Near-Infrared Upconversion Controls Photocaged Cell Adhesion

Wen Li,[†,‡] Jiasi Wang,[†,‡] Jinsong Ren,[†] and Xiaogang Qu*,[†]

[†]Division of Biological Inorganic Chemistry, State Key Laboratory of Rare Earth Resource Utilization, Laboratory of Chemical Biology, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China
[‡]University of Chinese Academy of Sciences, Beijing 100039, China

S *Supporting Information*

ABSTRACT: Dynamic control of cell-surface interactions with near-infrared (NIR) light is particularly attractive for regeneration medicine and cell-based therapy. Herein we successfully achieve NIR-controlled cell adhesion with upconversion nanoparticles (UCNPs) based programmable substrate. The UCNPs can harvest the biocompatible NIR light and convert it into local UV light, which results in cleavage of the photocaged linkers and on-demand release of adhesive cells. The strategy also enables the feasibility of deep-tissue photocontrol of cell adhesion on substrate. Our work may open a new avenue for design of UCNP-based cell scaffolds to dynamically manipulate cell—matrix and cell—cell interactions.

Molecular approaches to regulate cell—material interactions are desirable for cell biology and medical application.[1] Among them, dynamic control of cell adhesion on substrates is the fundamental issue because cell adhesion has profound effects on the cell fate and diverse cellular responses.[2] So far, switchable substrates that are responsible for various external stimuli have been explored.[3] Photoactived cell adhesion has attracted much attention because it can allow noninvasively to control cells spatially and temporally.[3c−e] However, most of them have achieved limited success for in vitro study since UV—vis light used in the stimulus process is not friendly to cells, and it is also quickly attenuated in tissues. Compared with UV light, NIR light is expected to cause minimal cell damage and has remarkably deep tissue penetration.[4] Recently, NIR-controlled cell adhesion on a surface by using photothermal effect of graphene and DNA was reported.[5] However, the thermosensitive DNA was susceptible to nuclease degradation in body fluid. To bridge the gap between UV light and NIR stimulus for cell adhesion, converting NIR light into local UV light can be a good way to control cell adhesion.

Recently, an appealing candidate for the application of NIR light has emerged, which is based on lanthanide-doped upconversion nanoparticles (UCNPs).[6] These UCNPs are usually made of host lattices of ceramic materials embedded with trivalent lanthanide ions. Due to the unique ladder-like energy level structures of lanthanide ions, UCNPs are able to absorb NIR light and convert it into high-energy photons in the UV, visible, and NIR regions.[7] Due to this fascinating photoluminescence property, UCNPs have been successfully used as NIR-induced mediators for photoisomerization of azobenzene group, photocleavage of compounds, and photo-

switch of ring opening—closing of dithienylethene molecule.[8] These works mainly highlight the application of UCNPs in biosensor,[8c] imaging,[8d] photodynamic therapy, drug delivery,[8e,f] and activation of DNA/siRNA.[8h] Inspired by these achievements, herein we found that UCNPs could also be successfully used in a dynamic substrate to regulate cell—surface interaction and cell adhesion with NIR light stimulus. This work may not only provide a solution to resolve UV light problem in photocontrolled cell adhesion but also may greatly extend the growing applications of UCNPs, especially for the fabrication of dynamic substrate for tissue engineering, cell-based therapy, and disease-related cell manipulation and analysis.

The general strategy was illustrated in Scheme 1. The substrate was first modified with UCNPs, which could harvest

Scheme 1. Schematic Illustration of the Using of UCNPs for NIR Light Controlled Cell Adhesion[a]
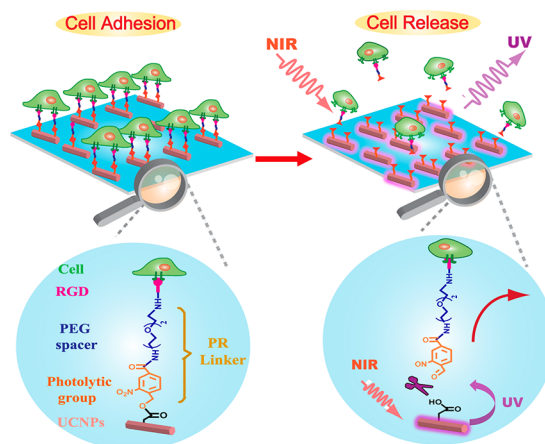
[a]Upon absorption of 980 nm light, the UCNPs can emit UV photons and activate the cleavage of the PR linker, resulting in the disassociation of RGD and cells from substrate.

the NIR light and convert it into the necessary UV light locally. Then the UV-photocleavable 4-(hydroxymethyl)-3-nitrobenzoic acid (ONA) molecule[9] was covalently attached to the UCNPs. After that, arginine-glycine-aspartic acid (RGD) was chosen here as the bioadhesive ligand to immobilize on the UCNPs modified surface to mediate cell adhesion. The RGD peptide motif can be specifically recognized by integrins, the

major receptors of cells for adhesion.[10] To ensure more efficient adhesion, a polyethyleneglycol (PEG) spacer was introduced between surface and RGD ligand to decrease nonspecific absorption and steric hindrance.[3e,7a] In our strategy, upon irradiation with NIR laser of 980 nm, the UCNPs could emit photons in the UV regions that, in turn, were absorbed by photolabile linkers. This resulted in the activation of the photocleavage reaction in the bioadhesive linker and dynamic change of cell binding state on surface. In addition, the UCNP-based system also showed potential in deep tissue activation of cell adhesion with the NIR stimulus.

For a starting point of our study, the lanthanide-doped $NaYF_4$:TmYb nanoparticles were synthesized as reported.[11] As shown in the SEM images (Figure 1a), the UCNPs possessed
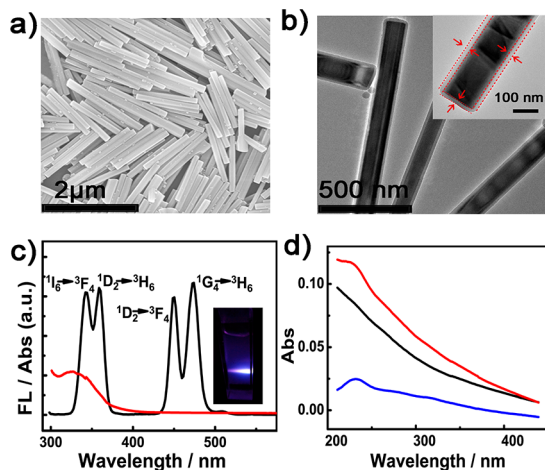

Figure 1. (a) SEM image of the $NaYF_4$:TmYb UCNPs. (b) TEM image of the UCNPs@SiO_2. The inserts showed the SiO_2 shell with red line. (c) Absorption spectrum of the ONA solution (red line) and the emission spectra of UCNPs@SiO_2 (black line). Insert photograph was the UCNPs@SiO_2 solution under NIR laser exposure. (d) UV−vis absorption spectra of UCNPs@SiO_2 modified surface before (black line) and after immobilization of PR linker (red line) and the changes in the absorbance after immobilization of PR linker (blue line).

uniform rod shapes with an average length of 1.45 μm and width of 125 nm (Figure S1). The powder X-ray diffraction confirmed the UCNPs were hexagonal in phase (Figure S2).[11] To increase their water solubility and biocompatibility, the thin silica shells with the thickness of 18 nm were coated on the surface of UCNPs (Figure 1b).[12] After silica coating, the UCNPs@SiO_2 nanoparticles were well dispersible in water and easy for surface modification. The nanoparticles functionalized with a carboxylic group (UCNPs@SiO_2-COOH) were obtained by treatment with 3-aminopropyltriethoxysilane and succinic anhydride, successively.[13] The surface modification processes were monitored with Fourier transform infrared spectra (Figure S3)[12,13] and ζ potential evaluations (Figure S4). Upon excited by a 980 nm CW laser, UCNPs@SiO_2-COOH displayed emission bands in the UV−vis region that were attributed to the transitions from the emitting energy levels of Tm^{3+} (Figure 1c). When the laser beam traveled through the UCNPs colloidal solutions, the purple-blue photoluminescence was also readily observed (Figure 1c inset). For current study, the UV light emitted from the UCNPs was appealing, since these wavelengths overlapped with the absorption band of ONA linker.

After synthesis and characterization of the functionalized UCNPs, the UCNP-based substrate was fabricated for control of cell adhesion. First, the UCNPs@SiO_2-COOH were attached to the amino group modified quartz surface after activating the carboxyl. The topography of the UCNPs@SiO_2 decorated substrate was observed with SEM (Figure S5a). Under the irradiation of NIR laser, the prepared substrate exhibited purple-blue photoluminescence (Figure S5b). The photoresponsive linker (PR linker) contained the ONA group, and the PEG spacer was synthesized (Scheme S1) and then covalently conjugated on the UCNPs modified surface via ester bond. The detail modification process was provided in Scheme S2, and the obtained surface was defined as UCNPs@SiO_2-PR surface. As predicted in Figure 1d, the obtained surface revealed absorbance in UV region, which confirmed the successful attachment of o-nitrobenzyl contained linker on surface.

To examine whether the UCNPs on surface could be effective to trigger the photolytic reaction, the substrate was immersed into certain amount of solution and irradiated with NIR laser. The absorption spectral change of the surrounding solution is shown in Figure S6. After exposure to NIR laser, obvious absorption bands in 300−450 nm were detected, which were attributed to the formation of photocleaved nitro-sobenzaldehydes. UCNPs could harvest NIR light and emitted the UV light to photocleavage the o-nitrobenzyl groups, then the nitrosobenzaldehyde products were released from the surface and diffused to the solution. The spectral change after NIR irradiation had a similar trend as that recorded when the surface was exposed directly to UV light (360 nm). While after replacing the UCNPs@SiO_2 with SiO_2 nanoparticles, the NIR light exposure did not result in observable absorption change, thereby confirming the photolysis was, as expected, not susceptible to NIR light in the absence of the UCNPs. To further access the ability of photolysis for control the target molecule release, we used fluorescein isothiocyanate (FITC) as the model to visualize the detachment process directly. After immobilization of FITC on the amino terminus of PR linker, the substrate emitted bright green fluorescence (Figure 2a). When the substrate was treated with NIR light, the strong fluorescence dramatically diminished due to the photocleavage
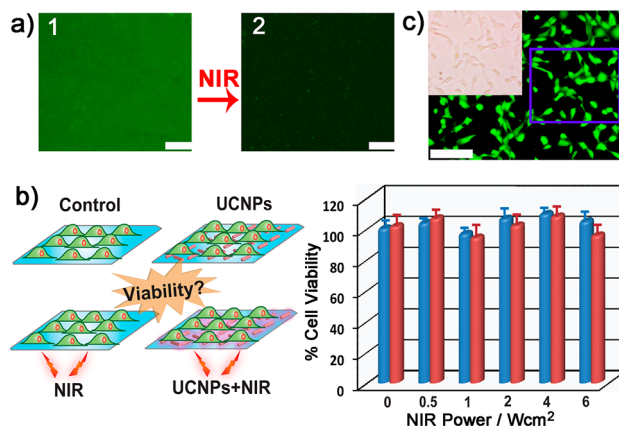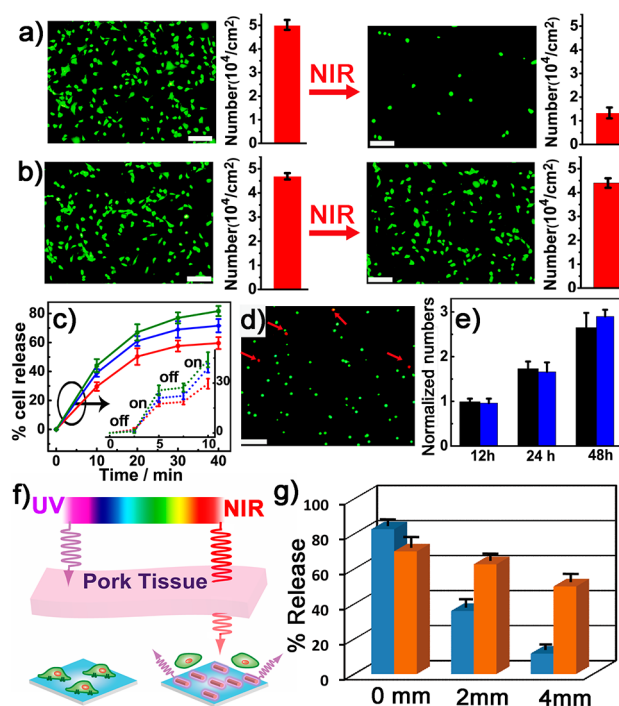

Figure 2. (a) Fluorescence images of substrate immobilized with FITC before laser exposure (1) and after 980 nm laser exposure (2). (b) Viability of cells on the substrate without (blue) and with (red) UCNPs exposed to various doses of 980 nm CW NIR laser for 30 min. (c) Fluorescence images of cells incubated on the substrate. Insert was the corresponding bright-field image of cells in the blue square. Scale bars are 100 μm.

of linkers. And the photocleavage efficiency was affected by the distance between ONA molecule and UCNPs (Figure S7), which suggested the effect of local UV light converted from UCNPs. To certify that the attenuation of fluorescence was not caused by the photobleaching, the control substrates without PR linker were fabricated. Irradiation with same NIR light did not cause obvious fluorescence change (Figure S8).

Prior to examining the NIR-controlled cell adhesion, we checked the biocompatibility of UCNPs as well as the safety of NIR light and especially the unconverted UV light from UCNPs. The NIH 3T3 fibroblast cells were cultured on UCNPs modified surface and then were irradiated with a different dose of 980 nm laser (2.5 min break after 2.5 min irradiation). Cells incubated on the surface without UCNPs were also exposed to NIR light and used as a control to study the particular toxicity of NIR light. The cell viability was assessed with MTT assay. As shown in Figure 2b, no obvious reduction in cell viability was detected in any of the cell samples treated with UCNPs, NIR laser, or the UCNPs + NIR laser. These results were consistent with previous reports, which have proved that the UCNPs have low toxicity and even can be used in vivo studies.[6] Additionally, the above results also showed that under our experimental conditions, not only the NIR light but also the upconverted UV had little damage to cells. The biocompatibility of upconverted UV light might be attributed to the localized UV radiation produced by the UCNPs compared to conventional whole-cell irradiation with direct UV light.[8h] Furthermore, the live/dead staining also exhibited that the cells were almost alive after exposure to NIR-to-UV UCNPs (Figure S9). Although the 980 nm laser can increase the water temperature, the short interval irradiation condition in our experiment could avoid this problem, and no obvious temperature increase (<3 °C) was observed. In addition, recently a new class of UCNPs with the excitation wavelength of ~800 nm has been successfully synthesized,[14] and it also provided an efficient solution to avoid the heating effect of 980 nm laser. Taken together, the UCNPs provided a safe approach for photoactivation of cell adhesion.

The ability of UCNPs for dynamic control of cell adhesion was then examined. For this purpose, the bioadhesive RGD ligand was covalently conjugated on the photosensitive substrate. Then, the cells were seeded on the substrate and monitored with fluorescence microscopy. After 5 h, the cells efficiently attached and spread on the surface with fully extended morphology (Figure 2c). After the cell adhesion, we studied whether the substrate could dynamically stimulate cell release with NIR laser. As shown in Figure 3a, after exposure to 4 W/cm² NIR light for 30 min, ~70% of the original adherent cells were released from the surface. The residual cells on the surface were mainly due to the photoresponsive linkers that were not completely cleaved by NIR laser. And clearly, the rate and amount of the cell release could be easily adjusted by the laser power intensity and exposure duration (Figure 3c). The on-demand release of cells from the responsive surface was demonstrated by alternating the NIR laser between "light" and "dark" conditions (Figure 3c inset). In addition, instead of the whole exposure to NIR light, the substrate was selectively irradiated with a laser through a mask. As designed, few cells were left on the irradiation site, while most of the cells on the nonirradiation region were still attached well (Figure S10). This showed that the NIR-responsive UCNPs could control of cell adhesion with high spatial resolution. For control, the cells on the substrate without UCNPs were almost not affected by laser

Figure 3. Fluorescence images of NIR light-induced cells release on (a) the substrate we designed and (b) substrate without UCNPs. Scale bars are 100 μm. (c) Percent of cell release after irradiation with NIR laser with 2 (red line), 4 (blue line), and 6 W/cm² (green line). 2.5 min break after 2.5 min irradiation. Inset was the detailed release process with laser on–off exposure for 0–10 min. (d) Viability of released cells assessed with a live (green)/dead (red) assay. Scale bars are 100 μm. (e) Normalized cell numbers of the released cells (black bar) and the controlled sample (blue bar) after proliferation of different times. (f) Illustration of in-deep tissue irradiation by covering the substrate with pork tissue. (g) Cell release induced by directed UV light irradiation (blue bar) and 980 nm NIR laser irradiation (orange bar) through 0, 2, or 4 mm of pork tissue.

irradiation (Figure 3b), indicating that the cell release was mediated by the upconverted UV light not the direct NIR laser. Additionally, to further confirm the cell detachment was caused by the cleavage of PR linker, the substrate without photosensitive ONA molecule was prepared. There was little reduction in cell numbers for it with the NIR light treatment (Figure S11). From the live/dead cell staining assay, most released cells were still alive (Figures 3d and S12). MTT assay was also performed which showed that our releasing condition did not affect the proliferation ability of cells (Figure 3e). In addition, after reincubated on the glass slide, the released cells remained functional and spread normally (Figure S13). These results confirmed that the UNCP-based photoactivation provided a biologically friendly platform for light control of cell adhesion.

Furthermore, we studied whether this system could be used for regulating cell adhesion in deep tissue, which was an important factor for further in vivo applications. For this purpose, pork tissues (muscle tissue) with different thickness were used as the tissue models plated on the top of the photoresponsive surface[15] (Figure 3f). The release of cells would occur only if the laser penetrated the tissue and reached the surface. Upon irradiation with NIR light, the cell release reduced with the increase of tissue thickness, but ~50% activation was still observed even with 4 mm tissue. When it

came to UV light, direct exposure to the light sources without the pork tissue resulted in a more efficient cell release than using NIR laser. However, the cell release was dramatically reduced to 35% when the UV light was blocked by 2 mm tissue, and only 10% was detectable once the light was blocked by 4 mm tissue (Figure 3g). The result was consistent with the absorbance difference of the tissue in UV and NIR region (Figure S14) and showed that the UCNP-based NIR light system offers significantly improved penetration depth and is preferred for the control of cell adhesion in deep tissue.

In summary, a new paradigm for NIR-controlled cell adhesion has been presented by using UCNPs acting as the nanotransducers. UCNPs can harvest NIR light and convert it into local UV light for cleavage of photocaged linker, thereby realizing on-demand cell release. This has been demonstrated in deep tissue penetration. To the best of our knowledge, this is the first example of using UCNPs to control cell adhesion, and rapid developments for UCNPs will further improve the performance of this platform, such as the use of a new kind of UCNP which can be irradiated with more suitable NIR light. Therefore, our work will facilitate the design of UCNP-based multifunctional cell scaffold for dynamic study of biological process, regeneration medicine, and disease-related cell isolation and analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
xqu@ciac.ac.cn

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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