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DNA-mediated biomineralization of rare-earth nanoparticles for simultaneous imaging and stimuli-responsive drug delivery

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ABSTRACT

A DNA-guided method for surface engineering of $NaGdF_4$:Ce/Tb hybrid nanoparticle has been proposed. In this study, the DNA molecules that retained after one-pot $NaGdF_4$:Ce/Tb synthesis is directly utilized as biotemplate for CaP heterogeneous nucleation, thus the dual-purpose function of DNA is realized in the current study which could afford a new type of pH-responsive theranostic platform to enhance the therapeutic efficiency while minimizing side effects. The introduction of another layer of aptamer molecules on CaP facilitated cellular uptake of the resulting nanocomposite into specific target cells via receptor-mediated endocytosis. After been taken by the target tumor cells, the $NaGdF_4$:Ce/Tb@CaP was found to be mostly accumulated in lysosome, which facilitated the dissolving of CaP coatings as non-toxic ions to initiate drug release and efficient cancer cell destruction. We envision that the hybrid nanocarrier may serve as practical and multifunctional probe for cancer therapy and the presented synthesis approach here may also benefit the preparation of many other types of multifunctional inorganic-biomolecular hybrid nanostructures based on the DNA nanotechnology.

1. Introduction

With the rapid advances of nano-biotechnology, there has been an explosion of interest in the use of biomolecules possessing sophisticated structures and outstanding functions as building blocks for the development of multifunctional nanomaterial [1–5]. This biomolecules-based strategy opens up a new avenue for rationally control the structures as well as physical and chemical features of the materials. Due to the conformational polymorphism, sequence-specific recognition and robust physicochemical nature, DNA has been extensively investigated in nanotechnology and material science [1,6]. The use of DNA as template appears to be one of the most promising avenues available for fabricating a variety of metallic-nanomaterials with potential applications ranging from electronics to biology [7–15]. DNA is rich of phosphate groups, amino groups and heterocyclic nitrogen atoms, it offers nucleation sites for metallic nanoparticles and provides control over the material growth and stability [6–15]. Meanwhile, the DNA attached on the prepared material could also be employed as recognition unit for the biological application or to organize the nanoparticles into periodic or discrete one-, two-, and three-dimensional architectures for material purpose [1,6–8]. For example, Kelley et al. recently reported that a chimeric DNA molecule could program both the growth and the biofunctionalization of the CdTe nanocrystals for bioimaging applications [1,7]. Although these DNA-based hybrid materials hold great promise in nanotechnology and nanomedicine, the exploring of further functions of the DNA anchored on the material surface still remains a big challenge in this field.

Porous inorganic nanoparticles with high specific surface area have emerged as appealing material recently for the development of delivery systems, where various guest molecules could be absorbed into the pores and later released into various solutions [16–19]. Meanwhile, due to the unique optical and magnetic behavior, the lanthanide ions doped-porous nanocrystals have been extensively employed for biomedical applications, including our recent development of DNA-based porous lanthanide doped $NaGdF_4$ nanocarriers for cell-specific drug delivery [20–25]. Compared with organic fluorophores that modified on the surface of material, the doped luminescent lanthanide cations provide complementary properties such as resistance to photobleaching, long luminescence lifetimes, absence of reabsorption and sharp emission bands in the visible and the near-infrared [26–30]. Moreover, it could be employed as a probe that combines therapy

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functions with multi-mode imaging methodologies which could provide complementary information in biomedical studies [31–35]. While the Ln^{3+} doped-porous nanoparticles (Ln^{3+}-pNP) have been adopted as promising nanomaterials for biological application, these delivery platforms might significantly compromise their efficiency because the entrapped drug may start leaking out of the carrier immediately after administration due to the lack of capping/blocking agent. Therefore, the ability to maintain optimum therapeutic efficacy, i.e. no premature release in blood circulation whilst having a rapid drug release in tumor tissues, remains a significant challenge for the development of Ln^{3+}-pNP based multifunctional delivery system.

During the past several decades, nature's strategies to the formation of a wide range of specially designed organic-inorganic hybrid materials such as bone, teeth, and shells have attracted increasing attention [36]. The mild condition and high efficiency of these preparation processes have inspired research to mimic them in vitro and a great deal of biominerals with tunable morphologies and properties has been prepared [37,38]. In terms of materials design, bio-inspired calcium phosphate (CaP) hybrid materials have attracted tremendous interest; this is mainly due to the fact that these hybrids have a wide range of very useful properties and potential applications [39–48]. For example, Epple et al. proposed multi-shell CaP-DNA nanoparticles and CaP-DNA/siRNA nanohybrids for effective transfection of cells [44]. Adair et al. developed CaP nanoparticles that encapsulated both fluorophores and chemotherapeutics for in vitro imaging and anticancer drug delivery [47,48]. Here inspired by the high binding affinity of CaP toward DNA, we propose to engineer the Ln^{3+}-pNP surface with a layer of natural CaP to overcome the big challenges in Ln^{3+}-pNP applications mentioned above. As shown in Scheme 1, followed by our previous report, porous $NaGdF_4$:Ce/Tb nanoparticles were prepared by using DNA as superior ligands. Then the hydrophilic DNA molecules that attached on the $NaGdF_4$:Ce/Tb nanoparticles were utilized as the nucleation sites for CaP heterogeneous nucleation. Thereafter, by precisely controlling the following growing process, CaP mineral nanoshells which possessed pH-dependent biodegradability could be formed (designed as $NaGdF_4$:Ce/Tb@CaP). Thus the dual-purpose function of DNA was realized in the current study which could afford a new type of pH-responsive theranostic platform to enhance the therapeutic efficiency while minimizing side effects. Moreover, an additional layer of aptamer (Apt) was attached to the mineral surfaces via chelation interactions, which produced a colloidal stabilized nanocomposite with biospecific properties (designed as $NaGdF_4$:Ce/Tb@CaP-Apt).

2. Materials and methods

2.1. Materials

$Ln(NO_3)_3$ and doxorubicin (DOX) were purchased from Sangon (ShangHai, China). NaF and dimethyl sulfoxide (DMSO) were purchased from Shanghai Chemical Factory (Shanghai, China). Fish sperm DNA was obtained from Sigma–Aldrich. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment.

The oligonucleotide used in this article was synthesized by Sangon Biotechnology Inc. (Shanghai, China). The sequence was as follows:

5′-GGTGGTGGTGGTTGTGGTGGTGGTGGT-3′ Aptamer (Apt)

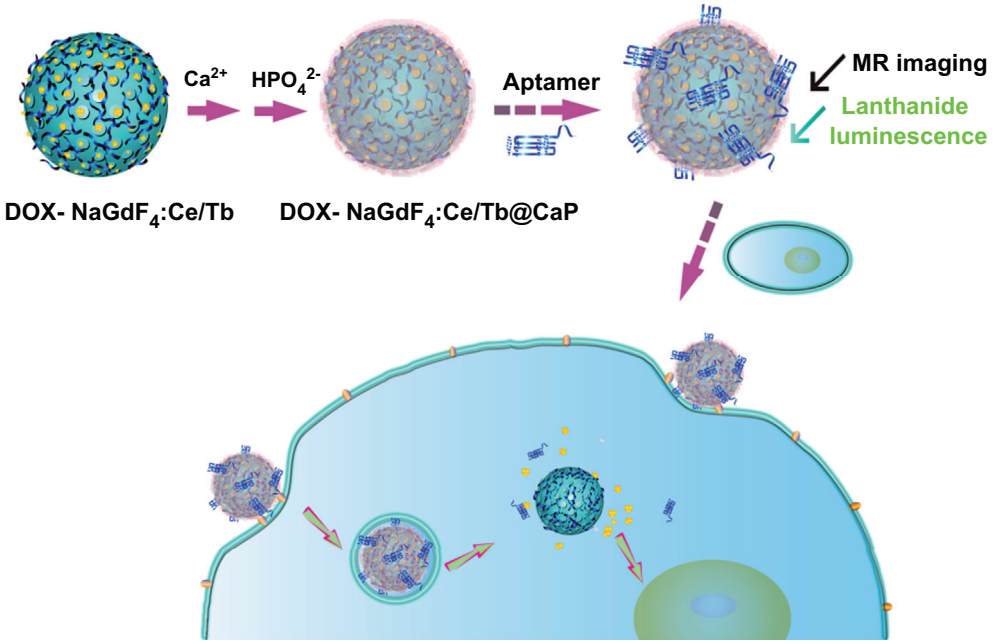
5′-TTAGGG TTAGGGTTAGGGTTAGGG TTA-3′ control DNA with randomized sequence (Rdm)

2.2. Measurements and characterizations

FT-IR analyze was carried out on a Bruker Vertex 70 FT-IR Spectrometer. SEM images were obtained with a Hitachi S-4800 FE-SEM. The UV–Vis absorption spectra and fluorescence spectra were recorded using a JASCO V-550 UV–Visible and a JASCO FP6500 spectrophotometer (JASCO International Co.,LTD., Tokyo, Japan). TEM images were recorded using an FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. The samples were degassed at 90 °C for 5 h. The magnetic properties of samples were collected on a MPM5-XL-5 superconducting quantum interference device (SQUID) magnetometer. N_2 adsorption–desorption isotherms were obtained on a Micromeritics ASAP 2020M automated sorption analyzer. The specific surface areas were calculated from the adsorption data in the low pressure range using the BET model and pore size was determined following the BJH method. The zeta potential of the nanomaterials in HEPES was measured in a Zetasizer 3000HS analyzer. Cell imaging was performed with a fluorescent microscope (Olympus, BX51).

2.3. Preparation of $NaGdF_4$:Ce/Tb nanoparticle

280 μL $Gd(NO_3)_3$ (0.2 M), 35 μL $Ce(NO_3)_3$ (0.2 M) and 15 μL of $Tb(NO_3)_3$ (0.2 M) were added to 100 mL beaker and diluted to 15 mL with water. Then 5 mL of the DNA stock solution (6.4 mM) was added quickly under magnetic stirring. Subsequently,



Scheme 1. Schematic illustration of the biomineralization process inspired DOX loaded $NaGdF_4$:Ce/Tb@CaP-Apt hybrid. DOX loaded $NaGdF_4$:Ce/Tb@CaP-Apt was internalized via receptor-mediated endocytosis. The DOX was released through the dissolving of CaP within intracellular endolyso-somal compartment.