Synthesis and Cytotoxicity of $NaYF_4$ Nanoparticles Coated in a Layer of Porous Silica

ABSTRACT

Qualitative (microscopy) or quantitative (spectroscopy) optical methods are one of the most prominent techniques in biology. In recent years rare-earth element doped upconversion nanoparticles have become a new subject of research in an ever-growing field seeking new and efficient methods of imaging and detection.

The upconversion process was discovered in the 1960s and by the 2000s, the first lanthanide-doped $NaYF_4$-based micro- and nanoparticles, with enhanced luminescent properties, were synthesized. These upconverting materials absorb two or more low-energy photons, typically from the infrared spectrum, and throughout 4f-4f transitions on the lanthanide ions they emit one higher energy photon, generally in the visible or ultraviolet spectrum.

Further enhancement of luminescence can be done by engineering an active shell capable of absorbing additional energy and transferring it into the active luminescent core.

Upconverting $NaYF_4$ nanoparticles suffer from a low quantum yield efficiency. Excitation at this spectrum generates considerable heat, which significantly rises the temperature of the system, to the point of being harmful to biological structures. This problem could be possibly mitigated by sensitizing antennas. An antenna would non-radiatively transfer the harvested energy to upconverting nanoparticle increasing the luminescence intensity. NIR-absorbing organic dyes (like IR-806) or quantum dots show promise as viable candidates for that role.

These lanthanide doped $NaYF_4$ nanoparticles have become a focus of study for new types of luminescent labels, which offer enhanced properties like excitation at deeper sections of the tissue, lower background noise, and higher photostability.

There are three main $NaYF_4$ upconversion nanoparticle synthesis approaches. These methods are the Ostwald-ripening method, hydrothermal synthesis, and the most prominent thermal decomposition, also known as thermolysis.

The thermal decomposition synthesis leaves oleic ligands on the surface of the nanocrystal which severely restricts biological applications. Hydrophilic properties can be gained by modifying the surface of the nanoparticle. These modifications can be presented in the following categories such as ligand exchange, ligand attraction, ligand oxidation, ligand removal, polymeric shell coating, Layer-by-layer assembly, Host-Guest self-assembly, and sialinization, which coats nanoparticles with a silica shell layer.

Surface-modified nanoparticles can be utilized in a broad gamut of biological research, which can be classified into categories like bioimaging, biosensing, tracking, drug and molecule delivery, photodynamic therapy, and optogenetics.

While UV and Vis excitations are restricted, the infrared spectrum provides few optical windows for efficient excitation within which around 50 to 90% of a signal can penetrate up to the depth of 2 centimetres or more for specific tissues. Deeper penetration into the layers of tissue could make utilization of upconverting labels as a valid strategy in observations of multi-cellular structures like tissues or whole organisms.

The uncertainty of cytotoxic and other potentially harmful side-effects of upconversion nanoparticles limits the use of lanthanide doped UCNPs. Reports show that bare $NaYF_4$ nanoparticles can degrade in bodily fluids. According to the current knowledge on the subject, due to the size, core- and core@shell-type nanoparticles can penetrate the cell membrane without the involvement of any active mechanisms of endocytosis. Nanoparticles of a more substantial size and with surface modifications are internalized by the cell through Caveolin- or Clathrin-mediated endocytosis. The necessity for thorough investigation stems from Clathrin- and Caveolin-mediated endocytosis being tightly related to the apoptotic pathways.

The argument for extensive research into the subject of cytotoxicity is additionally strengthened by *in vivo* studies suggesting high rates of accumulation in certain organs for specific kinds of $NaYF_4$ nanoparticles.

In conclusion, $NaYF_4$ upconverting materials require more investigation and some degree of an individual approach to thoroughly investigate their viability. The subject of composition, size, surface modification, and response from the cellular or immune systems necessitates this individualized approach for each kind of lanthanide-doped $NaYF_4$ upconversion nanoparticle.

This thesis presents the results of a preliminary investigation into the validity of the application of the 55 nm and 100 nm mesoporous silica-coated β-$NaYF_4$: Er^{3+}, Yb^{3+} @ β-$NaYF_4$: Nd^{3+}, Yb^{3+} nanoparticles as carriers of therapeutic agents and emission-enhancing organic molecules such as IR-806 used as an example of a carried molecule.

Synthesis of 28 nm β-$NaYF_4$: 2% Er^{3+}, 20% Yb^{3+} @ β-$NaYF_4$: 30% Nd^{3+}, 20% Yb^{3+} nanoparticles, was performed by a team in the Włodzimierz Trzebiatowski's Institute of Low Temperatures and Structure Research, Polish Academy of Sciences in Wrocław. These nanoparticles were used as a template for a synthesis of the silica shell *via* the modified Ströber method of silica condensation by the means of degradation of TEOS compound in a basic environment.

The protective properties of silica shells were studied in three different aspects. The impact of IR-806 on the overall chemical stability shows increased stability of the dye over two weeks from 21% for an aqueous solution to around 74% for silica-embedded IR-806. After seven months of storing this compound, it was shown that 16% of silica-embedded dye was still active while only around 1% remained in the soluble form. Analogous experiments concerned the study of light degradation by exposing to UV light of 254 and 366 nm. After 200 minutes of exposition, all water-dissolved molecules of IR-806 degraded while around 27% of the dye was left within the mesoporous silica shell. The last studied aspect of degradation was the effectiveness of preventing reactive oxygen species in the form of hydrogen peroxide from oxidizing molecules and rendering them spectrally inactive. Silica shell did not protect IR-806 molecules from the reach of H_2O2 and the rates of degradation are relatively similar between silica-embedded and aqueous dyes.

The macrophage-like THP-1, epithelial-like MDA-MB-231 cell line and epithelial-like A375 cell line from a patient with malignant melanoma were used for cytotoxic experiments with MTT assay and flow cytometry. Different silica-coated, as well as uncoated $NaYF_4$ nanoparticles, were used. For the macrophage-like THP-1 cell line, smaller core-type nanoparticles increased proliferation. The increase in proliferation of the core@shell-type UCNPs was even higher. For the epithelial-like MDA-MB-231 cell line, stimulatory properties for both core- and core@shell-type nanoparticles were not observed. Stimulation with silica-coated upconversion nanoparticles shows significant cell toxicity of these nanoparticles. The viability of the THP-1 cells decreased to 16%, while MDA-MB-231 dropped to 49%. THP-1 and A375 lines were selected for the experiments involving various sizes of silica shell and the potential cytotoxicity of IR-806 dye. Nanoparticles of the same composition, with two different sizes of silica shells, were chosen. For the thicker 96 nm silica-coated NPs, there was no major cytotoxic effect observed. Similarly, to the results of THP-1 and MDA-MB-231 experiments, the 55 nm silica-coated nanoparticles reduced the viability of the cells, but there was no additional cytotoxic effect from the silica-embedded dye. The media solution of the dye didn't impact the viability of the cell culture and any significant cytotoxicity of IR-806 was observed. Cytometric analysis of cell viability confirms MTT assay results and indicates induction of apoptotic processes in cells which leads to significant death of cells during 48 hours stimulation period.

These results combined with literature data, indicate existence of a spectrum range of diameter in which $NaYF_4$ nanoparticles are highly cytotoxic to the cells.