Application of Upconversion Nanoparticles in Biomedical Field

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Abstract. Upconversion nanoparticles (UCNPs) are currently one of the most concerned detection technologies. UCNPs are a new kind of luminescent material based on the anti-Stokes reaction of rare-earth ions. Their advantages lie in the doping of rare-earth ions in the luminescent material, which has a high signal to noise ratio and strong fluorescence intensity, has a long fluorescence lifetime of approximately 100 milliseconds, good bio-compatibility and low toxicity. The emission band is narrow and has high penetration power and there is less damage to the matrix and interface. Nowadays, detection technologies based on UCNPs and interdisciplinary integration have laid a solid foundation for the development of new detection methods and instruments in fields such as bioanalysis, bioengineering, and materials, and have been widely applied in various fields. To this end, this research will explain the basic principle of UCNPs and the influence of lanthanide factors. This research also discussed the practical applications of UCNPs in magnetic resonance imaging (MRI), photodynamic therapy (PDT), biomarkers, and immunoassays in biomedical field, and looked forward to future research on UCNPs.

1 Introduction

The upconversion nanoparticles (UCNPs) are a class of nonlinear anti-Stokes processes, where rare earth ions (such as Er\(^{3+}\), Eu\(^{3+}\), Yb\(^{3+}\), Tm\(^{3+}\) and Ho\(^{3+}\)) are added to inorganic substrates (e.g., oxide, fluoride and halides). They have the advantages of high signal to noise ratio and strong fluorescence intensity, a long fluorescence lifetime of approximately 100 milliseconds, good bio-compatibility and low toxicity. The emission band is narrow and has high penetration power. There is less damage to the matrix and interface. The upconversion process can take two or more continuously absorbed pumped photons, pass through non-radiative relaxation to reach the luminous level, and then jump to ground state to produce a high-energy emitted photon [1].

In 1966, Auzel formally proposed the concept of upconversion luminescence. Its characteristic is that two or more pumping photons are continuously absorbed by a long-lived medium, and emission at a wavelength below the pump wavelength. The upconversion luminescence process mainly includes three luminescence mechanisms: excited state absorption (ESA), energy transfer (ET) and photon avalanche (PA). The excitation source of UCNPs is near-infrared light. This technique can effectively inhibit the damage of external light to organisms and also does little damage to the organism. Multi-modal imaging of upconversion luminescent nanoparticles can be achieved by doping lanthanide up-conversion nanoparticles with similar particle size and chemical properties. By the 1990s, with the introduction of nanotechnology, scientists had synthesized many water-soluble and highly luminous nanoscale upconversion inorganic materials. This functional upconversion nanomaterial brings a revolutionary opportunity and development for the corresponding diagnosis, treatment, imaging, biological monitoring in the field of biomedicine.

At present, the multi-disciplinary fusion detection technology based on UCNPs has laid a solid foundation for the development of new detection methods and instruments in the fields of biological analysis, biological engineering and materials. UCNPs are widely used in various fields such as luminescence imaging, cell labelling, probe, MRI, PDT, chemotherapy, PET, single photon emission computed tomography (SPECT), X-ray computed tomography (CT), optical biosensor/biological detection, biomarker, immunoassay, drug delivery, temperature measurement, dye-sensitized solar cell (DSSC), fingerprint detector and photocatalysis. In addition to the dye-sensitized solar cell (DSSC), fingerprint detection and photocatalysis, the others are medical [2].

Compared with other biomaterials, UCNPs have been widely used and are expected to find more applications in biomedical fields due to their special structures and functions. In this research, the definition, mechanism and application of UCNPs are introduced, and its future development is prospected. Among them, light-emitting imaging, probe, MRI, PDT, PET, single-photon emission computed tomography (SPECT), X-ray CT and other technologies are relatively mature several technologies at present. At the same time, the application prospect, existing problems and development trend of the material are forecasted to lay a foundation for the application and development of up-conversion luminescent material. In this research, the mechanism of

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UCNPs and the application of various detection technologies will be introduced.

2 Applications of UCNPs

2.1. The physical and chemical characteristics of UCNPs

In upconversion luminescent materials, rare earth ions can generate high-energy anti-Stokes shifts due to their high energy absorption in the near-infrared band, thereby achieving upconversion luminescence. UCNPs are used as tracking agents. Unlike ultraviolet and visible light, UCNPs have low self-fluorescence backgrounds and capable of entering tissue without apparent effect on the organism. The upconversion luminescence is an anti-Stokes luminescence derived from Stokes's law. UCNPs, which are excited by near infrared (NIR) light can be used for many applications in biology and photonic devices. The UCNPs are mainly composed of host matrix, sensitizer (absorbtent) and activator. Typically, host base should meet the following 4 conditions. It can withstand the glare without difficulty. The phonon energy should be moderate and there should be no danger of radiation-free relaxation. It should require significant transparency to withstand free near-infrared photon migration in lattices. In order to maintain the original crystalline structure, good chemical and thermal stability is necessary.

Because there are multiple 4f transition zones, rare earths can be used as moderately concentrated activators to avoid activating them. The sensitizer has demonstrated that the absorption of energy is successfully transferred to neighboring activators. Yb is widely used as a photosensitizer because its band structure coincides with the f-e-f transition of rare earth ions. Recently, the UCNPs associated with NaYF₄ have been produced and applied extensively because of their remarkable upconversion ability [3]. Currently, there are five distinct mechanisms for regulating the upconversion: excited state absorption (ESA), photon avalanche (PA), energy transfer upconversion (ETU), synergistic upconversion (CUC) and energy transport mediated upconversion (EMU). In brief, UC processes are controlled by radiative transitions (absorption transitions and emission transitions), and due to the use of a faster non-radiative attenuation mode, such as: phonon coupling, surface quenching and so on. All these energy losses are attributed to material defects in UCNPs, and also provide a good support for the wide application of UCNPs.

2.2 Magnetic Resonance Imaging (MRI)

MRI is a non-invasive medical examination method. By collecting signals generated by the movement of water, oxygen, and other atomic nuclei within the human body in a magnetic field, the three-dimensional shape of the human body is obtained. The technology has wide application in detecting, diagnosing and treating diseases. The MRI scanner creates an image of the internal organs using a powerful magnetic field, a magnetic gradient and a radio wave. In most medical applications, a hydrogen atom made up only of protons emits a signal that is processed according to the density of the nucleus in a given region to form an image of the human body. MRI technology uses a kind of powerful magnet that can push protons from the human body into a magnetic field. The proton is excited by a high-frequency current flowing through the patient, upsetting the magnetic balance. When the RF is cut off, the MRI sensor can detect the energy released by the RF again. The timing required for the weight of protons and magnets depends on the surrounding environment and the chemical composition of molecules. This algorithm, based on technology, can stimulate and detect the rotation axis of protons in living water bodies. Depending on its superparamagnetic force, MRI contrast medium are usually split into two categories: T1 and T2, to facilitate clinical diagnosis. T1 weighted image, that is, the longitudinal relaxation imaging, the image represents low black signal, white high signal. T2 uses transverse relaxation, with white representing high signal and black representing low signal. Most lesions exhibit low signal in T1 area and high signal in T2 area. T2 is more sensitive to changes in organizational structure than T1, so it can be used to diagnose and evaluate diseases. In addition, high signal intensity typically represents significant changes in organisational structure, which can be determined by measuring T1 and T2. Among them, Gd³⁺ compounds have advantages such as thermodynamic stability, water solubility, and high relaxation, making them a common T1 contrast agent. Currently, Gd³⁺ nanoparticles are used as blood supply and irrigation agents, and are classified as tissue classification.

For its short residence time in the human body, combining two or more nanomaterials can extend their release time in the body. Researchers can adjust the appearance time of nanoparticles in the body by adjusting their composition, such as metals, semiconductors, and polymers. The fact that various gadolinium based paramagnetism nanoparticles have been extensively applied in MRI. These nanoparticles can be used to prepare contrast agents for MRI, as well as for other experiments such as nuclear magnetic resonance imaging (NMR) and magnetic resonance spectroscopy (MRS). In addition, these nanoparticles can also be used for magnetization measurement in NMR technology. They can be used to prepare magnetic resonance contrast agents, measure magnetic intensity, magnetic field intensity, and MRS, and have achieved good results, including Gd₂O₃, Gd₄(OH)₇, GdF₃, LiGdF₄, NaGdF₄, KGdF₄, BaGdF₅, GdPO₄, GdVO₄, GdBO₃, GdMO₃ and Gd₂MnO₄. UCNPs, on the other hand, they have their own characteristics that are good for MRI.

UCNPs and MRI can be used to diagnose and treat cancer, respectively, where a fragile atherosclerotic plaque can be detected in inflammatory MI macrophages [4, 5]. Wang et al. have prepared rare-earth UCNPs mainly composed of NaYF₄:Yb/Tm by solution-thermal method, and coated them with PEG functional metal-organic framework (MOFs) on the basis of optimized UCNPs to form UCNPs@MIL-PEG, which is
a multifunctional platform for MRI tracking. Liu et al. used a similar approach to construct MARCO-resistant NaGdF4:Yb, a promising dual-mode optical/MRI probe, which is able to reflect the study on non-invasive behaviour of M1 type macrophages, as shown in Fig. 1. In T2 weighted MR images, significant dose dependence increases with increasing contrast. This indicates that the potential effects of T2 MRI contrast agents may have a significant impact. In this case, we can better understand the impact of T2 MRI contrast agents. By combining specific doses, it can better evaluate the impact of contrast agents on T2 weighted MR images, thereby providing doctors with more accurate and effective diagnosis and treatment plans. In this study, the author designed a multifunctional nanoparticle based on UCNPs that can be applied for UCL/MR dual mode imaging and release of pH responsive drugs. This kind of UCNPs@MIL-PEG nanoparticles have excellent fluorescence performance and stability, and can be used in various biomedical applications. This study provides a promising research direction for medical applications based on UCNPs.

![Fig. 1. Schematic diagram of pH-responsiveness UCNPs@MIL-PEG](https://doi.org/10.1016/j.jallcom.2018.03.355).

**2.3 Photodynamic therapy (PDT)**

PDT is a new non-invasive treatment for tumor. It uses visible light, near-infrared or ultraviolet light as stimulating light source, irradiates and excites photosensitizer in tumor tissue, causes physical and biochemical reaction, produces single oxygen and other active oxygen substances (ROS) with biological toxicity, then oxidizes and damages tumor, virus-infected cells and other hyperproliferating cells, activates anti-tumor and anti-virus immunity, damages blood vessels, kills bacteria, fungi and viruses, and eliminates inflammation. When a certain wavelength of light irradiates endogenous or exogenous photosensitizable substances in biological tissues, this kind of photosensitive material absorbs the energy carried by photons and transforms from ground state to excited state. Photosensitizers in an excited state have very active properties. They quickly deexcite physically or chemically, releasing energy and returning to the ground state. In the case of physical deexcitation, it produces visible fluorescence, which can be used to deduce the disease associated with it. In the process of chemical deexcitation, a large number of reactive oxygen species are produced, in which singlet oxygen has a large proportion.

![Fig. 2. A model of photothermal and photodynamic treatment of 4T1 tumor cells in model animals by intravenous administration](https://doi.org/10.1016/j.bioadv.2022.213117).

Upconversion PDT is a nonlinear optical phenomenon, which can be transformed from low energy to higher energy by anti-Stokes process. Upconversion luminescent materials have been widely used in materials, energy, bio-imaging and biomedicine because of their moderate size and excellent optical properties. Nanoparticles have strong penetration ability, low fluorescence background, high photostability, and high signal to noise ratio. But, due to the hydrophobicity of photosensitizers (PS) that currently exist in PDT, which is not conducive to the distribution of drugs in the body, and the limited absorption wavelength of ultraviolet/visible light, there are the following problems in existing research: photosensitizers (PS) as photosensitizers bind to receptors in tumor cell membranes and mitochondria, release drugs, and enter cells; deliver photosensitizers to tumor tissue and achieve therapeutic goals through local aggregation (local administration) of tumor tissue; deliver photosensitizers to tumor tissue and kill tumor cells by releasing drugs and producing reactive oxygen species. In response to these issues, this project aims to select appropriate surface functional groups (such as hydrophobic interactions) and rare earth UCNPs with adjustable upconversion luminescence to improve the effectiveness of PDT.

In short, UCNPs can be modified to be a drug carrier that delivers hydrophobic PS to the tumor and acts as an optical converter that enables PS to be activated in the tumour for efficient PDT therapy in deep tissue. The synergistic effect of UCNPs and PS will greatly expand the application of photodynamic therapy in solid tumors, large area tumors and other non-surface diseases. A
novel up-conversion nano-heating material that can be simultaneously loaded with Bengal Rose (RB), is proposed to be anchored on fluoromagnetism using human transferrin (Tf) as a functional group, enabling multimode image-guided synergistic photothermal (PTT) and PDT. Near-infrared excitation of UCNPs-RB-Forster resonance energy transfer (FRET) produces reactive oxygen species (ROS) PDT, while due to the radiation-free phase transition of Er. This UCNPs@Tf-RB, used in conjunction with PTT-PDT, is expected to enhance the effectiveness of cancer treatment and provide new ideas for preclinical treatment of cancer [6], as shown in Fig. 2.

2.4 Biomarkers

Biomarkers are biochemical indicators that can reflect the occurrence or possible changes in tissues and organs such as systems, organs, tissues, cells and subcells. In clinical research and development, biomarkers serve as an important means to accurately identify beneficiary/risk populations, guide accompanying diagnostic development, and optimize research and development strategies, thereby improving the success rate of research and development. In the field of disease research, the biomarker is generally defined as an objective measurement and evaluation of the biochemical parameters of the normal physiological, pathological or therapeutic process. Measuring them gives us an idea of the current biological process that our body is in. The research related to biomarkers is currently a hot topic in scientific research. It can not only balance clinical work but also basic research, and has broad application prospects. There are various biomarkers in the human body, and with the continuous progress of molecular biology technology. The types of biomarkers include some specific molecules (such as prostate specific antigen PSA), gene mutations (such as BRCA mutations), cell markers (such as circulating tumor cells) and biological molecules (such as proteins or metabolites).

A biomarker is a signal indicator that an organism undergoes changes in the action of environmental pollutants at multiple levels, including molecular, cellular, and individual levels, before undergoing significant damage. The system can be used for early warning of serious poisoning injury. The cause of its occurrence may be related to changes in cellular structure and function, changes in biochemical metabolism, or abnormal metabolites. This enables detection and timely diagnosis of potential health issues.

UCNPs have different near-infrared radiation compared to ultraviolet and visible light, which allows them to reach tissues without significantly affecting organisms. Moreover, they have a low self-fluorescence background and can serve as tracers, making them a suitable choice as biomarkers. On this basis, Lu et al. conducted further research by using microRNA-10b (miRNA-10b) as a biomarker for early diagnosis of liver cancer [7], as shown in Fig. 3. This project proposes for the first time to use FRET technology to realize the highly sensitive detection of miRNA-10b, that is, on the basis of FRET, the fluorescence reduction degree of upconversion nanoparticles is used to quantitatively analyze miRNA-10b. On this basis, the miRNA-10b gene transcriptome was constructed to analyze the representation of miRNA-10b in human hepatocellular carcinoma and hepatocytes. Simultaneously, Wang and Sun synthesized a sensitive Biomarker (5-HIAA) sensitive probe (CFE) for the first time. It was loaded into MCM-41 microspheres containing UCNPs, which improved the light stability, self-quenching and cohesion of CFE molecules. Based on different expressions, a comparison was made between the Forster radius and emission lifetime, proving that the ability of the secondary excitation source UCNPs is effective in detecting CFE. Both of these applications have proved that UCNPs are feasible.

![Fig. 3. A schematic diagram of a miRNA-10b sensor between UCNPs and AuNPs using target-triggered CHA and LRET](https://doi.org/10.1016/j.talanta.2022.124032)

2.5 Immunoassay

Immunoassay is a method for quantitative analysis of certain chemical substances (like hormones, medicines, and certain proteins) by means of highly specific binding of antigens or haptons to homologous antibodies, including radioimmunoassay, enzyme immunoassay and fluorescence immunoassay. Because various antigen components, including small molecule haptons, can be used to prepare specific antiseras or monoclonal antibodies. By using this antibody as a reagent, the corresponding antigen in the sample can be detected. Therefore, the application range of immunoassay is very wide, and it can also be used in clinical testing to determine, including very small amounts of protein, small molecular weight glutin hormones, antibiotics and medications and pathogen antigens. In addition, purified antigens can also be used to detect antibodies in samples, such as anti HBs. UCNPs are widely used in upconversion linked immunosorbent assay (ELISA) and side flow immunoassay based on micro titration plates due to their advantages of simple operation and low cost. These tests are highly sensitive to different analytes. For example, schistosome circulating anode antigen, prostate specific antigen, Salmonella, HBV and HBV DNA, ochratoxin A, and Hg^{2+} ions. At the same time, due to all the advantages of UCNPs’s stable structure and low nano toxicity, it can be used for high sensitivity detection.
in immunoassay. Therefore, the application of UCNPs in immunoassay has become a hot topic in research.

Guo et al. developed a new FRET immunoassay technology based on UCNPs. This method can not only improve analysis efficiency but also reduce analysis costs, making it a very promising method for pesticide residue analysis [9]. As shown in Fig. 4, Shapoval et al. immobilized cardiac troponin I (cTnI) antibody covalently on UCNPs and prepared ULISA for detection [10], where the limit of detection is 0.13 ng/mL or 0.25 ng/mL.

![Fig. 4. Troponin in human heart was determined by ULISA [10, https://doi.org/10.1016/j.talanta.2022.123400].](image)

### 3 Conclusion

UCNPs are a new kind of functional materials which can convert low energy photon upconversion up into high energy photons. It has a high signal to noise ratio and strong fluorescence intensity, and the emission band is narrow and has high penetration power. In addition, there is less damage to the matrix and interface. It has greatly changed people's traditional understanding of the properties of luminescent materials. Rare-earth upconversion luminescent materials are widely used in many fields. This project takes rare-earth upconversion luminescent materials as the research object, and focuses on their applications in fields such as medicine, clinical, and bioengineering. However, with the increasing demand for research based on diseases such as tumors and cancer or biomedical fields, these interdisciplinary fusion detection technologies based on UCNPs are facing greater challenges. The surface upconversion functionalized nanoparticles should also have other functions. It can effectively prevent the agglomeration of UCNPs in the physiological environment. Due to the protection of the outer shell, UCNPs are not affected by chemicals in aqueous solutions. The core of UCNPs is protected by its outer shell from being damaged by chemicals in water. It should provide some functional groups that are connected to biological molecules such as FA, proteins and DNA. The chemicals on the surface of UCNPs can also affect their drug loading, drug release, and blood circulation cycle in cells. Therefore, the preparation, synthesis and surface modification of rare-earth upconversion luminescence materials are systematically studied in order to realize their wide application in biomedicine.

### References


