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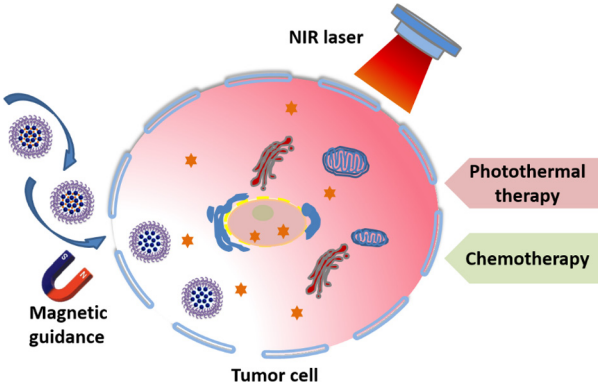
PEGylated magnetic Prussian blue nanoparticles as a multifunctional therapeutic agent for combined targeted photothermal ablation and pH-triggered chemotherapy of tumour cells

Peng Xue [a,b,1,*], Lihong Sun [a,b,1], Qian Li [a,b], Lei Zhang [c], Zhigang Xu [a,b], Chang Ming Li [a,b], Yuejun Kang [a,b,*]

[a] Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing 400715, China
[b] Chongqing Engineering Research Center for Micro-Nano Biomedical Materials and Devices, Chongqing 400715, China
[c] State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China

GRAPHICAL ABSTRACT

PEGylated Fe_3O_4-Prussian blue nanocomplex loaded with doxorubicin for combination tumor treatment with photothermal ablation and chemotherapy under magnetic guidance.



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ABSTRACT

Multifunctional nanoagents have become popular and valuable pharmaceuticals for effective cancer treatment. Moreover, there is an increasing tendency to develop therapeutic agents with excellent biocompatibility, high efficiency, specific targeting and combinatorial treatment effects. In this study, we proposed a facile technique to synthesize PEGylated (polyethylene glycol modified) magnetic Prussian blue (PB) nanoparticles with encapsulated doxorubicin (DOX), abbreviated as Fe_3O_4@PB/PEG/DOX NPs, for combined targeted photothermal ablation and pH-triggered chemotherapy of tumour cells. The PEGylation of Fe_3O_4@PB core-shell structure was achieved through a thin-film hydration process; DOX was loaded into the nanocapsule via hydrophobic interactions. An *in vitro* study indicated increased drug release under acidic conditions, mimicking mild acidic tumour microenvironments. Additionally, the nanocomposites exhibited superparamagnetism, contributing to an improved therapeutic effect guided by a localized magnetic field. Cytotoxicity studies demonstrated outstanding photothermal-chemotherapy combinatorial effects on HeLa cells, attributed to the targeted photothermic effect

* Corresponding authors at: Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing 400715, China.
E-mail addresses: xuepeng@swu.edu.cn (P. Xue), yjkang@swu.edu.cn (Y. Kang).
[1] These authors contributed equally to this work.

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mediated by the pH-triggered cellular uptake of DOX. Specifically, the viability of HeLa cells decreased to 8.5% after treatment with the nanoagent (DOX = 10 μg mL^{-1}) and near infrared irradiation, indicating an evident tumour inhibition effect *in vitro*. This study presented a nanoplatform for efficient and targeted cancer treatment, which may lead to the development of multifunctional nanodrug vehicles for cancer therapy.

1. Introduction

Vast genetic diversity, complex protein expression patterns and diversified cellular microenvironments can be attributed to high mortality rates in cancer diseases worldwide [1,2]. Therapeutic nanoparticles (NPs) have attracted much attention for their highly efficient cancer treatment results with minimal side effects [3–5]. Nanoagents for photothermal therapy (PTT) have been extensively investigated due to their outstanding capacity to convert the optical energy of near infrared (NIR) light irradiation into thermal energy [6–8]. There are many advantages of using NIR-laser-mediated PTT for cancer treatment, including low recurrence rates, rapid recovery, uncomplicated operation and minimal side effects to normal tissues [9]. Various NIR-absorbers with good photothermal conversion efficiency have been developed for tumour ablation, including gold nanospheres [10–12], copper sulfide nanoparticles [13,14], carbon-based nanoderivatives [15–17] and polymeric nanocomplexes [18–20]. However, monofunctional PTT agents usually fail to achieve optimal therapeutic efficacy because the distribution of NIR light-induced heating is not desirably homogeneous within tumour tissues [21–23]. To address this issue, recent efforts have been devoted to preparing novel nanocomplexes by combining multiple therapeutic modalities facilitated by PTT to achieve a synergistic anti-tumour effect, particularly for curing heterogeneous solid tumours [24–26].

Prussian blue (PB) is a typical synthetic pigment dispersed in colloidal form and has been approved by the US Food and Drug Administration (FDA) for the treatment of radioactive exposure in clinics [27]. Recently, various PB-based therapeutic agents have been investigated for highly efficient PTT due to their significant absorbance in the NIR optical regime [28–31]. Moreover, various modalities, such as magnetic fields [32], chemotherapeutic drugs [33] and pH adjustments [34], have been applied for manipulating precise and enhanced PTT based on Prussian blue nanoagents. Specifically, magnetic PB NPs were explored for improved magnetic resonance imaging and PTT [32]. PEGylated PB NPs were also designed for pH-controlled chemotherapy and PTT [34]. However, these auxiliary modalities have limitations. For example, the focusing of magnetic fields is typically insufficient and works only within short ranges, whereas pH-controlled drug release is highly dependent on the type of solid tumour and the pH-response behaviour of the nanoagents. Therefore, there exists a pressing demand to integrate these functions into a single drug nanocarrier with acceptable biocompatibility.

Superparamagnetic Fe_3O_4 NPs have been extensively utilized as nanocores for the construction of many core-shell structures to effectively transport nanodrugs into individual tumour cells [35,36]. Moreover, localized magnetic guidance can further enhance NIR light-induced tumour ablation effects mediated by internalized Fe_3O_4 nanocores [30]. As a typical anti-tumour drug model, doxorubicin (DOX) can be efficiently loaded onto functionalized nanocarriers to realize controlled drug release [37]. Furthermore, surface modification with polyethylene glycol (PEG) is a standard technique to improve interfacial biocompatibility and stability of nanoparticle composites in physiological environments [38,39]. Hence, the surface engineering of Fe_3O_4@PB NPs with DOX and PEG may contribute to a combined therapeutic approach for tumour ablation.

Herein, we present a facile strategy for modifying the surface of Fe_3O_4@PB NPs with PEG and DOX for combining NIR light-induced hyperthermia and chemotherapy of tumours to produce a synergistic therapeutic effect (Fig. 1). Fe_3O_4@PB NPs were synthesized by the consecutive addition of $[Fe(CN)_6]^{4-}$ into an NP dispersion under acidic conditions [32]. Based on Fe(III)–OH_2 functional moieties on the PB nanoshell, the surface modification of insoluble Fe_3O_4@PB NPs with oleylamine (OA-Fe_3O_4@PB NPs) was realized by a simple mixing process with OA-toluene dispersion [40,41]. To improve the biocompatibility of the drug carrier, hydrophobic OA-Fe_3O_4@PB NPs were modified with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000] (abbreviated as DSPE-PEG 2000) lipid through a thin film hydration process, resulting in hydrophilic PEGylated NPs [42]. To realize the combinatorial effects of photothermal-chemo therapy, DOX was selected as an antitumour drug model and encapsulated into the lipid portions of the Fe_3O_4@PB/PEG NPs (Fig. 1). We characterized the physicochemical properties of this nanocomplex, including morphology, stability, superparamagnetism and drug release behaviour. The photothermal effect, cell internalization and antitumour efficacy were further investigated *in vitro*. This study demonstrated that Fe_3O_4@PB/PEG/DOX NPs could work as a multifunctional nanomedicine to realize synergistic treatments for cancer therapeutics.

2. Materials and methods

2.1. Materials

Iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium hydroxide (NaOH), hydrochloric acid (HCl, 37%), potassium hexacyanoferrate(ii) trihydrate ($K_4[Fe(CN)_6]{\cdot}3H_2O$), oleyamine (OA, 70%), and DSPE-PEG 2000 lipid were obtained from Shanghai Advanced Vehicle Technology Pharmaceutical, Ltd. Doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Huafeng United Technology Co. Triethylamine (TEA) was obtained from Sigma-Aldrich, Dulbecco's modified Eagle's medium (DMEM), phosphate buffered saline (PBS), foetal bovine serum (FBS), penicillin/streptomycin, DAPI, Alexa Fluor 633 phalloidin and PrestoBlue cell viability assay kit were provided by Life Technologies (USA). Deionized (DI) water (18.2 MΩ cm) was collected from a purification system (Milli-Q Synthesis A10, Molsheim, France).

2.2. Synthesis of Fe_3O_4 NPs

The magnetic nanoparticles were synthesized by employing the alkaline precipitation method. First, $FeSO_4{\cdot}7H_2O$ (0.556 g) and $FeCl_3{\cdot}6H_2O$ (1.04 g) were dissolved in 5 mL DI water contained 0.17 mL of concentrated hydrochloric acid. Subsequently, this mixture was dropped into 50 mL NaOH solution (1.5 M) at 80 °C with vigorous mechanical stirring and N_2 protection. The obtained products were separated from the aqueous solution using a magnet and thoroughly purified with DI water.