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Dragon fruit-like biocage as an iron trapping nanoplatform for high efficiency targeted cancer multimodality imaging

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ABSTRACT

Natural biopolymer based multifunctional nanomaterials are perfect candidates for multimodality imaging and therapeutic applications. Conventional methods of building multimodal imaging probe require either cross-linking manners to increase its *in vivo* stability or attach a target module to realize targeted imaging. In this study, the intrinsic photoacoustic signals and the native strong chelating properties with metal ions of melanin nanoparticle (MNP), and transferrin receptor 1 (TfR1) targeting ability of apoferritin (APF) was employed to construct an efficient nanoplatform (AMF) without tedious assembling process. Smart APF shell significantly increased metal ions loading (molar ratio of 1:800, APF/Fe^{3+}) and therefore improved magnetic resonance imaging (MRI) sensitivity. Moreover, synergistic use of Fe^{3+} and APF contributed to high photoacounstic imaging (PAI) sensitivity. AMF showed excellent bio-stability and presented good *in vivo* multimodality imaging (PET/MRI/PAI) properties (good tumor uptake, high specificity and high tumor contrast) in HT29 tumor because of its targeting property combined with the enhanced permeability and retention (EPR) effect, making it promising in theranostics and translational nanomedicine.

1. Introduction

Non-invasive imaging techniques have attracted much attention due to their considerable roles in tumor diagnosis, prognosis and personalized treatment guidance [1–3]. Multimodality imaging provides comprehensive information and accomplishes synergistic advantages over any single modality alone [4–6]. The advance on nanomaterials research has offered numerous opportunity for designing multifunctional nanoprobes with unique chemical and physical properties [7,8]. Nanomaterials with various building blocks have been explored for multimodal imaging, however, it is usually contradictory to simultaneously satisfy expected demands and avoid intrinsic limitations. For instance, polymeric micelles are with easy synthesis and modification, but they are not suitable for loading of hydrophilic payloads [9–11]. Inorganic nanoparticles possess good biocompatibility, controllable size and shape, but accompanying poor *in vivo* stability [12–15]. Particularly, there is a growing need to accurately image biological targets (e.g. receptors over-expressed in tumor), which not only increases the cost of imaging probes, but also makes the preparation process troublesome. To address those issues, biological nanoparticles are perfect candidates because of their biodegradability and homogeneity. But their handling and further modification are very difficult [16–18]. Therefore, facile synthesis and construction of efficient multimodal imaging platforms is very significant.

As indispensable elements for living organisms, metal ions are also appropriate reporters for bioimaging because of their attractive physical characteristics such as magnetic, optical and radioactive properties [19,20]. However, free metal ions show weak *in vivo* stability and cause severe toxixicity, therefore metal ions are usually bound with or encapsulated into a carrier. Melanin nanoparticle (MNP) is such a carrier that not only shows intrinsic chelating ability with metal ions ($^{64}Cu^{2+}$, Fe^{3+}) for positron

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emission tomography (PET) and magentic resonance imaging (MRI) with a high loading capacity, but also possesses native optical properties for photoacoustic imaging (PAI) [21]. However, MNP complexed with metal ions could precipitate in aqueous solutions, and PEGylation was utilized to solve this problem and increase the water solubility of MNP. Moreover, in order to make naoparticles access to targeted tissues, apart from enhanced permeability and retention (EPR) effect, attaching a targeting moiety such as folic acid or RGD peptide is usually necessary [22–25], which makes the preparation process troublesome and also could result in heteogenous products.

Ferritin is a natural iron storage protein. It shows cage-like structure with small size (around 10 nm), large surface for modification, multi-channel (<1 nm) connecting the exterior with interior, dissociation-reassemble characters upon pH changes, making it a widely used nanoplatform for metal capture, drug delivery, bioimaging techniques and phototheramal therapy [18,26–28]. More importantly, it possesses targeting ability to transferrin receptor 1 (TfR1) which is overexpressed in numerous type of cancer cells [29]. Ferritin that is not combined with iron is called apoferritin, and it is extensively utilized for cargo loading. These features suggest ferritin a smart platform with intrinsic targeting ability.

Herein, we report the integrated endogenous nanomaterials for enhanced targeted multimodality imaging through ingeniously space matching, i.e., embedding ultrasmall MNPs into the cavity of apoferritin (APF). APF played synergistic roles of guaranteeing targeting and stability of the nanoplatform. First, we prepared ultrasmall and water-soluble MNPs and then encapsulated one MNP together with high concentration of ferric irons into the cavity of APF to prepare nanomaterials AMF with core–shell structures. $^{64}Cu^{2+}$ and Fe^{3+} can be easily loaded into AMF nanoparticles cavities without adding additional chelating agents. More importantly, AMF can achieve TfR1 targeted cancer trimodal (PET/MRI/PAI) imaging (Fig. 1). To the best of our knowledge, AMF represents the first organic nanoplatform integrating both melanin and apoferritin with high and stable metal ions load for targeted cancer molecular imaging.

2. Results and discussion

2.1. Preparation and characterization of AMF

The inside cavity diameter of APF is ~8 nm, thus, ultrasmall MNPs below 8 nm were synthesized from commercial melanin granules (Supporting Information) according to previous report [21]. The average sizes of MNPs were ~4.0 nm measured by transmission electron microscopy (TEM) (Fig. 2B). Hydrodynamic diameters of MNPs were ~5.6 nm determined by dynamic light scattering (DLS, Fig. 2E), and zeta potential of MNP was −31.8 mV (Table S1). The molecular weight of MNP was determined to be around 61 kDa by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Fig. S2).

APF cage shows reversible pH-responsiveness, it disassembles into discrete subunits in strong acidic environment (pH 2.0 or 3.5), and re-assembles to a cage-like structure under basic conditions (pH 8) [30–32]. This smart architecture renders it convenient to encapsulate MNP and Fe^{3+} within the cavity of APF to prepare AMF (Fig. 1). Usually, the loading yields of metal ions into apoferritin are very low; for example, it was reported that about 8–10 Gd-HPDO3A molecules and one molecule of [DFOFe] complex were trapped within an APF molecule [33,34]. Since MR signal is positively associated with Fe^{3+}, higher ferric ion capture is preferred. APF:Melanin:Fe^{3+} at different ratios were used to prepare AMF, and the optimal conditions were determined as 1:1:1000 (Supporting Information). The loading yield of Fe^{3+} to AMF was 71.36 ± 2.56%, achieving a molar ratio of 1:1:800 APF:MNP:Fe^{3+}. This was a very high loading. Previously, we found MNP quickly precipitated after adding Fe^{3+}. Even after PEGylation, the maximum ratio of Fe^{3+} to MNP-PEG was only around 90:1 [21]. In this study, the solution containing APF remained a clear brown solution at room temperature for at least 1 month (Fig. S3), whereas black precipitate was immediately observed in APF-free condition. On the other hand, in an MNP-free solution, Fe^{3+} was stably encapsulated in acid condition, but after transfer to neutral or basic condition, Fe^{3+} could leak freely from APF through channels between the exterior and interior cavities, resulting in red-brown precipitates. Therefore, in this circumstance, the final Fe^{3+} loading yield within APF alone was only 4%. In contrast, using the APF-MNP dual platform, Fe^{3+} ion loading increased more than 10 times and MNP inside APF dramatically enhanced the stability of Fe^{3+} loading.

The nanomaterials prepared in aqueous solution were examined by TEM and DLS. APF showed in Fig. 2A was stained with uranyl acetate, which cannot penetrate the protein shell and therefore cannot stain the cavity, revealing APF as lighter round patches, which was consistent with previous results [30–32]. The diameter was ~12.1 nm measured by TEM, the hydrodynamic diameter and zeta potential were ~13.6 nm and −17.2 mV respectively measured by DLS (Table S1). Fig. 2D showed that the AMF nanoparticle had the similar core–shell structure as APF, the diameter increased to

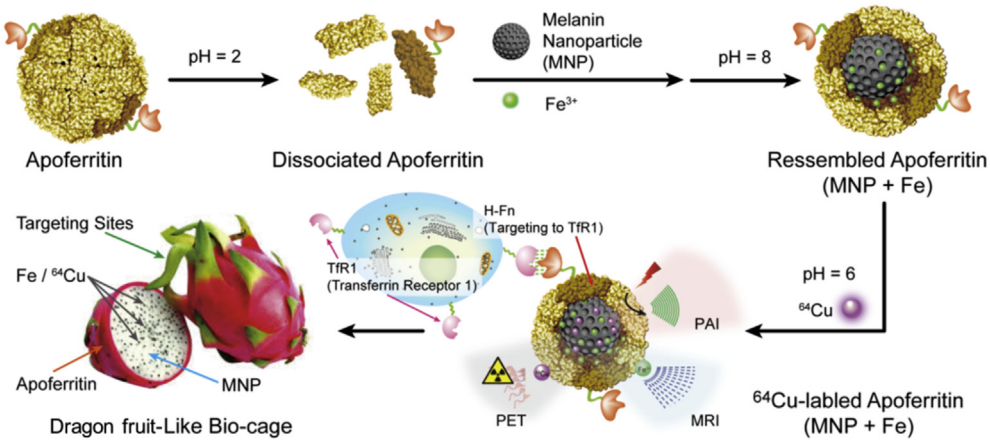

Fig. 1. Schematic illustration of AMF nanocage synthesis.