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Photo-tearable tape close-wrapped upconversion nanocapsules for near-infrared modulated efficient siRNA delivery and therapy

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ABSTRACT

RNA interference (RNAi) has become an appealing therapeutic approach for cancer and other diseases. One key challenge is the effective protection of these small fragile biomolecules against complicated physiological environments as well as efficient on-demand release. Here we design a photo-tearable polymer tape close-wrapped nanocapsule for efficient NIR modulated siRNA delivery. The photo-tearable nanocapsules comprise core-shell upconversion nanoparticles (UCNPs) coated with mesoporous silica layer for loading of photosensitizer hypocrellin A (HA) and small interfering RNA (siRNA) against polo-like kinase 1 (PLK1), and covalently bound thin membranes of polyethylene glycol (PEG) *via* a synthesized photocleavable linker (PhL). Upon irradiation at 980 nm, the UCNPs produce UV emissions to break PhL and tear out PEG membrane for siRNA release, and blue emissions to activate HA for generating reactive oxygen species (ROS). The close PEG membrane wrapping not only guarantees the efficient intracellular photocleavage, but also extends the circulation time and protects the loaded cargos from leakage and degradation. The ROS assists endosomal escape of the loaded cargos, therefore effectively improves the gene silencing efficiency and the suppressions of cell proliferation *in vitro* and tumor growth *in vivo*. The proposed photo-tearable tape-wrapped nanocapsules have promising potential application in precision medicine.

1. Introduction

RNA interference (RNAi), which utilizes exogenous small interfering RNA (siRNA) to specifically inhibit target gene expression [1–3], has become a tremendously appealing approach for innovative therapy [4]. However, one of the challenges for the broad clinical application of RNAi remains the efficient delivery of these small fragile biomolecules in target cells. The delivery vehicles must protect siRNA well from leakage and degradation in extracellular biological fluid, and controllably release siRNA in the cytoplasm of target cells [3,5]. Packing siRNA with nanoparticles, such as mesoporous silica [6], gold nanoshells [7], and polymers [8,9], has extended siRNA half-life in blood, enhanced tumor-specific cellular uptake and improved the gene transfection efficiency [10,11]. However, siRNA on the outer surface of nanoparticles is still exposed to the complicated physiological environment, which makes it amenable to RNase degradation [12,13]. Although mesoporous silica nanoparticles have been used to entirely encapsulate siRNA [14], it is difficult to turn the intracellular release of siRNA.

On-demand delivery and release using external stimuli are an important way for precise therapy since external triggers allow for better duration and dosage controls [15–17]. Light stands out among different external stimuli due to its noninvasiveness, easy generation and manipulation, and controllability with high spatiotemporal precision [18–20]. Some photoactive molecules containing *o*-nitrobenzyl moieties can be irreversibly cleaved under irradiation [21], and have been extensively applied for precise drug delivery [22] and optogenetic applications [23]. However, UV light is generally required for most of the photoreactions, which leads to phototoxicity [24] and quick attenuation in body tissues [25], and imposes the restrictions on its *in vivo* applications. Thus near-infrared (NIR) irradiation has attracted considerable attention in

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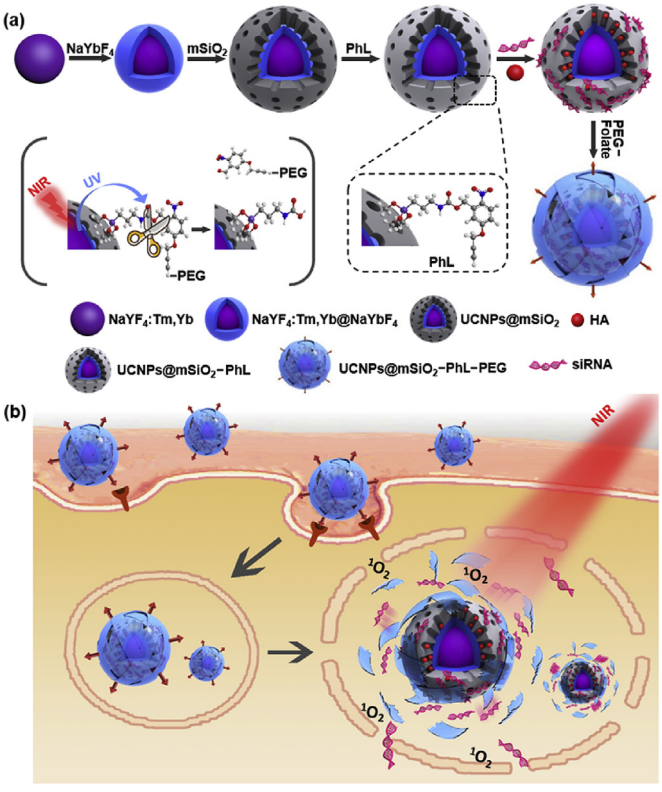
noninvasive therapy due to its significantly less damage to living tissues, remarkably deeper light penetration, and low autofluorescence background [26,27]. By combining the special natures of lanthanide-doped upconversion nanoparticles (UCNPs), which can adsorb two or more low energy photons to give out a higher energy photon [28], NIR irradiation can be upconverted to UV and visible radiations [29–32]. Accordingly, photoactivated molecules modified DNA [33], drugs [34,35] or targeting probes [36] have been incorporated with UCNPs to regulate intracellular gene expression and achieve light-activated cancer therapy, and incorporation of UCNPs with photodegradable polymers has been witnessed a rapid growth for drug delivery [37–39] and controllable macromolecules release [40–42].

Generally, the UCNPs and photocleavage sites are randomly distributed in the photodegradable polymers [37,39–41], which leads to a long travel distance of the upconverted UV light to reach all photocleavable cross-links, therefore lowers the utilization efficiency of light for intracellular application. To improve the photocleavage efficiency, here a photocleavable linker (PhL) was synthesized to covalently link polyethylene glycol (PEG) polymer to mesoporous silica coated UCNPs for design of a photo-tearable polymer "tape" and controllable release of siRNA from UCNP nanocapsules (Scheme 1a). The siRNA could be conveniently loaded on the surface of PhL modified silica *via* electrostatic adsorption, and the subsequent PEG encapsulation effectively extended the circulation time [43] and protected the loaded cargos from leakage and degradation. Upon irradiation at 980 nm, UCNPs produced two emissions at 345 and 365 nm to cleave the PhL and thus tore off the polymer tape to release siRNA intracellularly. However, this PEGylated system lacked the ability of membrane disruption for endosomal escape of siRNA to perform its gene silencing function. Thus a photosensitizer, hypocrellin A (HA), was loaded in the pores of mesoporous silica, which was activated by the upconverted emissions of UCNPs at 455 and 475 nm and generated reactive oxygen species (ROS). The ROS not only disrupted the endosomal membrane and liberated siRNA into cytoplasm (Scheme 1b) to boost the gene therapy efficiency, but also destructed cellular organelles to assist cell apoptosis. The designed photo-tearable tape-wrapped UCNP nanocapsules provided a universal strategy for efficient NIR-assisted gene therapy.



Scheme 1. Schematic illustrations of (a) synthesis of UCNP nanocapsules, and (b) folate receptor-mediated cellular uptake and NIR modulated intracellular siRNA delivery and therapy.

2. Materials and methods

2.1. Materials and apparatus

Anhydrous yttrium chloride (YCl_3) (99.9%), anhydrous ytterbium chloride ($YbCl_3$) (99.9%) and anhydrous thulium chloride ($TmCl_3$) (99.9%) were purchased from Sigma-Aldrich (USA). Sodium hydroxide, ammonium fluoride, cyclohexene, oleic acid (OA), 1-octadecene (ODE), (3-aminopropyl) triethoxysilane (APTES), cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), Cu(II) sulfate, L-ascorbic acid, potassium carbonate (K_2CO_3), N, N′-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), propargyl bromide, ethyl acetate (EtOAc), sodium sulfate (Na_2SO_4), petroleum ether, tetrahydrofuran (THF), triethylamine (Et_3N), methanol (MeOH), sodium borohydride ($NaBH_4$), 5-hydroxy-2-nitro-benzaldehyde (HNB), N, N′-disuccinimidyl carbonate (DSC), 1,3-diphenylisobenzofuran (DPBF) and acetonitrile (MeCN) were purchased from Aladin Ltd (Shanghai, China). 4-Dimethylaminopyridine (DMAP) was purchased from Adamas Reagent, Ltd. (Shanghai, China). Hypocrellin A (HA) was purchased from Biopurify (Chengdu, China). Four-armed polyethylene glycol modified with azido (PEG) (MW 2000) and two-armed polyethylene glycol modified with both azido and folate (PEG(FA)) or FITC (PEGF) (MW 2000) were purchased from Toyongbio (Shanghai, China). LysoTracker Red was purchased from Invitrogen (Carlsbad, CA, USA). Annexin V-FITC apoptosis detection kit and dihydroethidium were purchased from Keygen Biotech (Nanjing, China). RNA extraction kit, PrimeScript RT reagent kit and SYBR premix EX Taq kit were purchased from Takara (Dalian, China). PLK1 ELISA kit was purchased from Jin Yibai Biological Technology (Nanjing, China). PLK1 siRNA was purchased from GenePharma Ltd (Shanghai, China). The siRNA sequences were as follows:

siRNA: 5′-UGAAGAAGAUCACCCUCCUUAdTdT-3′,
siRNA-FAM: 5′-FAM-UGAAGAAGAUCACCCUCCUUAdTdT-3′,
siRNA-Cy5: 5′-Cy5-UGAAGAAGAUCACCCUCCUUAdTdT-3′,
Antisense: 5′-UAAGGAGGGUGAUCUUCUUCA-3′,
BHQ-antisense: 5′-UAAGGAGGGUGAUCUUCUUCA-BHQ-3'.

Transmission electron microscopic (TEM) images were captured on JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Dynamic light scattering (DLS) was conducted on ZetaPlus 90 Plus/BI-MAS (Brook haven, USA). Zeta potential analysis was conducted on Nano-Z Zetasizer (Malvern, UK). The UV–Vis absorption spectra were performed with Nanodrop-2000C UV–Vis spectrophotometer (Nanodrop, USA). Flow cytometric analysis was conducted on Coulter FC-500 flow cytometer (Beckman-Coulter, USA). The fluorescence spectra were acquired on F-7000 spectrofluorophotometer (HITACHI, Japan). The cell images were obtained on TCS SP5 confocal laser scanning microscope (CLSM) (Leica, Germany). Upconversion luminescence (UCL) spectra were collected on ZolixScan ZLX-UPL spectrometer with an external continuous-wave laser (980 nm) as the excitation source. MTT and ELISA assays were conducted on Hitachi/Roche System Cobas 6000