ICG-EPI NPs (without PEGylation) can be adjusted ranging from micrometer to nanometer by altering the mass ratio of ICG to EPI (Fig. 1A-B). Specially, the transmission electron microscopy (TEM) images, scanning electron microscopy (SEM) images, and dynamic light scattering (DLS) data revealed that the diameter of ICG-EPI NPs (without PEGyaltion) was 80–100 nm with an optimized mass ratio of ICG to EPI (1: 2), which was within the accepted range favoring passive tumor targeting by the enhanced permeability and retention (EPR) effect (Fig. 1A–C) [42,43]. The confocal laser scanning microscopy (CLSM) images in clearly showed that both EPI (false-color red) and ICG (false-color green) dyes were uniformly distributed throughout the hybrid NPs without obvious hierarchically core/shell structure (Fig. 1D). This result indicated the simultaneous self-assembly of both EPI and ICG into the intact and coherent ICG-EPI NPs. The zeta potential of ICG-EPI NPs (without PEGyaltion) in aqueous solution was negative (about − 20 mV) (Fig. 1E), which was beneficial for their stability because of the electrostatic repulsion. Furthermore, with this ratio, the encapsulation efficiency (EE) of EPI and ICG achieved > 95% determined by high-performance liquid chromatography (HPLC) (Table S1). Therefore, the optimized formulation prepared at a mass ratio of ICG to EPI (1:2) was selected from various different ratios for further surface PEGylation and the following *in vitro* and *in vivo* experiments.

Simple physical mixing of EPI·HCl and ICG in aqueous solution together resulted in precipitation/phase separation, which suggested that co-self-assembly could not be obtained spontaneously by simple physical mixing (Fig. 1F and Fig. S2). According to ^{1}H NMR nuclear magnetic resonance (NMR) spectrum, the two proton signals at ~ 14.0 and ~ 13.3 ppm which could be attributed to hydroxyl group in both EPI·HCl and ICG/EPI·HCl mixture, shifted to a high field of ~ 10.2 ppm in the ^{1}H NMR spectrum of ICG-EPI NPs (Fig. 1G). Furthermore, according to differential scanning calorimetry (DSC) thermogram and X-ray diffraction (XRD) pattern, compared with EPI·HCl or ICG/EPI·HCl mixture, the exothermic peak at ~ 188.6 °C of EPI molecule disappeared and the strong crystallization was also significantly restricted in ICG-EPI NPs (Fig. 1H-I) [20]. Additionally, the Fourier transform infrared (FT-IR) spectrum of ICG-EPI NPs was also recorded. Compared with ν(C = O) (~ 1716 cm^{-1}) and ν(C = C) (~ 1405 cm^{-1}, characteristic of the aromatic rings of anthracyclines [44]) signals of both EPI·HCl and ICG/EPI·HCl mixture, a high-wavenumber shift of ν(C = O) (~ 1745 cm^{-1}) and ν(C = C) (~ 1421 cm^{-1}) signals could be observed (Fig. 1J). Thus, these above results verified the existence interaction between ICG and EPI molecules.