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Red blood cell membrane camouflaged magnetic nanoclusters for imaging-guided photothermal therapy

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ABSTRACT

Along with intrinsic magnetic resonance imaging (MRI) advantages, iron oxide nanomaterials capable of photothermal conversion have been reported very recently and have again raised great interest in their designs among biomedical researchers. However, like other inorganic nanomaterials, high macrophage uptake, short blood retention time and unfavorable biodistributions have strongly hampered their applications *in vivo*. To solve these problems, a rational design of red blood cell (RBC) membrane camouflaged iron oxide magnetic clusters (MNC@RBCs) is presented in this paper. Our data show that by simply introducing an "ultra-stealth" biomimetic coating to iron oxide magnetic nanoclusters (MNCs), MNC@RBCs maintain the imaging and photothermal functionalities inherited from MNCs cores while achieving much lower nonspecific macrophage uptake and dramatically altered fate *in vivo*. MNC@RBCs with superior prolonged blood retention time, preferred high tumor accumulation and relatively lowered liver biodistribution are demonstrated when injected intravenously in mice, leading to greatly enhanced photothermal therapeutic efficacy by a single treatment without further magnetic force manipulation. Our study illustrates a well prepared integration of MNCs and RBCs, exploiting advantages of both functionalities within a single unit and suggests a promising future for iron-based nanomaterials application *in vivo*.

1. Introduction

Photothermal therapy (PTT) is an advanced hyperthermia strategy in cancer treatment that utilizes nanomediators converting light into heat to achieve optimal therapeutic effects. When accompanied by near infrared (NIR) light, PTT could locally ablate the cancerous region while minimizing damage to the non-targeted tissue [1,2]. Owing to its noninvasive nature, great temporal and spatial control and absence of reported resistance, PTT has garnered increasing interest in biomedical applications and a variety of nanomediators have been exploited for this purpose, such as gold-based nanostructures (e.g., nanoshells, nanorods, nanocages and hollow nanospheres) [3–6], NIR dyes (e.g., indocyanine green, IR780) [7–9], metal sulfides [10–12] and carbon-based nanomaterials [13–15]. Moreover, imaging modality integrated photothermal conversion (PTC) agents are also highly desired and have attracted tremendous interest because of their potential in diagnosis and simultaneously monitoring the therapeutic outcome [16,17].

Iron oxide (Fe_3O_4) nanoparticles, due to their low toxicity, good biocompatibility, high stability in the physiological environment, and most intriguingly, their intrinsic capability as magnetic resonance imaging (MRI) contrast agents, have been intensively studied for diagnostic and therapeutic applications [18,19]. Very recently, Fe_3O_4 nanoparticles (including individual Fe_3O_4 nanocrystals, Fe_3O_4 nanoclusters, and polymer-coated hydrophobic Fe_3O_4 nanocrystals), with broad light absorption in the NIR range, have been reported and demonstrated for cancer treatment both *in vitro* and *in vivo* as a novel class of PTC agents [20–23]. Chu et al. first reported differently shaped individual Fe_3O_4 nanocrystals exhibited

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photothermal effect at a relatively high concentration [20]. Chen et al. also found that, owing to their unique crystalline nature, highly crystallized iron oxide nanoparticles made by thermal decomposition with a delicate polymer coating could be used for efficient photothermal ablation of cancerous cells *in vivo* [22]. Moreover, our group previously presented a thorough comparative study on individual Fe_3O_4 nanocrystals and Fe_3O_4 magnetic nanoclusters (MNCs), and further revealed that MNCs out-performed those individual Fe_3O_4 nanoparticles with an enhanced PTT effect in mice [24]. However, due to their intrinsic alien nature, most Fe_3O_4 nanostructures, just like other exogenetic nanomaterials, are easily recognized and captured by the reticuloendothelial system (RES) and have a short blood retention time even for the PEGylated formulation, which is the most widely used polymer coating for building stealth nanoparticles [25,26]. The resulting impaired tumor accumulation of these nanomediators and therapeutic outcome, have strongly hindered Fe_3O_4 nanostructure applications in biomedical fields. Therefore, localized intratumoral injection is often used when applying Fe_3O_4 nanoparticles or Fe_3O_4-based nanomaterials [20,27–29], and sophisticated designs such as conjugating an active targeting moiety or magnetic manipulation are always required in order to achieve a good therapeutic effect [21,30,31]. How to alter Fe_3O_4 nanomediators fate *in vivo* and improve their therapeutic performance with a facile and reliable approach still remains a great challenge.

Red blood cell (RBC) engineering has been proposed as a biomimetic strategy to attain prolonged blood retention and reduced nonspecific macrophage uptake. RBCs with their natural surface "make-up" (e.g., CD47, various membrane proteins, acidic sialyl moieties and glycans) can effectively render their appendant or inner contents with extended blood retention time and immune-evading capabilities. Magnani et al. had successfully filled RBCs with magnetic particles to achieve overall better *in vivo* stability for imaging purposes [32,33]. Wang et al. had also illustrated a multifunctional platform decorating RBCs with different moieties to achieve improved blood retention time [34]. However, both of these approaches could only integrate RBCs stealth properties to platforms with sizes still retained in the micrometer level. Very recently, Zhang et al. developed a novel approach to tailor this "RBC costume" into the nanometer range [35–40]. It has been demonstrated that a variety of nanomaterials (such as polymeric nanoparticles and gold nanoparticles) can be encapsulated into the nano-sized RBC membrane-derived vesicles to obtain significantly prolonged systemic circulation as well as enhanced permeability and retention (EPR) effect which is a unique advantage of nano-sized materials in cancer research [41,42]. Moreover, this cell membrane encapsulation technique has been proved to render the inner nanomaterials with a superior blood half-life and reduced accelerated blood clearance compared to its PEGylated counterparts [43] and can also be integrated with active targeting ligands, indicating its promising potential for both passive and active targeting cancer therapy [44].

Inspired by novel RBC membrane camouflage strategy, here we report a rational design of RBC-derived membrane coated MNCs (MNC@RBCs) for imaging-guided photothermal anti-tumor treatment (Fig. 1). After confirming the successful membrane-MNC encapsulation process, we focused on testing whether the as-prepared MNC@RBCs had simultaneously integrated the MR imaging capability and PTC efficiency from their MNCs cores and the stealth properties from the camouflage membrane coating. To demonstrate the multi-functionalities from the MNCs cores, PTT assessments and T_2 mapping were carried out in aqueous medium and on cells while the stealth properties were studied by macrophage uptake and pharmacokinetics experimentation in an animal model. Furthermore, we illustrated that by simply introducing this biomimetic membrane coating, MNC@RBCs could be administered intravenously achieving greatly altered *in vivo* behavior, with well-preserved imaging and photothermal capabilities, leading to a significantly enhanced PTT performance in an animal xenograft model without complicated surface modification and external magnetic field manipulation.

2. Materials and methods

2.1. Synthesis of Fe_3O_4 magnetic nanoclusters

The Fe_3O_4 magnetic nanoclusters (MNCs) were synthesized by a solvothermal reaction with slight modifications [45]. Briefly, $FeCl_3 \cdot 6H_2O$ (1.08 g), NaOAc (1.20 g), and $C_8H_5Na_3O_7 \cdot 2H_2O$ (0.24 g) as a capping agent were first dissolved in ethylene glycol (20 mL) in a cornical beaker. Then the resulting mixture was carefully transferred into a 50 mL Teflon-lined stainless steel autoclave. After sealed tightly, the autoclave was heated at 200 °C for 10 h and cooled to room temperature. The acquired precipitates were washed with ethanol and then deionized water for several times and collected using a magnet. The final product MNCs were redispersed in water and stored at 4 °C for future use. All chemicals mentioned above were purchased from Shanghai Chemical Reagents Co., Shanghai, China.

2.2. Preparation of membrane-derived vesicles from RBCs

The vesicles derived from RBC membranes were prepared according to a well-established procedure with modifications [35]. Firstly, RBC ghosts were obtained via a so-called hypotonic treatment: fresh whole blood was withdrawn from imprinting control region (ICR) mice (male, bodyweight about 30 g) and was centrifuged at 3000 rpm for 20 min at 4 °C to collect the erythrocytes. The resultant RBCs were washed with PBS three times and recollected by centrifugation to remove plasma and other unwanted types of cells. Hypotonic treatment was conducted by gently mixing as-washed RBCs with excessive amount of 1/4× PBS (pH 7.4, 1.675 mM PO_4^{3-}) for one hour during which the intracellular component of RBCs was released. And then this mixture was centrifuged again at 9000 rpm for 15 min to collect RBC ghosts.

Secondly, vesicles derived from RBC ghosts were prepared by subsequential extrusions: the as-prepared RBC ghosts were modestly sonicated for 3 min in a bath sonicator (53 kHz, 100 W) and were subsequently extruded using an Avanti mini extruder (Avanti Polar Lipid Inc., Alabama, USA) through 1 μm, 800 nm, 400 nm and 200 nm polycarbonate porous membranes. The resultant RBC membrane-derived vesicles were stored in PBS at 4 °C before use.

2.3. Fusion of MNCs with RBC membrane-derived vesicles

200 μL RBC membrane-derived vesicles were mixed with 800 μL MNCs suspension at a final Fe concentration of 0.1 mg/mL and sonicated for 30 s (53 kHz, 100 W). The mixture was then extruded 20 times through 200 nm porous membranes using the same Avanti mini extruder. Excess vesicles were removed by centrifuging at 3500 rpm for 5 min at 4 °C, the MNC@RBCs as final product were collected at the bottom and redispersed for future use. Transmission electron microscope (TEM) (Tecnai G2 20 TWIN, FEI, USA), dynamic light scattering (DLS) (Zetasizer NANO, Malvern, UK) and UV–vis spectrometry (Synergy 2, BioTek Instruments Inc., USA) were used to characterize as-synthesized MNC@RBCs. In each TEM image, 30 random particles were picked and measured manually according to the scale bar to calculate the average particle size.