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Artificially controlled degradable inorganic nanomaterial for cancer theranostics

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ABSTRACT

Multifunctional nanomaterials for cancer diagnosis and therapy have recently prompted widespread concern. To avoid nanotoxicity, the development of novel degradable functional materials must be our main focus. In this study, we firstly developed ethylenediaminetetraacetic acid calcium disodium salt (EDTA)- and bovine serum albumin (BSA)-capped Mn_3O_4 nanoparticles (MONPs-BSA-EDTA) as a novel inorganic nanomaterials for multifunctional imaging-guided photothermal therapy, which can be degraded in a progress-controlled way by artificially introduced ascorbic acid. The degradation products can also be captured and their excretion accelerated. Careful studies suggested that the toxicity of the MONPs-BSA-EDTA and its degradation products is low. The degradation mechanism also suggests a new method of controlled drug release. The development of artificially controlled degradable inorganic nanomaterials also provides a new way to degrade nanomaterials and minimize ion release, which may have potential applications in cancer theranostics without nanotoxicity.

Because cancer is a major threat to public health, the importance of developing theranostics method cannot be overemphasized [1,2]. Magnetic resonance imaging (MRI) had been traditionally used for clinical diagnosis for many years, and the T_1–T_2 dual model selectively visualizes tumor tissues. Depending on the tissue site of interest, T_1-weighted MR images show the typical soft tissue anatomy, including fat, whereas T_2-weighted MR images reveal pathological phenomena, such as tumors or inflammation [3,4]. Therefore, because T_1–T_2 dual-model MRI collects complementary information that cannot be obtained with only one type of contrast agent, it allows a more precise diagnosis. Photothermal therapy (PTT) is a targeted noninvasive therapeutic intervention for cancer [5]. Compared with traditional cancer treatments, PTT is both noninvasive and less toxic to living systems. Because of the specific properties of metal elements, inorganic nanomaterials have received considerable attention in the field of cancer theranostics. Fe-, Gd-, and Mn-based nanomaterials have been explored as contrast agents for many years, and this has encouraged in-depth research of MRI [6–8]. PTT has also advanced rapidly with the intensive study of inorganic nanomaterials. Various inorganic photothermal conversion nanoagents have been investigated, including noble metal nanomaterials [9–12], semiconductor nanomaterials [13–18], carbon-based nanomaterials [19–23], and organic–inorganic nanocomposites [24–29]. Recently, a large number of studies have reported the development of metal oxides (such as Fe_3O_4 and $W_{18}O_{49}$) with broad absorption in the near-infrared (NIR) region as novel inorganic photothermal conversion nanoagents [30–32].

However, most inorganic nanomaterials cannot be degraded, and therefore pose potential threats, including the induction of mesothelioma [33]. With intensive research into inorganic nanomaterials, researchers expected that nanomaterials would be degraded to small particles (<10 nm) or ions for quick metabolism in living systems [34]. Recently, phosphate- and sulfide-based inorganic nanomaterials have been reported to biodegrade in living system [35,36]. However, the biodegradation of these inorganic nanomaterials depends on the environment of the living system and cannot be controlled, which limits their application. Recently, the groups of Liu and Hu reported alkalescence-degradable MoOx nanomaterials, which inspired the exploration of degradable inorganic nanomaterials for imaging-guided cancer therapies [37]. The next step in the development of degradable inorganic nanomaterials must be the development of true artificial control. Many previous studies have reported that manganese oxide nanoparticles (MONPs) can be degraded with a reduction process by reducible agents that can be artificially introduced into

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living systems. This may provide a new way to develop artificially controlled degradable inorganic nanomaterials [38,39]. Because ascorbic acid (AA) has been studied as an antioxidant and hepatic protectant for many years and its toxicity in living systems is low, it can be used as an inducing agent for artificially controlled degradation [40].

In this study, MONPs were developed as a novel efficient T_1–T_2 dual-model MRI contrast agent and PTT coupling agent, based on their relatively high electronic spin and strong broad absorption in the NIR region. The multifunctional MONPs were coated with bovine serum albumin (BSA) and ethylenediaminetetraacetic acid calcium disodium salt (EDTA) to improve their hydrophilicity and biocompatibility. *In vivo* MRI-guided PTT was first performed in tumor-bearing mice model. MONPs-BSA-EDTA was then degraded with an artificially induced reducible agent. The effects of various factors are discussed in detail. To confirm the potential toxicity of the heavy metals in nanoparticle degradation products to living systems, the concept of degradation product capture was proposed. Therefore, the biodistribution, metabolism, and period of toxicity of MONPs-BSA-EDTA and its degradation products were also examined to evaluate the safety of this artificially controlled degradation strategy.

1. Results and discussions

1.1. Synthesis and characterization

Oleylamine-capped MONPs (MONPs-OM) were prepared with a typical hydrothermal method [41] (Fig. 1A). Transmission electron microscopy (TEM) images suggested that the MONPs-OM were well dispersed on the copper grid, with a uniform spherical shape (Fig. 1B), a narrow size distribution of 50 nm, and a volumetric distribution of ~5.23×10^{-16} cm^3 (Fig. S1). The energy-dispersive X-ray analysis (EDXA) spectrum of MONPs-OM confirmed that the particles were composed exclusively of Mn and O (Fig. 1C), indicating the formation of manganese-oxide-based nanoparticles. The powder X-ray diffraction (XRD) pattern of MONPs-OM corresponded to the standard card of tetragonal phase of Mn_3O_4 (JCPDS: 24-0734), which confirmed that the obtained nanocrystal has high crystallization tetragonal phase (Fig. 1D). High-resolution TEM images (HR-TEM) showed d-spacings of ~0.157 nm, ~0.164 nm, and ~0.182 nm, corresponding to (321), (303), and (204), respectively, which also support the tetragonal structure (Fig. 1E). Selected-area electron diffraction (SAED) also supported to this result (Fig. S2). Therefore, the synthesized MONPs-OM was uniform in shape, with well-distributed sizes and high crystallinity. To generate hydrophilic MONPs, nitrosonium tetrafluoroborate ($NOBF_4$) was used to remove the OM on the particle surface and substituted with citric acid (CA) as the ligand, generating MONPs-CA.

It is well known that BSA-coated inorganic nanomaterials have many advantages, including good biocompatibility, and that they do not readily aggregate in the presence of salts [52]. To extend the biological applications of MONPs-CA, BSA was used to modify the MONPs-CA particles with a layer-by-layer (LBL) polymer coating method. The polymer coating was identified with zeta potential measurements and dynamic light scattering. The zeta potential of the nanoparticles increased from −22 mV for MONPs-CA to +26 mV for MONPs-polyallylamine hydrochloride (MONPs-PAH), and then decreased to −13 mV after they were coated with polyacrylic acid (MONPs-PAA), which confirmed the LBL polymer adsorption onto MONPs-CA (Fig. 1F). Finally, BSA was conjugated to the surface of MONPs with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and N-hydroxysuccinimide active amide formation. The hydrodynamic size increased from 70 to 150 nm with the successful layer-by-layer coating method (Fig. 1G). The BSA coating showed positive charges on its surface, which benefit the passive target to tumors (Table S1). EDTA was loaded onto MONPs-BSA, and the amount loaded (37.8%, w/w) was determined with inductively coupled mass spectroscopy (ICP-MS) by measuring the Ca^{2+} content. With this surface modification, MONPs-BSA-EDTA was well dispersed and stable in water, phosphate-buffered saline (PBS), serum, and Dulbecco's modified Eagle's medium (DMEM) (Table S2). As the storage time increasing, the MONPs-BSA-EDTA can still remain high colloidal stability and solubility.

The ultraviolet–visible–NIR (UV–vis–NIR) spectrum suggested that MONPs-BSA-EDTA has strong broad absorption, similar to that of other reported photothermal agent [42] (Fig. 2A), and the absorption properties were concentration dependent (Fig. S3). The molar extinction coefficient was calculated with equation (1).

$$\varepsilon = \frac{AV_{NPs}\rho N_A}{LC} \tag{1}$$

Based on the average volume and density of MONPs-BSA-EDTA, its molar extinction coefficient at 785 nm was calculated to be 6.6×10^8 M^{-1} cm^{-1}, which is clearly higher than that of $Cu_{2-x}Se$ [43] (7.7×10^7 M^{-1} cm^{-1} at 980 nm), but similar to that of $Cu_{2-x}S$ [44] (9.53×10^8 M^{-1} cm^{-1} at 808 nm). Its high molar extinction coefficient makes MONPs-BSA-EDTA a promising novel photothermal agent.

1.2. Measurement of photothermal properties

Because MONPs-BSA-EDTA has a high molar extinction coefficient and strong absorption in the NIR region, we examined its potential application in photothermal cancer therapy with irradiation at 785 nm at a harmless low power density (0.64 W cm^{-2}). We used water as the control. After irradiation for 8 min, the water temperature increased <1.0 °C, whereas the temperature of the MONPs-BSA-EDTA solution (50–500 ppm) increased by 7.8–26.3 °C (Fig. 2B and C). From Fig. 2C, a positive relationship between the MONPs-BSA-EDTA concentration and temperature was identified (Fig. 2D). As the concentration increased to 200 ppm, the solution temperature reached ~50 °C (room temperature was 25 °C), which can kill ordinary tumor cells, as reported previously [45]. The photostability, standing stability, and stability of MONPs-BSA-EDTA at various physiological solutions were also investigated with UV–vis–NIR spectrometry (Figs. S4 and S5). MONPs-BSA-EDTA was robust to photothermal heating after five cycles of NIR-laser-induced heating, when indocyanine green (ICG) was used for comparison (785 nm laser at 0.64 W cm^{-2}, laser irradiation for 3 min in each cycle; Fig. 2E).

For compare MONPs-BSA-EDTA with other reported photothermal agents, we calculated its photothermal conversion efficiency with equation (2).

$$\eta = \frac{hA\Delta T_{max} - Q_s}{I(1 - 10^{-A_\lambda})} \tag{2}$$

According to equation (2), the η value for MONPs-BSA-EDTA was 34.7% under 785 nm irradiation (Fig. S6). In contrast, Au nanorods, which are widely used for cancer therapy, showed a much lower η value of 23.7% [46]. The higher η value of MONPs-BSA-EDTA makes it greatly superior to Au nanorods as a PTT coupling agent.

1.3. Toxicity studies

MONPs-BSA-EDTA was designed for cancer theranostics, so its toxicity must be clarified. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay suggested that the toxicity of MONPs-BSA-EDTA to both HCT116 (cancer cells: Figs. 3A, S7A, S8A)