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Improving acceptor efficacy rather than energy transfer efficiency: Dominant contribution of monomers of acceptors modified on upconversion nanoparticles

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ABSTRACT

Upconversion nanosensitizers have been widely considered to have important applications in the treatment of major diseases such as tumors and the utilization of solar energy. Majority of the efforts so far have been focused on improving the efficiency of energy transfer (ET) between upconversion nanoparticles (UCNPs) and the anchored sensitizers with premise that high ET efficiency will lead to high acceptor efficacy. This premise is, however, proved by our current work to be invalid for commonly used load. Interaction between adjacent sensitizing molecules was found to be critical which undermines the amount of excited monomer sensitizers and thus fades the efficacy. Here NaYF4:Yb3+,Er3+ UCNPs and rose bengal (RB) photosensitizer molecules were used as the model energy donors and acceptors, respectively. Contrary to monotonous increase of the ET efficiency from UCNPs to RB species with increasing RB loading, acceptor efficacy characterized by the reactive oxygen species, as well as the RB fluorescence, exhibits bizarre dependence on the RB loading. The underlying mechanism was well studied by the steady-state and time-resolved spectroscopy of a series of samples. RB aggregates are believed to be responsible for the severe deviation between the ET efficiency and acceptor efficacy. The conclusion was validated by in vitro test where the photodynamic therapy with the most monomer RB in UCNPs-RB nanosensitizers kills 35.8% more cells than that with the highest RB loading. This understanding sheds light on construction of new ET based nanosystems for broad applications, such as medicine, solar energy utilization and optical storage.

1. Introduction

Rare earth-doped upconversion nanoparticles (UCNPs) have aroused considerable attention in recent years due to their superiority in the large anti-Stokes shift, the strong resistance to photobleaching, the deep penetration of near-infrared (NIR) excitation light to biotissues and the absence of background fluorescence interference.1–3 In their vast applications, such as biomedical fields and photonic devices, energy transfer (ET) plays a significant role.4–9 The UCNPs generally act as the energy donors by transferring the upconverted excitation energy to their adjacent energy acceptors, diverse functions of the energy acceptors, such as fluorescing, generating heat and undergoing photochemical reactions, are realized.8–12 The construction of highly efficient UCNPs-based ET systems is of crucial importance for applications.
Up to now, great efforts have been devoted to improving ET efficiency, which is generally characterized by the quenching of the upconversion luminescence (UCL) and/or the shortening of its decay lifetime.\textsuperscript{2,3,10} The commonly used optimizing strategies include the bare-core design of the UCNPs to shorten the donor–acceptor distance, the optimization of the spectral overlap between the emission of the UCNPs and the absorption of the energy acceptors, and so forth.\textsuperscript{12–14} However, for application, the efficacy exhibited by the energy acceptors is of great interest rather than the ET efficiency. Typical examples include the photodynamic killing effect of the photosensitizer acceptors in UCNPs-photosensitizer therapeutic nanoplatforms,\textsuperscript{15} the NIR responsiveness of photochromic materials in optical storage devices,\textsuperscript{10} and the emission intensity of the acceptor fluorophores in UCNPs-based fluorescent sensors.\textsuperscript{16} In order to obtain high acceptor efficacy, people usually adopt the way of increasing the loading amount of the acceptors, such as through introducing excessive acceptors when constructing the ET systems.\textsuperscript{17–19} Such strategies are based on the premise that high ET efficiency will monotonously lead to high acceptor efficacy. The scope of validity of the premise, however, is not rigorously established. Concomitant effects like aggregation of the acceptors are not well explored, which impedes designing and optimizing ET based functionalized upconversion nanoplatforms.

Aiming to unravel this issue, a model ET system was constructed in this work, in which Na\textsubscript{3}YF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} UCNPs acted as the energy donor and a typical photosensitizer, rose bengal (RB), as the energy acceptor. It was found that high ET efficiency did not bring about high acceptor efficacy, including the RB fluorescence intensity and the amount of produced reactive oxygen species (ROS). The underlying mechanism was revealed based on the steady-state and temporal spectroscopic behaviors. Furthermore, the conclusions were validated by in vitro tests, in which photodynamic therapeutic efficacy of the UCNPs-RB conjugates on cancer cells exhibited the great impact of the RB monomers, rather than the RB loading, on RB performance.

2. Experimental

2.1. Materials and synthesis of Na\textsubscript{3}YF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} UCNPs

The Na\textsubscript{3}YF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} UCNPs with oleate ligand were synthesized through solvent-thermal method. The details of materials and the exhaustive synthetic procedures are in the Supporting Information (SI).

2.2. Coupling of the UCNPs with RB

The UCNPs were firstly modified with amino groups by two steps. In the first step, oleate ligand was removed from the surface of the UCNPs. Briefly, 1 mL of oleate-capped UCNPs (10 mg/mL) was mixed with 2 mL of HCl (0.05 mol/L). After stirring for 24 h vigorously, the UCNPs were transferred to water layer. Subsequently, the UCNPs were washed twice with isopropanol and dispersed in 2 mL of water. In the second step, the bare UCNPs were modified with poly (allylamine) (PAAM) solution. The reagent of PAAM (50 μL) was added into the UCNPs and stirred for 20 h. After being washed with water for several times, the PAAM-UCNPs were dispersed in water. Then, the UCNPs were coupled with RB photosensitizers covalently. Hexanoic acid ester (RB-HA), which was synthesized according to the reported protocol,\textsuperscript{20} was used to endow RB with carboxyl group. N-(3-dimethylaminopropyl)-N-ethylenediamine hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (NHS), and RB-HA with the mass ratio of 10:10:1 were firstly mixed in dimethyl formamide (DMF) and stirred for 2 h. Then, specified amounts of RB-HA (0, 10, 15, 30 and 50 μg) were mixed with 0.5 mL of PAAM-UCNPs (2 mg/mL), respectively. After continuous stirring for 24 h, the UCNPs-RB composites were collected by centrifugation and washed with dimethylsulfoxide for several times in order to remove the unbound RB-HA. The amount of RB loaded on the surface of the UCNPs was calculated according to the absorption spectra of the centrifugal supernatant. Its concentration was 0, 2.9, 4.8, 10.6 and 15.0 μmol/L, respectively. The number of RB molecules bound to each UCNP was calculated to be about 0, 120, 200, 400 and 600, respectively.

2.3. Characterizations

X-ray diffraction (XRD) pattern of the UCNPs was acquired using a Rigaku D/MAX diffractometer (Japan) with Cu K\textsubscript{α} radiation. The morphology of the UCNPs was characterized by a transmission electron microscope (TEM, JEM-2100 Plus, Japan). Fourier transform infrared (FTIR) transmittance spectra were recorded with a Nicolet IS10 spectrometer under the liquid nitrogen environment. Absorption spectra of RB were collected using a Shimadzu UV2600 spectrometer (Japan). Luminescence spectra of the UCNPs were recorded using a fluorescence spectrometer (Horiba (Photon Technology International) QuantaMaster/TimeMaster 400, USA). Steady-state UCL spectra were obtained under excitation of a 980 nm laser diode (Hi-Tech Optoelectronics Company Limited), and the temporal behaviors were recorded with a nanosecond tunable laser (Quanta-Ray LAB-170–100H/PR IMOSCAN/ULD-240). Temporal behaviors of RB emission were acquired using a transient fluorescence spectrometer (JY Horiba FluoroLog-3, France).

2.4. Detection of ROS

A chemical probe, 1,3-diphenylisobenzofuran (DPBF), was used to confirm ROS production by monitoring its absorption intensity at 417 nm. Typically, DMF solution of DPBF (20 μL, 10 mmol/L) was firstly mixed with 2 mL of the UCNPs-RB solution. The loading amount of RB is 0, 2.9, 4.8, 10.6 and 15.0 μmol/L, respectively. The mixed solution was then irradiated with NIR light (1 W/cm\textsuperscript{2}) for different time (5, 10, 15, 20, 25 and 30 min). The whole process was carried out in the dark.

2.5. Cytotoxicity of the UCNPs-RB

In order to evaluate the cytotoxicity of the UCNPs-RB, glioma cells (U87MG) were cultured in a constant temperature incubator containing 5 vol% CO\textsubscript{2} at 37 °C. The culture medium was Dulbecco’s modified eagle’s medium (DMEM) containing 100 μg/mL of streptomycin, 100 U/mL of penicillin and 10 vol% fetal blood serum (FBS). U87MG cells were firstly seeded into 96-well plates (1 × 10\textsuperscript{4} cells per well) and incubated for 24 h. After removing the culture medium, the UCNPs-RB with specified concentrations (0, 20, 50, 100, 200, 500 and 1000 μg/mL) in the culture medium were added to the wells, and the cells were cultured for another 4 h. Subsequently, 10 μL of cell counting kit-8 (CCK-8) reagent was added to each well. After incubating for 2 h, the absorbance at 450 nm was measured by a microplate spectrophotometer (Wellscan MK 3, Labsystems Dragon, USA).

2.6. Observation of the cells with and without UCNPs-RB conjugates attached

U87MG cells (1 × 10\textsuperscript{5} cells/mL) were added to the culture dishes with a silicon slide inside and cultured for 12 h to attach the cells on the silicon slides. For the observation of the cells with UCNPs-RB conjugates attached, the culture medium was replaced by the
UCNPs-RB conjugates (1000 µg/ml) dispersed in the culture medium, and further cultured for 4 h. Afterwards, both the cells with and without the UCNPs-RB conjugates attached were rinsed with phosphate buffered saline (PBS, 0.01 mol/L) for three times, and fixed with glutaraldehyde (2.5 vol%) at 4 °C for 2 h. The fixing agent was then removed, and the cells were rinsed with PBS (0.01 mol/L) for three times, followed by dehydration in ascending concentrations of ethanol. The cells without UCNPs-RB conjugates attached were prepared by dropping the culture medium containing the UCNPs-RB conjugates (1000 µg/ml) on the silicon slides. After drying naturally, the silicon slides with the UCNPs-RB conjugates were also rinsed with PBS for three times, and dehydrated in ascending concentrations of ethanol. The three samples were then characterized with a Quanta FEG-250 scanning electron microscope (SEM) and a fluorescence spectrometer configured with a 980 nm laser.

2.7. Intracellular ROS production of the UCNPs-RB

The ability of the UCNPs-RB to produce ROS intracellularly was investigated with U87MG cells by using a fluorescent reagent 2,7’-dichlorodihydrofluorescein diacetate (DCFH-DA). U87MG cells were seeded into 96-well plates (1 × 10^4 cells per well) and incubated for 24 h. And then, the U87MG cells were divided into 16 groups: (1) control group, (2) NIR light group, (3) RB + NIR light group, (4) UCNPs + NIR light group, (5) the UCNPs-RB (RB concentration is 2.9 µmol/L) + NIR light group, (6) the UCNPs-RB (RB concentration is 4.8 µmol/L) + NIR light group, (7) the UCNPs-RB (RB concentration is 10.6 µmol/L) + NIR light group, (8) the UCNPs-RB (RB concentration is 15.0 µmol/L) + NIR light group, (9) the UCNPs-RB (RB concentration is 2.9 µmol/L) group, (10) the UCNPs-RB (RB concentration is 4.8 µmol/L) group, (11) the UCNPs-RB (RB concentration is 10.6 µmol/L) group, (12) the UCNPs-RB (RB concentration is 15.0 µmol/L) group, (13) the UCNPs-RB (RB concentration is 2.9 µmol/L) + 532 nm light group, (14) the UCNPs-RB (RB concentration is 4.8 µmol/L) + 532 nm light group, (15) the UCNPs-RB (RB concentration is 10.6 µmol/L) + 532 nm light group, and (16) the UCNPs-RB (RB concentration is 15.0 µmol/L) + 532 nm light group. The fresh culture medium containing RB, the UCNPs and the UCNPs-RB (1000 µg/ml) were used to stain the cells for 20 min. Then, the cells were washed with PBS for three times. The cells were finally observed with a fluorescent microscope.

2.9. Statistical analysis

The data were expressed as mean ± standard deviation. Student’s t-test was used to evaluate the statistical significance. *p < 0.05, **p < 0.01, and ***p < 0.001 were considered to be statistically significant.

3. Results and discussion

3.1. Construction of ET system

The ET system is constructed by coupling typical NaYF₄:Yb³⁺,Er³⁺ UCNPs with a popular photosensitizing molecule RB. The UCNPs are of hexagonal phase and exhibit a uniform size of about 25 nm (Fig. S1). The coupling of PAAM-modified UCNPs and carboxyl-modified RB molecules was confirmed by FTIR technique. As shown in Fig. S2, the absorption peaks of the UCNPs-UCNPs at 1530 and 1050 cm⁻¹ originate from the bending vibration of N-H and the stretching vibration of C-N, respectively. It indicates the successful modification of the UCNPs with amino-abundant PAAM. For carboxyl-modified RB molecules, the C=O stretching vibration and −OH deformation vibration of carboxyl group appear at 1725 and 950 cm⁻¹, respectively. Through coupling with the UCNPs, the two vibrational peaks of RB from the carboxyl group basically disappear, and the C = O stretching vibration mode of amide bond appears at 1632 cm⁻¹. It demonstrates that the UCNPs and RB molecules are conjugated by covalent interaction.

3.2. Emission behavior of the UCNPs-RB conjugates

The effect of the energy acceptor quantity on its efficiency was firstly investigated from the perspective of emission behavior. The emission spectra of the UCNPs-RB conjugates under the excitation of 980 nm are shown in Fig. 1(a). Three characteristic emission peaks of the NaYF₄:Yb³⁺,Er³⁺ UCNPs centered around 520, 540 and 655 nm are observed. They correspond to electronic transitions of Er³⁺ ions from 2H₁₁/₂, 4S₃/₂ and 4F₉/₂ to 4I₁₅/₂, respectively. An additional weak emission band centered around 590 nm is observed from the surface-bound RB molecules. As RB molecule cannot be excited directly by 980 nm to emit fluorescence, the RB emission band is ascribed to the ET from the UCNPs. As shown in Fig. S3, the emission spectrum of the NaYF₄:Yb³⁺,Er³⁺ UCNPs overlaps well with the absorption spectrum of RB in the green region (515–565 nm), while there is little overlap in the red region of the UCL (640–685 nm). Therefore, ET can occur from the green emission bands of Er³⁺ ions to RB, whereas the red emission band cannot participate in the ET efficiently. Accordingly, the green UCL is quenched by the RB acceptors, and the red UCL hardly changes.

The efficiency (η) of the UCNPs-RB conjugates was calculated by using Eq. (1) [19]:

\[
  \eta = 1 - \frac{I_{DA}}{I_D}
\]

where I_D and I_DA are the green emission intensity of the UCNPs in the presence and absence of RB, respectively. As shown in Fig. 1(b), the ET efficiency increases monotonically with increase of RB amount. It should be noted that this ET efficiency includes the contribution of both nonradiative resonant ET and radiative ET. The nonradiative ET is confirmed by the shortened decay lifetime of green UCL (Fig. 1(c) and Table S1) and nearly unchanged decay...
lifetime of red UCL (Figs. S4 and Table S1) with increasing RB amount. The nonradiative ET efficiency (Fig. S5) was calculated by Eq. (2):

$$\eta_{\text{nonradiative}} = 1 - \frac{\tau_{\text{DA}}}{\tau_D}$$ (2)

where $\tau_{\text{DA}}$ and $\tau_D$ are respectively the decay lifetime of the green UCL in the presence and absence of RB, is always lower than the total ET efficiency obtained from the UCL intensity. The discrepancy results from the existence of radiative ET.

Both the nonradiative resonant ET and radiative ET pathways can contribute to RB emission. With increase of RB amount, the emission intensity of the RB acceptors under 980 nm excitation firstly increases and then decreases (Fig. 1(a)). There is an optimal loading amount of the acceptors, with which the emission of the acceptors is the highest. This result is conflicting with the general perception, i.e., the more the acceptors are loaded, the better the acceptor efficacy becomes.

In order to reveal the dependence of the RB emission intensity on the acceptor amount, the emission spectra of RB acceptors on the UCNPs and temporal behaviors of RB emission under direct excitation (540 nm) are recorded. As shown in Fig. 2(a), with increasing loading amount of the acceptors, the emission intensity first increases and then decreases, and a red shift of the emission peak appears. It is related with the formation of RB aggregates, which is proved by the reduced absorbance ratio of 570 to 530 nm in the absorption spectra of the UCNPs-RB conjugates (Fig. S6). The fluorescence decay curves of the RB acceptors on the UCNPs are presented in Fig. 2(b). Considering the coexistence of RB monomers and aggregates, the decay curves are fitted by using bi-exponential function, and the fitting results are shown in Table 1. It can be seen that a relatively short time constant ($\tau_1 \approx 0.37$ ns) and a relatively long time constant ($\tau_2 \approx 1.60$ ns) exist for all the samples. The amplitude $A_1$ increases gradually from 0.55 to 0.95 with increase of the RB loading amount, while the amplitude $A_2$ exhibits a contrary variation tendency (from 0.45 to 0.05). It is consistent with increasing amount of RB aggregates with increase of the RB loading amount. The enhanced interaction between RB molecules when aggregates appear accelerates nonradiative dissipation of the excited states, thus leading to a shorter lifetime ($\tau_1$) than RB monomers ($\tau_2$).

The number of RB monomers on the surface of each UCNPs was estimated (Fig. 2(c)) according to the RB loading amount on the surface of the UCNPs and the ratio of RB aggregates and RB monomers ($A_1/A_2$). It firstly increases and then declines with the increasing RB acceptors. The energy transferred to the surface RB monomers is proportional to the product of the ratio of RB monomers to all the RB molecules and the quenched UCL intensity. Its variation tendency agrees well with that of the RB emission intensity under 980 nm excitation (Fig. 2(d)), indicating that RB monomers are mainly responsible for the fluorescence of RB acceptor.

3.3. ROS production from the UCNPs-RB conjugates

To confirm that fluorescence intensity of RB does represent the ROS production, the effect of the energy acceptor quantity on its efficacy was studied from the perspective of ROS generation. DPBF, which can be oxidized by ROS and exhibit reduced absorbance at 417 nm, was used to monitor the amount of produced ROS by the UCNPs-RB conjugates under NIR irradiation. Fig. 3(a) presents the relationship between DPBF consumption and the 980 nm irradiation time. It can be seen that neither the photosensitizer RB nor the UCNPs alone generate ROS under 980 nm irradiation. The UCNPs-RB conjugates produce ROS efficiently under NIR irradiation, indicating the indirect excitation of the photosensitizer RB by the NIR light excited UCNPs via ET. As the loading amount of RB on the UCNPs increases to 4.8 μmol/L, the amount of ROS increases. With further increase of the loading amount of RB, less ROS is generated. The highest ROS amount appears with the RB loading amount of 4.8 μmol/L corresponding to the strongest fluorescence of RB. The loading amount is not the highest.

The capability of generating ROS was then quantitatively evaluated by using DPBF consumption rate ($k$) according to Eq. (3):

$$\ln(\frac{[\text{DPBF}]_0}{[\text{DPBF}]}) = kt$$ (3)

where $[\text{DPBF}]_0$ is the concentration of DPBF before irradiation and $[\text{DPBF}]$ is the concentration of DPBF after irradiation for a specified time. $k$ is obtained by linear fitting (Fig. S7). As shown in Fig. 3(b), $k$ follows much similar variation tendency to the energy transferred to the RB monomers. It means that ROS is mainly generated by RB monomers. Combined with the results of RB emission, it can be concluded that RB monomers are mainly responsible for RB emission and ROS production, and RB aggregates contribute little to obtaining high efficacy.

3.4. Photodynamic therapy of cancer cells with the UCNPs-RB conjugates

The great impact of RB monomers to ET performance was further verified by intracellular production of ROS and photodynamic killing effect of the UCNPs-RB conjugates on cancer cells.
Human glioblastoma U87MG cell line, which is commonly used for research on the most common and lethal malignant tumor in central nervous system, was chosen as the cancer cell model. The cytotoxicity of the UCNPs-RB conjugates on U87MG cells was firstly evaluated by the standard CCK-8 method. As shown in Fig. 4(a), the survival rate of U87MG cancer cells remains above 95% when the concentration of the UCNPs-RB conjugates increases to 1000 μg/mL, indicating negligible cytotoxicity of the UCNPs-RB conjugates. Therefore, the nanoconjugate concentration of 1000 μg/mL was chosen for the following cell experiments. The cells, the UCNPs-RB conjugates and the cells incubated with the UCNPs-RB conjugates were also fixed on the silicon slides. Their SEM images and emission spectra are presented in Figs. S8 and S9. It can be seen that the size, shape, and UCL properties of the nanocrystals hardly change with and without UCNPs-RB conjugates attached to the cells.

The intracellular production of ROS was visualized by using DCFH-DA as the indicator. DCFH-DA can be transferred to DCFH after entering the cells, and then oxidized to DCF by ROS to emit green fluorescence under excitation. Fig. 4(b) shows the images of the U87MG cells under various treatments. There is no green fluorescence from the cells untreated, only treated with NIR laser, or simultaneously treated with NIR laser and the photosensitizer RB or NIR laser and the UCNPs. Yet, green fluorescence is observed after the UCNPs-RB conjugates with various RB loading amounts are injected into the cells and the NIR laser exposure is used. The intensity of the green fluorescence is closely related with the RB loading amount of the UCNPs-RB conjugates. It follows similar variation tendency to that under direct excitation of RB with 532 nm laser (Fig. S10). Consistent with the RB emission intensity and ROS production amount detected by DPBF, the UCNPs-RB conjugates with RB of 4.8 μmol/L produce the strongest green fluorescence by using the intracellular ROS probe. It demonstrates that ROS can be generated in the cancer cells by the NIR light excited UCNPs-RB conjugates, and the UCNPs-RB conjugates containing the most RB monomers produce the highest amount of ROS.

The photodynamic killing effect of the UCNPs-RB conjugates on U87MG cells was then investigated by the standard CCK-8 method. The viabilities of the cancer cells under various treatments are shown in Fig. 4(c). It can be seen that except for the experiment groups treated with the UCNPs-RB conjugates and NIR irradiation at the same time, the average cell viabilities of the other groups remain above 95%. It indicates that neither the NIR laser nor the

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Table 1
Fitting results of fluorescence decay curves of the UCNPs-RB conjugates at 590 nm under 540 nm excitation.

<table>
<thead>
<tr>
<th>Concentration (μmol/L)</th>
<th>A_1</th>
<th>( \tau_1 ) (ns)</th>
<th>A_2</th>
<th>( \tau_2 ) (ns)</th>
<th>( \tau_{\text{average}} ) (ns)</th>
<th>K^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9 μmol/L</td>
<td>0.55</td>
<td>0.36</td>
<td>0.45</td>
<td>1.61</td>
<td>0.92</td>
<td>0.9991</td>
</tr>
<tr>
<td>4.8 μmol/L</td>
<td>0.68</td>
<td>0.36</td>
<td>0.32</td>
<td>1.60</td>
<td>0.76</td>
<td>0.9990</td>
</tr>
<tr>
<td>10.6 μmol/L</td>
<td>0.89</td>
<td>0.38</td>
<td>0.11</td>
<td>1.61</td>
<td>0.52</td>
<td>0.9988</td>
</tr>
<tr>
<td>15.0 μmol/L</td>
<td>0.95</td>
<td>0.37</td>
<td>0.05</td>
<td>1.60</td>
<td>0.43</td>
<td>0.9988</td>
</tr>
</tbody>
</table>

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Fig. 2. Emission spectra (a) and temporal behaviors of RB emission at 590 nm (b) from the UCNPs-RB conjugates with various amounts of RB under 540 nm excitation. IRF is the instrument response function; (c) Estimated number of RB monomers on the surface of each UCNP; (d) Integrated RB emission intensity of the UCNPs-RB conjugates in the range of 565–630 nm and the energy transferred to the RB monomers by the UCNPs under 980 nm excitation.
Fig. 3. (a) The absorption intensity of DPBF at 417 nm as a function of 980 nm laser exposure time; (b) The consumption rate of DPBF (k) by the UCNPs-RB conjugates with various amounts of RB.

Fig. 4. (a) Viability of U87MG cells treated with the UCNPs-RB at different concentrations; (b) Detection of ROS produced in U87MG cells stained by DCFH-DA: (I) Blank; (II) NIR irradiation; (III) RB + NIR irradiation; (IV) UCNPs + NIR irradiation; the UCNPs-RB conjugates with 2.9 μmol/L (V, IX), 4.8 μmol/L (VI, X), 10.6 μmol/L (VII, XI) and 15.0 μmol/L (VIII, XII) RB without (V–VIII) and with (IX–XII) NIR irradiation. The green fluorescence represents the production of ROS; (c) Viabilities of U87MG cells under different treatments. Gray: with NIR irradiation. White: without NIR irradiation. Data are presented as mean ± standard deviation (n = 3). **P < 0.001; (d) Fluorescence images of U87MG cells under various treatments ((I–XII) are consistent with those in (b)) stained with calcein-AM (green, live cells) and PI (red, dead cells).
materials themselves can kill the cancer cells efficiently. Only the UCNPs-RB conjugates can exhibit apparent killing effect towards the cancer cells under the 980 nm laser irradiation. This result agrees well with the production amount of the intracellular ROS. Moreover, with increase of the RB loading amount, the cell viability first decreases and then increases. When the highest RB loading amount of 15 μmol/L is used, the cell viability is 58.6%. When the optimal loading amount of 4.8 μmol/L is used, the lowest cell viability of 22.7% is obtained, indicating that 35.8% more cells are killed in this case than that with the highest ET efficiency.

The photodynamic therapeutic efficacy of the UCNPs-RB conjugates on U87MG cells was further visualized by staining the RB loading amount of 4.8 μmol/L is used, the lowest cell viability of 22.7% is obtained, indicating that 35.8% more cells are killed in this case than that with the highest ET efficiency.

The UCNPs-RB conjugates with the RB loading amount of 4.8 μmol/L is used, the lowest cell viability of 22.7% is obtained, indicating that 35.8% more cells are killed in this case than that with the highest ET efficiency.

The simplified ET system was constructed by anchoring RB photosensitizer molecules on NaYF4:Yb3+·Er3+ UCNPs covalently. The best acceptor efficacy indicated by the strongest emission intensity of RB and the most produced ROS is achieved with a specified RB loading amount, while the ET efficiency is not the highest with the same amount of RB. In other words, high ET efficiency does not induce high acceptor efficacy. It is related with the formation of RB aggregates. RB monomers are demonstrated to be mainly responsible for ROS generation. The UCNPs-RB conjugates with the RB loading amount of 4.8 μmol/L exhibit the highest therapeutic effect. The highest loading amount of the photosensitizer RB does not guarantee the best efficacy. It highlights the importance of increasing the monomers of the energy acceptors when designing relevant ET systems for various applications.

4. Conclusions

In summary, an ET system was constructed by anchoring RB photosensitizer molecules on NaYF4:Yb3+·Er3+ UCNPs covalently. The best acceptor efficacy indicated by the strongest emission intensity of RB and the most produced ROS is achieved with a specified RB loading amount, while the ET efficiency is not the highest with the same amount of RB. In other words, high ET efficiency does not induce high acceptor efficacy. It is related with the formation of RB aggregates. RB monomers are demonstrated to be mainly responsible for ROS generation. The UCNPs-RB conjugates with the highest RB loading amount of 4.8 μmol/L exhibit the best therapeutic effect. The highest loading amount of the photosensitizer RB does not guarantee the best efficacy. It highlights the importance of increasing the monomers of the energy acceptors when designing relevant ET systems for various applications.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jre.2021.04.008.

References