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Ferritin-based drug delivery systems: Hybrid nanocarriers for vascular immunotargeting

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ABSTRACT

Ferritin subunits of heavy and light polypeptide chains self-assemble into a spherical nanocage that serves as a natural transport vehicle for metals but can include diverse cargoes. Ferritin nanoparticles are characterized by remarkable stability, small and uniform size. Chemical modifications and molecular re-engineering of ferritin yield a versatile platform of nanocarriers capable of delivering a broad range of therapeutic and imaging agents. Targeting moieties conjugated to the ferritin external surface provide multivalent anchoring of biological targets. Here, we highlight some of the current work on ferritin as well as examine potential strategies that could be used to functionalize ferritin via chemical and genetic means to enable its utility in vascular drug delivery.

1. Introduction

Medical goals for drug targeting are extraordinarily diverse. Potential utility of drug delivery systems transcends all medical specialties. Pathophysiological context and biological factors defining specifications for a suitable drug delivery system (DDS) are unique in every pathological condition and patient. Multitudes of natural, synthetic, and hybrid DDS using different principles and materials are needed for these purposes [1,2].

Among the key features are amenable routes of administration and delivery, therapeutic target, DDS and drug cargo and their features – pharmacokinetics, size, biocompatibility, durability, etc. In addition to these investigational parameters, important translational parameters to consider in designing an efficient targeted drug delivery system include amenability to scale-up, quality control and cost of production.

Natural carriers include biomolecules and their assemblies, as well as cells and their fragments. Biological nanoparticles assembled from natural or modified biomolecules exhibit various unique architectures and functional properties that render them strong contenders for targeted drug delivery. Numerous self-assembled biological nanoparticles exist in nature such as ferritin, virus-like particles (VLPs), heat-shock protein cages, chaperones, carboxysomes, and enzyme complexes [3–9]. These natural nanoparticles each provides a unique set of characteristics that could be applied in biotherapeutics.

Here we consider ferritin nanoparticles as carriers for vascular drug delivery. In this review we highlight some of the progress in the area of ferritin-based nanocarriers and their applications in therapeutics and imaging. Their potential as modular and stimuli-responsive drug delivery platform, as well as strategies for conjugation by genetic and chemical conjugation means will be discussed. Finally, we outline methods for pulmonary targeting, advancements in targeted pulmonary drug delivery systems, and the potential use for ferritin in pulmonary therapeutics and imaging.

2. Ferritin nanoparticles

Ferritin is a major iron-storage protein in the body consisting of 24 subunits that self-assemble to form spherical nanocages of around 12 nm in diameter with an interior cavity of 8 nm [10,11]. Two types of subunits make up the ferritin nanocages, heavy (21 kDa) and light (19 kDa) chains [12,13]. The proportion of these subunits varies in different tissues [14]. The heavy chain has catalytic ferroxidase activity that oxidizes Fe(II) to Fe(III) which is insoluble and nucleates at the core [15,16]. Ferritin nanocages can load up to 4500 iron atoms in their interior cavity [12,13]. Endogenous ferritin is a non-enzymatic antioxidant that plays a cytoprotective role inside the cells by sequestering iron and preventing its harmful pro-oxidative effects [17].

Elevated serum ferritin levels have been observed during inflammatory conditions as well as in some cancers [18]. The primary role for circulatory ferritin is unclear, however it has been reported to bind to and be internalized by a range of cells. Human ferritin binding occurs preferentially via its heavy chain rather than light chain [19].

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The ferritin heavy chain interacts with the human transferrin receptor 1 (TfR1) leading to its endocytosis. This natural TfR1 targeting capability has been used to target TfR1-overexpressing cancer cells as a nanoparticle-assisted anti-cancer drug delivery strategy [19,20]. Other ferritin receptors have been discovered in mice, such as T cell immunoglobulin and mucin domain protein-2 (TIM-2) which binds to heavy chain ferritin, and scavenger receptor class A, member 5 (SCARA5) which binds selectively to light chain ferritin. However, in humans, TfR1 remains as the primary ferritin receptor [19,21,22].

Ferritin has quite a broad range of applications that span from therapeutics to imaging, diagnostics, bioelectronics, water purification, and even bioactuators for potential development as artificial muscle. The natural capacity of ferritin to encapsulate metals provides a platform that can be easily modified to give it unique functional characteristics. Biomineralization of ferritin with metals such as Co, Pt, Mn, Ni, and others have been used in bioelectronics because of their good electron transfer and storage capacity for development bionanobattery, biofuel cells, biosensors, and others [23–25].

The iron-loading capacity of ferritin can also be used for complexation with oxyanions such as orthophosphates. The Dutch company BiAqua (www.biaqua.nl) has developed an innovative water treatment strategy using hyperthermophilic ferritin to adsorb oxyanion (phosphate and arsenic) water contaminants. This strategy circumvents the low affinity and biofouling issues that face other water purification strategies. Ferritin has even been proposed as a delivery platform in the nutritional field. Li M. et al. [26] developed calcium-loaded soybean seed phytoferritin nanocages for use as edible calcium supplement. Encapsulation inside ferritin was to protect calcium ions from dietary absorption inhibitors such as tannic acid, oxalic acid, and zinc ions. Aside metal and mineral loading, ferritin has been incorporated into hydrogels for use as bioactuators with potential application in artificial muscle development. Nanocomposite hydrogel actuators were developed by embedding ferritin in poly(vinyl alcohol) (PVA) nanofibers. The ferritin acted to reinforce the nanofibers resulting in 230% increase in the elastic modulus compared to PVA hydrogels alone. Reversible bioactuation was observed by switching the pH, with contraction at pH 4 and expansion at pH 9 [27].

2.1. Ferritin as modular drug delivery platform

Numerous groups have utilized the ferritin as a drug delivery platform by loading it with small molecule therapeutics and incorporating targeting moieties on the surface either chemically or genetically. Ferritin forms a natural self-assembled oligomeric protein complex with uniform size and structure amenable to chemical and genetic modification for attachment of various cargos and targeting moieties.

Some characteristics that make ferritin a promising drug delivery candidate include remarkable thermal stability (withstanding temperatures up to 80–100 °C) and pH stability (pH 3–10), monodispersity, small uniform size, biocompatibility, biodegradability, low cost large-scale production, hollow cavity (nanocage) with reversible assembly and disassembly for encapsulation of drugs and imaging agents, ease of conjugation by chemical procedures (ample surface area with reactive moieties for conjugation) and genetic means (reported genetic sequence available for modification by classic recombinant cloning strategies).

A number of groups have reported on ferritin as carrier for drug delivery. Ferritin-based nanotherapeutics have been developed by loading ferritin nanocages with small molecules such as doxorubicin [28,29], cisplatin [30–32], curcumin [33], carotenoids [34], and cerium oxide [35]. Ferritin also provides robust strategy for metal nanoparticle encapsulation [36–38]. Strategies used drug loading include pH- or salt-induced disassembly/reassembly of ferritin, diffusion-based encapsulation, and direct conjugation of drug to ferritin surface (Fig. 1a).

Some have incorporated certain functionalities into ferritin to make it more modular and hence a simpler modifiable platform (Fig. 1b). In order to avoid complex chemical conjugation strategies, Kang H.J. et al. [39] incorporated Fc-binding peptide between the D and E helices of ferritin from hyperthermophilic archaeon, as modular ferritin platform for binding Fc-region of antibodies in a non-covalent and orientation-specific manner. The platform was used to complex with anti-HER2 trastuzumab antibody and an antibody against folate receptor, demonstrating cell-specific in vitro binding. Another modular apoferritin platform strategy developed by Hwang M.P. et al. [41] was to genetically incorporate His tag and protein G in apoferritin for detection of cancer biomarker. Apoferritin was combined with antibodies that interact with their Fc region to protein G, as well as nanoconstructs (such as quantum dots, gold nanoparticles, or magnetic nanoparticles) that were surface-functionalized with Ni-NTA derivatives for interaction with His-tags on apoferritin. Lee E.J. et al. [42] developed a ferritin-based siRNA delivery system called Proteinticles. Proteinticles were designed by genetically engineering human ferritin to display peptides on its surface such as cationic peptide (CAP) for binding siRNA, tumor targeting peptide, cell penetrating peptides, and an enzymatic cleavage site for releasing siRNA inside tumor cells. The poly-siRNA-proteinticle complexes demonstrated effective gene silencing in tumor cells. The modular apoferritin platform provides a simple strategy to switch between various cargo probes and antibodies.

Most ferritin drug delivery reports encapsulate the therapeutic agents which requires the breakdown of ferritin for drug release. In order to allow for rapid drug release from ferritin, Kwon C. et al. [40] site-specifically conjugated 24 β-cyclodextrins (β-CD) molecules per ferritin for potential loading of small hydrophobic molecules. Fluorescein isothiocyanate-adamantane (FITC-AD) was loaded onto a ferritin-coated β-CD molecule. The study revealed drug release half-life of 3 h, suitable for rapid drug delivery purposes, nonetheless release time could be increased with further fine-tuning.

Modifying ferritin to become stimuli-responsive adds greatly to its multifunctionality and controlled drug delivery capability. One group genetically modified ferritin to dissociate at pH 6 and reassemble at neutral pH. To do so, repeats of the GALA cell penetrating peptide were incorporated into *E*-helix truncated ferritin. The reversible transition of GALA from random coil to α-helix at acidic pH results in ferritin disassembly [43]. This may have applications in drug delivery, for inducible drug release at acidic pH as well as a method for drug loading in milder weak acid rather than the usual disassembly in a more damaging strong acid solution of pH 2.

For development of a multifunctional stimulus-responsive delivery system, Kang Y.J. et al. [44] incorporated thrombin cleavable peptide between the D and E helices of ferritin subunit. Thrombin cleavage induces the release of helix E and formation of 1.5 nm holes in the 4-fold axis of ferritin. A fluorophore was conjugated to the C-terminal genetically incorporated cysteine and NHS-PEG4-biotin was conjugated to the amine groups on the surface of ferritin for use as a targeting ligand for delivery to target cells over-expressing biotin receptors such as cancer cells. The fluorescent probe was released upon administration of thrombin. Various small molecules can be incorporated inside ferritin or genetically incorporated with peptides such as cytotoxic and apoptotic peptides for controlled release at target site.

3. Medical applications of ferritin

Ferritin has found wide-ranging utility in both therapeutics and imaging. The therapeutic applications of ferritin have primarily been focused on cancer therapy and vaccines.

3.1. Ferritin in cancer therapy

There are numerous studies on the use of ferritin for cancer therapy. Some have used the natural transferrin receptor targeting capability of ferritin to target transferrin-overexpressing cancer cells, while others have attached more cancer-specific targeting ligands. Falvo E. et al.