CHAPTER

UPCONVERSION NANOPARTICLES 13

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13.1 INTRODUCTION

A flurophore is a substance that can reemit light upon excitation. Organic flurophores are used as contrast agents in bioimaging for real-time visualization of biological process. However, they suffer from several limitations like photodegradation and unsuitable broad emission spectra for multiplex biolabeling. Another alternative for these materials is quantum dots (QDs). Although QDs have narrow emission bandwidth and high quantum yield, they are highly toxic. Also, QDs suffer from intermittent emission. These drawbacks are rectified by using a new class of lanthanide-doped nanomaterials called as upconversion nanoparticles (UCNPs) [1]. Upconversion is an optical process in which two or more photons absorb light, get excited, and then emit light at a wavelength lower than excitation wavelength (Figure 13.1). Mostly d-block and f-block elements undergo photon upconversion. QDs and organic flurophores got excited in the ultraviolet and visible light, in which the biological samples induced autofluorescence. UCNPs did not face this problem as they emit radiation in the near-infrared region in which biological molecules are transparent. They exhibit low toxicity, high photostability, and show sharp emission wavelength [2].

13.2 PROPERTIES OF UCNPs

Most of the UCNPs are highly crystalline materials. Unlike conventional luminescence, upconversion processes involve multiple intermediate states to accommodate low energy excitation photons. UCNPs consist of inorganic host and lanthanide dopant ions embedded in the host lattice. They depend on the ladder like arrangement of energy levels of lanthanide dopant ions. The crystal structure and optical property of host materials play prominent role. The host materials absorb excited energy of the dopant ions through lattice vibrations. When the crystal structure of host material changes, the crystal field around dopant ions varies, attributing to different optical properties of UCNPs. Highly crystalline UCNPs exert strong crystal field around dopant ions and minimize energy loss of dopant ions arising from crystal defects [4].

S. Thomas, Y. Grohens, N. Ninan: Nanotechnology Applications for Tissue Engineering. ISBN: 978-0-323-32889-0
 DOI: http://dx.doi.org/10.1016/B978-0-323-32889-0.00013-3

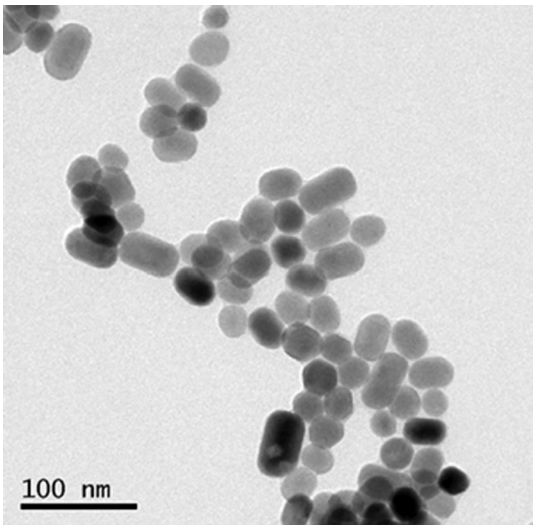

FIGURE 13.1

TEM image of UCNPs.

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In case of lanthanides, the 4f electrons are completely shielded by 5s and 5p subshells and hence they don't interact with host lattice. Therefore, the absorption and emission spectra of lanthanide-doped UCNPs depict sharp lines, which are spectroscopic fingerprints. The chemical composition of the host material does not affect the emission peak. The colors emitted by UCNPs can be varied by changing the dopant concentration. These emissions do not involve chemical bond breakage and are thus stable against photobleaching.

Surface functionalization of UCNPs is necessary to improve aqueous solubility and biological functions. Some of the functionalization techniques include surface silanization, ligand exchange, ligand attraction, oxidation, and electrostatic layer by layer assembly. Among them, silanization has gained lot of importance as silica coating is applicable to both hydrophilic and hydrophobic materials. Besides these, nonsilane reagents like polyethyleneimine are also used for surface modification.

Cytotoxicity is an important factor to be analyzed before conducting biomedical applications. Wang et al. proved that PEGylated UCNPs are not cytotoxic and can be successfully used as suitable drug carriers [3]. Very limited toxicity is reported in case of carboxyl and amino functionalized UCNPs. PEG-modified $BaGdF_5$:Yb/Er UCNPs are reported to have low toxicity and long circulation time [5].

13.3 APPLICATIONS IN DRUG DELIVERY

The main goal of targeted drug delivery is to deliver drug to diseased cells and spare the normal cells [6,7]. Wang et al. functionalized UCNPs with PEG-grafted amphiphilic polymer. They were loaded with a chemotherapy drug like doxorubicin by simple adsorption for intracellular drug delivery.

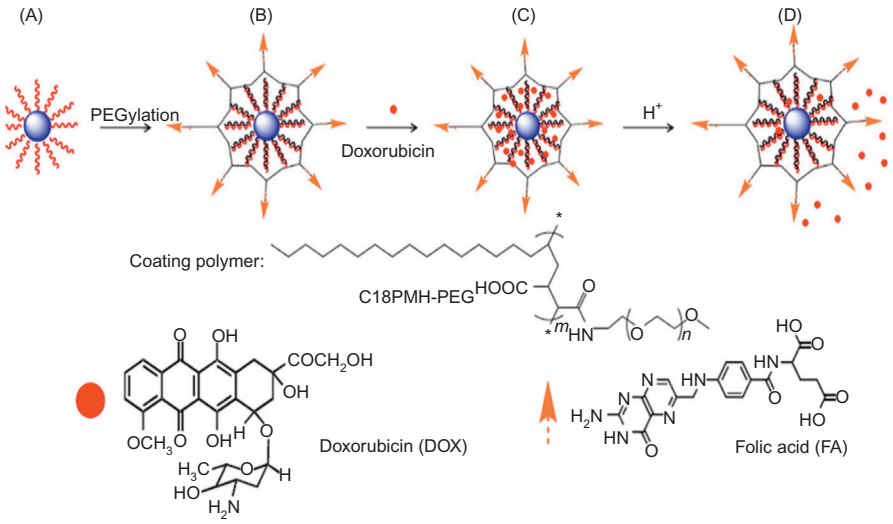

FIGURE 13.2

Schematic diagram showing UCNP-based drug delivery system.

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The release of doxorubicin was pH controlled with an increased drug dissociation rate in acidic environment. It was observed that DOX was shuttled into cells by UCNPs and released in cells by endocytosis. The nanoparticles were conjugated with folic acid so that they can target cancer cells (Figure 13.2) [3]. Yang et al. formulated doxorubicin-loaded ultra-small sized $BaGdF_6Yb^{3+}/Tm^{3+}$-based UCNPs. The drug was released by cleavage of hydrazine bonds in acidic environment. The nanoparticles proved to be toxic to anticancer cell lines (HeLa cells). Around 10 mg/kg of UCNPs were injected in mice which survived for 40 days without any adverse health effects [8]. Xu et al. have encapsulated hydrophobic UCNPs along with iron oxide nanoparticles using amphiphilic block polymer by microemulsion method. They were loaded with doxorubicin and fluorescent dye and were found to be cytotoxic on HeLa cells [9]. Liu et al. reported on the development of multifunctional UCNPs that can target cancer cell nuclei and deliver anticancer drug to the nuclear region. The nanoparticles were made of Er/Yb-doped $NaYF_4$ core and $NaGdF_4$ shell and enhanced the efficacy of doxorubicin by direct drug delivery to nucleus of HeLa cells [10].

13.4 APPLICATIONS IN BIOLOGICAL IMAGING

Biological imaging is a technique developed that allows noninvasive study of biological processes in small lab animals [11,12]. UCNPs are gaining lot of attention in biological imaging due to their photostability, deep tissue reaching, and autofluorescence (Figure 13.3). A number of articles have reported the use of UCNPs as bioimaging agents. Chromophoric ruthenium complexes based nanophosphors were synthesized as highly selective water-soluble probes for upconversion

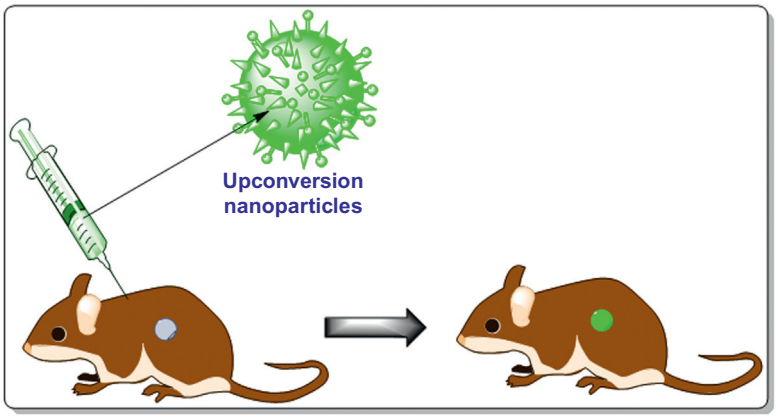

FIGURE 13.3

Schematic diagram showing the use of UCNPs for bioimaging.

luminescence sensing of intracellular mercury ions. This nanoprobe could detect lower levels of mercury (1.95 ppb) in drinking water. The maximum level of mercury was 2 ppb, as set by US EPA. It could detect changes in the distribution of mercury in living cells [13]. Chen et al. developed biocompatible core/shell ($NaYbF_4$:Tm^{3+})/CaF_2-based UCNPs for high contrast and deep imaging. These particles emitted photoluminescence at 800 nm, when they are excited at 980 nm. Around 700 pmol/kg of UCNPs were intravenously injected into BALB/c mice. High contrast images were obtained by using a nanoparticle-loaded synthetic fibrous mesh wrapped around the femoral bone of rat [14]. Zhou et al. prepared multihydroxy dendritic UCNPs with enhanced water dispersibility and surface functionality for bioimaging. They mainly consist of multihydroxy hyperbranched polyglycerol shell. They showed low cytotoxicity, deep light penetration depth, and high luminescent contrast [15]. Yi et al. synthesized dual model nanoprobes for synergistic upconversion luminescence and X-ray imaging in a single system functionalized by amine. These water-soluble UCNPs produced green and dominant red emissions. High contrast images of HeLa cells labeled by these particles were obtained. The *ex vivo* upconversion images showed that UCNPs traveled from lungs into liver. Both upconversion and X-ray images were obtained at the same region of nude mouse showing its dual role. They exhibited low cytotoxicity and did not show autofluorescence.

13.5 APPLICATIONS IN BIOLOGICAL DETECTION

UCNPs assist in biological detection through two mechanisms namely, fluorescence resonance energy transfer (FRET) and non-FRET. In FRET process, energy is transferred between donor and acceptor at a distance of 10 nm, through Coulombic interactions. Li et al. developed an ultrasensitive FRET aptasensor

for detection of kanamycin using UCNPs as the energy donor and graphene as the energy acceptor [16]. UCNPs were modified using oleic acid and synthesized via hydrothermal process followed by ligand exchange with hexanedioic acid. UCNPs were tagged with kanamycin aptamer through EDC-NHS procedure. The aptamer and graphene were brought closer by $\pi-\pi$ interaction which initiated FRET process leading to quenching of UCNPs fluorescence. When kanamycin was added to UCNPs−aptamer−graphene complex, energy transfer was blocked by the conformation change of aptamer into a hairpin structure. The UCNP-based aptasensor showed good specificity towards kanamycin without getting disturbed by other antibiotics. Another FRET system was designed for determining thrombin, using $NaYF_4$:Yb,Er UCNPs as donor and gold nanorods as acceptor [17]. The UCNPs were carboxyl functionalized and conjugated with thrombin aptamers. The fluorescence emission band of UCNPs overlapped with absorption band of gold nanorods. The fluorescence quenching efficiency increased with concentration of thrombin and the aptasensor was successful in measuring thrombin in blood plasma.

For non-FRET based detection, UCNPs were used as luminescent reporter and luminescence from these nanoparticles were observed directly. Zhang et al. synthesized lanthanide-doped upconverting phosphors for detecting glutathione. Their unique NIR excitation nature can overcome interferences from complex samples [18]. Upconverting phosphors and dopamine quinone are linked through hydrogen bonding and electrostatic interaction. Dopamine quinone quenched upconverting fluorescence while glutathione reduced dopamine quinone tuning on fluorescence. This fluorescence method broadened the scope of UCNPs in complex biological detection.

13.6 CONCLUSION AND FUTURE OUTLOOK

This chapter presents the recent developments of UCNPs in biomedical field including its properties and surface characteristics. Though several progress has been achieved, scientists still face some challenges. While preparing small UCNPs (10 nm), scientists have observed reduction in its luminescence efficiency. To maintain luminescence efficiency, several parameters (temperature, pH, concentration) need to be controlled. Most of the UCNPs do not easily get dissolved in water and poses several problems. So, surface functionalization of UCNP is a prime factor for biomedical applications. The realization of multiple functionalities on the surface of UCNPs is challenging. Although near-infrared radiation has better penetration depth than UV and visible, yet it failed to perform imaging in larger animals or humans. Thus, researchers need to bring about more reforms to face the challenges faced by UCNPs and make them promising materials in future.

ACKNOWLEDGMENTS

We thank the Brittany region, The European Union (FEDER), and the French Ministry for Research for rendering financial support.

REFERENCES

[1] Wang F, Banerjee D, Liu Y, Chen X, Liu X. Upconversion nanoparticles in biological labeling, imaging, and therapy. Analyst 2010;135(8):1839–54.

[2] Lin M, Zhao Y, Wang S, Liu M, Duan Z, Chen Y, et al. Recent advances in synthesis and surface modification of lanthanide-doped upconversion nanoparticles for biomedical applications. Biotechnol Adv 2012;30(6):1551–61.

[3] Wang C, Cheng L, Liu Z. Drug delivery with upconversion nanoparticles for multi-functional targeted cancer cell imaging and therapy. Biomaterials 2011;32(4):1110–20.

[4] Yang Y, Sun Y, Cao T, Peng J, Liu Y, Wu Y, et al. Hydrothermal synthesis of NaLuF4:153Sm,Yb,Tm nanoparticles and their application in dual-modality upconversion luminescence and SPECT bioimaging. Biomaterials 2013;34(3):774–83.

[5] Zeng S, Tsang M-K, Chan C-F, Wong K-L, Hao J. PEG modified BaGdF5:Yb/Er nanoprobes for multimodal upconversion fluorescent, *in vivo* X-ray computed tomography and biomagnetic imaging. Biomaterials 2012;33(36):9232–8.

[6] Ninan N, Thomas S, Grohens Y. Zeolites incorporated polymeric gel beads—promising drug carriers. Mater Lett 2014;118(0):12–6.

[7] Narayanan D, Ninan N, Jayakumar R, Nair SV, Menon D. *O*-carboxymethyl chitosan nanoparticles for controlled release of non-steroidal anti-inflammatory drugs. Adv Sci Eng Med 2014;6(5):522–30.

[8] Yang D, Dai Y, Liu J, Zhou Y, Chen Y, Li C, et al. Ultra-small BaGdF5-based upconversion nanoparticles as drug carriers and multimodal imaging probes. Biomaterials 2014;35(6):2011–23.

[9] Xu H, Cheng L, Wang C, Ma X, Li Y, Liu Z. Polymer encapsulated upconversion nanoparticle/iron oxide nanocomposites for multimodal imaging and magnetic targeted drug delivery. Biomaterials 2011;32(35):9364–73.

[10] Liu J-N, Bu W, Pan L-M, Zhang S, Chen F, Zhou L, et al. Simultaneous nuclear imaging and intranuclear drug delivery by nuclear-targeted multifunctional upconversion nanoprobes. Biomaterials 2012;33(29):7282–90.

[11] Sebastian M, Ninan N, Elias E. Nanomedicine and cancer therapies. Toronto: Apple Academic Press; 2013.

[12] Ninan N, Thomas S, George A, Sebastian M. Second world conference on nanomedicine and drug delivery. Ther Deliv 2011;2(6):711–5.

[13] Liu Q, Peng J, Sun L, Li F. High-efficiency upconversion luminescent sensing and bioimaging of Hg(II) by chromophoric ruthenium complex-assembled nanophosphors. ACS Nano 2011;5(10):8040–8.

[14] Chen G, Shen J, Ohulchanskyy TY, Patel NJ, Kutikov A, Li Z, et al. (α-$NaYbF_4$:Tm^{3+})/CaF_2 core/shell nanoparticles with efficient near-infrared to near-infrared upconversion for high-contrast deep tissue bioimaging. ACS Nano 2012;6(9):8280–7.

[15] Zhou L, He B, Huang J, Cheng Z, Xu X, Wei C. Multihydroxy dendritic upconversion nanoparticles with enhanced water dispersibility and surface functionality for bioimaging. ACS Appl Mater Interfaces 2014;6(10):7719–27.

[16] Li H, Sun D-E, Liu Y, Liu Z. An ultrasensitive homogeneous aptasensor for kanamycin based on upconversion fluorescence resonance energy transfer. Biosens Bioelectron 2014;55:149–56.

[17] Chen H, Yuan F, Wang S, Xu J, Zhang Y, Wang L. Aptamer-based sensing for thrombin in red region via fluorescence resonant energy transfer between NaYF4:Yb,Er upconversion nanoparticles and gold nanorods. Biosens Bioelectron 2013;48:19–25.

[18] Zhang Y, Tang Y, Liu X, Zhang L, Lv Y. A highly sensitive upconverting phosphors-based off–on probe for the detection of glutathione. Sens Actuators B Chem 2013;185:363–9.