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Applications of Upconversion Nanoparticles in Molecular Imaging: A Review of Recent Advances and Future Opportunities

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Molecular imaging (MI) is visualization and characterization of biological processes at the sub-cellular level. Early diagnosis and monitoring of treatment procedure are main advantages of this approach. Molecular probe with high affinity is one of the important requirements of MI. Within the extensive group of nanoparticles, upconversion nanoparticles that play a prominent role in optical MI cab be used as an MI probe. Upconversion phosphors are ceramic materials in which rare earth atoms are embedded in a crystalline matrix structure. The materials absorb light in the near infrared region and emit in the visible region via several mechanisms. This agent can be used for optical imaging, gene or drug delivery and photodynamic therapy. The present study reviews the recent development in synthesis and modulating the characteristics of upconversion nanoparticles and their applications in MI. Furthermore, recent research approaches and future opportunities regarding the development of these nanoparticles are discussed.

Key words: Molecular Imaging, Fluorescent Nanoparticles, Upconversion Nanoparticles, Contrast Agents.

Molecular imaging (MI) that is one part of molecular medicine is defined as a non-invasive, quantitative and repetitive imaging of targeted macromolecules and biological processes in living subjects at the cellular and molecular levels[1, 2]. In this field, instead of diagnosing and classifying disease by symptoms or systemic changes, tests are being developed for the characteristic biological and biochemical markers and processes which occur in various types of disease[3]. The ability of determination of pathologies tissues without invasive biopsies or surgical procedures is the main advantages of this process[4]. Tumors may be spatially and temporally heterogeneous in terms of gene expression[5], metabolism[6], hypoxia[7], angiogenesis[8], cell proliferation[9], apoptosis[10, 11], and other phenotypic features, but this technique can help investigators to better understand these features[12]. These features are important for tumor detection, characterization, staging, prognosis assessment, treatment planning, and early treatment monitoring, as well as for monitoring of cell trafûcking and new drug development[13, 14]. MI for performance, have two basic requirements include: (i) high affinity molecular probes with the ability to overcome biologic delivery barriers and (ii) a sensitive, fast, high special and temporal resolution imaging modality to detection this probe[15]. MI probes that provide imaging signal are referred by many different names such as molecular beacons, reporter probe, tracers, smarts probe, activatable probe, contrast agent, and nanoparticles[16]. The rapid growth of nanotechnology and nanoscience could greatly expand the clinical opportunities for MI[17]. The basic rationale is that nanometer-sized particles have functional and structural properties that are not available from either discrete molecules or bulk

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materials[18, 19] When conjugated with biomolecular affinity ligands, such as antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumors with high specificity[20]. Structurally, nanoparticles also have large surface areas for the attachment of multiple diagnostic and therapeutic agents. Recent advances have led to the development of biodegradable nanostructures for drug delivery[21]. As discussed above, one of the most important requirement of MI is an imaging modality[22]. Different modalities can be used in MI include: single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), ultrasound (US) and optical imaging. Sensitivity, spatial resolution, temporal resolution, depth of signal penetration and cost of these modalities are different[23]. Between all of them, optical imaging is the safest method. Possibility of real-time imaging, fast data acquisition (minutes), relatively high spatial resolution and low cost are the most important advantages of this technique[24, 25]. Optical MI is an imaging discipline that measures light released from either endogenous sources or exogenously administered agents that, encoded within its signal, bears information about biological processes on a microscopic scale[26]. For improved signal-to-background ratio (SBR) and targeting of speciûc biological activity, this imaging technique relies on the excitation and detection of ûuorescence from an exogenous contrast agent[27]. During the early 1990s, Huffman and Kraetschmer discovered how to synthesize and purify large quantities of fullerenes[28]. Now, there are two different types of fluorescent imaging contrast agents that can be used in optical MI: endogenous and exogenous agent. Endogenous agents to produce the optical signal, work by enzyme-mediated process in cells and tissues. Two important examples for endogenous probes include: fluorescent proteins[29] and luciferin/luciferase systems[30]. Need for genetical modification of targeted cells and low emission wavelength (510 nm) are the most important limitations of fluorescent protein. In addition, luciferin/luciferase systems suffer from inhomogeneous scattering and limited light penetration[31]. The exogenous contrast agents which are inserted into a biological system from outside can be classified into three different types: (1) organic dye, (2) quantum dots (QDs) and (3) upconversion particles.

Organic dyes are widely used as ûuorophores in biomedical imaging and detection, However they are also not good for multicolor imaging, because of two inherent properties: (1) organic dyes have relatively broad emission spectra and hence result in the signal overlap from different dyes; and (2) one organic dye can only be suitably excited by the lights within a certain narrow wavelength range and it thus needs nearly the same numbers of excitation light sources as the dyes used[32]. also most organic dyes suffer from low quantum yields, rapid photobleaching, poor stability[33, 34]. Another novel optical nanoparticles, are quantum dots (QDs) that have broad excitation proûles, narrow and symmetric emission peaks (commonly 25–35 nm full width at half maximum)[35] and minimal spectral overlap[36]. Therefore, these nanocrystals can be used for multiplexed detection of molecular targets[37, 38]. However, the use of quantum dots for biological recognition has several drawbacks[39]. The potential toxicity of quantum dots is an important concern for health and environment[40]. In addition, their use for labeling individual biological molecules are limited by intermittent emission (blinking)[41]. In addition, both the organic ûuorophores and quantum dots are generally excited with ultraviolet (UV) and visible light exposure. Absorption of these radiations by the biological samples often induces autoûuorescence phenomenon interfering with ûuorescent signals obtained from exogenous biomarkers. Prolonged exposure of the biological samples to UV radiation can also cause photodamage and mutation in the sample[42]. These limitations have necessitated development of a new type of high-quality and well-shaped nanomaterials known as upconversion nanomaterials (UCNs)[43] during the mid 1960s[8]. Upconversion phosphors (UCPs) are ceramic materials in which rare earth atoms are embedded in a crystalline matrix. The materials absorb light in the near infrared (NIR) region and emit in the visible region via a two-photon or multi-photon mechanism[43]. The aim of this article is review of recent advances and future opportunities of UC nanoparticles in MI.

Mechanism of Upconversion

Conventional ûuorophores exhibit the downconversion phenomenon, i.e., higher energy

photons are absorbed while lower energy ones are emitted due to internal energy loss[44]. Compared with downconversion, Upconversion is a nonlinear optical phenomenon characterized by conversion of low energy radiation like NIR to a high energy radiation like visible light (Fig. 10)[45]. This process requires the absorption of two or more photons to provide the sufficient energy for the UC emission to occur. There are four different classes of UC mechanisms[46]. These four basic mechanisms are discussed in details in following.

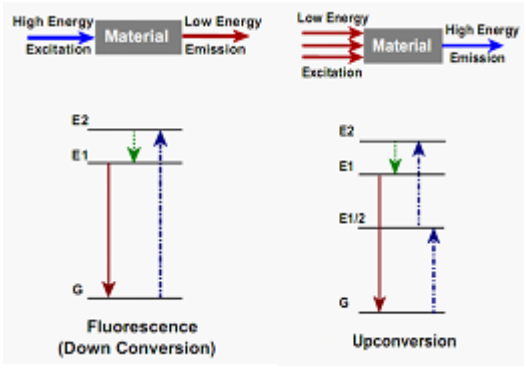

Fig. 10. Schematic diagrams of two different optical phenomena and corresponding energy levels: downconversion process (left) and upconversion process (right)[47].

Excited State Absorption:

Excited State Absorption (ESA) upconversion mechanism is a single ion mechanism based on a sequential absorption of two photons. This mechanism was first proposed by Bloembergen in 1959[48]. The general energy scheme of ESA is shown in figure 11 and involves the successive absorption of two photons. An electron is excited from a ground state (E_0) to a metastable intermediate state (E_1) during a ground state absorption (GSA) process. A second photon then promotes this electron from intermediate state () to the higher excited state () in optical transition and results in UC emission, when electron is transferred from back to[49].

Energy Transfer Upconversion:

Energy Transfer Upconversion (ETU) mechanism was studied extensively in the mid 1960's. The pioneering contributions of Auzel resulted in the observation of the ATPE effect[51], which was later termed energy transfer upconversion. Compared to ESA, its upconversion efficiency is at least two orders of magnitude higher than ESA and in this mechanism two ions participate in producing the upconversion emission, as shown in figure 12. The first ion, sensitizer (energy donor), is responsible for absorption of excitation photons, and the second ion, activator (energy acceptor), emits the upconversion emission. First, by a ground state absorption, the sensitizer ion is excited to energy state E_1. Second, the sensitizer ion energizes the activator ion to E1 state through a nonradiative transfer, then itself relaxes to the ground level. Third, the excited sensitizer transfers another photon to the activator through a second nonradiative transfer which excites the activator from E_1 to E_2. Consequently, the upconversion emission relaxes the activator ion back to ground state(E_0)[52].

Photon Avalanche:

The third upconversion mechanism is photon avalanche (PA) which was first introduced by Chivian in 1979 [46]. In the PA process, after

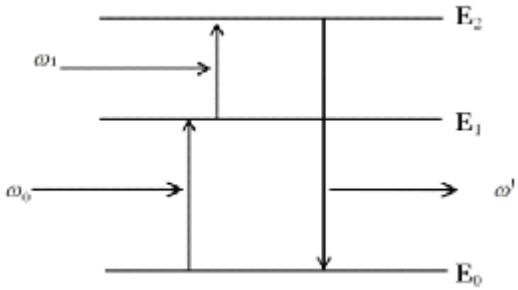

Fig. 11. Schematic diagram of excited-state absorption (ESA)[50].

Fig. 12. Schematic diagram of Energy Transfer Upconversion (ETU)[47].

absorption of the excitation radiation, an ion is excited. The excitation radiation is usually not resonant with the absorption transition from the ground state to the intermediate states, but a little higher than E_2. Through the cross relaxation, it goes down to state. Energy transfer occurs between the state electron and the state electron, resulting in the formation of two ions in the state. One of them absorbs the excitation radiation and is excited to the E state, in which it interacts with state electrons and energy transfer II occurs to form three E1 electrons. Here, the excitation radiation is resonant with the absorption transition from E1 to E. By repeating the whole steps again and again, the number of electrons in the E state increases dramatically. When the electrons go back to the E0 state, a strong upconversion emission is emitted (fig 13). PA is one of the most efficient upconversion processes, but it suffers from drawbacks such as its dependence on the excitation power and the delay in response to excitation (up to several seconds) because of the numerous ESA and cross relaxation processes[43].

Energy Migration-mediated Upconversion (EMU):

In this process four ions participate in producing the upconversion emission, include sensitizers (Ion 1), accumulators (Ion 2), migrators (Ion 3) and activators (Ion 4) (fig 14). The sensitizer is used to harvest pump photons through GSA absorption, which promotes a neighbouring accumulator ion to an excited state. The accumulator ions receive electrons from the sensitizer or rarely by absorbing the pump photon to reach the highest excited level from which energy is transferred to a migrator ion thorough a non-radiative relaxation. This step is followed by a series of random hops of energy between migrator ions until the activator ion receives the energy thorough a non-radiative transfer from migrator to activator. Finally, the radiative relaxation of the activator ion results in an upconversion emission. An important feature of EMU is that the excitation energy collected by sensitizer can be amassed in the accumulator ions by successive energy transfers, while enabling one step energy transfer to the activator. Second point is that EMU provides high conversion efficiency for low energy excitation. And finally it provides tunable upconversion emission possible by replacing activator ions with different activator ions[53]. EMU is a really efficient method especially for low energy excitation, but it needs state of the art structural engineering of the particles with core shell structure. Therefore, ETU is the preferred and practical mechanism for producing upconversion with more than one dopant ions[49].

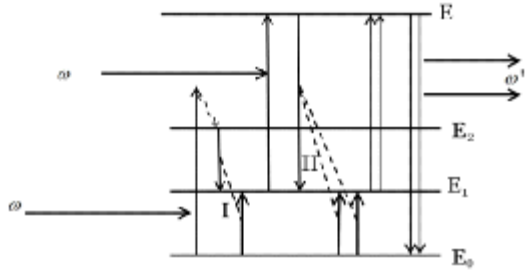

Fig. 13. Schematic diagram of photon avalanche[50].

Contrast Stains for Optical Imaging:

Zijlmans et al. in 1999 for the first time have reported upconverting properties of lanthanide-doped particles that can be useful in bio imaging. Their finding demonstrated that by using IR radiation for excitation of submicron-sized $Y_2O_2S:Yb/Tm$ particles, to study the distribution of prostate-speciûc antigen (PSA) in parafûn-embedded sections of human prostate tissue, non-speciûc autoûuorescence signal associated with short-wavelength excitation are completely eliminated. In addition, they have reported that UC particles do not bleach after continuous exposure to high excitation energy levels. Therefore, UC particles labeled tissue samples can be conveniently stored for permanent records[89].

Another application of UPCNs has been reported by Zako et al for cellular imaging. They found that $Y_2O_3:Er$ nanoparticles modiûed with

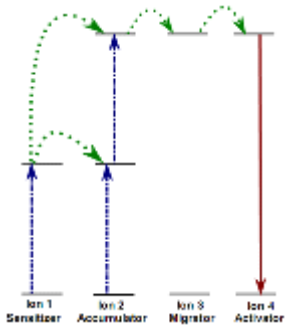

Fig. 14. Schematic diagram of EMU process[47]

cyclic arginine–glycine–asparatic acid (RGD) peptide can speciûcally bind to cancer cells with elevated integrin $\alpha_v\beta_3$ expression. This can provide new opportunities to document tumor integrin expression to evaluate treatment efûcacy in integrin-positive patients[90]. In addition, Wang et al. have used $NaYF_4:Yb/Er$ nanoparticles conjugated with antibody for highly speciûc staining and imaging of HeLa cells with antigen expressed on the cell membrane[91].

In addition, these UC particles can be used in tissue imaging. In 2008 for the first time the in vivo imaging of deep tissues in the Wistar rat using UCNPs has been reported by Chatterjee et al. in this study PEI (5 wt%) and 100 ¼l of resultant UCNPs (4.4 mg/ml) were injected subcutaneously into the groin and upper leg regions of the rat with a depth up to 10 mm. UPCNs injected below the abdominal skin, thigh muscles, and skin and translucent skin of the foot. The rat was then excited using a 980 nm laser. Furthermore, in control group, they injected QDs into the thicker skin or abdomen. As a result, the UCNPs injected below the abdominal skin, thigh muscles, and skin showed visible fluorescence under a 980 nm excitation. However IN control, they did not show any fluorescence under a UV excitation. Therefore UCNPs excited with NIR radiation therefore have great potential for in vivo imaging[92].

In summary, there are several reasons for the high potential of IR radiation in cell and tissue imaging: (i) a high signal-to-noise ratio, (ii) strong penetration ability and (iii) less photo damage to the cell/tissue under long-term irradiation. Therefore, RE doped UCNPs are promising alternatives to traditional fluorescent biolabels (such as organic dyes and QDs) for in vitro cell and in vivo tissue imaging.

Photodynamic Therapy (PDT) Using UCNPs:

Photodynamic therapy is a therapeutic option for cancer that relies on the interaction of light and drugs to kill targeted cancer cells[93]. PDT is effective in the treatment of early lung cancer[94], head and neck cancers[95], Barrett's esophagus[96], bladder cancer[97], and to be the ideal treatment for skin cancer[98]. due to a better selectivity for the tumor and a lower systemic toxicity for fewer side effects compared to radiation therapy and chemotherapy[99, 100]. This treatment modality has three basic components: light, photosensitizer (PS) molecules, and oxygen. With the absorption of light, a ground state PS is boosted into a high-energy state, leading to energy transfer to neighboring oxygen or other substrate molecules, and then the generation of singlet oxygen or other reactive oxygen species (ROS)(fig 16)[94]. The singlet oxygen generated can oxidize critical cellular macromolecules, including lipids, nucleic acids, and amino acids, thereby inducing cellular permeability alterations with the consequence of cell death by necrosis or apoptosis or both[101]. But one major drawback of this treatment modality in clinical applications is the low penetration of visible or even UV light. Thus this treatment is not suitable for large or internal tumors[102]. New approach to deliver light into deeper tissues for PDT treatment is using NIR-excitable upconversion nanoparticles (UCNPs) as an energy donor[103]. The first report of using UCNPs for PDT application was published by Zhang et al. In their study, UCNPs were coated with a silica shell, into which PS molecules were doped[104]. Since then, a number of papers have reported similar strategies for the PS loading on UCNPs[105-107]. Further efforts in this field may enable a new photodynamic therapeutic approach with greatly improved tissue penetration, suitable for treatment of relatively large or internal tumors[108-110].

Drug Release and Gene Delivery Using UCNPs:

In recently years, for remotely controlled release of therapeutic drugs at the site of interest such as tumors, photo responsive drug release systems have received significant interests. A

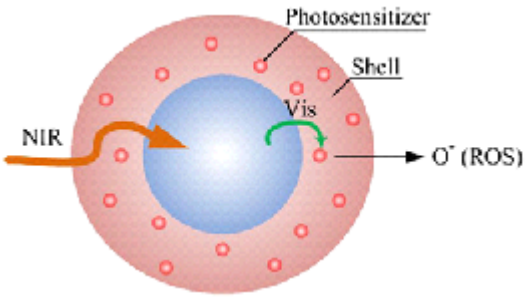

Fig. 16. Schematic design of UC nanoparticles-based PDT for the treatment of a tumor cell. The design is composed of a nanoparticles core and a porous silica or polymer shell impregnated with photosensitizers. The shell is also modiûed with functional groups for targeting a speciûc tumor cell[111]

specific class of UCNPs with Yb and Tm, are able to emit UV light under 980-nm NIR excitation, and could have great promise in the design of NIR-light responsive drug delivery systems[112]. For the first time Carling and coworkers demonstrated the use of NIR-to-UV UCNPs ($NaYF_4$: Yb Tm) for NIR laser triggered molecular releasing. A caged compound, 3´, 5´-d (carboxymethoxy) benzoin, which was usually photo activated by UV light, was conjugated to NIR-to-UV UCNPs. Under NIR laser irradiation, UV light generated by UCNPs could trigger the uncaging process and result in the release of organic molecules from UCNPs[113].

Recently, the use of NIR-to-UV UCNPs for photo-controllable gene expression has been reported by Jayakumar et al. the results of their experiments, prove that these nanoparticles has great potential in a number of fields including gene therapy for controlled and specific gene delivery/ knockdown, developmental biology for site-specific gene knockdown, and patterning of biomolecules using safe NIR light[114].

CONCLUSION

Fluorescent nanoparticles have excellent photostability, chemical stability and low toxicity. advantages of these NPs over visible QDs and organic dye doped NPs is their ability to be excited in the NIR region, where autoûuorescence is minimal, tissue penetration is maximum and there is minimum photodamage. They also do not exhibit photoblinking, which is a phenomenon observed in QDs. The upconversion ûuorescence output of UCNs is also higher than that of QDs. These particles can be applied for drug and gene delivery due to the ability to protect their encapsulated content and physical properties.

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