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Multi-responsive photothermal-chemotherapy with drug-loaded melanin-like nanoparticles for synergetic tumor ablation

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ABSTRACT

Photothermal-chemotherapy (PT-CT) is a promising strategy for cancer treatment, but its development is hindered by the issues regarding to the long-term safety of carriers and imperfect drug release profiles. In this article, we use polyethylene glycol-modified polydopamine nanoparticles (PDA-PEG) as an outstanding PT-CT agent for cancer treatment. PDA-PEG possesses excellent biocompatibility and photothermal effect, and could easily load anticancer drugs such as doxorubicin (DOX) and 7-ethyl-10-hydroxycamptothecin (SN38) via π-π stacking and/or hydrogen binding. Moreover, the drug-loaded PDA-PEG showed great stability and drug-retaining capability in physiological condition, and could respond to multiple stimuli including near infrared light, pH and reactive oxygen species to trigger the release of loaded anticancer drugs. The *in vitro* and *in vivo* studies demonstrated that PDA-PEG-mediated PT-CT showed synergetic effect for cancer therapy.

1. Introduction

The combination of photothermal therapy and chemotherapy (photothermal-chemotherapy, PT-CT) is considered as a promising strategy for the cancer treatment [1–3]. Photothermal therapy is subject to the heterogeneous distribution of hyperthermia and intermittent near-infrared (NIR) irradiations, thus leading to incomplete tumor ablation especially at the margins of tumor tissue [4,5], while chemotherapy suffers from the serious side effects and drug resistance [6,7]. The combination of PT-CT could achieve a complementary effect to improve therapeutic efficiency and reduce side effect [3,8,9]. Up to date, a host of nanomaterial-based carriers have been developed for the co-delivery of photothermal agents and chemical drugs to tumor site, which includes metal nanoparticles [2,3], carbon-based materials [10,11], silica nanoparticles [12], and polymeric nanoparticles [13]. Despite excellent therapeutic effect achieved, PT-CT agents are still confronted with critical issues regarding to the long-term safety of carriers and imperfect drug release profiles. Most of the current PT-CT agents are non-degradable and/or potential toxic. Their long retention in the body would cause unexpected safety problems. For instant, carbon-based nanomaterials including carbon nanotubes and graphene could induce systematic toxicity related to inflammation and thrombogenicity [14]; silica nanoparticles could cause pregnancy complications [15]; gold nanoparticles, although little toxicity is considered, are hard metabolized *in vivo*, and their redox activity may have unpredictable influence to normal tissues and organs [16]. The control release strategies with responding to external and/or internal stimuli could achieve a spatiotemporally controlled drug release in the tumor site to significantly enhance drug utilization and reduce side effect. For example, mesoporous silica-based drug carriers are decorated with diverse moieties such as gold nanoparticles [17], quantum dots [18], and peptides [19] outside of their surface or magnetic nanoparticles [20], coumarin [21], and azobenzene [22] inside of their channels to conduct stimuli-responsive drug release. However, these stimuli-responsive mechanisms lead that the drug carriers become more complicated along with increased cost and synthetic challenges. Besides, these off-test components integrated in the carriers may cause new safety problems.

Melanin-like biopolymers are widely existed in the human tissues

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and organs such as skin, hair, iris of eyes, and brain medulla [23,24], and they also have been widely used as polymer coating for diverse biomedical applications including diagnosis [25–28], biosensor [29,30], and tissue engineering [31–33], both of which suggests they have excellent biocompatibility. It is well demonstrated that melanin-like biopolymers could biodegrade into non-toxic components [34], which further guarantees their safety *in vivo*. Recent investigation demonstrated that melanin-like polydopamine nanoparticles (PDA) could efficiently convert NIR light into heat and kill cancer cells *in vitro* and *in vivo*, suggesting PDA are potential photothermal agent [13]. Moreover, PDA have plentiful aromatic rings on their surface, making it possible to load chemical drugs on their surface via $\pi-\pi$ stacking and/or hydrogen binding [28,35], and they also possess chemically-active catechol and quinone groups on their surface to facilitate the further modification for additional functions such as enhanced blood circulation or cell targeting [36]. These natural merits indicate PDA an optimal candidate for PT-CT.

In this article, we synthesized polyethylene glycol-modified PDA (PDA-PEG) and loaded them with anticancer drugs doxorubicin (DOX) and 7-ethyl-10-hydroxycamptothecin (SN38). PDA-PEG were primarily assessed for their photothermal efficacy, drug loading capacity and biocompatibility, and then the drug-loaded PDA-PEG (PDA-PEG/drug) were evaluated for their stability and drug-retaining capability in various conditions. The stimuli-responsive drug release from the PDA-PEG/drug was analyzed with the application of multiple stimuli including NIR light, pH, and reactive oxygen species (ROS). Finally, PT-CT based on PDA-PEG/drug was conducted *in vitro* and *in vivo* to determine their synergetic effect over cancer treatment.

2. Experimental section

2.1. Materials

Dopamine hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO). DOX, SN38, and mPEG-NH_2 (Mw = 2000) were obtained from Aladdin Reagent (Shanghai, China). For Cell culture, RPMI 1640 and DMEM media were purchased from Invitrogen (Carlsbad, CA), and fetal bovine serum was obtained from Clark Bioscience (Houston, USA). 3-(4,5-dimethylthiazol-2-yl)- 107 2,5-diphenyltetrazolium bromide (MTT) was purchased from Sangon Biotech (Shanghai, China). DMSO was obtained from Sinopharm Chemical Reagent (Beijing, China).

2.2. Synthesis of PDA-PEG

PDA was synthesized by using a method reported before [13]. In a typical synthesis, 0.6 mL ammonia aqueous solution (28–30%) was added into a mix of 8 mL ethanol and 18 mL deionized (DI) water in water bath at 30 °C under mildly magnetic stirring. 100 mg of dopamine hydrochloride dissolved in 2 mL deionized water was then injected into the above solution. The reaction was allowed to proceed for 12 h. The solution colors changed gradually from pale brown to dark brown. The product was collected by centrifugation at 15000 rpm for 15 min and then was washed three times with DI water. The concentration of PDA was determined by weigh after lyophilization.

PDA-PEG were prepared by simply mixing PDA with mPEG-NH_2 at a mass ratio of 1:2 in alkaline buffer solution (pH = 9) under magnetic stirring for 24 h. The product was purified by centrifugation and washed with DI water for three times.

2.3. Characterization

The transmission electron microscopy (TEM) images were taken using a Hitachi microscope (HT7700, Hitachi, Japan) operating at an acceleration voltage of 100 kV. The hydrodynamic diameters and zeta potentials were measured by DLS (Zetasizer Nano ZS90, Malvern Instruments, UK). The UV–Vis spectra were recorded by using a UV–Vis spectrometer (Cary60, Agilent Technologies, USA). The NMR analysis was performed on a Varian 699.804 MHz NMR spectrometer (Agilent Technologies, USA) at 298.2 ± 0.1 K.

2.4. Photothermal effect of PDA-PEG

To test the photothermal efficacy of PDA-PEG, 1 mL PDA-PEG in aqueous solution with different concentrations (0–200 μg/mL) was held in a quartz cuvette and irradiated with an 808-nm NIR laser at a power density of 3.6 W cm-2 for 10 min. The temperatures were recorded by using an infrared thermal camera (Magnity Electronics, China).

2.5. Drug loading and the stability of PDA-PEG/drug

DOX was loaded to PDA-PEG by mixing 2 mL of 0.8 mg/mL PDA-PEG suspension with 3.2 mL of different concentrations (0.125, 0.25, 0.5, and 1 mg/mL) of DOX in aqueous solution (pH = 7.0) under magnetic stirring at room temperature. After 12 h incubation, PDA-PEG/DOX was collected via centrifugation at 15000 rpm for 10 min, and then was washed three times with DI water. According to the previous reported method [37], different amount of SN38 (0.4, 0.8, 1.2, and 1.6 mg) were mixed with 2 mL of 0.8 mg/mL PDA-PEG that suspended in a mixture of water and DMSO (10:1, v/v). The mixed solution was incubated for 12 h under magnetic stirring. The undissolved SN38 was firstly removed by centrifugation at a relative low speed (2000 rpm) for 5 min, and then the supernatant was collected and centrifuged at a high speed of 15000 rpm for 10 min to precipitate PDA-PEG/SN38. The obtained PDA-PEG/SN38 was then washed three times with DI water. The drug loading capacity of PDA-PEG was determined by UV–Vis spectrometer. The typical absorption at 490 nm and 380 nm for DOX and SN38, respectively, was used to determine their loading capacity on PDA-PEG. The absorbance of PDA at the two peaks was deducted from the spectra of PDA-PEG/DOX and PDA-PEG/SN38.

The stability of PDA-PEG/drug was assessed via incubating them with different media including PBS, cell culture medium, and 50% fetal bovine serum in PBS for 1 h, and then the observation was conduct to check if there was agglomeration or sediment in the solution. Further, the drug-retaining capability of PDA-PEG/DOX was evaluated. PDA-PEG/DOX was incubated with 50% fetal bovine serum in PBS. At different time points, 0.1 mL of the solution was collected and then was centrifuged at a high speed of 15000 rpm for 10 min to remove PDA-PEG/DOX. After that, the supernatant was analyzed by high performance liquid chromatography (HPLC) to determine the concentration of released DOX. The drug retained in PDA-PEG was calculated by subtracting the released drug amount from the initially loaded one.

2.6. Stimulus-responsive drug release

To determine the light-triggered drug release kinetics, 3 mL of PDA-PEG/DOX or PDA-PEG/SN38 solution at a concentration of 80 μg/mL in a quartz cuvette was irradiated with an 808-nm NIR laser at a power density of 3.6 W cm^{-2} for different times. The PDA-PEG/drug was collected via centrifugation and washed three times, and then was analyzed by using UV–Vis spectrometer and HPLC to determine the drug release.

To assess drug release kinetics with the stimulation of pH and ROS, 1 mL of PDA-PEG/DOX or PDA-PEG/SN38 were packaged in a dialysis bag (MWCO = 3.5 kDa) and then immersed within 49 mL of