Light-responsive nanogated ensemble based on polymer grafted mesoporous silica hybrid nanoparticles†

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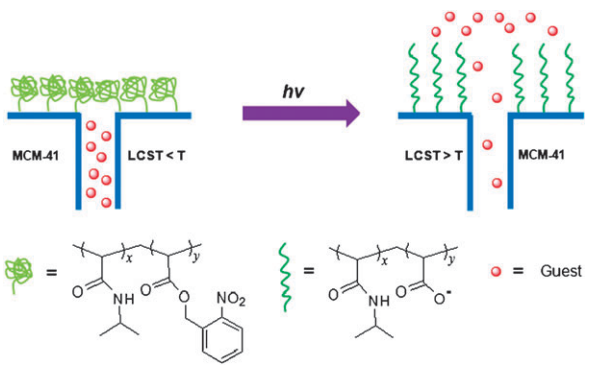
Mesoporous silica nanoparticles grafted with light-responsive polymer on the outer surface were developed as novel nanogated ensembles, which allow encapsulation and release of drug and biological molecules under light irradiation.

Controlled-release systems that integrate external stimulation with nanoscale carriers are promising vehicles for sensors and drug delivery applications.[1] Due to its unique features such as uniform and tunable pore structure and broad diversity in surface functionalizations, mesoporous silica (MS) has been employed as a versatile solid support for constructing a variety of hybrid materials for controlled drug delivery. Upon coating with molecular valves,[2] MS is able to encapsulate a payload of therapeutic compound and to transport it to specific locations in the body where it could be released upon either external or cellular stimulation. A series of MS based controlled-release systems have been developed that are responsive to distinct external stimuli such as pH,[2e–g] light,[2h–j] redox,[2k–m] competitive binding[2n] and enzyme.[2o] Among them, the light-responsive controlled-release systems are of more interest because of the non-invasive and high spatiotemporal resolution character of light.[3]

Poly(*N*-isopropylacrylamide) (PNIPAM), one of the most well known thermosensitive polymers and exhibiting a lower critical solution temperature (LCST) of *ca.* 32 °C in water,[4] has been widely applied for cell culturing,[5a] direction of protein adsorption,[5b] protein purification,[5c] and drug delivery.[5d–g] It is known that incorporation of hydrophobic or hydrophilic monomers into the PNIPAM backbone would lead to a decrease or increase in LCST, respectively. Therefore, when monomers bearing a light-responsive moiety such as azobenzene[6a,b] and 2-nitrobenzyl groups[6c,d] are incorporated in the PNIPAM backbone, the resultant polymers would be light-responsive; their LCST can be facilely modulated by applying light since the polarity of the light-responsive moieties changes under irradiation. The light-responsive character thereby endows the thermosensitive polymer with a unique property of modulating its phase state by UV irradiation at a chosen temperature.[6,7] We envisaged that the light-dependent phase state of the polymers can be employed as an attractive molecular valve for developing novel controlled-release systems.

Herein reported is a light-responsive nanogated ensemble based on light-responsive polymer grafted MS hybrid nanoparticles. The basic principle of the light-responsive nanogating is shown in Scheme 1. MS nanoparticles encapsulating guest molecules are functionalized with polymers on the surface. Copolymer poly(*N*-isopropylacrylamide-*co*-2-nitrobenzyl acrylate) (poly(NIPAMNBAE)), bearing photocleavable hydrophobic 2-nitrobenzyl (NBAE) groups, has a LCST below environmental temperature *T* (for biological study, this *T* is usually 37 °C). Thus, the polymer is in a collapsed (insoluble) state and the gate is "closed" so that the loaded molecules are locked in the pores. Upon UV irradiation, the hydrophobic 2-nitrobenzyl acrylate moiety is photolysed into hydrophilic acrylate which leads to an increase in the LCST of the resultant copolymers and it is now higher than *T*. As a consequence, the polymers change to their coil (soluble) conformation so that the gate is "opened" thereby allowing the entrapped molecules to escape.

Amine-terminated poly(NIPAMNBAE) was synthesized by chain transfer initiated free-radical polymerization in DMF at 75 °C for 24 h, using 2-aminoethanethiol as a chain transfer reagent and AIBN as an initiator (Fig. 1, for more details see ESI†).[6a] The amount of NBAE incorporated in the copolymers was designed in the preparation procedure to endow the polymer with LCST lower and higher than 37 °C before and after light irradiation, respectively.[6c,d] The precise content was determined by ^{1}H NMR to be 5.3% (Fig. S1, ESI†). Absorption spectroscopy was used to monitor the photolysis of the polymer under irradiation at $\lambda \geq 310$ nm, during which a decrease in the absorption at 265 nm and an



Scheme 1 Light-responsive nanogated ensemble based on polymer grafted mesoporous silica.

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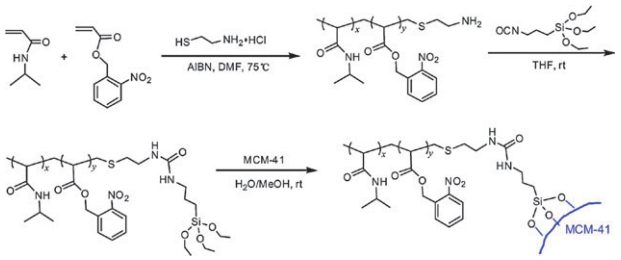

Fig. 1 Synthesis of light-responsive nanogated ensemble.

increase of it at 325 nm were observed, indicating the breakage of the photolabile ester bond and the generation of hydrophilic acrylate (Fig. S2). Accordingly, the LCST of the prepared poly(NIPAMNBAE), which was 12.5 °C in 10 mM pH 7.4 phosphate-buffered saline (PBS) before photolysis, was found to increase to 45 °C after UV irradiation (Fig. S3). These observations confirm that light indeed modulates the LCST of poly(NIPAMNBAE) and the polymer is suitable for biological application because of its suitable LCSTs around 37 °C.

MCM-41-type MS nanoparticles were synthesized by condensation of tetraethyl orthosilicate in the presence of a CTAB micelle template.[8] Amine-terminated poly(NIPAMNBAE) was first coupled to a functionalized silane, 3-(triethoxysilyl)-propyl isocyanate, then grafted to the pore outlet of MS nanoparticles to afford MS@polymer hybrid nanoparticles (Fig. 1). The observation of a FTIR band at 1650–1710 cm^{-1} and a peak at 1540 cm^{-1}, characteristic of amide C=O and N–H stretching, respectively (Fig. S4), confirmed the successful grafting of polymer onto MS. X-ray powder diffraction (Fig. S5) of MS and the hybrid material both show three distinct peaks at 2θ = 2.24°, 3.85° and 4.48°, that index to (100), (110) and (200) planes, respectively, revealing both the nanoparticles have ordered MCM-41-type 2D hexagonal (*P6mm*) symmetry. TEM images shown in Fig. 2 reveal a uniform pore structure of MS. A 4-nm thick polymer coating was observed around the silica particle after grafting (Fig. 2B). Both the MS and the hybrid nanoparticles have a diameter of *ca.* 100–120 nm. The presence of nitrogen in the energy dispersive X-ray spectroscopy (EDX) further confirms the grafting of polymer (Fig. S6). A comparison of the thermogravimetric analysis (TGA) data for MS, polymer and hybrid materials suggests that 43% by weight of the hybrid is due to the grafted polymer (Fig. S7). Dynamic light scattering data reveal the hybrid nanoparticle has a LCST of 14 °C, which is slightly higher than that of random poly(NIPAMNBAE).

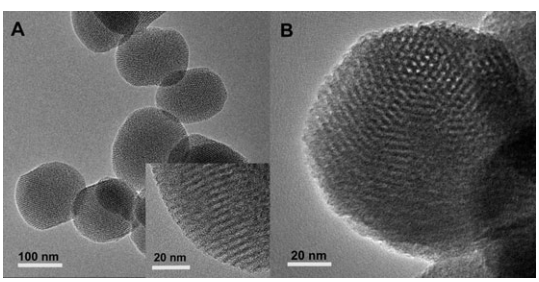

Fig. 2 TEM images of MCM-41-type MS nanoparticles (A) and MS@polymer hybrid nanoparticles (B).

UV irradiation led to an increase in the LCST to 46 °C (data not shown). This result indicates that tethering of polymer to the MS surface has not suppressed its light responsive property.

To investigate the light-responsive gating behavior of the hybrid nanoparticles, fluorescein was utilized as a model drug molecule and was loaded by stirring with MS@polymer nanoparticles in a pH 7.4 PBS solution in an ice bath for 12 h. The bath temperature was then increased to 37 °C and the excess fluorescein was removed by centrifugation and repeated washing with PBS buffer at 37 °C. The fluorescein loaded hybrid nanoparticles were collected and redispersed in PBS buffer at 37 °C to test their controlled release profile. The results are shown in Fig. 3. Since the setting temperature was higher than the LCST of hybrid nanoparticles (14 °C), the poly(NIPAMNBAE) was insoluble and the entrances and outlets of the MS pores were blocked by the collapsed polymer chains; the release of fluorescein was thereby significantly obstructed at this temperature. Only 8% leakage of the entrapped fluorescein molecules was observed after 24 h. As UV irradiation was shown to increase the LCST of polymer to 46 °C, the resulting polymer was soluble at 37 °C, thus the gate being opened allowed a gradual release of the dye molecules. 82% of the loaded fluorescein was released after 24 h.

Finally, to evaluate the biological compatibility and intracellular light-triggered release property of the hybrid nanoparticles, MHCC97H cells were incubated with fluorescein loaded MS@polymer nanoparticles for 24 h on a plate and then washed with PBS buffer to remove the non-internalized nanoparticles. Fluorescence microscopy images of the incubated cells are shown in Fig. 4. Significant fluorescence was observed inside the cells, but outside the nucleus before UV irradiation (Fig. 4C), indicating that the fluorescein-carrying hybrid nanoparticles were cell permeable but were blocked from entering the nucleus by their large sizes. However, the cells were fully fluorescent after UV irradiation (Fig. 4F), which strongly suggests the light-triggered release of fluorescein from the nanoparticles and its diffusion into the nucleus. Meanwhile, there was negligible difference in the viability of cells incubated with or without hybrid nanoparticles evaluated by MTT experiments (ESI†). These results confirm

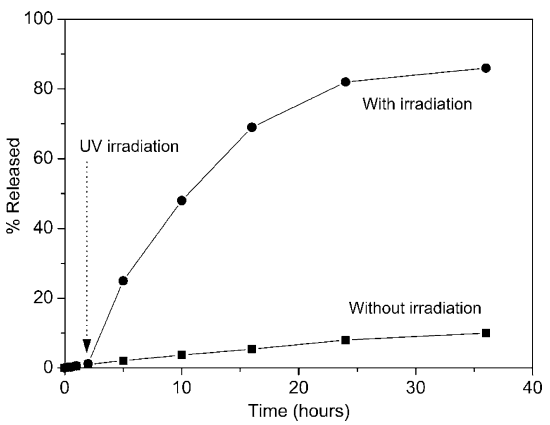

Fig. 3 Time profiles of fluorescein release from hybrid nanomaterials with and without UV irradiation.

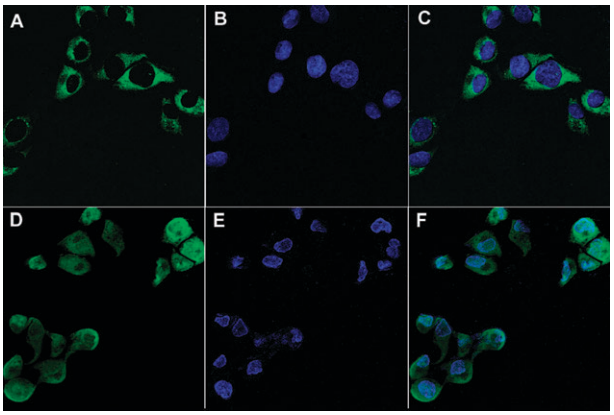

Fig. 4 Confocal fluorescence microscopy images of MHCC97H cells incubated with fluorescein loaded MS@polymer nanoparticles (5 μg mL^{-1}) before (A–C) and after (D–F) UV irradiation. (A) and (D) show the emissions of fluorescein excited at 488 nm; (B) and (E) show the emissions of Hoechst 33258 excited at 405 nm, which indicate the cell nucleus; (C) and (F) show the overlay images.

that the hybrid nanoparticles are cell permeable and biocompatible so they are suitable for intracellular light-controlled drug delivery. However, at this stage the dispersion in aqueous solution of the hybrid nanoparticles in pore-closed state is not high due to the hydrophobic nature of the polymer shell (Fig. S8). We hope that modification of the silica surface or polymer shell with hydrophilic macromolecules such as PEG would improve this parameter.[8]

In conclusion, we have developed a novel controlled-release system that uses mesoporous silica nanoparticles as nanocontainers and polymer as light-responsive valve. Light-responsive polymer that has a LCST of 14 °C, below 37 °C, was tethered to the surface of MCM-41 type MS nanoparticles to lock the loaded molecules within the MS pores. Upon UV irradiation, the hydrophobic NBAE groups in the polymer backbone were photolysed into hydrophilic acrylate which led to an increase in the LCST of the resulting copolymers to 46 °C, now higher than 37 °C. Thus the polymer changed its phase state at 37 °C and the loaded molecules were released from the hybrid nanoparticles. The system possesses advantages such as non-invasive and high spatiotemporal resolution resulting from the using of light stimulus, as well as good biocompatibility and ease in functionalization. The results reported here suggest that it can be applied in stimulus controlled drug and gene delivery systems for biological applications.

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