Upconversion Nanoparticles: Biological Applications

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Abstract

Upconversion nanoparticles (UCNs) are a recent class of fluorophore that incorporate physico-chemical principles to enhance low-energy photons to higher energy levels. As new luminescent nanomaterials, upconversion nanoparticles demonstrate superior properties compared to conventional fluorophores, including large signal-to-noise ratio and high photo stability. Additionally, as these particles have near-infrared excitation wavelengths, they present further advantages, including deep tissue penetration and low photodamage to biological specimens. Upconversion was first introduced in the 1960s, and soon received widespread attention due to its various optical applications, including infrared quantum counter detectors and compact solid-state lasers. In recent years, preparing high-quality lanthanide-doped nanoparticles is turning into a usual procedure, emphasizing the importance of upconversion in biological science and practices. Upconversion nanoparticles are biocompatible and small in size; therefore, they can readily be coupled to proteins or other biological macromolecular systems and implemented in several assay formats, from bio-detection to cancer treatment. Also, the intense visible emissions generated by upconversion nanoparticles under near-infrared excitation are more penetrative and less biologically damaging compared to ultraviolet excitation currently in use. This makes these particles excellent candidates as cent stains for bio-imaging processes. The present review investigates new aspects of UCNs in terms of definition, development, and application and their use in targeted drug delivery, bioimaging, detection, and Photodynamic Therapy (PDT) and focuses on the approaches employed in the process.

Key words: Upconversion nanoparticles, Photodynamic Therapy, Imaging, Detection, Drug Delivery.

1. Introduction

Upconverting nanoparticles (UCNPs) are particles with a diameter in the range of 1–100 nm that display photon upconversion. Photon upconversion involves the absorption of two or more incident photons with low levels of energy and their conversion into a single emitted photon with a higher energy level. These particles usually absorb infrared (IR) radiation and emit visible light or ultraviolet (UV) radiation [1]. In this important nonlinear optical phenomenon, a low-energy excitation is converted in an anti-Stokes process to a high-energy emission. Ever since its discovery in the 1960s, upconversion has accelerated the development of efficacious optical devices, including temperature sensors, the compact solid-state lasers, and the infrared quantum counter detectors [2]. After the emergence of nanoscience and nanotechnology in the late 1990s, interests in UCNP began to rise in the fields of material science, energy, bioimaging,

and biomedicine [3]. This is a result of the suitable Nano size and singular optical properties of UCNPs (especially in comparison with the first organic dyes and second quantum dots) and their other benefits such as the non-autofluorescence background with large signal-to-noise ratio, large anti-Stokes shifts for the significant separation of the UCL from the excitation, narrow emission bandwidths for an easy multiplex imaging, non-blinking and non-bleaching of upconversion luminescence (UCL) for long-term repetitive fluorescence, and the NIR excitation for efficient penetration in deep tissue [4].

In general, upconversion nanoparticles comprise inorganic host molecules and a lanthanide dopant embedded inside the host lattice [5]. Lanthanides are all capable of upconversion in one form or another. However, the absorption and promotion to the intended levels of the visible and UV ranges can only be achieved with Erbium (III) (Er^{3+}), Holmium (III) (Ho^{3+}), and thulium (III) (Tm^{3+}). This is because the inner shell electrons of these elements are protected by the $5s^2 5p^6$ sub-shells, which creates numerous defined energy states. Extra doping of Ytterbium (III) (Yb^{3+}) might have a positive effect on a number of nanoparticles; however, doping must always be lower than % 2 mol as to prevent loss of excitation energy during cross-relaxation processes [6,7]. Typically, UCNPs consist of rare earth-based lanthanide- or actinide-doped transition metals and feature greatly effective cellular uptake and large optical penetrating power with little background noise in the deep tissue level, making them excellent options for using in vivo bio-imaging, bio-sensing, and nanomedicine.

UCNPs are also highly processable and amenable to surface functionalization. Therefore, it is easy to inject them into animal bodies or integrate them into optoelectronic devices, thus advancing further research. Upconversion, although promising in various applications, suffers from inadequate emission intensity, which greatly hinders their progress for further utilizations. Small extinction coefficients of the lanthanide ions result in the UCNPs' poor absorption of excitation light. Also, as the energy levels of the lanthanide ions are extremely complicated, many nonradioactive deactivation routes may appear [7]. Recently, extensive attempts have been made to develop new structures and compositions to improve the emission intensities of UCNPs. In the present review, biological applications of Upconversion Nanoparticles in recent years are investigated.

2. Upconversion Mechanisms

The upconversion nanoparticles' mechanism of action is different from other luminescence mechanisms. Other luminescence processes usually involve promoting one electron from the ground state to the excited state [5,8]. In contrast, upconversion nanoparticles work with several low-energy pump photons (intermediate meta-states) to gather the low-energy excitation photons.

Specific lanthanides with a 3+ charge are the only ions that can be upconverted. Therefore, a partially filled 4f electron sub-shell remains from ionization. The lanthanide ions can act as direct emitters of light, or a sensitizer that absorbs, upconverts, and transfers excited light to an emitter (mostly the Ytterbium dopant). Figure 1a−e shows the five different processes that, through transferring the energy to the emitter, enable the lanthanide ions to upconvert the light. These processes are as follows: (a) excited-state absorption (ESA); (b) energy transfer upconversion (ETU); (c) cooperative sensitization upconversion (CSU); (d) cross relaxation (CR); and (e) photon avalanche (PA) [9].

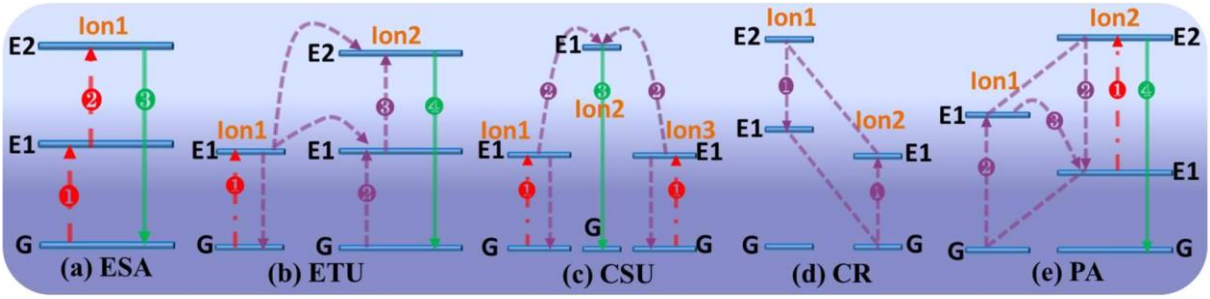

Figure 1. Principal UC processes for lanthanide-doped UCNPs: (a) excited-state absorption (ESA), (b) energy transfer upconversion (ETU), (c) cooperative sensitization upconversion (CSU), (d) cross-relaxation (CR), and (e) photon avalanche (PA). The red, violet, and green lines represent photon excitation, energy transfer, and emission processes, respectively. Reprinted with permission from ref 9. Copyright © 2014 American Chemical Society

2-1. Excited-State Absorption (ESA)

Excited-State Absorption is formed as a successive absorption of pump photons by a single ion because a simple multilevel system is structured as a ladder. This is shown by a three-level system for two sequential photon absorption in Figure 1a. This mechanism is the result of the equal separation from G to E1 and from E1 to E2, and also the reservoir capability of the intermediate level E1. When an ion is excited from the ground state to the E1 level, it is highly possible that another pump photon promotes the ion from E1 to the higher-lying state E2 before it decays back to the ground state as the E1 state has a longer lifetime. As a result, upconverted emission will begin from the E2 level. To achieve an effective ESA, it is necessary for the energy states of lanthanide to be arranged in the form of a ladder. The required energy structure for these processes exists only in a few ions (e.g., Er^{3+}, Ho^{3+}, Tm^{3+}, and Nd^{3+}). These ions also suitably match in excitation wavelength match with the output of diode lasers (at ~975 and/or 808 nm) in commercial use [5, 9,10].

2-2. Energy Transfer Upconversion (ETU)

The Energy Transfer Upconversion shown in Figure 1b incorporates two neighboring ions. This process, therefore, differs from ESA in Figure 1a that takes place within one lanthanide ion. An ETU process begins with the excitation of an ion 1 (known as the sensitizer) from the ground state to its metastable level E1 through absorbing a pump photon. Subsequently, its collected energy is transferred to the ground state G and the excited-state E1 of ion 2. The ion 2 (called the activator) is then excited to its upper emitting state E2, and the sensitizer ion 1 relaxes back to ground-state G for the second time. The average distance of the sensitizer from the activator, determined by the concentrations of dopants, influences the effectiveness of an Energy Transfer Upconversion process [10]. In contrast, the dopant concentration does not affect ESA as the process involves only one ion. The ETU is an extremely important process for UCNPs because the hitherto best theranostic UCNPs employ ion pairs (i.e., sensitizer/activator) of Yb^{3+}/Tm^{3+}, Yb^{3+}/Er^{3+}, and Yb^{3+}/Ho^{3+} to achieve a higher excitation at ~975 nm. It should be noted that the scattering and absorption rate of biological tissues is rather small (975 nm) and within the "optical transparency window" of tissue. Yb^{3+} is an excellent sensitizer here as its absorption cross-section is large enough in the NIR region at ~975 nm. Also, its optimized concentration does not require evoking deleterious cross-relaxations (see section 1.2.4) to remain high (20−100% for fluoride nanoparticles) as Yb^{3+} has only two energy levels in its structure [11]. The focus of investigations has so far been on developing Yb^{3+}-sensitized UCNPs that are pumped at around 975 nm. Moreover, single lanthanide-doped systems that also employ the

lanthanide ion as the sensitizer have demonstrated efficient ETUs. For instance, high ETU efficiency has been observed in Ho^{3+}-doped $NaGdF_4$ nanoparticles excited at 1200 nm, or Er^{3+}-doped $LiYF_4$ excited at a telecom wavelength of 1490 nm. Using other elements as sensitizers can further the quenching and enhancement of some emission bands; for example, Nd^{3+}, Ce^{3+}, and Ho^{3+} have been employed as sensitizers to improve the blue emission band of Tm^{3+}, red emission band of Ho^{3+}, and NIR emission band of Tm^{3+}, respectively [4, 9].

2-3. Cooperative Sensitization Upconversion (CSU)

The Cooperative sensitization upconversion (CSU) process (Figure 1c) involves the interaction of three ion centers. Ions 1 and 3 usually have the same type. Following the absorption of excitation photons, both ion 1 and ion 3 can reach the excited state, respectively [6]. Afterward, ion 1 and ion 3 can interact with ion 2 at the same time and cooperate to transfer the contained energy in order to excite ion 2 to a higher state. By emitting an upconverted photon, the excited ion 2 can relax back to the ground state. The efficacy of CSU is in general orders of magnitude lower than either the ESA or ETU processes. This is due to the presence of quasi-virtual pair levels during transitions, described through quantum mechanics in higher perturbation. However, as confined excitation is needed to counterbalance the low efficiency, efforts will be concentrated on achieving high-resolution imaging that cannot be provided by the other UC mechanisms. The CSU mechanism has been observed in Yb^{3+}/Tb^{3+}, Yb^{3+}/Eu^{3+}, and Yb^{3+}/Pr^{3+} ion pairs [9, 11, 12].

2-4. Cross Relaxation (CR)

Cross relaxation (CR) energy transfer process (Figure 1d) is the result of ion−ion interaction where the excited ion 1 transfers part of its energy to ion 2 through the following process: E2 (ion 1) + G (ion 2) → E1 (ion 1) + E1 (ion 2). Ions 1 and 2 can be the same or different, and it is possible for the ion 2 to also be excited. As an essential outcome of the ion−ion interaction, the efficiency of the CR process is closely associated with the dopant concentration. The widely-known “concentration quenching mechanism” of emission is primarily caused by CR. However, it is still possible to be intentionally applied in tuning the color output in UCNPs or constructing effective photon avalanche mechanisms [4, 9, 10].

2-5. Photon Avalanche (PA)

The process of Photon avalanche (PA) (Figure 1e) generates UCs’ higher than a specific threshold of excitation power. The up-converted fluorescence generated below this threshold is very small. On the other hand, the PL intensity is increased by orders of magnitude above the pump threshold. In reality, the PA is a looping process and contains the ESA process for excitation light and an effective, feedback-producing CR. At first, the level E1 of ion 2 is populated by nonresonant weak ground-state absorption. The PA loop begins with the ESA process elevating ion 2 at level E1 to the emitting level E2. This is followed by this efficient CR process between ion 1 and ion 2: E2 (ion 2) + G (ion 1) → E1 (ion 2) + E1 (ion 1). Finally, the loop is completed by ion 1 transferring its energy to ion 2 and populating its level E1. The net effect of the looping process is one ion 2 at metastable E1 state generating two metastable E1 ion 2s. Following this looping process, the two ion 2s at the E1 state will produce four; four will produce eight, and so on, creating an avalanche effect for populating ion 2 in its E1 state, and therefore the PA UC from the emitting E2 level. PA can easily be identified as it usually necessitates a pump threshold and its buildup time is long (seconds). Also, UC PL becomes massively dependent on the pump power around the threshold pumping power [8, 9, 11, 13].

3. Biological Applications

Upconversion nanoparticles are biocompatible and small in size and are, therefore, best employed at the point of interaction of biology, medicine, and nanotechnology. The technology of these particles is an innovation in the interdisciplinary field of nanomedicine. In this section, the benefits and advantages of upconversion nanoparticles in biological applications are discussed.

3-1. Drug Delivery

The unique optical properties of upconversion nanoparticles, including production of emission and absorption spectra via a forbidden 4f-4f electron transition, resulting in them having long-lifetime and tunable emissions [13]. They are therefore suitable for drug delivery, and there are proven systems of drug delivery that involve upconversion nanoparticles combined with other biocompatible materials (either hydrophobic pockets, a mesoporous silica shell, or hollow spheres with a mesoporous surface). The exceptional properties of these systems are of essential help in solving the existing issues in imaging probes used today and are prospective in achieving multifunctional nanoplatforms featuring imaging and therapeutic modalities at the same time [14]. In this section, using UCNPs in drug delivery applications is discussed.

The advantages of drug delivery systems include improvements in the efficiency of pharmaceutical payloads and providing better stability, solubility, biodistribution, and pharmacokinetics to drugs. The literature presents three approaches of creating UCNP-based systems for drug delivery: (i) hydrophobic pockets, (ii) mesoporous silica shells, and (iii) hollow spheres with the mesoporous surfaces [9, 15, 16].

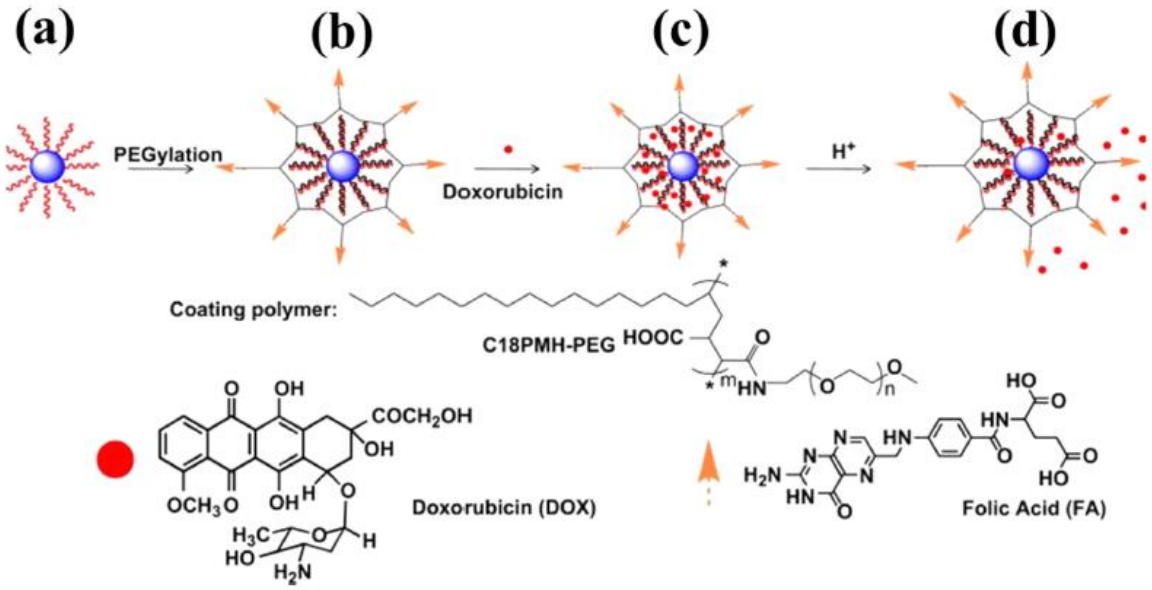

Figure 2. Schematic illustration of the UCNP-based drug delivery system: (a) As-synthesized oleic acid capped UCNPs; (b) C18PMH-PEG-FA functionalized UCNPs; (c) the loading of DOX on UCNPs; DOX molecules are physically adsorbed into the oleic acid layer on the nanoparticle surface by hydrophobic interactions and (d) release of DOX from UCNPs triggered by decreasing pH. Reprinted with permission from ref 9. Copyright © 2014 American Chemical Society

The hydrophobic−hydrophobic interaction between the hydrophobic ligand on the particle surface and the drugs is used to create “hydrophobic pockets” from hydrophobic drugs on the UCNP surface [17]. For instance, PEGylated amphiphilic polymer onto the OA capped $NaYF_4:Yb^{3+}/Er^{3+}$ UCNPs forms a hydrophobic pocket on the particle surface to partition anticancer drug molecules of doxorubicin (DOX) on (Figure 2a). As DOX has a slower release rate at higher pH, its release was controlled by changing the pH of the solution; thus contributing to the controlled drug release in tumor cells. Lately, a similar approach in incorporating the $NaYF_4:Yb^{3+}/Er^{3+}$ and iron oxide nanocomposites has been used to the same effect, enabling optical imaging and magnetic-targeted drug delivery be performed simultaneously [9, 18].

The second method (Figure 2b) involves depositing drugs in the pores of mesoporous silica shells that are coated onto the surface of UCNPs. As the surface area and pore volume of mesopores in the silica shell are large, it is possible to deposit larger amounts of drugs. An instance is adding ibuprofen to mesoporous silica-coated β-$NaYF_4:Yb^{3+}/Er^{3+}$ UCNPs fibers that had been fabricated through electrospinning [9, 20]. In this case, the researchers succeeded in ameliorating the ability of ibuprofen-loading by creating mesoporous silica-coated α-$NaYF_4:Yb^{3+}/Er^{3+}$ nanospheres using a simple two-stage sol−gel modification. Regulating the ibuprofen load was possible by changing the thicknesses of mesoporous SiO_2 layers. Multifunctional nanocarriers incorporating the UC luminescent nanoparticles of $NaYF_4:Yb^{3+}/Er^{3+}$ core (UCNPs) and thermo/pHcoupling sensitive polymer poly [(N-isopropylacrylamide)-co-(methacrylic acid)] (P(NIPAm-co-MAA)) gated mesoporous silica shell have been considered for use in controlled drug release and cancer theranostics (e.g., including UC PL imaging) [9, 21].

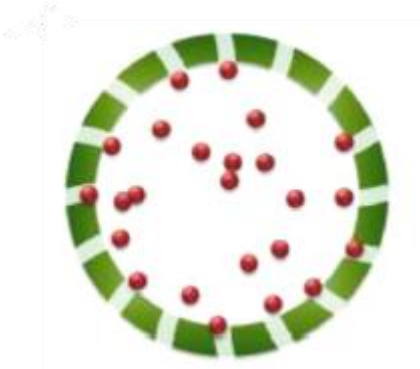

Figure 3. Schematic representation of current approaches to construct UCNP-based drug delivery systems: hollow mesoporous coated spheres. Reprinted with permission from ref 9. Copyright © 2014 American Chemical Society

The third method (Figure 3) involves loading drugs into a hollow UCNP with a mesoporous shell. As the UCNP is hollow, sufficient drug loading is possible without compromising UCPL imaging ability [9]. Monodisperse core/shell structured upconverting Yb(OH)-CO_3@$YbPO_4:Er^{3+}$ hollow spheres can be used as carriers of the antineoplastic drug DOX. DOX has been shown to travel into cells by core/shell hollow spheres carrier and to release inside cells following endocytosis. The DOX-loaded spheres were found to be much more cytotoxic than free DOX. Also, $Y_2O_3:Yb^{3+}/Er^{3+}$ hollow nanospheres have been synthesized for delivering DOX into HeLa cells, producing high contrast images of cells and tissues without the harmful effects of radiation. Lately, a more significant nanorattle system has been introduced for magnetic-guided chemotherapy through an ion-exchange process [22]. This multifunctional

mesoporous nanostructure comprises a hydrophilic lanthanide-doped $NaYF_4$ shell and an inner magnetic nanoparticle, featuring, therefore, both upconverting luminescent and magnetic properties. A successful DOX-loading into the hollow volume of the nanorattle was carried out through the porous UCNP layer. In vivo experiments with doxorubicin showed promising shrinking effects on tumors and an extremely elevated tumor targeting in the presence of an applied magnetic field [9, 23].

3-2. Upconversion Nanoparticles for Photodynamic Therapy

Photodynamic therapy (PDT) is a non-invasive method used in the treatment of several diseases, including cancer. Recently, integrating upconversion nanoparticles (UCNPs) in PDT has attracted the interest of many researchers. When UCNPs are excited under a near-infrared (NIR) light, they emit high-energy visible light [24]. This high-energy light can activate the photosensitizer (PS) molecules in its vicinity and generate singlet oxygen, eventually killing cancer cells. Compared to conventional visible- or ultraviolet (UV) light-induced PDT, the large penetrative reach of NIR light enables NIR-excited UCNPs to activate PS molecules far deeper in the tissue. Besides providing energy for PDT, UCNPs are also applicable in the NIR light-triggered drug release, activation of 'caged' imaging, or therapeutic molecules in a similar manner [25].

PDT is associated with several limitations; for instance, the hydrophobicity of PSs commonly affects their pharmacokinetics and bodily biodistribution, and their absorption of UV/visible wavelengths is a restrictive factor for the how deeply the excitation light can penetrate. This paves the way for the UCNP to solve these problems with its favourable surface functional groups and controllable upconverted emissions. In summary, it is possible to use UCNPs as drug carriers of hydrophobic PSs accumulation into the tumor and, simultaneously, act as light-transducers activating PSs in tumor for an efficacious PDT process in deep tissues [26, 27].

By synergistically combining the UCNPs and PSs, PDT can achieve a much more extensive capability in the treatment of several non-superficial diseases, including solid and large tumors. A detailed discussion of various designs of UCNP-PS systems for different applications is provided below [28].

The upconversion efficacy, the surface-functionalization of UCNPs, and the matching wavelengths of UCL and PSs absorption present in UC-PDT systems are contributing factors to an effective PDT treatment. Firstly, peak absorption is necessary for PS to maintain its consistency with the UCNP emission. Secondly, for the energy to be efficiently transferred, PS needs to be near this luminous inner core [29, 30]. The entrapment of PS onto UCNP for efficient ROS production is usually performed through the following three methods: covalent conjugation, physical adsorption, and silica encapsulation. Considering the above-mentioned mode of PS attachment or loading, the acquired UC-PDT features the benefits of (1) integration with functional compounds or targeting agents does not permit the premature release of PS, improves tumor-targeting and accumulating and, therefore, lowers the overall phototoxicity level; (2) The considerable surface-to-volume ratio of UCNP greatly improves PS loading; (3) UCNP-PS system can exhibit amphiphilicity, which many excellent PS systems lack; this allows a straightforward journey for UCNP-PS through the blood stream and into tumor tissue; (4) Enhanced permeability and retention (EPR) effect induced by the abnormal tumor neovasculature with leakage and inadequate lymphatic drainage of the tumor tissue, helping achieve a facile diffusion of UCNP-PS into tumor tissue and their retention afterward [31, 32].

3-3. Upconverting nanoparticles Bioimaging

In addition to low cytotoxicity levels, deep tissue penetration, low background signals, sharp emission bands, large Stokes shifts, and low photobleaching are among the exclusive luminescent properties of upconversion nanoparticles that make them excellent candidates for use in bioimaging and biolabeling [33]. As upconversion nanoparticles work through a two-photon absorption mechanism, their produced energy emission and upconversion efficacy are higher. This is particularly observed in comparison with other technologies that, for instance, involve organic dyes and quantum dots [34]. Also, these particles are capable of surface functionalization, meaning that their luminescence performance will not change after solubilization for application in the body. Imaging of various types of breast and ovarian cancer cells, HeLa cells, KB cells, HepG2 cells, and AB12 mice mesothelioma cells have been carried out using upconversion nanoparticles [35].

In UCNP bioimaging, a laser is employed for exciting the UCNPs in a sample and the emitted frequency-doubled light is detected afterward. The UCNPs' narrow emission spectra, desirable chemical stability, low level of toxicity, reduced autofluorescence background, prolonged luminescence lifetime, and elevated resistance against photoquenching and photobleaching result in their excellent application in imaging. Conventional bio labels use the Stokes-shift mechanism and, therefore, require high levels of photon energy. In contrast, the anti-Stokes mechanism employed by UCNPs results in decreased energy consumption, a lower level of damage, and deeper light penetration into the tissue [36]. Multimodal imaging agents consist of several signal reporting modes. UCNPs with Gd^{3+} or Fe_2O_3 can be used as luminescent probes and MRI contrast agents. UCNPs have also been applied for the configuration of photoluminescence and X-ray computed tomography (CT), and preparation of trimodal UCNPs combining photoluminescence, X-ray CT, and MRI have also been carried out involving these particles [26]. The favorable interaction between fluoride and lanthanide ions is useful for the application of UCNPs as imaging agents in imaging lymph nodes and staging for cancer surgery on the basis of single-photon emission computed tomography (SPECT) [30, 37]. As UCNPs are targeted fluorophores and in conjugation with ligands, they can form over-expressed receptors on malignant cells as a photoluminescence label for selective cell imaging. UCNPs are also applicable in functional imaging, including providing assistance in cancer surgery by targeting lymph nodes and the cardiovascular system. UCNPs incorporate dopant modulation to enable multiplexed imaging and shift emission peaks to resolvable wavelengths. Single-band UCNPs conjugated to antibodies have a much better performance in the detection of breast cancer cells than conventional fluorophore labeling of antibodies, which also cannot be subject to tests and multiplexed analysis [32, 38].

One of the essential and extensively used methods in biology is fluorescent labeling. Downconversion fluorescence labels excited with ultraviolet or short-wavelength that are currently in use have poor autofluorescence and inadequate signal-to-noise ratio and can harm living organisms with incident photo damage. In contrast, upconverting fluorescent nanoparticles produce detectable, higher-energy photons in the near-infrared (NIR) or visible region under NIR light irradiation in a process known as upconversion [35, 39]. These nanoparticles are capable of removing the issues associated with traditional downconversion labels, boasting advantages such as near-negligible levels of autofluorescence, no photo damage to living organisms, good detection sensitivity, and deep light penetration. They are, therefore, excellent choices as fluorescent labels in bioimaging applications [23].

Upconversion materials have applications in photostable biological labels, cell imaging, and also photodynamic therapy (PDT) of cancer and several other diseases [17]. A biological system does not exhibit fluorescence under infrared radiation; luminescence is produced only by

upconversion. This therefore greatly darkens the background and adds to the precision and sensitivity of biological detection. Moreover, UCNPs boast great chemical stability, large SNR, low levels of potential biological toxicity, and other favorable properties, making them preferable for biological imaging marking [40].

3-3-1. Targeted upconversion materials

Wang et al. in Fudan University, China, conducted extensive studies on applying targeted upconversion materials probes in small-animal imaging. Folic acid (FA) was used for the hydrothermal synthesis and functionalization of the amino-functionalized surface of $NaYF_4$:Yb,Er [27]. The resulting material was employed to evaluate the folate receptor expression of FR (+) cervical carcinoma (HeLa) cells with FR (−) human breast cancer cells (MCF-7) as control. Red light (500– 560 nm) and green light (495–570 nm light) were observed at 980 excitations, which significantly overlapped in HeLa cells. It can therefore be confirmed in photographs with bright and dark fields. UCNPs-FA and UCNPs-NH_2 were next injected in vivo into the tail vein. A HeLa tumor existed on the right flank of the same mouse [8]. One day later, a UCNPs-FA signal was observed in the tumor; the untargeted probe did not accumulate confirming molecular imaging of the folate. Similar experiments incorporating U87 MG tumor model and the RGD peptide and $\alpha_v\beta_3$with $NaYF_4$: 20% Yb, 1.8% Er, 0.2% Tm as a fluorescent probe have been performed. A desirable tumor targeting function has been exhibited by UCNPs RGD peptides. Tissue slice imaging was found to have a 600 μm depth and small autofluorescence interference. The Region of Interest (ROI) analysis indicated that a 24 SNR exists between tumor and background lighting signal conversion. Li et al. evaluated the toxicity of polyacrylic acid (PAA)-modified $NaYF_4$:Yb,Tm by injecting it intravenously into mice. The results indicated that the main targets of PAA-UCNPs are the spleen and liver [41].

3-3-2. Upconversion materials with core-shell structure

A silicon-layer core-shell structure of nanocrystalline $NaYF_4$: Yb, Tm @ Fex Oy (core 20 nm, shell 5 nm) has recently been synthesized which boasts a strong NIR emission at 800 nm with continuous 980 nm laser excitation manifested through a 12 emu g^{-1} magnetization intensity [38]. So far, KB cells and mouse lymphatic system upconversion imaging have benefited from the successful application of core-shell nanocrystals [31]. A core-shell structured $NaYbF_4$:Tm/$NaGdF_4$ with the size of 12 nm was also proposed by Ohulchanskyy et al. The emission intensity of their $NaGdF_4$-coated $NaYbF_4$:Tm was three times higher than that of pure $NaYbF_4$:Tm core. Core-shell nanoparticles were prepared by coating the UCNPs with SiO_2 and amino modifiers. Afterward, surface functionalization was performed and the rabbit antibodies and UCNPs were conjugated [14]. The antibody-UCNP conjugates were utilized in detecting a cancer biomarker carcinoembryonic antigen. As the antibody and UCNPs are connected, the UCNPs can become attached to the HeLa cells. This helps further fluorescent imaging and detection of the HeLa cells. These findings show that the amino-functionalized UCNPs are applicable as fluorescent probes in cell imaging and immunolabeling [14, 29, 42].

3-3-3. Bifunctional upconversion materials

A method to activate caged small interfering RNAs (siRNAs) was proposed by Zhang et al. that employed NIR-to-UV upconversion process for higher spatial and temporal gene interference patterns. Light-sensitive molecules (4,5-dimethoxy-2-nitroacetophenone, DMNPE) were used to cage siRNA molecules against the anti-apoptotic gene surviving [43]. In this case, NIR-to-UV $NaYF_4$:Yb,Tm UCNPs acted as delivery and activators. Liu has shown the synthesis of

tetragonal-phase $LiYF_4$ nanoparticles doped with upconverting lanthanide ions. It has been observed that the microwave hydrothermal method is capable of a faster synthesis of $NaYF_4$:Yb^{3+},Tm^{3+} microtubes, and a better upconversion fluorescence intensity can be achieved. Moreover, the authors were able to conclude the probable reasons and mechanisms for improving the fluorescent intensity [44]. The mentioned bifunctional nanoparticles can be applied in numerous fields such as florescence imaging, targeting, bioseparation, cancer diagnosis and treatment, DNA separation, and magnetic resonance imaging (MRI). In the previous decade, the therapeutic application of UCNPs has substantially advanced; however, concerns regarding their safety and toxicity encumber their widespread use [14].

3-4. Biosensors and temperature sensors

As nanothermometers, UCNPs have been employed in identifying intracellular temperature differences. ($NaYF_4$: 20% Yb^{3+}, 2% Er^{3+}) @$NaYF_4$ core–shell structured hexagonal nanoparticles are able to measure the physiological temperatures ranging from 25 °C to 45 °C with an accuracy rate of lower than 0.5 °C in HeLa cells [40]. Also, the functions of UNCPs as biosensors can be diversified when combined with recognition elements such as antibodies or enzymes. UCNPs combined with MnO_2 nanosheets were able to detect intracellular glutathione. The addition of MnO_2 nanosheets reduces the degree of UCNP luminescence, which is then selectively restored by glutathione via reduction of MnO_2 to Mn^{2+}. The combination of SYBR Green I dye and $NaYF_4$: Yb^{3+}/Tm^{3+} nanoparticles is capable of in vitro examination of Hg^{2+} with a 0.06 nM limit of detection. In vivo detection of Hg^{2+} and other heavy metals have been reported. It is possible to simultaneously detect various species due to the tunable and multiplexed emissions [18, 27, 32].

3-5. Detection and assay

Several forms of biochemical tests such as immunoassay, affinity assay, and DNA hybridization assay implement upconversion nanoparticles as their luminescent reporters. Their excellent signal-to-noise ratios improve detection limits beyond conventional luminescent reporters. There have been examples of detection limits of 10 pg human chorionic gonadotropin in a 100 mL sample [45]. Another report showed that upconversion nanoparticles succeeded in detecting a detection limit of 1 ng/mL probe DNA. This is four times higher than the detection limit of organic dye cyanine 5 [23].

4. Summary and Future Perspective

Reports have shown that UCNPs can be successfully applied in biological practices such as drug delivery, light-regulated drug release, and photodynamic therapy. Experiments on rodents have demonstrated a favorable prospect for UCNP therapy [20, 34, 41]. The effectiveness of UCNPs in chemotherapy can be improved through developing new UCNP agents with a higher drug payload and the ability of targeted drug release. Also, theranostic agents are necessary for simultaneous medical diagnosis and therapy [43, 45]. A comprehensive study of drug delivery with guided imagery in treating cancers or malignancies is highly recommended. Due to the present difficulties and limitations, future research is necessary to maximize the usefulness of these particles in various applications [8, 34]. In the field of bioimaging, UCNPs have achieved targeted tumor labeling in cell cultures and animal models [5]. So far, however, most imaging investigations have concentrated on their desirable modification of particles and characterizing their selectivity, internalization, and biodistribution [9]. Although UCNs are highly biocompatible, their success in vivo diagnosis should be ascertained. Any alteration in the size,

composition, coating, and other characteristics of particles will modify their biological behavior; for instance, the biodistributions, cytotoxicity levels, and excretion routes. These factors and their relationship need to be systematically investigated [11, 28].

Also, UCNs can be of great help in disease understanding. This is especially true where biodistribution of the pathogens or affected cells has great repercussion to the disease progression and treatment efficacies such as cancer and infectious diseases. UCNs can track these biodistributions for a long time and reveal the yet unknown aspects of disease manifestations. Submicron UC particles have made detection and sensing only possible through the device formats of LFT and microarrays as LRET is usually used in the homogeneous assay in solution. Therefore, the particles' size can be problematic because distance has a substantial effect on LRET, and a longer distance between the donor (that is, particles) and the acceptor results in far less effectiveness [19].

As smaller UCNs are successfully synthesized, homogeneous UCN-based assays are set to become more developed and advanced, thus presenting as new, more favorable assays [20]. Also, future UCN-based assays are likely to advance following newer and more efficient target recognition partners and go beyond mere antibody-antigen interactions or DNA hybridizations and use, for instance, carbohydrate-protein or aptamer-protein interactions as substitutes. As a result, UCNs present the excellent potential of developing the next generation of POC diagnostics. Moreover, as capable therapeutic agents, UCNs can play the role of Nano transducers for PDT and delivery vehicles for genes and drugs [9, 45].

It is necessary to carry out systematic assessments of PDT efficacy, particularly regarding parameters such as photosensitizer-UCNs ratio, tumor-targeting specificity, and optimal light dose in order to prepare UCN-based PDT for use in clinical cancer treatments [12]. In vivo penetration depth should also be examined to practically assess the feasibility of UCN-based PDT for treating deep tumors [6]. Moreover, non-oncological diseases can also benefit from the mentioned PDT strategy as it can also be effective on pathogens. In contrast, using UCNs as carriers for genes and drugs is still in its early stages of development [45]. The focus of future developments should be on exploiting the exceptional optical properties inherent in these particles to advance their tracking and on-command release capabilities as Nano-carriers.

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