Tumor-Targeting Upconversion–Nanoparticle-Based Unimolecular Micelles for Simultaneous Chemotherapy, Photodynamic Therapy, and Fluorescence Imaging

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Statement of Purpose: Lanthanide ion (such as Er^{3+}, Yb^{3+}, Tm^{3+})-doped upconversion nanoparticles (UCNPs) have attracted much attention in recent years for biomedical applications due to their unique ability in converting near-infrared light (NIR) to higher-energy photons in the UV, visible, and NIR regions. NIR light (e.g., 980 nm) can penetrate deeper into biological soft tissues than UV or visible light. UCNPs can also enable high quality imaging since the background autofluorescence is significantly reduced under NIR excitation. In this study, we developed a unique tumor-targeting UCNP-based unimolecular micelle (Fig. 1) for combined chemotherapy, photodynamic therapy, and fluorescence imaging. The $NaYF_4$:Yb/Tm/Er UCNPs emit light in the UV, visible, and far-red regions. As shown in Fig. 1, the hydrophobic core of the unimolecular micelle formed by a photosensitive poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) (PNBMA) polymer undergoes a hydrophobic-to-hydrophilic transition under UV light emitted by NIR-activated UCNPs due to photo-induced polymer side-group cleavage, thereby triggering a rapid drug release (AB3, an HDAC inhibitor) for effective chemotherapy. The photosensitizer Rose Bengal (RB) conjugated directly onto the UCNP core when excited at the 540 nm emitted by NIR-activated UCNPs can effectively generate singlet oxygen for photodynamic therapy. The far-red emission (650 nm) of UCNPs can be used for micelle/nanoparticle imaging. The unimolecular micelle was also conjugated with KE108 peptide, a novel active-tumor-targeting ligand, which specifically target the somatostatin receptors overexpressed by medullary thyroid cancer cells.

Methods: The $NaYF_4$:Yb/Tm/Er UCNP core was prepared using the thermal decomposition method. Both the amphiphilic PNBMA–poly(ethylene glycol) (PEG) block copolymer arms and RB were conjugated onto the UCNP core as shown in Fig. 1. KE108 peptides were also selectively conjugated onto the terminal ends of the PEG segments for active tumor-targeting. The hydrophobic drug AB3 was loaded into the photosensitive hydrophobic core of the resulting UCNP–RB/PNBMA–PEG–KE108 unimolecular micelles. The effect of NIR excitation on singlet oxygen generation and *in vitro* drug release of the UCNP-based micelles were studied. Medullary thyroid cancer cells, MZ–CRC–1, with overexpressed somatostatin receptors were treated with the AB3-loaded micelles. The effect of the KE018 peptides on the cellular uptake of the micelles was studied by two-photon microscopy based on the 650 nm luminescence band of the UCNPs.

Results: The UCNP-based unimolecular micelles were developed for targeted cancer therapy and imaging. As shown in Fig. 2, the UCNPs emit multiple luminescence bands under NIR. In particular, the luminescence bands in the UV region overlap with the photocleavable hydrophobic polymer segment (i.e., PNBMA), thereby providing rapid drug release (Fig. 3) resulting from the hydrophobic-to-hydrophilic transition of the micelle core. Meanwhile, the luminescence band at 540 nm can effectively activate the photosensitizer (RB) to generate singlet oxygen for photodynamic therapy (data not shown). Lastly, the 650 nm luminescence band was conveniently used for fluorescence imaging to localize the micelle *in vitro* (Fig. 2 (B)) or *in vivo*. As shown in Fig. 2 (B), KE108 increased the cellular uptake of the UCNP-based micelle drastically due to its strong binding affinity for all five subtype somatostatin receptors (SSTR1-5).

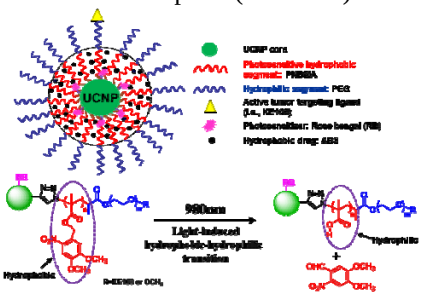

Fig. 1. Tumor-targeting UCNP-based unimolecular micelles for simultaneous chemotherapy, photodynamic therapy, and fluorescence imaging.

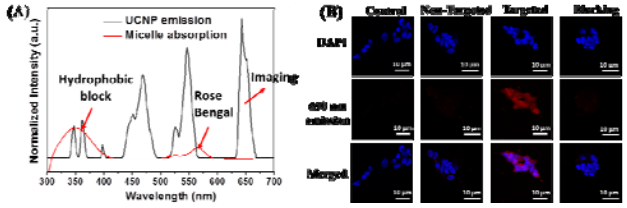

Fig. 2. (A) The UNCP emission spectrum and the absorption spectrum of the hydrophobic PNBMA polymer segment and RB photosensitizer. (B) The KE108 peptides enhance the cellular uptake of the micelles. Images were taken with a two-photon microscope based on the 650 nm luminescence of the UCNPs.

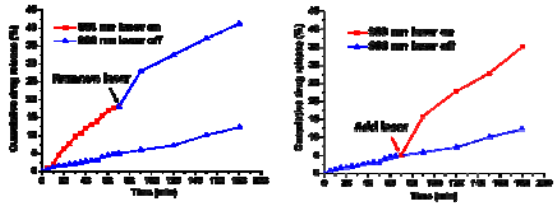

Fig. 3. NIR light-triggered *in vitro* drug release profiles.

Conclusions: The novel KE108 targeting ligand drastically enhanced the cell uptake of the medullary thyroid cancer cells. The multifunctional UCNP-based unimolecular micelles were able to perform fluorescence imaging, photodynamic therapy, and controlled drug release simultaneously under the same NIR light irradiation, suggesting that this unique nanoplatform is promising for targeted cancer theranostics. Several studies on this nanoplatform, including cell viability, *in vivo* anticancer efficacy, and *in vivo* imaging, are on-going.