Yolk-shell structure for upconverting nanoparticles: Bioimaging, drug delivery, and photodynamic therapy

Abstract

Upconverting Nanoparticle (UCNP) has recently received many attentions from theranostics and nanomedicine fields because it can be designed for multi-functional targeted nanomedicines with multi-modal imaging. One of popular UCNPs is NaLnF₄: Yb,Er/Tm, which absorbs infrared and releases visible or ultraviolet light to trigger drug release or to produce singlet oxygen for therapy. Lantanides doping enhances upconversion luminescence and enable magnetic resonance imaging. In addition IR-regulating drug release reaches deep without harm. Core-shell nanostructures have been applied for the most of UCNP applications, but the therapeutic efficacy is still far away from the desired levels in nanomedicine. First, loading space is limited by the thickness or porosity of shell, so enough loading isn’t guaranteed in most of core-shell structures. Thicker shell is better for higher loading, but a bigger particle size is unavoidable. Porosity isn’t a parameter to control simply. Second, only outer shell surface is offered for surface modifications to specific binding or properties, which is critical of targeted therapy. However, when UCNP is housed in a yolk-shell structure, the void, which isn’t available in core-shell structures, can be a solution for loading both drugs and photo triggers in drug delivery, or photosensitizers in photodynamic therapy. In addition, both inner and outer surfaces can be modified as any desired purposes. Third, the movable yolk UCNP has more chance to contact with photo triggers and photosensitizers in the void. All the benefits with yolk-shell structure are resulted in high therapeutic efficacy. In this mini review, some of yolk-shell UCNP examples are introduced for in vivo multi-modal bioimaging with high contrast, IR-regulated drug release, and high efficacy in photodynamic therapy.

Keywords: Upconverting Nanoparticles (UCNP); Yolk-shell; Bioimaging; Drug delivery; Photodynamic therapy; Theranostics

Graphical Abstract

nanomedicine meets theranostics and improves its therapeutic level. UCNPs exhibit the photon upconversion that convert two incident lower energy photons to one higher energy photon [2]. They are commonly composed of lanthanide or actinide-doped transition metals, because they have multiple 4f electrons with long enough excitations for upconversion [2]. In biomedical applications, one of popular UCNPs is NaLnF\textsubscript{4}::Yb,Er/Tm [3,4]. Ytterbium-erbium or ytterbium-thulium are sensitizers and doped to NaLnF\textsubscript{4} nanoparticles for absorbing infrared (IR) and releasing visible (Vis) or ultraviolet (UV) light [2,5,6]. Sodium (Na\textsuperscript{+}) is a cation with a similar radius, while fluoride (F\textsuperscript{-}) has a low phonon energy with a good stability. Their synthesis has been already reviewed in many literatures [1,4,7]. For the practical applications such as water soluble or binding to functional groups, core UCNPs need to be coated with inorganic materials or capping polymers [8,9]. UCNP@silica core-shell nanostructure is one of popular forms because of easy preparation [10], water-soluble, and biocompatible features. Titania, [11] drug-conjugated, [12] and capping ligands [13] are also available for the shell materials. No matter what the material is, the main challenge would be the loading ability to deliver drugs, photo triggers, or photosensitizers to targets. The therapeutic efficacy with core-shell nanostructures is limited by the loading space in the shell. Pores or pore size distribution could not be adjusted simply from the synthetic procedures. Simply thicker shell or larger dose is required for higher loading, but a bigger particle size or any side effect caused by heavy doses would be inevitable.

Yolk-shell structure is so-called “rattle” representing yolk@void@shell configuration [14-16] and has more benefits than core-shell structure. First, the void can be filled with anything to deliver or carry. It is a much larger amount than any loading in core-shell structures. Second, both inner and outer surfaces can be modified as desired (e.g. one is hydrophobic, the other is hydrophilic). It is quite useful when keeping undesired chemicals inside of the shell, but releasing drugs only to outside targets. Core-shell has an option at outer surface only. Third, yolk is movable and can have more chance of contact with anything in void. For the applications in nanomedicine, UCNP is commonly placed at yolk position, and mesoporous silica has been one of frequent materials for shell. Photo trigger-conjugated drug or photosensitizer can be stored in both void and pores of shell, which are better than only pores of core-shell structure. In addition, much higher energy transfer efficiency in photodynamic therapy is available with movable UCNP yolk. Both inner and outer surfaces of shell are modified with desired surface properties, or tethered to any specific binding to tumor or cancer cell surfaces. When UCNPs meet yolk-shell nanostructure, theranostics can be achieved as multimodal imaging, target specificity, and multifunctional therapeutic properties.

Radiotherapy and chemotherapy have been widely used in cancer treatments [17,18]. However, radiotherapy fails to eradicate hypoxic tumors and high doses of irradiation unavoidably because damage to normal cells [19]. Chemotherapy is limited for drug resistant cells [20]. Its efficacy may be improved by high doses, but side effects would cause other diseases. Although both therapies destroy cancer DNA structures, the DNA can self-repair to reproduction and regrowth. In photodynamic therapy, [21,22] cytotoxic singlet oxygen (O\textsuperscript{2}.) can be more efficient at killing cancer cells, as it inhibits DNA repair and elicits to cell death. UCNPs convert NIR to Vis or UV light, and continuous wave near-infrared (CW NIR) is the ideal excitation source for photosensitizer to generate singlet oxygen. In addition, NIR lights penetrate deeply into tissues with no harm.

In this mini review, only some of selected UCNP examples built in yolk-shell nanostructures are introduced to prospect for practical applications in nanomedicine. In vivo tri-modal bioimaging is available with upconversion luminescence (UCL), magnetic resonance imaging (MRI), and computed tomography (CT) [23]. Chlorambuchil drug release is regulated by IR light and amino-coumarin phototrigger [24]. High efficacy in photodynamic therapy using singlet oxygen (O\textsuperscript{2}.) is obtained from NaLuF\textsubscript{4}:Gd/Yb/Er encapsulated in amino-terminated organ silica when both IR and photosensitizer are present [25].

**Bioimaging**

Various technologies using ultrasonic, optical, luminescent, magnetic, or X-ray sources are available for bioimaging. The main requirements in nanomedicine include spatial resolution, sensitivity to tumor/cancer cells, penetration depth, etc. Recently rare-earth UCNPs have been considered as promising fluorescent imaging probes [26]. UCL imaging has a deeper penetration depth with NIR than conventional photoluminescence using organic dyes/quantum dots and UV light. In addition, neither harm nor auto fluorescence in bioimaging. MRI has a much deeper penetration depth and a much better contrast. If magnetization recovers before MR measurement, the image weighting is denoted by T\textsubscript{2}. If it decays before the measurement, that is denoted by T\textsubscript{1}. Tumor signal is high in T\textsubscript{2}-weighted, but low in T\textsubscript{1}-weighted MR images [27]. CT images are preferred when high special resolution is required [28].

In UCNPs, Gd\textsuperscript{3+} doping enhances UCL [29,30] and allows application as a contrast agent for in vivo MRI [31]. Hence magnetic/luminescent dual-mode imaging is available with Gd-doped UCNPs. **Figure 1** shows one of examples [32]. UCNP is NaYF\textsubscript{4}:Yb/Er, and additionally Gd is doped via seed-mediated process for Gd-UCNP (NaYF\textsubscript{4}:Yb/Er@NaGdF\textsubscript{4}). UCSN is the final yolk-shell structure in **Figure 1A**. A nude mouse bearing HeLa tumor was used for bioimaging experiments, and MR (**Figures 1B,C**) and UCL (**Figures 1E**) images were measured before and after the intratumoral injection of UCSN as contrast agents in vivo. High signal intensity at the tumor site indicates not only targeting folate receptors in HeLa tumor cells [33], but also enhanced MRI. In addition, in vivo UCL signal were also observed under a CW NIR laser (980 nm), demonstrating dual-mode (MR/UCL) imaging of UCSN in vivo.

**Trimodal (UCL/MRI/CT) imaging** is also possible. Zhu et al. [23] reported Yb\textsuperscript{3+} and Er\textsuperscript{3+}/Tm\textsuperscript{3+} co-doping to NaLuF\textsubscript{4}:Fe\textsubscript{15}O\textsubscript{17} nanoparticles are yolks for the magnetic manipulation without shielding UCL signal. HF/NaF solution converts Fe\textsubscript{15}O\textsubscript{17}/SiO\textsubscript{2}/Lu\textsubscript{2}O\textsubscript{3} core-double shell nanostructure to multifunctional Fe\textsubscript{15}O\textsubscript{17}/NaLuF\textsubscript{4}:Yb/Er/Tm yolk-shell UCNP. After intratumoral injection of UCNPs, in vivo UCL images were measured in bright and dark fields. Excitation source is CW NIR at 980 nm, while 800 nm signal is measured with a high contrast. In vivo T\textsubscript{1}-weighted MR and CT images were also obtained. After the injection, about 50% weaker (darker) signal was observed in the tumor area, demonstrating UCNPs as a good MRI contrast agent. High contrast of tumor area was also observed well in volume-rendered and coronal CT images. Volume rendering is a computer technique to get a 2-D projection of a 3-D sample. Tridimensional imaging is applicable with yolk-shell structured UCNPs, although further research is required for smaller particle sizes, better sensitivity and deeper penetration depth.
Drug Delivery

Drug release can be triggered by photolysis, [34] pH responses, [35,36] redox, [37] enzymes [38] or temperature [39]. Among them, NIR light trigger using UCNPs has been succeeded in drug delivery with convenient manipulation and improved therapeutic efficacy. Traditional photo-regulated drug release uses UV light, which has a shorter penetration depth and is harmful to living tissues. In contrast to UV, CW NIR light penetrate deeper without damage [3]. Yb\(^{3+}\) and Tm\(^{3+}\) doped lanthanide upconversion nanoparticles convert CW 980 nm to UV light that drives photosysis to release drugs.

Zhao et al. [24] reported a photo-triggered drug release as shown in Figure 2. Yolk is NaYF\(_4\), Tm,Yb/NaLuF\(_4\), which convert NIR to intense UV emission, and shell is mesoporous silica that has a large pore volume to be loaded with hydrophobized phototrigger-conjugated drug (Figure 2A,B). Lipase is blocked by the mesoporous shell, hence enzymolysis is not allowed in yolk-shell nanostructures. Amino-coumarin is the phototrigger that releases chlormebucil (an anticancer drug, denoted as ACCh) upon photosysis under UV adsorption at 380 nm. The phototrigger has hydrophobic two octanyl chains, which prevent from being released together with the drug. Drug release from the YSUCNP-ACCh was carried out in phosphate buffered saline (PBS, pH 7.5) solution. Figure 2C displays the drug release under CW NIR at 980 nm as a function of irradiation time. For the comparison, YSLnP-ACCh, in which the yolk is NaYF\(_4\):Yb@NaLuF\(_4\) without Tm\(^{3+}\), was also tested. Only YSUCNP-ACCh released the drug up to about 68%, while YSLnP-ACCh did nothing due to the absence of Tm\(^{3+}\). In addition, chlormebucil drug is released only when CW NIR laser is on (Figure 2D). Without light, the drug release stops. Kunming mice bearing S-180 tumor were used for photo-regulated drug delivery experiments. Figure 2E shows the photos of the mice injected with YSUCNP-ACCh, YSLnP-ACCh or saline on 1st, 3rd, 5th, and 17th day of NIR irradiation (980 nm, 50 mW/cm\(^2\), 20 min each day). No big difference was observed in tumor volume for YSLnP-ACCh and saline. In case of YSUCNP-ACCh, however, the tumor grew much slower, demonstraing successful drug release regulated by NIR light only.

Photodynamic Therapy

UCNPs can be applied for photodynamic therapy using photosensitizers under NIR light to produce cytotoxic singlet oxygen (\(^{1}{O}_2\)) that treat tumor cells in both in vivo and vitro. One of challenges for practical use is the efficacy of PDT, which depends on the efficiency of energy transfer from UCNPs to photosensitizers. Recently many core-shell typed UCNPs [21,22] have been developed for PDT, but sufficient photosensitizer loading adjacent to UCNPs prefers yolk-shell to core-shell nanostructures. Lu et al. [25] reported multifunctional nano-bioprobes based on organosilica-shell UCNP as depicted in Figure 3. UCNP is b-NaLuF\(_4\):Gd/Yb/Er capsulated in amino-terminated organosilica shell (ROS-ATF), which has a high affinity to urokinase plasminogen activator receptor (uPAR) as shown in Figure 3A. Mono-substituted b-carboxyphthalocyanine zinc (ZnPc-COOH) was used for a photosensitizer, as its absorption bands overlap with the red emission of Er\(^{3+}\). The mesoporous ROS-ATF shell accommodate the photosensitizer as high as 7.7 wt.%. H1299 (human lung cancer) cells were used to evaluate in vitro PDT efficacy. Figure 3B displays the viability of H1299 treated with UCNP@ROS-ZnPc-COOH or UCNP@ROS as a function of concentration under NIR irradiation (980 nm, 0.5 W/cm\(^2\), 10 min). The viability of H1299 by use of MTT assay decreased only when both NIR light and photosensitizer are present. The corresponding mi-
Figure 1: (A) Schematic diagram of UCNP synthesis, In vivo T1-MR images (B) before and (C) after intratumoral injection of UCNP@void@SiO$_2$. (D) Bright field and (E) in vivo upconversion luminescent image (UCL) after intratumoral injection of UCNP@void@SiO$_2$. (F) Overlay image of (D) and (E). Reproduced with permission from ref. [32].

Figure 2: (A) Schematic diagram of drug delivery, (B) Upconversion-based photolysis, (C) Drug release profiles of chlorambucil from YSUCNP-ACCh and YSLnP-ACCh in phosphate buffered saline solution (pH = 7.5) under CW 980 nm irradiation, (D) Release of chlorambucil drug from YSUCNP-ACCh as a function of 980 nm laser on and off, (E) Photos of tumor-bearing mice, to which are injected with YSUCNP-ACCh, YSLnP-ACCh, or saline after NIR radiation treatments on the 1st, 9th and 17th day. Reproduced with permission from ref. [24].

Figure 3: (A) Schematic illustration of amino-terminated organosilica-shelled UCNP (UCNP@ROS-ATF, noted for Rattle OrganoSilica-Amino Terminal Fragment), Viability of (B) H1299 and (C) HELF cells treated with UCNP@ROS-ZnPc-COOH and UCNP@ROS as a function of concentration with and without 980 nm irradiation, (D) and (E) Microscopic images of H1299 cells before and after photodynamic therapy treatment, respectively (scale bar = 25 µm). Reproduced with permission from ref. [25].

Figure 4: Upconversion luminescent spectra and lifetime of 4F9/2 for (A) UCNP@ROS, (B) UCNP@OS and (C) UCNP@RS with and without loading ZnPc-COOH. Reproduced with permission from ref. [25].

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References


