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Light-triggered reversible assemblies of azobenzene-containing amphiphilic copolymer with β-cyclodextrin-modified hollow mesoporous silica nanoparticles for controlled drug release†

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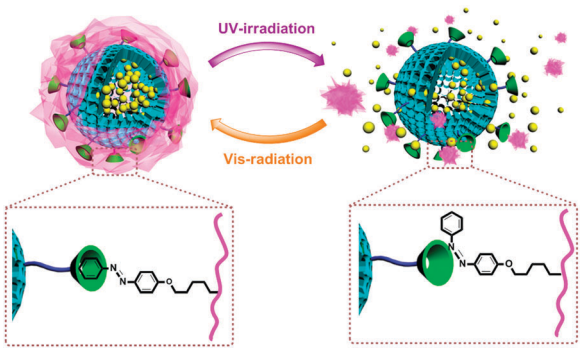
Hollow mesoporous silica nanoparticles (HMSs) were modified by β-cyclodextrin *via* a "click" reaction, an amphiphilic copolymer with a *trans*-azobenzene structure was then assembled onto β-cyclodextrin to cover the surface of the HMSs. The prepared nanocomposites can release drugs in a "release-stop-release" manner by converting light irradiation.

Mesoporous silica nanoparticles (MSNs) are generally considered as ideal drug delivery candidates due to their good biocompatibility and biodistribution, large surface area, high specificity due to modifiable Si–OH groups on the surface, and adjustable pores for sustained drugs release.[1] However, unmodified MSNs would prematurely release the loaded drug before reaching the site of action, which limit their practical application.[2] To overcome this disadvantage, researchers introduced "gate-keeper" systems to MSNs, which can not only avoid premature leakage of drugs, but also manipulate the drug release in a more controllable manner.[3]

So far most of these "gate-keeper" systems are activated by chemical triggers such as pH change, redox or enzymes[4] to achieve the desired controllable drug release. These triggers are usually designed based on the specific property of the target, such as weakly acidic environment of the tumor tissue,[5] highly reducing environment of cytosol.[6] However, these traditional "gate-keeper" systems are usually irreversible. These "gate-keeper" systems usually cannot be closed once they are open. This is a critical problem in MSNs based drug delivery systems. The drug loading of MSNs are usually very high and the drug release takes quite a long time (release of 80% of the loaded drugs takes about 60 h).[7] Most of drugs will thus go into blood circulation before being released completely at the target site (94.4% Si element could be excreted out through urine and faeces within 4 d).[8] MSNs equipped with irreversible "gate-keeper" systems would thus cause "secondary" side effects for normal tissues. It is desirable to design and synthesize an intelligent "gate-keeper" system, which can achieve reversible switching from "on" to "off" by external stimulation. This could be realized by host–guest interactions between azobenzene (Azo) and cyclodextrin (CD). It is known that *trans*- and *cis*- isomers of Azo can reversibly switched between each other upon light irradiation.[9] *Trans*-Azo can be well recognized by CD through hydrophobic and van der Waals interactions, while *cis*-Azo cannot. This process is fully reversible under alternating irradiation with UV and visible (Vis) light.[10] In addition, light is an attractive means of stimuli as it can be remotely controlled with high spatial and temporal accuracy easily.[11] Based on this principle, host–guest assembly and disassembly between Azo and CD hold great potential to manufacture a reversible "gate-keeper" system.

Here we designed a polymer-coated MSNs system as a drug carrier (see Scheme 1). Hollow mesoporous silica nanoparticles (HMSs) were used as drug delivery matrix owing to their high drug loading capacity.[12] HMSs were modified by β-cyclodextrin (β-CD) *via* a "click" reaction. An Azo-containing amphiphilic copolymer poly(PPHM-co-PEGMEM) (PPP) was synthesized by radical copolymerization and used as the light-stimulated "gate-keeper". We utilized light controlled inclusion and exclusion reaction of the PPP with β-CD on the surface of HMSs to reversibly control drug release.

The Azo-containing amphiphilic copolymer PPP was synthesized by radical copolymerization of 6-(4-(phenyldiazenyl)phenoxy)hexyl



Scheme 1 Schematic depiction of light-triggered drug release from HMS@β-CD@PPP.

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methacrylate (PPHM) and poly(ethylene glycol) methyl ether methacrylate (PEGMEM, 950) in cyclohexanone. The GPC data of the copolymer with different molar ratios was summarized in Table S1 (ESI†). A monomer feed ratio of [PPHM] : [PEGMEM] = 1 : 3 was chosen for detailed investigation. The structure of PPP was confirmed by ^{1}H NMR (Fig. S1, ESI†). The peaks of Azo (6.99–7.88 ppm) and PEGMEG (3.36 and 3.63 ppm) were clearly observed in spectrum, confirming that the amphiphilic copolymer PPP with a well-defined structure was successfully synthesized.

HMSs with a diameter of ~180 nm and low PDI (0.03) were prepared. HMS was first modified with an alkyne (HMS–alkyne). The azide modified β-CD was then bound to the surface of HMS–alkyne *via* a "click" reaction (HMS@β-CD). The FT-IR spectrum was used to characterize HMS–alkyne and HMS@β-CD (Fig. S2, ESI†).[13]

Ibuprofen (IBU) was used as the model drug and UV spectrophotometry was employed to monitor the drug loading and releasing processes. The drug loading was calculated to be as high as 782 mg g^{-1} (IBU/carrier) according to a calibration curve of IBU hexane solution (details in the ESI†). The reason for such high drug loading was primarily attributed to the hollow core of HMS that can store more drug molecules than normal mesoporous silica nanoparticles.[14]

The *cis*-Azo units were known to be recognized by β-CD.[10] The prepared *cis*-Azo containing amphiphilic copolymer PPP and HMS@β-CD will bind through multivalent inter-vesicular host–guest interactions. TEM images (Fig. 1) show the morphology of HMS@β-CD before and after binding with PPP. Compared to the pure HMS (Fig. 1a), there was little change in the morphology of HMS@β-CD (Fig. 1b) and the pores in the silica shell remained clearly visible, suggesting that modification with β-CD does not affect the porosity of HMS. After binding with PPP, a clear boundary between HMS and the polymer film was observed and the lineament of the nanoparticles become blurred (Fig. 1c). These results unambiguously confirmed that PPP has been successfully attached to the surface of HMS@β-CD.

Trans-Azo is known able to transform into *cis*-Azo under UV-irradiation.[9] Fig. S6 (ESI†) showed the comparison of ^{1}H NMR of PPP before and after irradiation with UV light. After irradiation with UV light for about 4 h, the peaks at δ = 6.99, 7.48, 7.88 ppm (Fig. S6-a, ESI†) that arise from the aromatic protons of the *trans*-Azo groups shifted to 6.72, 6.85 and 7.15 ppm (Fig. S6-b, ESI†), which can be attributed to the *cis*-Azo groups. This result confirmed that PPP underwent *trans*- to *cis*- conformational changes under UV-irradiation.

Trans-Azo is known able to form host–guest inclusion with CD, while *cis*-Azo cannot.[15] PPP is expected to undergo reversible light-responsive binding and unbinding from the surface of β-CD modified HMS. The photographs and change in hydrodynamic diameter under alternating UV and Vis light irradiation shown in Fig. 2 demonstrate this process. The HMS@β-CD dispersion (1) and PPP solution (2) alone were milky and orange (Fig. 2a) with particles size of ~183 nm (PDI = 0.033) and ~24.8 nm (PDI = 0.382) respectively. Upon mixing, HMS@β-CD and PPP assemble and form a yellow precipitate (Fig. 2b), the color of residue after washing and centrifugation was turned to yellow, the average particle size of the suspension was about 205 nm (PDI = 0.052), indicating the PPP aggregated to HMS@β-CD successfully and the product still kept a narrow size distribution. Then the sample was treated by irradiation of UV light for 2 h (Fig. 2c), the color of residue faded and the supernatant became pale yellow, because after UV-irradiation, some of the *cis*-Azo groups turned to *trans*-Azo, making the PPP leave the cavity of β-CD and re-dissolve in the supernatant. A new peak around 10 to 100 nm appeared in the particle size data of the suspension, which is strong evidence to prove that the PPP fell off and re-dissolved in the water. After stopping the irradiation of the UV light, *trans*-Azo would revert to *cis*-Azo and PPP would re-wrap the HMS@β-CD. The photograph in Fig. 2d verified this surmise because after stopping the UV-irradiation for about 2 h, the supernatant return to colorless. The peak of particle size attributed to PPP also decreased, suggesting that the PPP was re-wrapped to HMS@β-CD.

The above experiments confirm that HMS@β-CD and PPP can undergo a reversible binding and unbinding reaction under UV and Visible irradiation. Under Vis-light irradiation, *trans*-Azo groups in the polymer side chains can bind with β-CD through multivalent intervesicular host–guest interactions, resulting in formation of PPP wrapped HMS and shut the drugs inside the hollow core. After irradiation with UV light, *trans*-Azo was transformed into *cis*-Azo and consequently PPP was detached from the surface of HMS, allowing the release of the drug. Fig. 3 shows the results of drug release from IBU@HMS@β-CD@PPP *in vitro*. Two equal samples of IBU@HMS@β-CD@PPP were immersed in simulated body fluid (SBF) at 37 °C, one was placed under visible light and the other was treated by UV irradiation (λ = 365 nm). The drug releasing solution in a specified time was obtained by dialysis against SBF (MWCO = 3500) and the drug concentration was analyzed by UV-Vis spectroscopy. Fig. 3a shows that

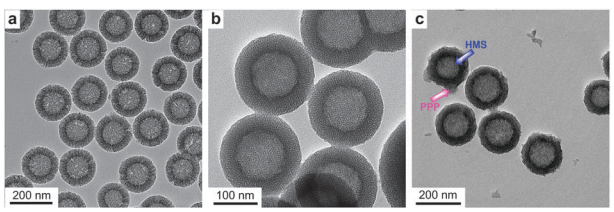

Fig. 1 TEM images of HMS (a), HMS@β-CD (b) and HMS@β-CD@PPP (c).

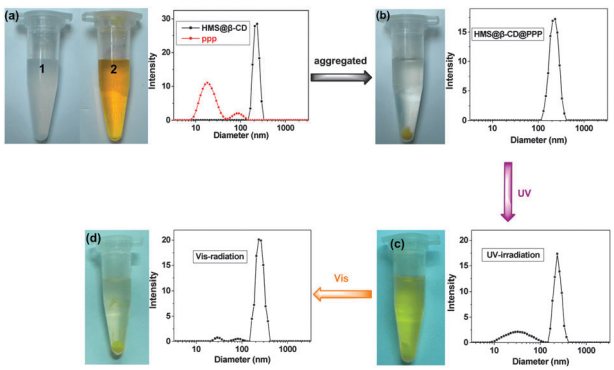

Fig. 2 The photographs and size distribution of HMS@β-CD and PPP (a), HMS@β-CD@PPP (b), HMS@β-CD@PPP under UV-irradiation (c) and HMS@β-CD@PPP under Vis-radiation (d).

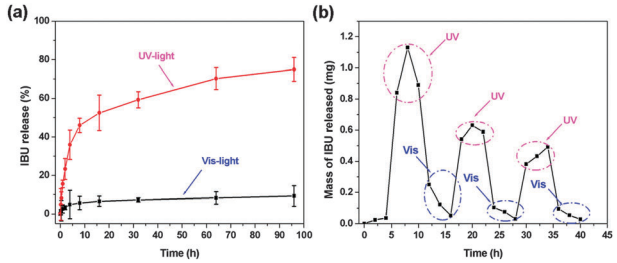

Fig. 3 Release of IBU *in vitro* from IBU@HMS@β-CD@PPP under different light irradiation at 37 °C (a) and under the conversion of UV and Vis (b).

80 wt% of IBU was released within 100 h under UV irradiation while less than 10 wt% of IBU was released under Vis-radiation. This result strongly demonstrated that HMS@β-CD@PPP would only release drugs by the stimulation of UV light.

To further investigate the reversibility of this "gate-keeper" system, an IBU@HMS@β-CD@PPP sample solution was treated with alternating UV and Vis irradiation every 6 h. The concentration of the released drug was analyzed by UV-Vis spectroscopy every 2 h and the results are summarized in Fig. 3b. It can be seen that the amount of drug release reduced significantly once the light irradiation switched from UV to Vis. This results strongly demonstrates that drug release could be initiated by UV light irradiation and shut off by Vis light irradiation.

In summary, we have obtained a reversible polymer "gate-keeper" system based on light-triggered binding and unbinding between Azo and β-CD-modified HMS. The UV light could transform the isomerism of the Azo groups from *trans* to *cis* conformation, which result in detachment of PPP from β-CD modified HMS, triggering the drug release. Most importantly, this drug delivery also could stop releasing by irradiation with Vis light. This strategy is expected to solve the problem of premature drug release in normal MSN based drug delivery and "secondary" side effects caused by the residual drug in the irreversible "gate-keeper" systems.

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