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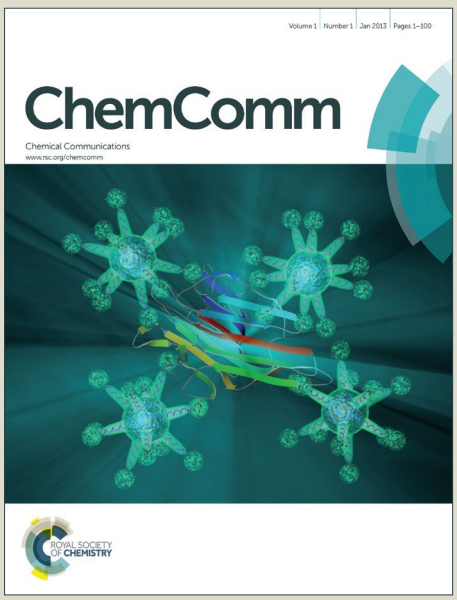

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Near Infrared-Caged D-Amino Acids Multifunctional Assembly for Simultaneously Eradicating Biofilm and Bacteria

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A nanodevice composed of an upconverting nanoparticle (UCNP) core and a thin TiO_2 shell with surface modified with D-amino acids was designed. Due to the UCNP core, the NIR light can be converted to high-energy UV photons. As a consequent, UV light could stimulate the TiO_2 shell produce antibacterial reactive oxygen species (ROS) and trigger the release of free D-amino acids (antibiofilm agents).

Most bacteria form multicellular communities known as biofilms in which cells are protected from environmental insults.[1] The cells within the biofilms are held together by a self-produced polymeric matrix. In biofilms, bacteria exhibits upward of 10−1000-fold more tolerant to antibiotic treatment antimicrobials than their planktonic counterparts, and are less susceptible to host immune defenses.[2] The inability to fully eradicate biofilms often forms the basis for chronic infections that can then lead to fatal outcomes. Biofilms can also be highly detrimental in industrial settings such as fouled immersed marine surfaces, clogged filtration membranes, or corroded pipes which act as reservoirs for pathogens in food and water processing.[3] Therefore, efficient agents specifically designed to inhibit biofilm formation or even disassemble biofilm are urgently needed across a wide range of applications.

Obviously, there is an unmet need of controlling biofilms in many settings. Up to now although much effort has been focused on the molecular mechanisms of biofilm formation,[4] the development of antibiofilm agents was rare.[2a,5] Significantly, a recent study reported that the incorporation of specific D-amino acids into the peptidoglycan of cell wall led to bacterium being released from the matrix of biofilm.[4a] Thus, the use of D-amino acids as anti-biofilm agents represents an excellent strategy due to their biologically benign. However, there are two problems before the successful utilization of D-amino acids: (1) the D-amino acids disperse the matrix of biofilms without any inhibition toward cell growth; (2) it is challenging to direct administer D-amino acids at an on-demand dose rate and for a sustained time period.

Herein, we designed a near-infrared (NIR) light-triggered multifunctional nanodevice for spatio-temporally controllable release of the D-amino acids to the biofilm infected site with combined generation of bactericides. The NIR light has been shown to penetrate tissue at depths beyond 1 cm with no observable damage to the intervening tissue.[6] In addition, light generated with a laser source are born to spatio-temporally controllable manipulation. In spite of those merits, the low-energy NIR light is powerless to achieve photo-activation of chemical reactions.[7] One way to surmount this obstacle is to convert NIR light to higher-energy photons in the UV, visible regions *via* upconverting nanoparticles (UCNPs).

As illustrated in Fig. 1, an UCNP was coated with a thin TiO_2 shell

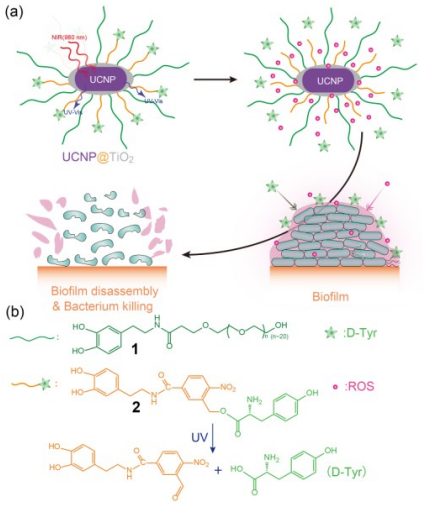

Fig. 1 Schematic diagram of multifunctional nanodevice for NIR-triggered biofilm disassembly and bacterium killing. UCNP was coated with a thin TiO_2 shell and the D-amino acids were linked to UCNP@TiO_2 surface with a UV cleavable linkage. Upon absorption of NIR light, the UCNP cores emit photons in the UV and visible regions that, in turn, are absorbed partially by the thin TiO_2 shell to release biocidal ROS and partially by the photo-cleavable moieties to release free D-amino acids.

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(namely UCNP@TiO_2), and the D-amino acids were linked to UCNP@TiO_2 surface with a UV cleavable linkage. The D-tyrosine (D-Tyr) was chosen because it could disperse biofilm at low micromolar concentrations. Upon absorption of NIR light, the nanodevices emit photons in the UV and visible regions that, in turn, are absorbed partially by the thin TiO_2 shell to release biocidal reactive oxygen species (ROS),[8] and partially by the photo-cleavable moieties to release D-Tyr (Fig. 1b). As shown below, our studies prove the efficacy of this approach, which represents one that is general and readily applicable to biofilm infection and pollution.

The UCNPs (β-$NaYF_4$:Tm^{3+} 0.5 mol%, Yb^{3+} 30 mol%) were first synthesized using a literature method.[9] Fig. S1 represents a typical transmission electron microscopy (TEM) image of the UCNPs that are uniformly distributed in size and dispersed well, and the mean size of these particles is about 30 nm in diameter. Upon the addition of specific amount of $Ti(OBu)_4$ into the UCNPs solution, a thin TiO_2 shell was evidently deposited around the UCNPs via the hydrolysis and condensation of $Ti(OBu)_4$, and forming the UCNP@TiO_2. The TiO_2 shells were amorphous before annealing. While hydrothermally annealed at 160 °C, the amorphous TiO_2 shells become anatase crystallized (Fig. 2a and Fig. S2).[10] Then, the UCNP@TiO_2 nanoparticles were modified with pre-synthesized compounds **1** and **2** (molar ratio=8:2; shown as Fig. 1b) to obtaining the ultimate multifunctional nanodevices (UCNP@TiO_2-D-Try). The synthesis and characterization details of **1** and **2** were shown in Electronic Supplementary Information (ESI). The **1** and **2** were readily linked to UCNP@TiO_2 through the chelation between Ti atom and enediol groups by forming stable five-membered rings.[11] The UCNP@TiO_2-D-Try nanodevices could readily be dispersed in water and culture medium due to the introduction of the hydrophilic PEG-ended **1**. Fig. 2b shows the photoluminescence spectra of UCNPs, UCNP@TiO_2, and UCNP@TiO_2-D-Try upon exposure to a 980 nm diode laser (10 W/cm^2). As compared to neat

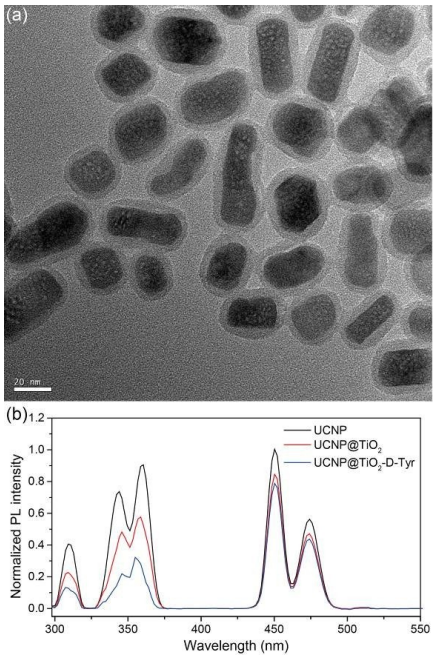

Fig. 2 (a) TEM image of UCNP@TiO2. (b) Photoluminescence spectra of neat UCNPs, UCNP@TiO2, and UCNP@TiO2-D-Try upon a 980 nm diode laser exposure.

UCNPs, the emission intensity of UV light around 350 nm of the UCNP@TiO_2 is much weaker, indicating that the UV photons delivered by the UCNPs are partially absorbed by the thin TiO_2 shell. Furthermore, the UV emission intensity was further decreased by comparing the spectrum of UCNP@TiO_2-D-Try to that of UCNP@TiO_2. This is in accordance with previously reported results that *o*-nitrobenzyl groups were photo-labile by adsorbing the UV light (Fig. 2b).[12]

We performed further experiments to confirm that NIR could trigger the release of D-Tyr and production of ROS from the UCNP@TiO_2-D-Try nanodevices. The former experiments were carried out by irradiating the UCNP@TiO2-D-Try water solution (2 mg/mL) with 980 nm NIR (5 W) for different time. The NIR irradiated solution was centrifuged and the supernatants were analyzed. As shown in Fig. S3, no free D-Try was found from the supernatant without NIR irradiation, and about 1 μM free D-Try was found within 10 min. This was distinct that the release of D-Try was a direct consequent of NIR triggering. For about 2 h NIR irradiation, the released D-Try reached a concentration beyond 5 μM, which was sufficient to disassemble biofilms.[4a]

On the other hand, the NIR triggered production of ROS was confirmed by fluorescence measurements. The TiO_2 crystals have been proven to produce ROS species (•OH) under UV irradiation.[8] Herein, we suppose that NIR could first be converted to UV *via* the UCNP core and the UV light could then induce the TiO_2 shell to produce •OH. Terephthalic acid (TA) was used as a fluorescence probe because it can react with •OH in basic solution to generate 2-hydroxy terephthalic acid (TAOH), which emits unique fluorescence signal with the spectrum peak around 426 nm. As shown in Fig. S4, significant fluorescence spectra associated with TAOH were generated upon NIR-irradiation of UCNP@TiO_2-D-Try. As a control, the neat UCNPs didn't produce any •OH under NIR. As we all know, •OH is highly antibacterial active. Therefore, UCNP@TiO_2-D-Try could be used for simultaneous biofilm disassembly and bacterial killing.

The NIR-activated biofilm dispersion and bacterial killing ability of UCNP@TiO_2-D-Try was tested against *Bacillus subtilis* strain NCIB3610, which tends to form visible biofilms on both semi-solid surfaces and air/liquid interface.[13] After 2 days of incubation in a biofilm-inducing medium, *B. subtilis* formed thick pellicles at the air/liquid interface of standing cultures (Fig. 3a). By incubating the

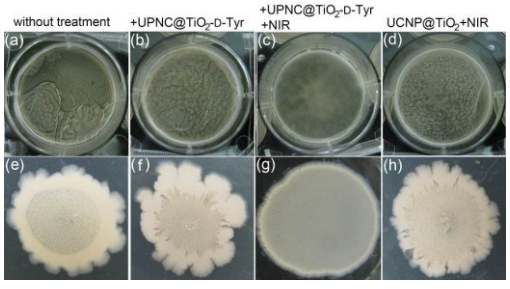

Fig. 3 *B. subtilis* biofilms at the air/liquid interface of standing cultures (a), and treated with UCNP@TiO_2-D-Try (b), UCNP@TiO_2-D-Try + NIR activation (c), and UCNP@TiO_2 + NIR activation. *B. subtilis* biofilms at semi-solid surface (e), and treated with UCNP@TiO_2-D-Try (b), UCNP@TiO_2-D-Try + NIR activation (c), and UCNP@TiO_2 + NIR activation.

biofilm with UCNP@TiO_2-D-Try overnight, it kept almost unchanged (Fig. 3b). Adding UCNP@TiO_2-D-Try to the biofilm culture and irradiated with NIR laser for 1.5 h, the biofilm was obviously disassembled with subsequent overnight incubation (Fig. 3c). As a control, UCNP@TiO_2 with NIR still cannot disperse the biofilms (Fig. 3d). Similarly, the UCNP@TiO_2-D-Try with NIR triggering can also cause pellicle breakdown on semi-solid surface (Fig. 3e-h). Control experiments were carried out by using a nanoparticle with SiO_2 nanoparticle as the core coated with TiO_2 shell (SiO_2@TiO_2) with the same surface chemistry as UCNP@TiO_2-D-Tyr. The results indicated that no free D-Tyr was released from SiO_2@TiO_2-D-Tyr nanoparticle under laser irradiation and the biofilm was not disassembled (Fig. S5).

We further investigated whether NIR-activated UCNP@TiO_2-D-Try can simultaneously kill the bacterial cells within biofilms. The same amount *B. subtilis* cells from biofilms with different treatment were collected and cultured in LB overnight. Then the absorbance at 600 nm of each cultured LB medium was measured. As shown in Fig. S6, just treatment of UCNP@TiO_2-D-Try + NIR can effectively kill the cells within biofilm. Without the disassembly of biofilm, the cells cannot be killed even with the generation of •OH. Meanwhile, treatment with D-Try alone or D-Try + NIR, the cells kept alive although the biofilm was disassembled. With the remained cells, they can re-form biofilms. This result proved the potent efficiency of our multifunctional nanodevices for the treatment of biofilm infection.

To make the results more convincing, the as-formed biofilm and UCNP@TiO_2-D-Try + NIR treated biofilm were investigated by TEM (Fig. 4). The cells were densely stacked and protected within the biofilm matrices (Fig. 4a & b). After treatment with UCNP@TiO_2-D-Try + NIR, the cell surfaces became smooth by detaching biofilm matrices, and the cells were broken (Fig. 4c). Moreover, the nanoparticles were attached to the surface of the cell (Fig. 4d, inset). This means that the released D-Tyr and ROS from the nanoparticles

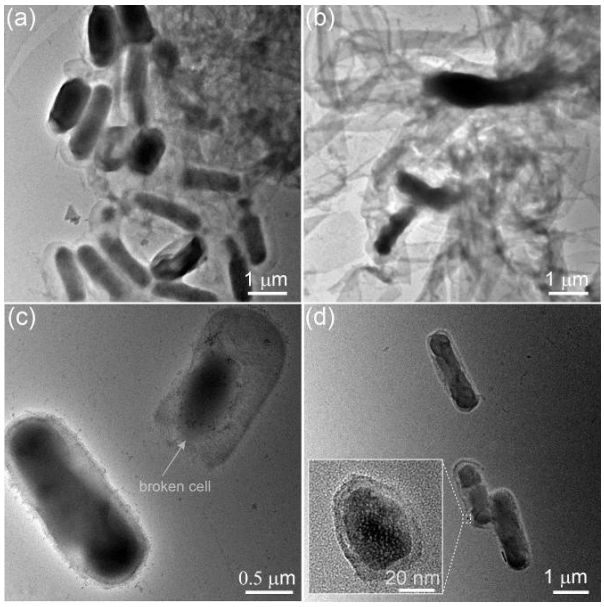

Fig. 4 TEM images of (a, b) as-formed, and (c, d) UCNP@TiO_2-D-Try + NIR treated *B. subtilis* biofilms. Inset, a core-shell structured nanoparticle attached to a bacterium surface.

can directly arrive to the biofilm and cell with highly local concentration. Therefore, the mechanism of the disassembly of biofilm of our nanodevices relies on the detachment of biofilm matrices from cell surfaces, which is in accordance to the previously observed results.[4a]

In conclusion, we developed a multifunctional nanodevice for the comprehensive treatment of biofilm infection with the activation of NIR light. Under the irradiation of NIR, the nanodevices can spatio-temporally release free D-amino acids (D-Tyr) and ROS (•OH) for biofilm dispersion and bacterial killing, respectively. Given that many bacteria produce D-amino acids, these amino acids may linked to nanodevice surfaces to provide a general strategy for biofilm disassembly. If so, then our mode of nanodevices might prove widely useful in medical and industrial applications for the prevention or eradication of biofilms.

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