Functionalized Upconversion Nanoparticles for Cancer Imaging and Therapy

K. Liu

Summary

Fluorescence imaging and photodynamic therapy (PDT**)** are very promising, and for some applications even clinically accepted, modalities for early stage cancer diagnosis and therapy. Unfortunately, their clinical value is still rather limited due to the strong autofluorescence background and the poor tissue penetration properties of short wavelength light, which needs to be used to activate the traditional UV-Vis fluorescence reagents. Recently, rare earth ions doped upconversion nanoparticles (UCNPs) have attracted considerable attention because of their unique optical properties such as the possibility to use relatively low excitation power densities (CW laser), low photobleaching rates, and multispectral (UV-Vis-NIR) emission. Despite many efforts to utilize UCNPs in biomedical applications, there are still major challenges to overcome to reach targeted imaging and therapy of cancer cells at an early stage. This thesis focuses on surface modification and functionalization of $NaYF_4$:Yb,Er UCNPs for improving their efficiency in cancer imaging and therapy.

Chapter 1 introduces the status quo and the major limitations of traditional down-conversion fluorescence imaging and photodynamic therapy of cancers. It then comprehensively demonstrates the advantages of utilizing lanthanide ions doped UCNPs for biomedical applications, such as bio-immunoassays, fluorescence imaging, and photodynamic therapy. The recent advances in the preparation and the surface chemistry of nanoparticles are also reviewed.

In **Chapter 2** we describe a multifunctional nanoplatform for simultaneous upconversion luminescence (UCL) imaging and photodynamic therapy (PDT) based on the selective fluorescence resonant energy transfer (FRET) from

multicolor luminescent $NaYF_4:Yb^{3+},Er^{3+}$ UCNPs to rose bengal photosensitizers (PS). Different from traditional approaches that employ electrostatic or hydrophobic interactions, we use a covalent bonding approach to load photosensitizing molecules on the UCNPs. This increases significantly the UCNPs-PS linkage stability and reduces the leakage/desorption probability of the PS. The efficiency of energy transfer is shown as we, for the first time, directly detect the 1270 nm emission of singlet oxygen from UCNPs-PS conjugates. *In vitro* tests performed on JAR choriocarcinoma and NIH 3T3 fibroblast cells verified the efficient endocytosis and photodynamic effect of the nanoplatform with 980 nm irradiation specificly to JAR cancer cells, which shows the promise of using UCNPs for image-guided photodynamic therapy of cancer. With the mature of UCNPs synthesis and functionalization, the integration of cancer imaging and cancer treatment might become truth in the future oncology researches.

In order to improve singlet oxygen production of the UCNPs-PS conjugates, further research is carried out on core/shell structured UCNPs in **Chapter 3**. $NaYF_4:Yb^{3+},Er^{3+}@NaYF_4$ core/shell UCNPs with different shell thickness are synthesized and covalently functionalized with photosensitizer molecules Rose Bengal. From the specific upconversion spectroscopic researches and the singlet oxygen measurements, we find that the optimal shell thickness is a trade-off between the conflicting optimal conditions for upconversion and energy transfer efficiency. On one hand, the shell coating can efficiently protect the emission centers from non-radiative decay caused by surface defects or high-energy vibrational modes outside the UCNPs and therefore enhances the upconversion luminescence intensity. On the other hand, the shell also increases the distance between the donor and acceptor and thus impedes excitation of the PS via a FRET process. It has been determined that the optimal shell thickness for PDT is 4 layers, corresponding to a thickness of 5.7 nm. Analogous researches can be expanded to FRET

based immunoassays, in which the UCNPs shell thickness also plays a critical role in energy transfer, an advanced detection sensitivity might be achieved if optimal shall thickness is reached.

The properties of the surface coating (ligand materials, charges, and hydrodynamic diameters *etc.*) play an important role in the interaction of nanoparticles with cells. In **Chapter 4,** ligand-free $NaYF_4$:Yb^{3+},Er^{3+} UCNPs (bare UCNPs) are synthesized and further modified with different ligand molecules: aminoethyldihydrogenphosphate (AEP), polyallylamine (PAAm) and polyacrylic acid (PAA). We systematically investigate their interaction with *in vitro* cultured mammalian cells. All nanoparticles show a good dispersibility and upconversion luminescence intensity in aqueous solution, but the hydrodynamic diameter and surface charges are different. A cellular uptake and cytotoxicity study on MCF-7 breast cancer cells and 3T3 normal fibroblast cells reveals that the PAAm coating can efficiently enhance the cellular uptake and endocytosis efficacy of UCNPs. On the other hand, it is more toxic than the others, and this should definitely be taken into account when considering further *in vivo* applications.

The *in vivo* detection of early stage cancer, *i.e.* smaller than 2 mm, is presently still a huge challenge. In **Chapter 5** a sensitive luminescence imaging method is demonstrated for targeted labeling of cancer spheroids transplanted on a chick embryo chorioallantoic membrane (CAM) using antibody functionalized upconversion nanoparticles (UCNPs-Ab). Benefitting from the high sensitivity and photostability of UCNPs, the behavior of the particles in the microcirculation could be systematically investigated using intravital microscopy. We show that the UCNPs-Ab conjugates can extravasate from the CAM blood vessels and specifically label the xenografted tumor spheroids (~500 um), which indicates that UCNPs have a great potential for *in vivo* target labeling and diagnosis of cancer at an early stage. Besides, the CAM also provides us an ideal model to research the tumor invasion and

angiogenesis process, more indepth researches on tumor metastases need to be carried out in the future with the aid of non-photobleaching UCNPs.

The lack of depth information during traditional single color fluorescence imaging is another major issue that restricts the accuracy of this imaging method for *in vivo* cancer diagnosis. **Chapter 6** presents the use of multispectral upconversion luminescence imaging for ascertaining the tissue depth based on the relative intensity changes of different emission bands. We first use a liquid phantom as atissue-mimicking model for a quantitative assessment of the attenuation of the $NaYF_4$:Yb^{3+},Er^{3+} UCNPs emission bands at 540 nm and 650 nm under 980 nm NIR excitation. The 540/650 intensity ratio (G/R) is calculated at each depth and plotted in a logarithmic diagram. We then find an exponential decay with increasing tissue depth. Finally we employ this method in practice using layered pork muscles to verify the quantitative relation of the G/R ratio. We show that thicknesses up to a centimeter can be determined with an accuracy of less than 1 mm. The G/R upconversion intensity ratio itself, proposes a simple method to critically localize the cancer leision depth without extensive mathematically modeling. And if combined it with tomography, more informations about the cancer 3D structures can be obtained, this might become a research tendency in cancer imaging.

In summary, this thesis describes the surface modification and functionalization of upconcersion nanoparticles for improving the efficacy of these nanoparticles in cancer imaging and therapy. Although a large amount of *in vivo* animal research and preclinical research is still needed, the UCNPs have already opened a new path in early stage cancer imaging and therapy, and hold great promise for the future.