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Synthesis of upconversion nanoparticles conjugated with graphene oxide quantum dots and their use against cancer cell imaging and photodynamic therapy

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ABSTRACT

Multifunctional nanocomposite has a huge potential for cell imaging, drug delivery, and improving therapeutic effect with less side effects. To date, diverse approaches have been demonstrated to endow a single nanostructure with multifunctionality. Herein, we report the synthesis and application of core-shell nanoparticles composed with upconversion nanoparticle (UCNP) as a core and a graphene oxide quantum dot (GOQD) as a shell. The UCNP was prepared and applied for imaging-guided analyses of upconversion luminescence. GOQD was prepared and employed as promising drug delivery vehicles to improve anti-tumor therapy effect in this study. Unique properties of UCNPs and GOQDs were incorporated into a single nanostructure to provide desirable functions for cell imaging and drug delivery. In addition, hypocrellin A (HA) was loaded on GOQDs for photo-dynamic therapy (PDT). HA, a commonly used chemotherapy drug and a photo-sensitizer, was conjugated with GOQD by π-π interaction and loaded on PEGylated UCNP without complicated synthetic process, which can break structure of HA. Applying these core-shell nanoparticles to MTT assay, we demonstrated that the UCNPs with GOQD shell loaded with HA could be excellent candidates as multifunctional agents for cell imaging, drug delivery and cell therapy.

1. Introduction

Lanthanide-doped upconversion nanoparticles (UCNPs) have been attracted a great interest in various fields because of their unique optical properties reported previously (Chen et al., 2014). Thus, these nanostructures have a specific value for biological applications in various therapeutic fields such as *in vivo* imaging and photodynamic cancer therapies (Shen et al., 2013). In other words, lanthanide-doped UCNPs with ladder-like energy level structures have an ability to convert low energy excitation to high-energy emission via two or more photon absorption or energy transfer. Upconversion refers to sequential absorption of multi-photons leading to the emission shorter than the excited wavelength, which is anti-stokes type emission (Junwei et al., 2008). UCNPs have been differentiated from others in terms of very little auto-fluorescence and scattering on multiphoton absorption. Consequentially, they have a low background signal and high signal-to-noise ratio offering outstanding advantages in application of therapeutic fields. Therefore, UCNPs have become efficient candidates for the analysis of biological and environmental samples due to their bio-compatibility, especially for *in vivo* and *in vitro* fluorescence imaging (Haase and Schafer, 2011; Park et al., 2015; Seo et al., 2015). The similar atomic size and chemical properties of lanthanide elements make them more biocompatible and stable among other elements.

Photodynamic therapy (PDT) is a clinical tumor treatment using nontoxic light-sensitive agents that are toxic to target malignant and other diseased cells (Idris et al., 2012). When photosensitizing agents are exposed to a particular type of light, they produce cytotoxic reactive oxygen species (ROS). ROS leads nearby cells to death with minimal invasiveness, toxicity and side effect (Sharman et al., 2000; Martin and Barrett, 2002; Chatterjee et al., 2008). PDT requires photosensitizer (PS), light source, and oxygen. PS helps production of singlet oxygen by absorbing ultraviolet or visible region and transferring it to adjacent molecules (Konan et al., 2002). The PS transforms tumor cells with excited singlet state by intersystem crossing as a result of an irradiation at a

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specific wavelength of light. The excited triplet state can transfer hydrogen atom or electron to form radicals interacting with oxygen. These radicals produce oxygenated products like hydrogen peroxide, super oxygen, and hydroxyl ion or can transfer from oxygen to singlet oxygen, which is highly reactive. As a result, the ROS leads tumor cell to death.

Hypocrellin A (HA) is one of PSs isolated from natural fungus, *Hypocrella bambusae Sacc.*, which was discovered in China (Zhang et al., 1998). HA has low toxicity in an inactivated form (Li et al., 2015) and can be metabolized *in vivo*. Thus, HA can be used in some capillary disease treatment due to higher PDT effect under presence of singlet oxygen than many widely used PSs (Diwu et al., 1990; Shan et al., 2013).

Graphene oxide quantum dot (GOQD) is a class of carbon-based dots. The GOQD has received tremendous attention, due to its outstanding advantages such as low toxicity, low cost, excellent biocompatibility, resistance to photobleaching and ease of fabrication (Dong et al., 2012, 2013; Jiang et al., 2015; Lu et al., 2015; Zhang et al., 2016). Both GOQD and HA can easily form the HA/GOQD complexes through π-π stacking due to same planar sp^2 structures (Thomas et al., 2010; Jang et al., 2015) without complicated synthetic process which can break the structure of PS (Guo et al., 2013).

Other materials such as organic dyes and semiconductor quantum dots (QDs) have been used as biological luminescent labels, but they have certain limitations. For example, organic dyes exhibit rapid photobleaching, and QDs are less chemically stable, inherently toxic and show fluorescence intermittence. Hence, NIR-to-visible UCNP seems to be a promising alternative fluorescent material for bio-detection based on their advantages such as high chemical stability, low toxicity, economic instrument cost, and tunable optical properties. In this study, we report ytterbium and erbium ions-doped sodium yttrium fluoride ($NaYF_4$:Yb^{3+}, Er^{3+}), the most efficient NIR-to-visible UCNP in solid-state materials doped with rare-earth ions. $NaYF_4$:Yb^{3+}, Er^{3+} UCNP has been synthesized and analyzed their unique advantages.

We designed multifunctional nanostructure composed of UCNPs, GOQDs and HA for cell imaging drug delivery and PDT of cancer cell (Scheme 1). First, ytterbium and erbium ions-doped sodium yttrium fluoride ($NaYF_4$:Yb^{3+}, Er^{3+}) nanoparticles were synthesized as UCNP. Polyethylene glycol (PEG) was coated on the surface of the UCNPs additionally for bio-compatibility. The PEGylation was performed to make UCNP more effective in disease monitoring and drug delivery system. Next, we attached GOQDs to the PEG-coated UCNPs for drug delivery carrier. Finally, multifunctional material, GOQD-attached UCNP were synthesized with a great potential for both upconversion luminescence (UCL) imaging and PDT in cancer diagnostics. Furthermore, HA as a PS was loaded on the surface of the GOQD-attached UCNP (Oleinick et al., 2002; Zhou et al., 2015). This drug-delivery system was proposed to minimize side effect, achieve a delivery system for PDT and help *in vitro* imaging (Zheng et al., 2013). These nanocomposites were designed due to their intrinsic properties for drug-delivery from GOQD, therapy from HA and imaging capability from UCL.

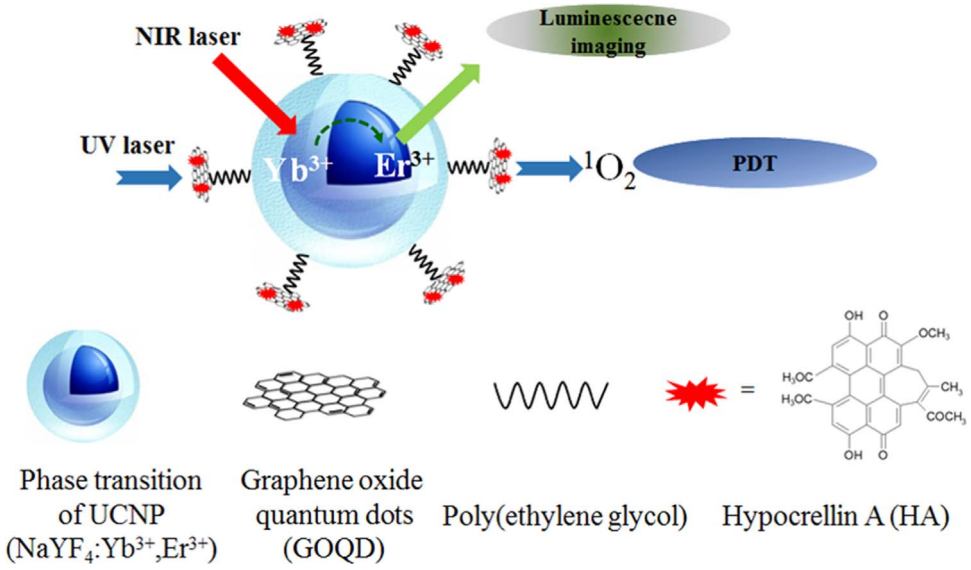
2. Experimental

2.1. Materials

Yttrium nitrate hexahydrate, ytterbium chloride hexahydrate, erbium nitrate pentahydrate, sodium fluoride, and nitric acid were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium citrate was obtained from APS Biotech (Seoul, Korea). Ethyl alcohol was acquired from Emsure (Billerca, MA, USA). Cetyltrimethylammonium bromide (CTAB) was purchased from Daejung (Seoul, Korea). All solutions were prepared with deionized (DI) water (Direct-Q® Water Purification System, Millipore, Billerica, MA, USA). Graphite nanoparticle (GNP) (93%, 3–4 nm) was obtained from SkySpring Nanomaterials (Houston, TX, USA). Sulfuric acid (95.0%) purchased from Junsei (Tokyo, Japan) and Nitric acid (60.0%) obtained from Samchun (Seoul, Korea). HA (> 77%) was purchased from abcam (Cambridge, UK).

2.2. Instruments and characterizations

The UCL spectra under 980 nm continuous wave laser excitation were analyzed using Shamrock Spectrograph (Shamrock 303i, Andor Technology, Belfast, Ireland). The absorbance was analyzed by UV/Visible/NIR spectrophotometer (V-670, Jasco, Tokyo, Japan). Their fluorescence properties were also analyzed using the Multimode microplate reader (BioTek Synergy H1, Winooski, VT, USA), absorbance on methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The particle size was determined by transmission electron microscopy (TEM, Tecnai G2 F30 S-TWIN, FEI, Hillsboro, OR, USA) and particle size analyzer (Otsuka ELSZ-1000, Osaka,



Scheme 1. The action procedure of HA/GOQD/UCNP nanoparticle.