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Red blood cell membrane-camouflaged melanin nanoparticles for enhanced photothermal therapy

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ABSTRACT

Photothermal therapy (PTT) has represented a promising noninvasive approach for cancer treatment in recent years. However, there still remain challenges in developing non-toxic and biodegradable biomaterials with high photothermal efficiency *in vivo*. Herein, we explored natural melanin nanoparticles extracted from living cuttlefish as effective photothermal agents and developed red blood cell (RBC) membrane-camouflaged melanin (Melanin@RBC) nanoparticles as a platform for *in vivo* antitumor PTT. The as-obtained natural melanin nanoparticles demonstrated strong absorption at NIR region, higher photothermal conversion efficiency (~40%) than synthesized melanin-like polydopamine nanoparticles (~29%), as well as favorable biocompatibility and biodegradability. It was shown that RBC membrane coating on melanin nanoparticles retained their excellent photothermal property, enhanced their blood retention and effectively improved their accumulation at tumor sites. With the guidance of their inherited photoacoustic imaging capability, optimal accumulation of Melanin@RBC at tumors was achieved around 4 h post intravenous injection. Upon irradiation by an 808-nm laser, the developed Melanin@RBC nanoparticles exhibited significantly higher PTT efficacy than that of bare melanin nanoparticles in A549 tumor-bearing mice. Given that both melanin nanoparticles and RBC membrane are native biomaterials, the developed Melanin@RBC platform could have great potential in clinics for anticancer PTT.

1. Introduction

As a noninvasive cancer treatment, photothermal therapy (PTT) has earned much attention in recent years due to its localized tumor ablation and minimal heating damage to adjacent normal tissues [1]. The mechanism of PTT is that photothermal agents strongly absorb and covert near infrared (NIR) light into thermal energy after their adequate accumulation within tumors [2]. Currently, various materials have been explored as effective photothermal agents, including inorganic nanomaterials (e.g. gold-, carbon-, palladium-, and magnetic nanoparticles) [3–6], NIR dye (indocyanine green, IR825) [7,8], polymer nanoparticles (e.g. polydopamine, polyaniline, polypyrrole, poly(-ethylenedioxythiophene) (PEDOT)) [9–12] and other nanomaterials (e.g. porphysomes, Prussian blue) [13,14]. Despite their extraordinary photothermal effect, the long-term safety of these synthetic nanomaterials *in vivo* may still limit their further clinical application in cancer treatment. For instance, metallic nanoparticles, especially those non-degradable metallic nanoparticles, would retain in the biological system for a very long time and cause metal-related cytotoxicity [15], while carbon-based nanomaterials have been widely reported to induce serious toxicities such as oxidative stress and lung inflammation after administration [16]. Therefore, it will be of great clinical value to develop biocompatible and biodegradable photothermal nanomaterials with high photothermal efficiency for *in vivo* cancer PTT.

Melanin, is a ubiquitous natural biopolymer, which is widely distributed in many organisms including human skin [17]. It has

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been a new class of biomaterials for biomedical application because of its intrinsic properties and various biological functions [18]. For example, Lu et al. synthesized melanin-like colloidal nanospheres as effective photothermal agents for *in vivo* cancer therapy and achieved as high as 40% photothermal conversion efficiency [19]. Fan et al. synthesized water-soluble melanin nanoparticles and developed a melanin-based theranostic platform for multimodality imaging of melanoma and tumor drug delivery by loading sorafenib on their surfaces through strong binding interaction between melanin and aromatic structures of sorafenib [20,21]. Compared with those synthetic melanin nanoparticles described above, the natural melanin nanoparticles extracted from living organisms attracted more alluring attention for cancer PTT because of their native biocompatibility and biodegradability, which could effectively eliminate the side-effects as well as default metabolism in biological system [22]. Chu et al. successfully extracted natural melanin nanoparticles from black sesame seeds for lymph node imaging and cancer PTT [23]. After intratumoral injection into tumor-bearing mice, the as-obtained natural melanin nanoparticles presented appealing performance in tumor growth inhibition with considerable PTT efficacy and low toxicity. However, although the "enhanced permeation and retention (EPR)" effect of tumors could provide passive targeting, like other nanoparticles, melanin nanoparticles still could be easily recognized and rapid cleared by the reticuloendothelial system as exogenous invaders when injected *via* tail-vein, and eventually resulted in poor tumor accumulation and limited therapeutic effect [24]. Therefore, it will be of great significance in strategies that can promote the accumulation of melanin nanoparticles at target sites as well as improve their PTT performance for *in vivo* cancer therapy.

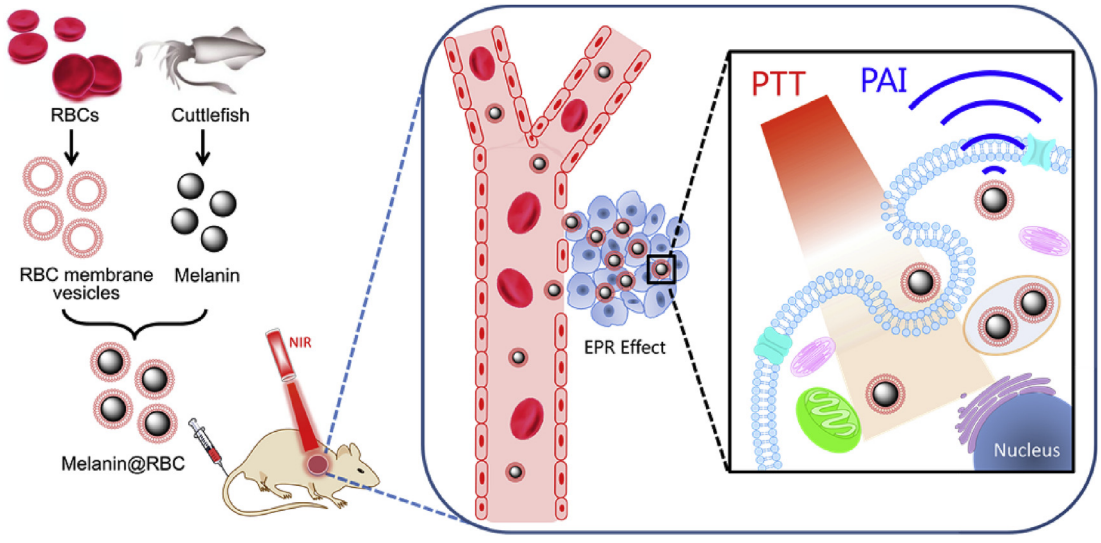
Recently, functionalization of nanoparticles through a top-down naturally derived bio-membranes coating approach represents an emerging strategy to construct biomimetic system [25–27]. Red blood cell (RBC), of which surface makeup composed of many "self-makers" (e.g. CD47 proteins, peptides, glycans, acidic sialyl moieties), is a native long circulating carrier that enables nanoparticles to effectively escape immune recognition in the body [28]. RBC membrane-camouflaged nanoparticles have been strongly verified as promising candidates for biomedical application, which benefit from the prolonged blood circulation, high biocompatibility, immune-evasion, and reduced accelerated blood clearance effect endowed by RBC membrane [29,30]. For example, Zhang and co-workers have engineered RBC membrane with various functional nanoparticles for *in vivo* drug delivery, blood detoxification, antibacterial vaccination and anticancer therapies [31–34]. Moreover, our group previously fabricated RBC membrane-coated Fe_3O_4 particles as a platform for cancer PTT, and *in vivo* studies revealed that Fe_3O_4 particles coated with RBC membrane performed superior PTT efficacy than bare Fe_3O_4 particles [35]. Very recently, Liu et al. achieved outstanding synergistic combination therapy *in vivo* by cloaking doxorubin (DOX)-loaded hollow mesoporous Prussian blue (HMPB) nanoparticles with RBC membrane (DOX@HMPB@RBC) [36]. The developed DOX@HMPB@RBC nanoparticles exhibited excellent immune evading capacity and longer blood retention compared with bare HMPB nanoparticles. These studies indicated that erythrocyte membrane coating strategy could be a facile and effective way to enhance tumor accumulation and tumor ablation effect of natural melanin nanoparticles.

In this work, we extracted natural melanin nanoparticles from living cuttlefish and firstly explored them as effective photothermal agents. The photothermal property, biocompatibility as well as degradability of as-obtained natural melanin nanoparticles were investigated. Afterwards, RBC membrane-camouflaged melanin nanoparticles (Melanin@RBC) were developed as a platform for *in vivo* cancer PTT (Scheme 1). With the inherited photoacoustic imaging (PAI) characteristics from melanin nanoparticle core [37] and promoted immune evasion capability and enhanced tumor accumulation from RBC membrane, the developed Melanin@RBC was employed as a PAI contrast for tumor imaging and its *in vivo* PTT efficacy in A549 tumor-bearing mice after intravenous administration was investigated.

2. Materials and methods

2.1. Materials

Fresh cuttlefish (~400 g) were purchased from Tongren Fishes Wholesale Market (Shanghai, China). Dopamine hydrochloride (DA-HCl) was ordered from Sigma-Aldrich (St. Louis, USA). Ethylene glycol, sodium hydroxide (NaOH), nitric acid (HNO_3), hydrogen peroxide (H_2O_2, 35%) and ammonia ($NH_3 \cdot H_2O$, 25%–28%) were from Aladdin (Shanghai, China). Ethyl alcohol was purchased from Shanghai Heqi Chemical Co. Ltd (Shanghai, China). Roswell Park Memorial Institute (RPMI-1640) medium and Dulbecco's modified



Scheme 1. Schematic illustration of red blood cell membrane camouflaged melanin nanoparticles for enhanced photothermal therapy.