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Near infrared light-mediated photoactivation of cytotoxic Re(I) complex by lanthanide-doped upconversion nanoparticles †

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Platinum-based chemotherapy, although has been well proven effective in the battle against cancer, suffers from limted specificity, severe side effect and drug resistance. The development of new alternatives with potent anticancer effect and improved specificity is therefore urgently needed. Recently, there are some new chemotherapy reagents based on photoactive Re(I) complexes have been reported as promising alternatives to improve specificity mainly attributed to the spatial and temporal activation process by light irradiation were reported. However, most of them respond to short wavelength of light (e.g. UV, blue or green light), which may cause unwanted photo damage to cells. Herein, we demonstrated a system for near-infrared (NIR) light controlled activation of Re(I) complex cytotoxicity by integration of photoactivatable Re(I) complex and lanthanide-doped upconversion nanoparticles (UCNPs). Upon NIR irradiation at 980nm, the Re(I) complex can be locally activated by upconverted UV light emitted from UCNPs and subsequently leads to enhanced cell lethality. Cytotoxicity studies showed effective inactivation of both drug susceptible human ovarian carcinoma A2780 cells and cisplatin resistant subline A2780cis cells by our UCNPs based system with NIR irradiation, and there was minimum light toxciity observed in the whole process, suggesting that such system could provide a promising strategy to control localized activation of Re(I) complex and therefore minimize the potential side effects.

1 Introduction

Transition metal based antitumor complexes, especially platinum drugs such as cisplatin, oxaliplatin and carboplatin have been studied for their abilities in cancer treatment for almost half a century.[1] Some of these complexes have gained worldwide applications in chemotherapy against various types of cancers. However, the use of these metal complexes in treatment process has been mostly associated with severe side effects such as limited tumor specificity, intrinsic or acquired multi-drug resistance.[2] Therefore, the rational design through the development of new alternatives with potent anticancer effect and improved specificity will be thus the main concern, and extensive studies have been carried out so far. [3-16] Recently, some photoactivatable Re(I) complexes which demonstrated the great potential to inactivate tumor growth based on increased tumor recognition have been investigated as possible candidates in chemotherapy.[17-22] In these cases, light irradiation, which has impressively high spatiotemporal resolution, is required to activate the complexes for enhanced cytotoxicity within targeted tumor area. Despite their initial success, most of these photoactivatable Re(I) complexes are triggered by short-wavelength of light, such as UV, blue and green light. The application of short-wavelength light may cause undesired damage of tissue, protein and DNA.[23] In addition, limited penetration depth in tissue making it difficult to reach tumors which locate beneath skin surface.[24] Therefore the application of short wavelength light in activating Re(I) complexes for better antitumor effect could be largely restricted. As comparison, long-wavelength light irradiation has been considered to cause less photo-damage and have deeper tissue penetration, making it more suitable for applications in biological system. Therefore, considering the potential significance of photoactive Re(I) complexes in antitumor treatment, the rational design of a simple yet specific strategy which allows activation of Re(I) complexes by long-wavelength light selectively in tumor region with minimum biological damage is highly desirable, and relevant investigations toward this direction still need to be further conducted[25]. Recently, Lanthanide-doped upconversion nanoparticles (UCNPs) as a promising tool to gain precise control of light irradiation, have drawn much attention for its application in sensing[26-28], imaging[29-33], drug delivery[34-41],

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phototherapy[42-47] and theranostic[48-51] because of its unique optical properties. Generally, UCNPs can absorb long wavelength near infrared (NIR) light and give out multiple emission ranging from UV to visible and even NIR region[52-58]. Such appealing photo-physical property enable the local activation of photosensitive metal complexes for therapeutic purpose by remotely controlled long-wavelength light irradiation which is suitable for living system[16, 43, 44, 49, 59-71]. For example, the successful NIR photoactivation of Pt metal drug complexes has been reported effective in suppressing the viability of cancer cells and inhibiting the tumor growth in living animals.[16, 44, 49, 71] Similarly, UCNPs have also been utilized for NIR photoactivation of Ru complex to control the release of antitumor drug.[61, 63, 65, 67, 69] Moreover, some other transition metal complexes such as Zn, Fe, Cr and Mn have also been reported for the purpose of photodynamic therapy or the light-mediated delivery of gaseous molecules such as NO and CO.[43, 59, 62, 64, 66, 68, 70] Inspired by these pioneer studies, herein, we demonstrate a system for NIR light controlled activation of Re(I) complex cytotoxicity by combining photoactivatable Re(I) complex with lanthanide-doped upconversion nanoparticles. Upon NIR irradiation, the Re(I) complex can be locally activated by upconverted UV light emitted from UCNPs and subsequently leads to enhanced cell lethality, therefore minimizing the potential side effect.

2 Experimental Section

2.1 Instruments and general methods

^{1}HNMR spectrum was recorded on a Bruker AVANCE AV 300MHz spectrometer. ESI-MS spectrum was acquired on a Thermo LCQ DECA XP Liquid Chromatography-Mass Spectrometer. UV-VIS spectra were recorded on a SHIMAZU UV-1800 UV spectrophotometer. Fluorescence spectra were obtained on a SHIMAZU RF-5301PC spectro-fluorophotometer. The fluorescence emission spectra of UCNPs was recorded at an angle of 90° to the excitation laser (980 nm) and an optical SEC-2000 spectrometer coupled 2048 pixels CCD array (ALS Co., Ltd). Transmission electron microscope (TEM) images were recorded using a FEI EM208S TEM (Philips) operated at 100 kV. Dynamic light scattering (DLS) measurements were performed by Brookhaven 90 plus Nanoparticle Size Analyzer. Rhenium content was measured on an Agilent 7700 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Cell imaging was carried out on a fluorescence microscope (Nikon, Eclipse TE2000-E).

2.2 Synthetic procedure of [Re(DIP)(CO)$_3$(cpy)](PF$_6$):

Typically, the starting material, [Re(DIP)(CO)$_3$Cl], was prepared according to a literature procedure.[72] The prepared molecule was then converted to [Re(DIP)(CO)$_3$](OTf). Basically, AgOTf (0.235 mmol) was added to a suspension of [Re(DIP)(CO)$_3$Cl] (0.157 mmol) in 50 mL of CH_3CN. The mixture was refluxed under nitrogen overnight in the dark. After removed off-white AgCl precipitate, CH_3CN was evaporated under reduced pressure. The resulting solid was dissolved in 50mL of THF containing 3-(Hydroxymethyl)pyridine (1.5 mmol) and refluxed under nitrogen atmosphere for 20h in the dark. The mixture was then evaporated to dryness and the solid obtained was further dissolved in dichloromethane with 5mL of thionyl chloride and heated to reflux under nitrogen atmosphere for 5h. After removal of dichloromethane and excess amount of thionyl chloride by nitrogen flush, the resulting yellow solid was dissolved in CH_3CN and added dropwise into saturated NH_4PF_6 solution. The yellow precipitate was collected and washed with distilled water and diethylether followed by recrystallization from diethylether and dried under vacuum to afford the final product. (Yield: 77%) ^{1}H NMR (300 MHz, DMSO) δ 9.85 (d, *J* = 5.4 Hz, 2H; H of Ph_2-phen), 8.68 (s, 1H; H of pyridine), 8.52 (d, *J* = 4.9 Hz, 1H; H of pyridine), 8.22 (d, *J* = 5.4 Hz, 2H; H of Ph_2-phen), 8.14 (s, 2H; H of Ph_2-phen), 7.99 (d, *J* = 8.1 Hz, 1H;H of pyridine), 7.69 (s, 10H; C_6H_5 at Ph_2-phen), 7.42 (t, *J* = 7.9, 5.7 Hz, 1H;H of pyridine), 4.70 (s, 2H; CH_2). ESI-MS: m/z 729.9 [M-PF_6^-]$^+$ Elemental Analysis (%) Calcd: $C_{33}H_{22}ClF_6N_3O_3PRe{\cdot}5H_2O$: C,41.06; H,3.34; N,4.35; C/N,9.41. Found:C,39.79; H,2.97; N,4.11; C/N,9.68.

2.3 Preparation of NaYF$_4$:Yb^{3+}/Tm^{3+} core nanoparticles

$NaYF_4$:Yb^{3+}/Tm^{3+} core nanoparticles were synthesized according to the method reported previously.[73] In general, 1mmol of RE(CH_3 CO_2)$_3$ (RE= 59.5% Y + 40% Yb + 0.5% Tm), 12 mmol of NaF, and 20mL of oleic acid (OA)/1-octadecene (ODE) (v/v = 1:1) mixed solvent were added to the round bottom flask and heated to 110°C under vacuum with magnetic stirring for 30 min to remove residual water and oxygen. Then, the temperature was further increased to 320°C and kept for 1.5h under nitrogen atmosphere. The resulting UCNPs were obtained by centrifugation for the following application.

2.4 Fabrication of NaYF$_4$:Yb^{3+}/Tm^{3+}@NaYF$_4$ core-shell nanoparticles

A cyclohexane solution of as-prepared $NaYF_4$:Yb^{3+}/Tm^{3+} core nanoparticles (200mg) and 20mL mixture of OA and ODE (OA/ODE=v/v=1:1) were added to a round bottom flask and heated to 120°C under vacuum with magnetic stirring for 1h and flushed with nitrogen. Then, the temperature was further heated to 310°C. The mixture of 3mL of OA and ODE (OA/ODE= v/v = 1:1), Y(CH_3CO_2)$_3$ (1mmol) and Na(CH_3CO_2) (1mmol) were added to the solution immediately. The reaction was kept at 310°C for 1h under nitrogen atmosphere. The resulting core-shell UCNPs were isolated by centrifugation for the further surface modification.

2.5 Synthesis of PAA-functionalized UCNPs (UCNP-PAA)

A ligand exchange process was performed using poly(acrylic acid) (PAA) to replace the original hydrophobic ligands on the nanoparticle surface.[74] PAA (50mg) was dissolved in ethanol (5mL) and mixed with 2mL of as-prepared core-shell UCNPs dispersion in chloroform (20mg). The resulting mixture was kept overnight with magnetic stirring. The solution was then centrifuged at 2 × 10^4 rpm for 10 minutes. The precipitate was

washed 3 times with ethanol to obtain the UCNP-PAA particles.

2.6 Surface coating of chitosan

The reagents EDC (28.7mg) and NHS (11.6mg) were added to an aqueous dispersion (10mg, 2.5mL) of as-prepared UCNP-PAA particles to activate carboxyl group on UCNP-PAA for 30min. Then an aqueous solution of chitosan (4mg/ mL, 2.5mL) was added and kept overnight with magnetic stirring. The solution was then centrifuged at 2×10^4 rpm for 10 minutes. The precipitate was washed 3 times with deionized water to obtain the UCNP-PAA-chitosan (UPC) particles.

2.7 Loading of Re(I) complex on UPC:

The previously prepared Re(I) complex (0.8mg) was dissolved in CH_3CN 20μL to give a yellow solution. The resulting solution was added into PBS (1mL, 10mM, pH7.4) containing UCNP-PAA-chitosan particles (5.2mg). The mixture was then stirred overnight. The resulting Re(I) loaded UCNPs (Re-UPC) was obtained by centrifugation at 2×10^4 rpm for 10 minutes and washed with PBS/CH_3CN (20:1) three times to remove excess amount of the Re(I) complex. Loading of the Re(I) complex was determined by both fluorescence spectroscopy and ICP-MS method.

2.8 Stability evaluation of Re-UPC:

The Re-UPC (50uM) were incubated with PBS (10mM, pH 5.0 or 7.4) at 37 °C of different time intervals. Released Re(I) complex from Re-UPC was obtained by centrifugation at 2×10^4 rpm for 10 minutes. The amount of released Re(I) complex in the resulting supernatant was measured by fluorescence spectroscopy.

2.9 Cellular uptake

Cellular uptake was investigated by using fluorescence microscope. Drug susceptible human ovarian carcinoma A2780 cells or cisplatin resistant human ovarian carcinoma A2780cis cell were seeded at a density of 10^5 cells per well in a 35mm u-dish (ibidi) containing RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS, HyClone) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) at 5% CO_2 and 37 °C for 24 hours prior to treatment with Re-UPC or Re(I) complex. After 2 hours incubation, the media were removed and cells were washed. The images were acquired on fluorescence microscope (Nikon Eclipse TE2000) with the excitation filter at 364nm.

2.10 Cell viability assay

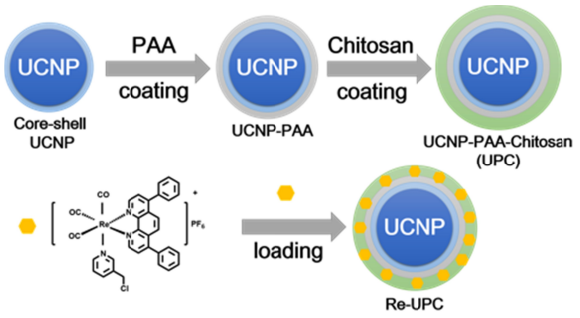
In cell viability assay, cells were seeded in 96-wells plates with a density of 10^4 cells per well. After 24 hours of incubation at 5% CO_2 and 37°C, the medium was replaced with fresh medium containing Re(I) complex or UPC, Re-UPC. After another 24-hour incubation at 37°C, culture media were removed, cells were washed and then incubated with 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) containing culture media. After 5 hours incubation, media were removed and 100μL of DMSO was added. The absorbance at 570 nm was measured by a Tecan's Infinite M200 microplate reader. For NIR light irradiation experiment, cells were incubated with Re-UPC for 2 hours. Subsequently, the drug-containing medium was replaced with fresh medium and cells were irradiated with NIR (980nm, 1.5W/cm^2, 5min break after 5min irradiation). After 24 hours of incubation cell viability was evaluated by standard MTT assays. In phototoxicity tests, cells were exposed to NIR for different period of time followed by 24 hours incubation and MTT assays to investigate cell viability.

3 Results and discussion

3.1 Synthesis and characterization of Re-UPC:

Scheme 1 illustrates the design of NIR-mediated activation of Re(I) complex on chitosan-coated UCNPs (UCNP-PAA-chitosan). The Re(I) complex was obtained through a facile synthesis by refluxing a mixture of $[Re(DIP)(CO)_3(CH_3CN)](PF_6)$ and 4-(Chloromethyl)pyridine in THF under nitrogen atmosphere. The compound was characterized by ^{1}HNMR and ESI-MS (Fig.S1 and S2†). To establish our platform, first, $NaYF^4$:Yb^{3+}/Tm^{3+} core nanoparticles were prepared using thermal decomposition method, followed by coating of the $NaYF_4$ shell on the surface to afford the core-shell UCNPs. TEM images (Fig.S3†) demonstrate that, the as-prepared core nanoparticles have a narrow size distribution of around 25nm. After coating with $NaYF_4$, the size of particles increased to about 35nm. The as-prepared core-shell particles are terminated with oleic ligands which is hydrophobic. To improve the solubility of particles in water, the core-shell UCNPs were transfer from nonpolar solvent to aqueous environment by using poly(acrylic acid) (PAA) as functional ligands to replace the hydrophobic oleic ligands.

Moreover, to facilitating the loading of Re(I) complex, as well as biocompatibility of the nanoparticles, carboxyl groups of PAA on the PAA-coated UCNPs (UCNP-PAA) were activated using EDC/NHS method, and followed by further conjugation with amine groups on amphiphilic chitosan structures. The size of the obtained PAA-coated UCNPs and chitosan-coated UCNPs were determined by dynamic light scattering (DLS) and transmission electron micrographs (TEM). As given in Fig.1, the obtained UCNPs (UCNP-PAA and UCNP-PAA-chitosan) have good mono-dispersity and uniform morphology. The inner core-shell structures of both UCNP-PAA and UCNP-PAA-chitosan were ~35nm. The hydrodynamic diameter distribution, measured by



Scheme.1 Schematic representation for the synthesis of Re(I) complex loaded UCNPs (Re-UPC).

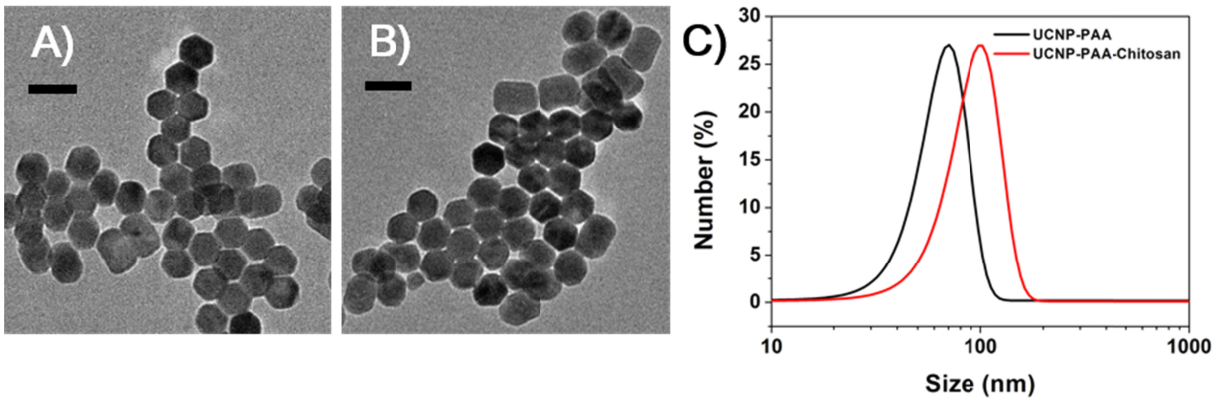


Fig.1 Characterization of prepared nanoparticles. A) and B) TEM images of PAA-coated UCNPs (UCNP-PAA) and chitosan-coated nanoparticles (UCNP-PAA-Chitosan, UPC), respectively. Scale bar =50nm. C) Size distributions of the prepared UCNP-PAA and UCNP-PAA-Chitosan (UPC) determined by DLS.

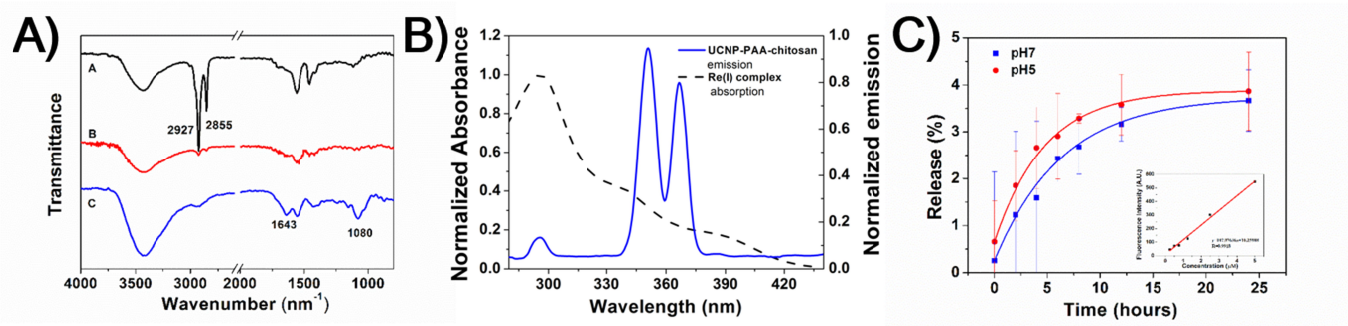


Fig.2 A) FT-IR spectra of core-shell UCNP (black), UCNP-PAA (red) and UCNP-PAA-Chitosan(UPC) (blue). B) Absorbance spectra of the Re(I) complex (black dash line) and emission spectra of UPC (blue line, λ_{ex}=980nm). C) Cumulative release of Re(I) complex from Re-UPC at 37°C in PBS buffer. pH5.0 (red) and pH7.4 (blue). Inset: the calibration curve used to determine the concentration of released Re(I) complex.

DLS is ~76nm for UCNP-PAA and ~118nm for UCNP-PAA-chitosan, respectively. In addition, the successful coating of PAA and chitosan was further confirmed by Fourier transform infrared (FT-IR) spectra. As shown in Fig.2A disappearance of vibration of -CH_3 at 2927 cm^{-1} and 2855 cm^{-1} suggests the successful removal of oleic acid from particle surface. Further conjugation of chitosan on the particle surface can be confirmed by peaks situated at 1080 cm^{-1} and 1643 cm^{-1} corresponding to the C-O stretching and formation of amide between carboxyl group on PAA and amine group on chitosan. Moreover, spectroscopy studies indicated that the absorption of the Re(I) complex overlaps the emission of UCNP-PAA-chitosan (Fig.2B and Fig.S4†) at 291nm ($^1I_6$-$^3H_6$), 350nm ($^1I_6$-$^3F_4$) and 365nm ($^1D_2$-$^3H_6$), suggesting that the as-prepared UCNP-PAA-chitosan can be utilized for activation of the Re(I) complex. Next, to afford the final product Re-UPC (Re(I) complex loaded UCNP-PAA-chitosan), the Re(I) complex was dissolved in aqueous solution (PBS/CH_3CN =50:1) in which UCNP-PAA-chitosan (UPC) was suspended to facilitate the incorporation of the complex onto the particles surface (UPC). Loading of the Re(I) complex was confirmed (Fig.S5†) by fluorescence emission of Re complex on Re-UPC. Loading amount of the complex was quantified by fluorescence spectroscopy and ICP-MS analysis. The amount of the complex

on Re-UPC was determined to be around 6.14 wt %, which is comparable to the value in the nano-platforms reported previously.[75] Furthermore, in order to exam the stability of Re-UPC under biological relevant condition, the possible release of the Re(I) complex was evaluated. In general, the aqueous suspensions containing Re-UPC were incubated at 37°C (pH 5.0 and 7.4) for different time duration within 24 hours. The release of Re(I) complex was quantified by measuring fluorescence intensity of the supernatant after centrifugation.[17] As shown in Fig.2c, there was no obvious release of the Re complex observed under different pH conditions (e.g. pH 5.0 and pH 7.4) in PBS buffer at 37°C, suggesting that loading of Re complex is stable under both conditions.

3.2 Cellular uptake monitored by fluorescence microscopy:

To evaluate the cellular uptake of Re-UPC, fluorescence imaging study was carried out. In this typical study, A2780 cell, a well characterized cisplatin susceptible human ovarian carcinoma cell line, and its cisplatin resistant subline, A2780cis were chosen as the target cells. Both cells were incubated with Re-UPC (10μM) in the dark for 2 hours before fluorescence imaging. As shown in Fig.3 and Fig.S6† the green fluorescence signal of the Re(I) complex loaded on Re-UPC was observed within both cell lines, indicating the successful internalization of Re-UPC into cells. To further investigate the possible mechanism of cellular uptake of Re-UPC, both type of cells were treated with Re-UPC (10μM) at 4°C in the dark for 2 hours. It can be observed that (Fig.3 and Fig.S6†) at lower temperature (~4°C), there was little green fluorescence inside cells, suggesting the inhibited internalization of Re-UPC. These results indicated that the uptake of Re-UPC followed a temperature dependent endocytotic pathway.[76] Moreover, the uptake of free Re(I) complex was investigate as control. It can be observed that the fluorescence intensity of Re-UPC inside cell is much higher than that of free complex (Fig.S6and S7†), demonstrating more cell uptake via Re-UPC. Moreover, it suggested that UPC can serve as a reliable platform for effective delivery of Re(I) complex, which can potentially lead to better antitumor effect in vitro and in living cells.

3.3 Cytotoxicity Assay upon Light Irradiation:

Moreover, we evaluated the cytotoxicity of our system in living cells. Both drug susceptible human ovarian carcinoma A2780 cells and its cisplatin resistant subline A2780cis were chosen as target cell lines. Basically, the two types of cells were treated with Re-UPC and 1 hour of NIR irradiation (980nm) followed by 24 hours incubation before cell viability assays. Similar cellular incubation in the absence of NIR irradiation was used as control.

Tumor cells alone and cells treated with UPC were irradiated under NIR to assess the potential phototoxicity. As indicated in Fig.4A and Fig.4B, no significant toxicity was detected in Re-UPC treated A2780 cells and A2780cis cells without NIR irradiation. However, when cells were treated with Re-UPC and 1h of NIR irradiation (at 980nm) more potent cytotoxicity could be achieved. Cell viability of A2780 cells dramatically decreased from 89% to around 55% (0.5μM) exhibiting enhanced cytotoxicity of Re-UPC upon NIR irradiation (Fig.4A). Similarly, improved cell lethality was also achieved in cisplatin resistant cell line A2780cis. There was 47% cell viability observed in A2780cis cells treated with Re-UPC (1μM) and NIR irradiation, which was much lower than (~ 74%) the similarly treated cells without NIR activation (Fig.4B). These results clearly suggested that NIR irradiation of Re-UPC is essential for activated toxicity.

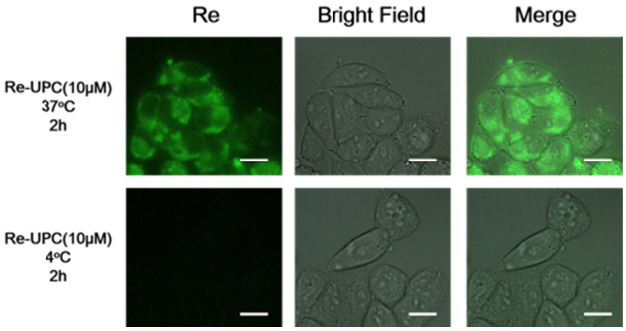


Fig.3 Fluorescence microscope images of A2780 cells incubated with Re-UPC (10μM) at 37°C and 4°C for 2h, λ_{ex}=364nm. All scale bars are 10μm.

In addition, the light-mediated cytotoxicity in both A2780 and A2780cis cell lines exhibited dependence on concentration of Re-UPC and duration of NIR irradiation. In particular, the increased cytotoxicity could be achieved when the higher concentration of Re-UPC was applied. As indicated in Fig.4A and Fig.4B, under 1h NIR light irradiation, Re-UPC (0.5μM) was found to exhibit obvious cytotoxicity against both A2780 cells and A2780cis cells, and there were 55% and 86% cell viability detected respectively. Moreover, the more potent cell lethality (e.g. 32% and 47% cell viability) of A2780 and A2780cis cells was observed when the higher concentration of Re-UPC (1μM) was used. Similarly, the more significant cell lethality could be also observed when the prolonged NIR irradiation was applied. It can be observed that (Fig.4C and Fig.4D) cell treated with 0.5μM of Re-UPC under 2h NIR irradiation exhibited more distinct toxicity towards A2780 and A2780cis cells with cell viability of 40% and 59% respectively than that under 1h NIR illumination. The potent NIR-triggered cytotoxicity in both A2780 and A2780cis cells demonstrated that Re-UPC can be successfully used in activating the Re(I) complex for effective inactivation of both drug susceptible and cisplatin-resistant tumor cells. As control, the similar free Re(I) complex under UV irradiation also exhibited photoactivatable cytotoxicity which was found to be dependent upon the concentration of the complex and duration of UV irradiation (Fig.S8A and S8B†). Although photoactivated cytotoxicity can be achieved by both NIR and UV irradiation, NIR activated Re-UPC showed slightly better antitumor effect in comparison to free Re(I) complex irradiated by UV light. For instance, A2780 cells incubated with 1μM of Re-UPC followed by 1h NIR irradiation result in more obvious toxicity (32% cell viability, Fig.4A) than cells treated with 1μM of Re(I) complex and 5min UV illumination (43% cell

viability, Fig.S8A†) Similarly in A2780cis cell line, 1μM of Re-UPC with 1h NIR irradiation caused more cell death (47% cell viability, Fig.4B) than 1μM of Re(I) complex and 5min UV illumination (60% cell viability, Fig.S8B†). Despite the fact that it is possible to obtain improved cytotoxicity of Re(I) complex with prolonged UV irradiation, overexposure to UV light may cause drastic photo-damage to cells (Fig.S8C and S8D†). In contrast, more potent cytotoxicity of Re-UPC can be achieved by prolonged NIR irradiation (Fig.4C and Fig.4D) without obvious cell lethality. Therefore, these

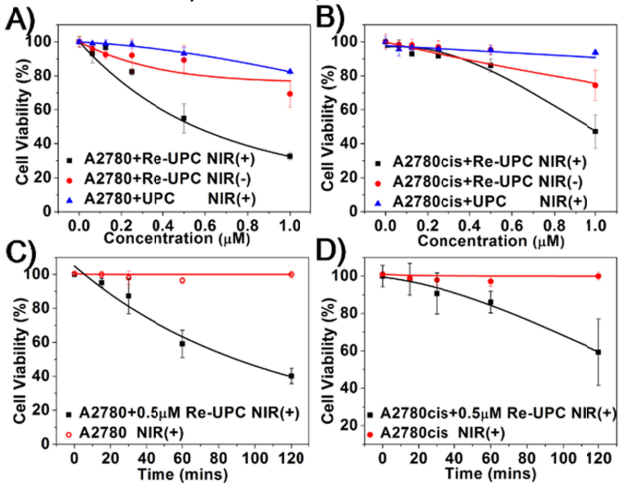


Fig.4 The cytotoxicity assays of the Re-UPC (black) and UPC (blue) at different concentration with 1 h of NIR irradiation (1.5 W/cm^2) in A) A2780 and B) cisplatin-resistant A2780cis cells. The cells treated with the Re-UPC but without light illumination were used as controls (red). C) A2780 cells and D) A2780cis cells under different exposure time of NIR with treatment of Re-UPC (0.5μM, black) or without Re-UPC (red).

results suggested that the combination of UCNPs and photoactivatable Re(I) complex of cytotoxicity for inhibition of targeted tumor cells in both drug susceptible cells and cisplatin resistant cells. Moreover, the use of NIR illumination, compared to UV light, can largely reduce photo-damage to cells.

Furthermore, to study the mechanism of photoactivated cytotoxicity, N,N-dimethyl-4-nitrosoaniline (RNO) imidazole assay was carried out. In general, PBS solution containing RNO, imidazole and the Re(I) complex or Re-UPC were irradiated in fluorescence quartz cuvettes. Absorbance of N,N-dimethyl-4-nitrosoaniline at 440 nm was monitored by UV-VIS spectrometer. As indicated in Fig.S9†, compared to sample without light irradiation, an obvious decrease in the absorbance of RNO was observed when Re(I) complex was activated by UV. Similarly, Re-UPC under NIR irradiation also caused decreased absorbance of RNO, suggesting that the photo-activated cytotoxicity observed was probably associated with ROS generated during photoactivation.[19, 77] In addition, to better understand the feasibility concerning photoactivation of the Re(I) complex, the further studies have also been carried out to investigate the possible ligand dissociation of the complex before and after light treatment.[17, 78, 79] Typically, the myoglobin assay[77] and NMR analysis were performed to study the photochemistry of the rhenium (I) complex. By following standard Myoglobin assay to determine CO release, a solution containing 50μM myoglobin(Mb), 10mM dithionite and 15μM of the Re(I) complex in PBS solution was prepared and irradiated by UV light (8.9mW/cm^2). The possible CO dissociation was tracked by monitoring the absorbance of Mb at 557nm.[80, 81] Compared to sample without photoactivation (in Fig.S11†), the decreased absorbance band at 557nm of Mb was observed upon light irradiation. Meanwhile, two bands of MbCO adduct also appeared at around 540nm and 577nm. Similar spectra changes were also observed when Re-UPC conjugate was irradiated under NIR light (Fig.S12†). These results indicated the conversion of Mb to MbCO, suggesting the possible dissociation of CO from the complex upon light irradiation.[80] Moreover, although the detailed mechanism remains under further investigation, the further NMR analysis demonstrated that there are no observable peaks found corresponding to the control molecule (e.g. free pyridine) after light irradiation (Fig.S13†), suggesting that the pyridine ligand was most likely kept in the Re complex and there was no obvious dissociation of pyridine from the whole molecule structure.[63, 82]

Conclusions

In summary we have demonstrated upconversion nanoparticle-based system for NIR activated cytotoxicity of Re(I) complex. Typically, UCNPs can be used to activate the complex with upconversion luminescence triggered by NIR. The system can be effectively uptake by tumor cells via endocytosis. In addition, upon NIR irradiation, the locally activated drug exhibited enhanced cytotoxicity against both drug susceptible A2780 cells demonstrating that NIR irradiation combined with our system can achieve potent photoactivated cytotoxicity while minimizing unwanted photodamage to cells. Therefore, such combination of UCNPs and photoactivatable drug can provide a promising strategy to remotely control the activation of cytotoxicity to inhibit targeted tumor cells growth in both drug susceptible cells and cisplatin resistant cells and thus minimize the potential side effects.

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Near infrared light-mediated photoactivation of cytotoxic Re(I) complex by lanthanide-doped upconversion nanoparticles †

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