Rare Earth Upconversion Luminescent Nanomaterials for Lymphatic Imaging in Small Animals

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Abstract: Lymphography is a hot issue in oncology, the accurate location of sentinel lymph nodes can provide guidance for the diagnosis and treatment of tumors. Fluorescence imaging is the only way to visualize cells and small animals in vivo. A key problem in this field is how to further improve the penetration depth and signal-to-noise ratio. Rare earth nanomaterials are rich in optical, magnetic, X-ray absorption and radioactive energy, are a kind of important multi-functional materials. In order to apply them to biomedical imaging and biological detection, people need to develop new rare earth nanomaterials. Integrating its special properties, it can be used in multi-mode imaging and biological detection. This paper aims to explore the application of rare earth upconversion luminescent nanomaterials in lymph node imaging of small animals. Rare earth upconversion luminescent nanomaterials were used in many small animal models, such as mice and rabbits. Up-conversion luminescence in vivo lymphography of shallow cervical, deep cervical and axillary multilocus lymph nodes was realized. Using different polymers as synergistic ligands, rare earth upconversion luminescent nanomaterials labeled with radioactivity and less than 10 nm were synthesized directly, the distribution of rare earth nanomaterials coated with different polymers in vivo was observed by SPECT imaging. The results show that rare earth is a conversion luminescent nanomaterial (CUNPs) with high lymphatic tropism and high signal-to-noise ratio. In addition, we found that water-soluble nanomaterials with a wide range of sizes can be used for lymph node imaging; however, the matrix and surface coating of CUNPs had no significant effect on lymphatic imaging.

Key words: Rare Earth Upconversion, Luminescent Nanomaterials, Small Animal Lymphatic System

1. Introduction

At present, people have a strong interest in early diagnosis of diseases, and researchers are constantly working on the research and development of new analytical techniques. Especially in recent years, early detection and diagnosis of tumors and their lymphatic metastasis by tracer technology have become the focus of attention in the fields of molecular biology, medicine and imaging in the world [1]. The lymphatic system is an important part of the body's immune system and plays an important role in the body's fight against tumors and foreign antigens. Lymph nodes activate cells for immune response [2]. The lymphatic network recirculation system, which is spread throughout the body like the blood circulatory system, not only transports and renews extracellular matrix components, but also maintains tissue fluid balance and homeostasis, providing a pathway for tumor cells to metastasize to lymph nodes. Among them, tumor lymphatic metastasis is a new hot spot in oncology and external scientific research.

The unique luminescent properties of rare earth upconverting luminescent materials determine their many advantages in the application of bioluminescent labels. If the emission band is narrow, the luminescence lifetime is relatively long, the chemical stability is high, and the light stability is good [3]. In addition, near-infrared continuous wave lasers bring many advantages. For example, large back-shift and deep light, these characteristics make rare earth upconversion luminescent materials have great application prospects in the field of bioimaging [4-6]. However, in the past few years, upconverting materials have been concentrated on bulk glass and crystalline materials, but few have been applied to life science research. Because the use of upconverting luminescent materials in bioimaging requires small size nanomaterials with strong upconversion luminescence, which are typically water soluble. With the rapid development of nanotechnology in the past decade, upconverting nanomaterials has attracted much attention in the field of bioanalysis and imaging, and has rapidly become the frontier of interdisciplinary research in photochemistry, materials science and biology [7]. However, the application of rare earth upconversion materials in bioimaging is still in its infancy compared to organic dyes. These methods can synthesize nanoparticles having a uniform particle size and a controlled morphology, and have high up-conversion luminescence efficiency. Sometimes it is necessary to combine several methods to synthesize high quality nanoparticles.

Berthod and Caliper are the oldest and most professional brands to work on small animal living visible light

imaging systems. The former has bioluminescence and high fluorescence sensitivity. The bioluminescence sensitivity of the latter is relatively high, and the fluorescence is slightly insufficient. With the development of optoelectronic technology and bioengineering technology, as well as the deepening of life science research, living imaging technology is also progressing [8, 9]. However, regardless of the same brand upgrade development, the structure between different brands is similar, divided into two main parts (including CCD, black box, gas anesthesia system) and operational analysis software. Bioluminescence imaging is the luminescence caused by the chemical reaction between luciferase and its substrate, and the oxidation of alcohol requires oxygen, so bioluminescence can occur only in living cells and tissues [10]. Luciferases commonly used in molecular imaging include luciferase from Sargassum, luciferase from bacteria, and luciferase from Renilla. Luciferase molecular probes are commonly used for preclinical molecular imaging in small animals because they do not require an excitation source and have good biocompatibility and low background noise [11]. Fluorescence imaging uses an external excitation source to illuminate fluorescent groups, such as fluorescent protein GFP and ATP. The fluorescent signals of these fluorophores are much stronger than bioluminescence. They can form in vivo tropical optical imaging probes by coupling with specific ligands or biomolecules, which has important application prospects in early cancer diagnosis.

Fluorescence imaging is a method of tracing the fluorescent signal of a probe, which is a method for in vivo observation at the subcellular level. Heavy metal phosphorescent complexes and rare earth upconversion luminescent nanomaterials are two new types of luminescent marking materials [12]. The poor water solubility of phosphorescent complexes and the lower luminescence efficiency of rare earth upconversion luminescent nanomaterials limit their bioimaging to some extent application. In this paper, the application of rare earth upconversion luminescent nanomaterials in different small animal models such as mice and rabbits, respectively, achieved up-conversion luminescence lymphography of the superficial neck, deep neck and axillary lymph nodes. The rare earth up-converting luminescent nanomaterials with radioactive labeling and less than 10 nm were directly synthesized by using different polymers as synergistic ligands. The difference of distribution of different polymer-coated rare earth nanomaterials in vivo was observed by SPECT imaging. The results show that rare earth is a luminescent nanomaterial (CUNPs) for lymphatic imaging with high lymphatic tropism and high signal-to-noise ratio. In addition, we found that water-soluble nanomaterials with a range of sizes can achieve lymph node imaging, while matrix and surface coating of CUNPs have no significant effect on lymphoid imaging [13].

2. Proposed Method

2.1. Biomedical Imaging Technology

For centuries, human beings have been committed to understanding their own life phenomena and life processes. With the continuous development of science and technology, people's research is also deepening. Visual imaging technology combines physics, chemistry, medicine and other disciplines, and is the core of life sciences and basic medical research and clinical diagnosis. Biomedical imaging refers to a visualization technique that presents tissue structure information or function information of a living being in the form of an image. According to different imaging principles, the main biomedical imaging technologies include X-ray computed tomography (X-ray CT), ultrasound imaging, magnetic resonance imaging (MRI), radionuclide imaging, including single photon emission computed tomography (SPECT) and positive Electron emission tomography (PET), photoacoustic imaging (PAT) and optical imaging. and many more. These imaging techniques have been widely used in medical treatment and basic research in life sciences. Bioimaging technology can be divided into anatomical imaging and physiological functional imaging according to imaging functions. Traditional ultrasound imaging and imaging methods are used to display the anatomy of the body. Optical imaging, including and including nuclear medicine imaging techniques, can display the physiological functions of an organism. In addition, there are two biological imaging modalities: in vitro and in vivo. The in vitro test method uses a small amount of tissue for imaging analysis, such as incineration microscopy for cell and tissue analysis, but this method can only partially reflect the state of the organism and cannot dynamically reflect biological information. In vivo imaging is a real-time and non-invasive imaging of an organism that directly reflects the state of the organism.

Ultrasound imaging (US) uses a difference in different echo or acoustic impedance characteristics of an ultrasound beam between tissues to receive a reflected signal to obtain an image of an internal organ. This method is widely used in the diagnosis of obstetrics and gynecology and cardiovascular system. X-ray computed tomography (CT) is a high-resolution 2D and 3D imaging image that uses tissue of varying density and thickness to measure X-ray depletion coefficients in an organism [14]. CT angiography is the ability to absorb and consume X-rays by contrast agents, improve the contrast between pathological tissues and normal tissues, and more clearly show the structure and extent of pathological tissues. Magnetic resonance imaging (MRI) is based on the precession principle of the atomic nucleus spin angular momentum in an external magnetic field. At present, clinical magnetic resonance imaging mainly uses the hydrogen molecules of human water molecules in an external magnetic field to generate signals through radio frequency pulses, and then reconstructs images. It is

suitable for soft tissue. Morphological structure is displayed. Radionuclide imaging uses different tissues to selectively absorb or aggregate radioactive compounds and detect the concentration distribution of radionuclides in the tissue and track it over time by detectors. Radionuclide imaging includes single photon emission computed tomography (SPECT) and positron emission computed tomography (PET). The radionuclide image not only reflects the morphological structure of organs and tissues, but also provides physiological and biochemical information of living tissues. In vivo near-infrared fluorescence imaging relies on an external source to excite fluorescent probes. In one aspect, the fluorescence of the excitation and fluorophores is absorbed or scattered by the biological tissue, which reduces the depth of light penetration and the intensity of the fluorescent signal. On the other hand, the laser produces tissue autofluorescence, which results in high background noise. Affects the sensitivity of imaging. Hemoglobin, water and lipids are the main absorbents in biological tissues. They have the lowest near-infrared absorption coefficient at 650-900 nm. Near-infrared light penetrates deeper than visible light. The maximum penetration depth of near-infrared light in the breast or lung tissue is 12 cm. Near-infrared light is 6 cm away from muscle tissue. The near-infrared light in adult brain tissue is 5 cm. Therefore, the wavelength range of NIR is 650-900 nm. It is called the "bio-optical imaging window." Near-infrared photoluminescence imaging is one of the key techniques for obtaining high-resolution and high-sensitivity in vivo imaging. Photoacoustic imaging (PAI) means that the tissue is stimulated to emit ultrasound, and then the light absorption distribution information in the tissue is reconstructed from the collected sound waves. Photoacoustic imaging has high contrast and resolution and has great potential for early diagnosis.

2.2. Fluorescence Imaging

In vivo optical imaging of living animals mainly uses two techniques of bioluminescence and fluorescence, Table 1 and Figure 1. Bioluminescence is the labeling of cells with the luciferase gene, while fluorescent techniques are labeled with fluorescent reporter groups. Using a very sensitive optical inspection instrument, researchers can directly monitor cellular activity and genetic behavior in living organisms. Traditional animal experiment methods require slaughtering experimental animals at different time points to obtain data, and experimental results at multiple time points are obtained. In contrast, in-vivo imaging is performed by recording the same set of subjects at different time points, tracking the movement and changes of labeled cells and genes of the same observed target, and the obtained data is more authentic. This technology has been widely used in life sciences, medical research and drug development in the few years since its development. Compared to bioluminescence imaging, fluorescence imaging has the advantages of ease of operation, relatively inexpensive price, intuitive results, diverse target targets, and ease of acceptance by most researchers. However, in the process of fluorescence imaging, many substances in the organism produce fluorescence when excited by excitation light, such as skin, hair, various tissues and food. Especially when the labeled target is deeply hidden inside the tissue, high-energy excitation light is required, and the non-specific fluorescence generated is strong. Although different techniques are used to separate the background light, it is difficult to completely eliminate the background noise due to the limitation of the fluorescence characteristics. These background noises cause low sensitivity to fluorescence imaging. Therefore, how to avoid background fluorescence interference of biological samples becomes a key scientific issue in the field of fluorescence imaging.

Fluorescent labeling is the most important step in fluorescence imaging. Therefore, the design and synthesis of fluorescent probes is very important. Fluorescent probes are specific to a particular molecule. A labeled compound capable of fluorescent tracing in vivo and in vitro can reflect the amount or function of a target molecule in vivo and in vitro. With the development of materials science and nanotechnology, in addition to traditional fluorescent proteins and organic dyes, a series of new nano-luminescence probes, such as organic and inorganic hybrid luminescent nanomaterials, semiconductor quantum dots, diamond nanoparticles, have been developed. Carbon nanomaterials and rare earths. Luminescent nanomaterials. So far, organic fluorescent dyes are still the most widely used luminescent markers, and their research and development are relatively mature. They have high fluorescence quantum efficiency and an adjustable luminescence spectrum. However, organic dyes have poor photochemical stability, broad absorption and emission bands, severe photobleaching and photolysis, which greatly limits their use. In addition, organic dyes have a short fluorescence lifetime and relatively small organic dye displacements. Therefore, the sensitivity of organic dyes is easily affected by interference. Rare earth complexes have a long fluorescence lifetime, but their absorption wavelength is shorter, the energy requirement of excitation light is higher, and the light damage to cell samples is large, which greatly limits their wide application. At the same time, rare earth ions have strong binding ability to water, and the stability of rare earth complexes in water is not good enough, which limits their use. Semiconductor quantum dots (QDs) have received much attention in recent years due to their high fluorescence quantum yield, narrow emission spectrum, tunable spectrum and excellent anti-bleaching properties. However, their chemical stability is still unsatisfactory and their biological toxicity cannot be completely avoided. It is worth noting that fluorescent dyes and quantum dots typically use high energy or visible light as the excitation light, and then they emit low energy and long wavelength photons. The use of high-energy light as an excitation source introduces some significant drawbacks, such as a lower depth of light penetration, which can destroy the biological tissue being tested, and the organism

itself also produces autofluorescence.

2.3. Rare Earth Luminescent Materials According to the Luminescence Mechanism

The luminescence mechanism of the rare earth luminescent material is divided into down-conversion luminescence and up-conversion luminescence according to different energy conversion modes. Down-conversion luminescence refers to the luminescence phenomenon in which one or more low-energy photons are emitted after the rare earth ions absorb a high-energy photon. Generally, the rare earth activators used for down-conversion luminescence are Eu^{3+}, Tb^{3+} and the like. Upconversion luminescence refers to the fact that rare earth ions absorb one or two low-energy photons and emit a high-energy photon. The non-linear luminescence phenomenon usually refers to the conversion of near-infrared light into visible light. Compared to organic dyes and quantum dots, rare earth upconversion luminescent materials have many advantages as a new generation of bioluminescent labels, such as high chemical stability and long luminescence lifetime. Preparation of Upconversion Luminescent Materials the main synthetic methods are hydrothermal, solvothermal, and pyrolysis.

Proper nano-size and uniform morphology are prerequisites for nanomaterial bioimaging. Currently, hydrothermal methods, pyrolysis methods and solvothermal methods are widely used. Typically, UCNPs having controlled size and morphology are prepared by hydrothermal methods. The basic process flow is as follows: the rare earth source and the gas source are dissolved in the solution, placed in the high pressure reaction liquid, sealed, and treated at high temperature and high pressure. The rare earth source is usually an oxide of a nitrate, chloride or rare earth ion. LnF3 nanoparticles were prepared by NH_4F as a fluoride source. In the preparation of MLnF4 nanoparticles, NaF or KF is usually used as a fluoride source. A simple method for preparing UCNPs is based on the interfacial effects of liquid, aqueous and aqueous (LSS). A series of fluorine-based upconversion luminescent nanomaterials having different substrates, crystal phases, sizes and morphologies, such as NaYF4, can be prepared by the LSS method. In the hydrothermal reaction process, many experimental parameters affect the growth of nanomaterials, such as the concentration of raw materials, the type and concentration of doping ions, hydrothermal temperature and time, pH and so on. In the hydrothermal process, the generated rare earth nanoparticles have a high degree of crystallization by high temperature and high pressure reaction, but the reaction environment is more intense, and thus the resulting particle size has a slightly higher dispersion.

Suitable nano-size and uniform morphology are prerequisites for bio-imaging of nanomaterials. More methods are now used for hydrothermal, pyrolysis and solvothermal methods. Hydrothermal methods usually use raw materials to prepare UCNPs with controlled size and morphology. The basic process is as follows. The rare earth source and gas source are dissolved in the solution, placed in a high pressure reaction dad, sealed, and treated at high temperature and high pressure. The rare earth source is typically a nitrate, chloride or oxide of a rare earth ion. LnF_3 type nanoparticles are prepared, and the fluorine source is usually NH4F. MLnF4 type nanoparticles are prepared, and the fluorine source is usually NaF or KF. A simple method for preparing UCNPs is based on the interfacial action of liquid phase, in-phase and aqueous phase (LSS). A series of fluorine-based upconversion luminescent nanomaterials with different matrix, crystal phase, size and morphology can be prepared by LSS method, such as $NaYF_4$. During the hydrothermal reaction, many experimental parameters affect the growth of nanomaterials, such as the concentration of the reaction materials, the type and concentration of the ions, the hydrothermal temperature and time, and the pH. In the hydrothermal process, high temperature and high pressure, the reaction is intense, and the rare earth nanoparticles produced are highly crystallized, but the reaction environment is more intense, so the particle size dispersion is slightly higher.

Generally, the particle size control method can be analyzed based on the nucleation growth model curve. As the monomer concentration increases, the first stage is in a non-precipitating state. Due to the high energy of spontaneous homogeneous nucleation, it cannot be nucleated even under the condition that the raw material is supersaturated. As the energy of the system increases over time (usually increasing the temperature), when the energy exceeds the second phase at full time, the entire reaction solution system nucleates and forms a stable nucleation center, typically within a few nanometers. As the monomer nucleates, the concentration of the monomer in the entire reaction system decreases immediately, below the critical concentration of nucleation. At this level, the system enters a third stage of growth. At this stage, the system no longer nucleates and the remaining monomers provide only nucleated particles for growth until the concentration of monomer in the system is below the saturation concentration. Therefore, according to the curve, the method of controlling the particle size is to effectively control the size and size monodispersity of the rare earth nano material by adjusting the temperature, the concentration of the reactant to rapidly nucleate the nanoparticles, and suppressing the growth by adjusting the time and surface ligands

3. Experiments

3.1. Construction of Upconversion Luminescent Small Animal Living Imaging System

A steady-state laser beam generated by a semiconductor laser (center wavelength of 980 nm) is introduced

through the optical fiber. The beam expander lens and the beam shaping lens are sequentially placed in the forward direction of the laser beam. The beam expander lens is connected to the connecting arm to freely adjust the angle of the fiber port beam. Place the sample platform underneath the two laser beams. This object is located on the sample table. A 980 nm cut-off filter, lens, emission filter, and EMCCD were placed in turn over the sample stage. The semiconductor laser (center wavelength is 980 nm) [15], the beam expander lens, the beam shaping lens, the sample stage, the 980 nm cut-off filter, the lens, the emission filter and the EMCCD are sequentially connected into an optical path. By adjusting the articulated arm, small animals can be illuminated 360 degrees in all directions. Signals in a specific wavelength range can be selected by transmitting filters and receiving them by EMCCD. The computer is connected to the EMCCD for image storage and scan control. In addition, the stepper motor drives the sample stage to move along the Z axis. This system has the following advantages over existing in vivo fluorescence imaging techniques. Since endogenous fluorescent substances and common organic fluorescent dyes in biological samples cannot be stimulated by steady-state near-infrared lasers, the system eliminates interference from background fluorescence such as autofluorescence of biological samples and organic fluorescent dyes, and is a highly sensitive system. Since the steady-state laser-pumped upconversion luminescent material is difficult to photobleach under steady-state near-infrared laser excitation, the steady-state near-infrared laser has a weak photobleaching effect on the organic fluorescent dye, and has little damage to the organism. With a small sample size, the system is an imaging technique that allows continuous observation of biological samples over time. The 980 nm CW laser is cheaper than the femtosecond laser used in the two-photon fluorescence probe, so the system is easy to generalize.

3.2. Experimental Materials

The four substrates listed in Table 1 are the rare earth upconversion luminescent nanoparticles with surface ligands of glutaric acid GA, citrate ligand Cit, polyacrylic acid PAA, and dopamine, respectively. It is 110~150, 20~30, 25~35, 36~42. The relevant properties of these four materials are summarized in Table 1.

Table 1. Physicochemical properties of UCNPs for lymphoid imaging

UCNPs	Surface ligand	Particle size (nm)	Crystal phase	surface
GA-UCNPs	Glutaric acid (GA)	110~150	α	Dicarboxyl
Cit-UCNPs	Citric acid ligand Cit	20~30	β	Tricarboxyl
PAA-UCNPs	Polyacrylic acid (PAA)	25~35	β	Polycarboxyl
UCNPs@Fe_3O_4	Dopamine	36~42	β	Amino

The small animal experiment procedure follows the guidelines of the Animal Protection and Use Committee. 5~10 weeks old female mice and 1 month old female bunny. The mice were intraperitoneally injected with chloral hydrate (body weight, average anesthesia, subcutaneous injection of conventional lymphatic contrast agent methylene blue in the tongue, left and right ears and left and right front paws of the same mouse, after injection, the mice were sacrificed and cut The neck and underarm skin were opened and a blue-stained lymph node was photographed using a digital camera.

The particle size and morphology of the synthesized OA-UCNP were characterized by transmission electron microscopy (TEM). The nanoparticles were dispersed in a cyclohexane solution or water, and then 10 L of the solution was added to the copper mesh to be naturally dried. Energy dispersive X-ray analysis (EDXA) of nanoparticles was performed on a JEOL JEM-2010 FTEM. The upconversion luminescence (UCL) spectrum was measured on a PTI QM40 fluorescence spectrometer using a 0-3W adjustable external 980 nm continuous excitation source instead of a xenon lamp [16]. Fluorescent in vivo imaging instruments are in vivo imaging instruments. All characterization tests were performed at room temperature.

4. Discussion

For the study of lymphatic imaging, four rare earth upconversion luminescent nanoparticles with different particle sizes, matrices and surface ligands were tested as follows. Surface amino-modified UCNPs are synthesized in one step using a modified hydrothermal microemulsion method with a diameter of approximately 20 nm and a length of 20-40 nm. Due to the presence of the amino moiety on the surface, NH_2-UCNPs samples are readily dispersed in water and some polar organic solvents such as MDF or DMSO. An aqueous solution of NH_2-UCNPs emits visible light under excitation of a 980 nm continuous laser. Specifically as shown in Figure 1. The up-conversion spectrum of the NAYF4: Yb, Er sample exhibits three characteristic emission bands of Er^{3+}.

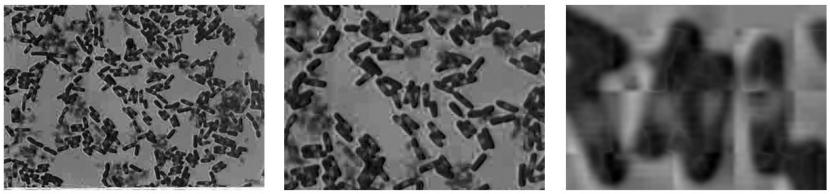

Figure 1. TEM image of NAYF4: Yb, Er sample

Importantly, the amino functional groups on the surface of the NH_2-UCNPs sample facilitate the further attachment of biologically active molecules, in particular the condensation between the amino functional groups on the UCNPs and the shuttle functional groups in the active molecule. The TEM morphology of the nanoparticles in Figure 2 shows that the four substrates with different substrates (LaF_3 and $NaYF_4$) and surface ligands (glutaric acid GA, citrate ligand Cit, poly) The rare earth up-converting luminescent nanoparticles of acrylic acid PAA and dopamine have uniform morphology and good monodispersity. The four nanoparticles have different particle sizes: 110~150, 20~30, 25~35, 36~42. A 4.8 nm thick Fe_3O_4 coating layer exists on the surface of UCNPs@Fe_3O_4. Among them, GA-UCNPs are α phase, while the other three are β phase. All four have good biocompatibility and are stably dispersed in aqueous solution.

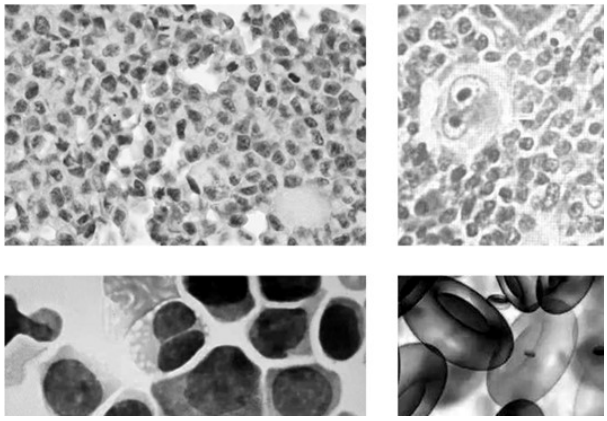

Figure 2. TEM image for lymphatic imaging

Further, we tested the upconversion luminescence properties of UCNPs. As shown in Figure 3, under the excitation of a 980 nm laser, the four nanoparticles emit stable bright blue light in water, indicating that the nanoparticles have good upconversion luminescence properties. The peak position of the emission spectrum and the energy level transition corresponding to the ion sum, respectively. The size of NaYbF4:Tm@CaF_2 particles observed under electron microscope is consistent. The interplanar spacing after coating CaF_2 was 0.27 nm, which was identified as the (200) crystal plane of cubic phase UCNPs. X-ray powder diffraction (XRD) further confirmed the composition and lattice structure of the UCNPs, and the position of the diffraction peak corresponds to the cubic phase CaF_2 standard card. X-ray energy spectrum further confirmed the elemental composition of NaYbF4:Tm@CaF_2, and Na, Yb, Tm, F and Ca elements were detected in the synthesized UCNPs.

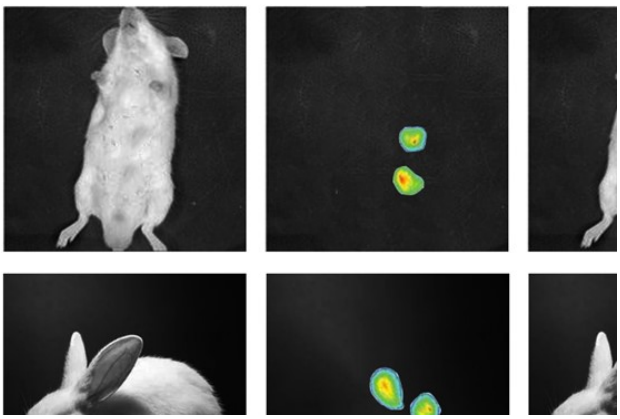

Figure 3. Dynamic light dispersion measurement

In general, the lymph nodes of small animals are not easily found because they are too small. In addition,

because lymphatic metastasis is an important tumor metastasis pathway, lymphatic imaging is of great significance for tumor metastasis diagnosis and surgical guidance. The citric acid-modified $NaYbF_4$:Tm@CaF_2@$NaDyF_4$ aqueous solution produced a distinct near-infrared up-conversion emission peak at 800 nm under 980 nm excitation. Since the excitation and emission wavelengths are all in the first optical window, Cit-$NaYbF_4$:Tm@CaF_2@$NaDyF_4$ nanomaterials are expected to be used for small animal in vivo upconversion luminescence as shown in Figure 4.

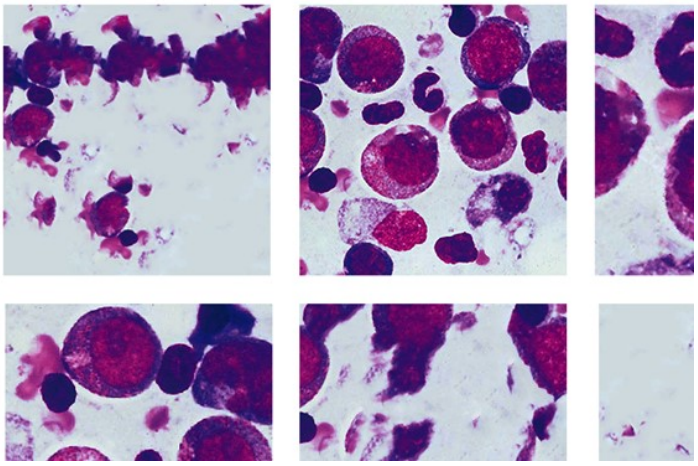

Figure 4. Z-scan upconversion luminescence image of Cit-UCNP.

As shown in Figure 4, the superficial neck, deep neck and axillary lymph nodes of the rabbit were stained with blue. The successful establishment of a multi-site lymph node methylene blue imaging model confirmed the path of drainage to the corresponding lymph nodes in different regions. The tongue drains the shallow cervical lymph nodes, the ear drains the deep cervical lymph nodes, and the forepaw drains the axillary lymph nodes. This model not only provides evidence for the study of regional draining lymphatic system, but also establishes the basis for the application of UCNPs for lymphatic imaging. But as shown in Figure 4. Methylene blue must be cut into the rabbit fur to see lymph nodes, and no living body detection can be achieved. And the blindness of the skin anatomy leads to a larger wound. At the same time, there are shortcomings such as peripheral blue staining, short lymph node imaging time and low tissue resolution, which makes the development of lymph node imaging technology have many obstacles. As a new fluorescent staining agent, rare earth upconversion luminescent nanomaterials in lymph node imaging The application in it has obvious advantages.

5. Conclusions

Rare earth up-converting luminescent nanomaterials (UCNPs) have rich optical, magnetic and X-ray absorption and depletion properties, and have broad application prospects in biomedical imaging and biological detection. Functional recombination will provide a broader application space for rare earth upconversion luminescent materials. Fluorescence imaging can visually show in vivo gene expression and cellular activity, and can avoid the lack of gas caused by large-scale trauma caused by imaging of bioreactive dyes. It is a powerful tool for molecular imaging technology and is widely used in medical and biological research. However, in cell and in vivo imaging processes, there are a large number of endogenous fluorescent substances in biological samples, and their autofluorescence causes very severe interference to the prominent signals of the label molecules. This paper focuses on the application of rare earth upconversion luminescent nanomaterials in the imaging detection of small animal lymphatic system. Two new multifunctional rare earth upconversion luminescent nanostructure materials were designed and synthesized for imaging and tracking of animal lymphatic system. The main conclusions are as follows:

(1) The synthesized NaYbF4:Tm@CaF_2@$NaDyF_4$ multifunctional rare earth luminescent nanoprobe was used for three-mode lymphography of mice. Intermediate CaF_2 in the nanostructure design acts as a shield and successfully prevents surface hardening effects. Up-conversion imaging of cells and up-conversion imaging of lymph nodes in live mice were obtained.

(2) Rare-econversion luminescent nanomaterials with different sizes, surface ligands and shell coatings were designed from three aspects: particle size, surface ligand modification and shell coating. Rare earth up-conversion luminescence is used to guide lymphatic tissue clearance studies.

(3) Rare earth upconversion luminescent materials have many advantages such as high chemical stability, excellent optical stability, and narrow band emission. In addition, the near-infrared laser (980 nm) is used as an excitation source, and the rare earth up-converting luminescent material emits nearly 800 nm of near-infrared light, which brings many advantages. For example, deep light penetration depth, almost no damage to biological tissues,

no autofluorescence of biological tissues, and the like. These features make it possible to become a new generation of lymphographic imaging contrast agents.

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