808 nm-activable core@multishell upconverting nanoparticles with enhanced stability for efficient photodynamic therapy

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Abstract

Background: The unique upconversion properties of rare-earth-doped nanoparticles offers exciting opportunities for biomedical applications, in which near-IR remote activation of biological processes is desired, including in vivo bioimaging, optogenetics, and light-based therapies. Tuning of upconversion in purposely designed core-shell nanoparticles gives access to biological windows in biological tissue. In recent years there have been several reports on NIR-excitable upconverting nanoparticles capable of working in biological mixtures and cellular settings. Unfortunately, most of these nanosystems are based on ytterbium's upconversion at 980 nm, concurrent with water's absorption within the first biological window. Thus, methods to produce robust upconverting nanoplatforms that can be efficiently excited with other than 980 nm NIR sources, such as 808 nm and 1064 nm, are required for biomedical applications.

Results: Herein, we report a synthetic method to produce aqueous stable upconverting nanoparticles that can be activated with 808 nm excitation sources, thus avoiding unwanted heating processes due to water absorbance at 980 nm. Importantly, these nanoparticles, once transferred to an aqueous environment using an amphiphilic polymer, remain colloidally stable for long periods of time in relevant biological media, while keeping their photoluminescence properties. The selected polymer was covalently modified with two FDA-approved photosensitizers (Rose Bengal and Chlorin e6), which can be efficiently and simultaneously excited by the light emission of our upconverting nanoparticles. Thus, our polymer-functionalization strategy allows producing an 808 nm-activable photodynamic nanoplatform. These upconverting nanocomposites are preferentially stored in acidic lysosomal compartments, which does not negatively affect their performance as photodynamic agents. Upon 808 nm excitation, the production of reactive oxidative species (ROS) and their effect in mitochondrial integrity were demonstrated.

Conclusions: In summary, we have demonstrated the feasibility of using photosensitizer-polymer-modified upconverting nanoplatforms that can be activated by 808 nm light excitation sources for application in photodynamic therapy. Our nanoplatforms remain photoactive after internalization by living cells, allowing for 808 nm-activated ROS generation. The versatility of our polymer-stabilization
strategy promises a straightforward access to other derivatizations (for instance, by integrating other photosensitizers or homing ligands), which could synergistically operate as multifunctional photodynamic platforms nanoreactors for in vivo applications.

Full Text
Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures
Figure 1

a) Transmission EM (TEM) micrographs of the layer by layer process from cores (left) to the multishell particles (right). Scale bars correspond to 50 nm. The corresponding histograms
for the synthetic steps are depicted in green bars below each image. b) Table reflecting UCNPs composition, including the diameter or thickness of the core or the layer based on TEM images, and the individual core and shell composition as determined by ICP-MS. c) Photographs of solutions of cores (left) and core@multishell UCNPs (right) under with a 980 nm- and 808 nm-excitation lasers, respectively. d) Emission spectra of cores (black lines) and core@multishell UCNPs (green lines) in chloroform (~ 5 nM particles) under 808 nm and 980 nm excitation (1 W·cm-2), respectively.
Figure 2

a) Absorbance of core, core@multishell, and UC-PMA NPs in chloroform and water. b) Overlay of the Rose Bengal and Chlorin e6 absorbance and UC-PMA emission in water. c) Summary of the PSs attached per UCNP. d) DLS and LDA values of the different ligands’ combinations attached to the UCNPs; each bar is labeled with the corresponding polydispersity index (PDI). e) Hydrodynamic diameter values (number-weighted) overtime in different media (water, blue; supplemented ALF, green; supplemented DMEM, orange) of UC-PMA (white dots) and UC-PMA-RB,Ce6 (black dots) (raw data showed in Table S9).
Figure 3

a) Uptake of UC-PMA-RB,Ce6 (2 nM) by HeLa cells at different incubation times.  
b) Viability studies of UC-PMA and UC-PMA-RB,Ce6 after 24 h of NPs incubation under dark conditions, and IC50 values.  
c) Irradiation controls with untreated cells (only cells and laser) and cells loaded with UC-PMA (2.5 nM) after 3 h of incubation and different irradiation times using 5 or 10 W·cm$^{-2}$.  
d) Impact on the cell viability of UC-PMA-PS (2.5 nM, 3 h of incubation) under different laser irradiances and different irradiation times.  
e) Comparison in the viability impact of free PS and its combinations at a 250 nM, and the PS linked to the UCNPs incubated at 2.5 nM, under dark conditions, and after 20 minutes of irradiation with an 808 nm laser with a irradiance of 5 W·cm$^{-2}$. 

Figure 4

a) Intracellular localization of UC-PMA-RB,Ce6 in HeLa cells after 3 h of incubation (2.5 nM of NPs). From left to right: Red (RB, Ex. 561, Em 620/60), Green (Ce6, Ex. 405, Em. 725/40), merged Red + Green (yellow color indicated colocalization of RB and Ce6), merged image of bright field (BF) + red + green. b) Intracellular colocation of UC-PMA-RB and lysosomes and mitochondria in HeLa cells after 3 h of incubation. From left to right: Red (RB, Ex. 561, Em 620/60), Blue (Lysotracker Blue, Ex. 405, Em. 450/50), Green (Mitotracker Green, Ex. 488, Em. 525/50), merged image of blue + red + green. Purple indicates lysosomes and UCNPs colocalization. Scale bars represent 20 µm.
Figure 5

a) Degradation rate of DPBF at 407 nm upon 5 W·cm-2 irradiation with an 808 nm laser at 2.5 nM concentration of UCNPs or 250 nM of free PS. b) Cells treated with UC-PMA- Rb,Ce6; 2.5 nM for 3 hours under dark conditions after TMRE exposure (upper raw), and after 40 minutes of 5 W·cm-2 irradiation (lower raw). c) Corrected-total cell fluorescence (CTCF) of cells incubated for 3h with 2.5 nM UC-PMA-RB,Ce6 and TMRE with and without PDT.

Supplementary Files

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