Stimuli-responsive biocompatible nanovalves based on β-cyclodextrin modified poly(glycidyl methacrylate)$^\dagger$

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β-Cyclodextrins (β-CDs) were grafted onto star-shaped poly(glycidyl methacrylate)s (5S-PGMAs) with a straightforward and efficient ring-opening addition of amine groups to result in PGMA–CDs, which not only possess good water-solubility and biocompatibility, but also can serve as polymeric supramolecular hosts to form inclusion complexes with suitable guests. They can be easily assembled on the surface of azobenzene-functionalized mesoporous silica nanoparticles (MSNs) via host–guest interactions to obtain MSN@PGMA–CD hybrid nanoparticles, which have been fully characterized by Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), transmission electron microscopy (TEM) and elemental analysis. The experimental results showed that these types of inorganic–organic hybrid mesoporous nanocomposites possess good cargo encapsulation and release properties, as compared with the simple supramolecular nanovalves with β-CD itself as the gating component, upon activation by light, temperature variation, and competitive binding agents. In addition, the extremely low cytotoxicity of the nanocomposites demonstrated by MTT assay can further broaden their applications in controlled drug release.

Introduction

As human health problems are becoming increasingly serious, much research has been focused on nanomaterials with biomedical potential. Especially, stimuli-responsive nanocontainers for drug delivery and release have recently attracted widespread interest in chemical and biological fields. Self-assembled polymeric micelles are traditional nanocontainers that are formed under some certain conditions and destroyed when the environments (pH, temperature, light, etc.) are changed to realize controlled drug release.$^{1-10}$ In the face of extremely complicated physiological environments, their dependency on the surroundings is a sign of instability and limits the applications. Other traditional nanocarriers are functionalized inorganic frameworks with large surface areas and suitable pore volumes, such as mesoporous silica nanoparticles (MSNs),$^{11-16}$ metal–organic frameworks (MOFs),$^{17,18}$ and zeolite imidazolate frameworks (ZIFs).$^{19-21}$ MSNs are always the ideal candidates as drug carriers because of their special characters, easy preparation and surface-modification, good stability and biocompatibility.$^{11,16}$

Nowadays, many researchers have devoted themselves to build new stimuli-responsive systems for drug release by combining the advantages of silica nanoparticles (NPs) and polymers. Different kinds of stimuli-responsive polymers have been functionalized on the surfaces of MSNs via direct covalent bonds$^{22-27}$ or the method of assembly.$^{28-31}$ Narain et al. used the thiol–ene click chemistry to fabricate pH and temperature responsive polymer functionalized silica NPs.$^{26}$ Zhao et al. constructed nanocomposites successfully through strong complexation of adamantane grafted onto poly(ethylene glycol) polymers and β-cyclodextrins (β-CDs) functionalized on MSNs.$^{29}$ These previously reported systems mostly relied on the stimuliresponsiveness of polymers themselves that did not involve supramolecular assembly and disassembly of macromolecular host-containing copolymers with stalk components in the surfaces of MSNs.$^{32}$ In addition, poly(glycidyl methacrylate)s (PGMAs) are a unique type of polymers that are inexpensive and easy to be functionalized with a straightforward and efficient ring-opening addition method, generating different kinds of PGMA derivatives.$^{33-36}$ Especially, star-shaped (4- to 8-arms) PGMAs in addition to simple linear shaped ones offer more potential as compared with traditional polymers. However, to
the best of our knowledge, there is no report on nanovalve systems based on CD-containing PGMA, no matter linear shaped or star-shaped, for controlled delivery.

On the other hand, azobenzene derivatives mostly present in their trans-configurations in the ground state, while turn into cis-configurations upon irradiation by UV-light. According to their isomerization capability, they are usually tethered to the inner pore walls of MSNs as nanoimpellers, which have been studied by the Zink group and us. However, they can also serve as stalk components encircled by macrocyclic receptors to constitute [2]rotaxanes or [2]pseudorotaxanes in nanovalve systems when they are tethered to the outer surfaces of MSNs. β-CD shows a higher binding affinity towards trans-azobenzene derivatives compared with cis-azobenzene derivatives in aqueous solutions.

Herein, we chose 5-arm PGMA (S5-PGMA) and utilised ring-opening addition of amine groups to generate β-CD modified S5-PGMA (PGMA–CD) which not only has good water solubility and biocompatibility, but also serves as polymeric supramolecular hosts to form inclusion complexes with suitable guest compounds. Therefore, we designed an inorganic–organic–polymeric hybrid mesoporous nanocomposite fabricated through the assembly of β-CD incorporated S5-PGMA with azobenzene-functionalized MSNs (MSN-azo) and further studied its controlled cargo release in response to external stimuli such as light, temperature variations, and competitive binding agents (Scheme 1). Its biological compatibility was also analysed by the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

**Experimental section**

**Materials and methods**

Cetyltrimethylammonium bromide (CTAB) was purchased from Sinopharm. Glycidyl methacrylate (GMA), 2-bromoisobutyryl bromide, bipyridyl and CuBr were bought from Shanghai Adamas Reagent Co. Ltd. (Shanghai, China). Other starting materials and reagents, such as tetraethoxysilane (TEOS), (3-aminopropyl)triethoxysilane (APTES), and dicyclohexyl-carbodiimide (DCC), were purchased from Aladdin. Toluene was dried using calcium hydride before use. 2-[4-(p-Tolylazo)phenoxy]acetate ethyl ester (M-2, ESI†) was synthesized according to the method reported in the literature. Atom transfer radical polymerization (ATRP) initiator, i.e., 1,2,3,4,6-penta-O-isobutyrylbromide-α-D-glucose, was synthesized using a convergent route developed by us. Mono(6-ethyleneamidine)-6-deoxy)-β-CD (EDA-CD) was synthesized according to the literature reports.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 300 MHz NMR spectrometer. The mass spectrum was recorded on an Agilent 1290 MicrOTOF QII instrument. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Vertex 80 V spectrometer. Elemental analysis was performed using an Elementar vario micro 45 cube. Small-angle powder X-ray diffraction (XRD) measurements were carried out using a Rigaku SmartLab III powder diffractometer. The radiation source was copper (Kα = 1.39225 Å). Scanning electron microscope (SEM) images were collected on a JEOL JSM 6700F instrument. Au coating of the particles used for imaging was
carried out by sputtering for 2 min. Transmission electron microscopy (TEM) images were collected on a Hitachi H-800 instrument, and the accelerated voltage was 200 kV. Thermogravimetric analysis (TGA) was done using a Q500 instrument. Dynamic light scattering (DLS) measurement was performed on a Zetasizer Nano ZS instrument. N₂ adsorption and desorption isotherms (BET and BJH) were carried out using a Micromeritics Gemini instrument. The controlled release profiles were obtained via UV-vis spectroscopy using a Shimadzu UV-1800.

The materials were stimulated using an Nd:YAG laser (λ = 355 nm, pulse width: 5 ns, frequency: 10 Hz) whose energy was adjusted by a set of neutral filters.

**Synthesis of PGMA-CD**

S5-PGMA was synthesized via ATRP. Bipyridine (1.5 equiv.), ATRP initiator (5-amin initiator of 2-bromoisobutyl bromide) (1.0 equiv.) and CuBr (1.0 equiv.) were added into the THF solution (0.30 M) of GMA (105 equiv.). The reaction was carried out under argon for 15 min at room temperature and then heated at 90 °C for 24 h. After cooling down, the mixture was passed through a silica gel column with THF as an eluent to remove copper. After evaporation of the solvent, the product was dissolved in a small volume of THF and precipitated twice in diethyl ether.

S5-PGMA (0.10 g) and EDA-β-CD (1.00 g) were dissolved in DMF (20 mL). The mixture was heated at 80 °C for 12 h. Then, ethylenediamine (8 mL) was added to the flask and reacted for another 12 h in order to complete ring-opening reaction of epoxy groups. The resulting crude PGMA–CD product was purified by dialysis against deionized H₂O, followed by lyophilization.

**Synthesis of 2-[4-(p-tolylazo)phenoxyl]acetic acid (M)**

NaOH (aq., 100 mL, 0.25 M) was added to a solution of 2-[4-(p-tolylazo)phenoxyl]acetate ethyl ester (2.98 g, 8.6 mmol, 86%) of CD product was removed copper. A

**Azobenzene functionalization of MSNs (MSN-azo NPs)**

MSN-OH NPs were functionalized using our previously reported method.¹⁴ MSN-OH NPs (0.50 g) were suspended in anhydrous toluene (50 mL), and APTES (1.2 mL, 5.0 mmol) was added to the mixture. The solution was refluxed under a nitrogen atmosphere for 24 h. After it was cooled down to room temperature, the product was centrifuged and washed thoroughly with anhydrous toluene and MeOH, and dried at 50 °C overnight under reduced pressure to give MSN–NH₂ NPs.

**Construction of supramolecular nanocomposites composed of MSN-azo NPs and PGMA–CDs**

MSN-azo NPs (20 mg) were dispersed in distilled H₂O (12 mL) by soaking them at room temperature. After stirring, the PGMA–CD solution (2 mL, 40 mg mL⁻¹) was added dropwise to the mixture solution and stirred for 20 h.

**Controlled release experiments**

The probe molecule, rhodamine B (RhB), was loaded into the mesopores of MSN-azo NPs by soaking the NPs (10 mg) in an aqueous solution of dye (6 mL, 3 mM) at room temperature. After stirring for 20 h, the solution was centrifuged and concentrated to 2 mL. Then, the PGMA–CD solution (1 mL, 20 mg mL⁻¹) was added to the mixture containing NPs and dyes and stirred for another 20 h. The RhB-loaded PGMA–CD-capped MSNs were separated with centrifugation and washed with distilled H₂O more than 5 times. Finally, the red products (RhB-loaded MSN@PGMA–CD) were dried in a vacuum oven overnight.

RhB-loaded MSN@PGMA–CD NPs (1.5 mg) were placed in the bottom corner of a cuvette, and 1-adamantamamine hydrochloride (AH) solution was carefully added. To monitor the release of RhB, the UV absorption spectrum of the solution was recorded with the change of time. Instead of AH solution, we added distilled H₂O into the cuvette. And then a 355 nm UV-light laser was directed into the solution on the assembled NPs to activate the nanovalves. Its responsiveness to temperature was also investigated at an elevated temperature (50 °C). Meanwhile, its cargo encapsulation and release property were compared with those of the RhB-loaded, CD-capped MSNs (RhB-loaded MSN@CD) that were prepared under the same conditions through competitive binding of AH.

**In vitro cytotoxicity**

The cytotoxicities of MSN-azo NPs and MSN@PGMA–CD in vitro were evaluated by the standard MTT assay. Two types of cells, namely, normal human embryonic kidney (HEK) 293 cells and human cervical adenocarcinoma (HeLa) cells, were chosen. The cells were cultivated in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin
(100 units per mL), and streptomycin (100 mg mL\(^{-1}\)) at 37 °C in tissue culture flasks in a moist atmosphere (5% CO\(_2\)/95% O\(_2\)). After three generations, they were sowed into 96-well plates with 4000 cells per well and incubated for 24 h under the same conditions as before. For toxicity studies, the materials before and after assembly were added into 96-well plates at different gradient concentrations, i.e., 5, 10, 25, 50, 100 mg mL\(^{-1}\), and cells were allowed to grow for 44 h. Then MTT solution (20 μL, 5 mg mL\(^{-1}\)) was added to each well and cells were incubated for 4 h. The resulting formazone crystals were solubilized by adding DMSO (150 μL) to each well. The extent of cell survival was determined at 490 nm using an automated microplate reader. The cell viability was calculated by using the following formula: viability (%) = (mean absorbance value of treatment group/mean absorbance value of control) × 100.

Results and discussion

The average molecular weight of S5-PGMA was about 9 kDa by Gel Permeation Chromatography (GPC) (see ESI, Fig. S6†). EDA-CD and ethylenediamine were added into the DMF solution of S5-PGMA in succession to avoid polymers from crosslinking, generating the water-soluble and biocompatible PGMA-CD. The conjugate ratio of β-CDs to the side chain of PGMA was 11.8% calculated using phenol-sulphuric acid assay (see ESI, Fig. S7†), indicating that there is one CD in every eight repeating units of PGMA-CD. \(^1\)H NMR spectra of polymers are given in Fig. S4 and S5†.

We can deduce that MSN-OH NPs possess uniform mesoporous channels, narrow pore size distribution and high specific surface area (948 m\(^2\) g\(^{-1}\)) from the N\(_2\) adsorption/desorption measurement (Fig. S8, ESI†). Functionalized MSNs modified by the azobenzene derivatives onto its outside surface were prepared through a post-synthetic grafting method. The morphology of MSN-azo by SEM (Fig. 1) showed that MSN-azo NPs are mainly homogeneous spherical in shape, with an average size of ca. 210 nm in diameter, which was also confirmed by DLS (Table S1, ESI†) and TEM (Fig. 1). The size measured by DLS was a little bigger because it was the NPs' hydration diameter instead of the real diameter. TEM also reveals the existence of ordered 2D hexagonal arrays of cylindrical mesopores (ca. 2.4 nm in diameter) of the MSN-azo NPs. And after PGMA-CD capping, the MSNs exhibited less smooth surface and the mesopores became blurry, visually demonstrating that PGMA-CDs were successfully assembled on the surface of MSN-azo NPs and could thus effectively block their por orifices.

The microcrystalline structure of silica NPs was verified by small-angle powder XRD (Fig. 2). The intermediate MSN-OH NPs showed clear standard Bragg peaks of (100), (110), (200) and a faint peak of (210), reflecting a highly ordered 2D hexagonal array, in accordance with the results demonstrated by TEM (Fig. S9, ESI†). The distance between adjacent pores and pore diameters of these MSNs were variables related to 2\(θ\).\(^{14}\) Although the materials were surface-modified with azobenzene derivative M and encased by PGMA-CD polymers, they still maintain an ordered 2D hexagonal structure, and the distance between adjacent pores and pore diameters of materials have just negligible changes. All data presented above indicated the good stability of the materials we prepared so far.

FT-IR spectroscopy (Fig. 3) has been used to monitor the progress of MSNs’ functionalization based on MSN-OH NPs. There were peaks at 1641 cm\(^{-1}\) and 1562 cm\(^{-1}\) in the spectrum of MSN-NH\(_2\) NPs, which are the characteristic absorption of the amino group. After modification of MSN-NH\(_2\) with azobenzene derivatives, the absorption band at 3461 cm\(^{-1}\) disappeared and a new sharp band at 1670 cm\(^{-1}\) was shown, providing further evidence of azobenzene functionalization on the surfaces of MSNs. Those peaks at 1530 cm\(^{-1}\), 1447 cm\(^{-1}\) and 1381 cm\(^{-1}\) belong to the benzene ring’s skeletal vibration of
the azobenzene moiety. The growth of the peaks of the RhB-loaded MSN@PGMA–CD curve at 3288 cm\(^{-1}\), 2939 cm\(^{-1}\) and 1661 cm\(^{-1}\) compared with the curves of MSN-azo and PGMA–CD is indicative of the fact that PGMA–CDs have encased on the surface of MSN-azo NPs through hydrophobic interactions between hosts and guests. Since MSNs are thermally stable, the lost weight of materials in TGA curves (Fig. 4) at high temperature (>100 °C) was ascribed to the loss of its surface groups. There was about 5.75% weight loss of MSN-azo NPs except for the loss of MSN-NH\(_2\) and we can conclude that about 0.289 mmol g\(^{-1}\) of azobenzene derivative \(M\) was modified onto MSN-NH\(_2\) NPs. Then a number of PGMA–CDs were successfully assembled on the surface of MSNs inferred by the spare 7.73% of weight loss of MSN@PGMA–CD NPs. There were significant changes of the average contents of C element and N element though elemental analysis (Table S2, ESI†). About 4.90% of azobenzene derivatives were grafted onto the surface of MSN–NH\(_2\) NPs, which is consistent with the numerical analysis of TGA (5.75%) according to the change of N element content.

The fiber optical spectrum of MSN-azo NPs (Fig. S11, ESI†) compared with MSN–NH\(_2\) shows three bands concentrated at 235 nm, 343 nm, and 433 nm, which belong to the absorptions of azobenzene entity grafted onto the surfaces of MSNs. The strongest absorption at 343 nm indicated that azobenzene entities mainly presented in the trans-isomerization. But there is also a small portion of cis-isomers deduced from the weak absorption at 433 nm, which is negligible. According to the fiber optical spectrum of MSN-azo, laser light at 355 nm can be used to realize the trans-to-cis isomerization of azobenzenes modified on the surfaces of MSNs.

In order to further test the operation of this system, AH was chosen as a competitive binding agent, because of its stronger binding affinity towards β-CD as compared with azobenzene. The probe (RhB) was loaded into MSN-azo NPs and then capped by PGMA–CD in two kinds of solvents, i.e., distilled H\(_2\)O or EtOH. The cargo loading capacity in distilled H\(_2\)O is ca. 1.5 times of the loading in EtOH calculated through the release curves of competitive binding provided in Fig. S12, ESI.† So the samples of RhB-loaded MSN@PGMA–CD used in other experiments were all prepared in distilled H\(_2\)O. A laser beam (355 nm) was chosen to study this polymeric nanovalves’ responsive ability to UV light. When the light was focused on the NPs, the trans-to-cis isomerization of azobenzene units partly occurred, leading to the dissociation of β-CD in PGMA–CD from the trans-azobenzene-containing stalks. While star-shaped PGMA–CD functions as a net covering on the surface of MSNs, the dissociation of β-CD from the trans stalks only made the net become more loosened and have some distance from the orifices of MSNs, shown in Scheme 1. So, slow-release occurred as demonstrated in Fig. 5 and there is still 30.0% release (with 10.8% spontaneous leakage deducted) activated by light compared with a competitive binding method (defined as 100% effective release). In addition, we found that PGMA–CD can be more effective to reduce spontaneous leakage and the effective

**Fig. 3** FT-IR spectra of (a) MSN–OH (black), (b) MSN–NH\(_2\) (red), (c) MSN-azo (blue), (d) RhB-loaded MSN@PGMA–CD (green), and (e) PGMA–CD (pink).

**Fig. 4** TGA analysis of (a) MSN–NH\(_2\) (black), (b) MSN–azo (red) and (c) MSN@PGMA–CD (blue).

**Fig. 5** The release profiles of RhB dyes from MSN@PGMA–CDs (a) without activation, (b) with UV-light (355 nm) activation and (c) upon addition of a competitive binding agent (AH).
release of MSN-azo NPs capped by PGMA–CD was two times of that capped with only β-CD itself (Fig. 6). Significantly, the assembled nanocomposites were responsive to temperature because they were formed via host–guest interactions that would become weakened with an elevated temperature (Fig. S13, ESI†).

As shown in Fig. 7, with the increase of concentration of the two materials, the cell viabilities showed a declining trend. However, they only had slight toxicity to normal human cells, which was deduced from the fact that the cell viabilities were higher than 60%, even though their concentration was as high as 100 μg mL⁻¹. Hela cells had higher viability compared with 293 cells because of cancer cells’ tenacious vitality.³³ Over all, the materials, before and after PGMA–CD assembly, possess negligible cytotoxicity at low concentrations, allowing them to be used as nanocontainers for controlled drug delivery.

Conclusions

On reflection, we have demonstrated by the employment of multiple methods, such as TEM, FTIR, elemental analysis, and TGA, β-CD incorporated PGMA polymers can be successfully assembled on the surface of azobenzene-functionalized MSNs via host–guest interactions between the β-CD unit and azobenzene moiety. The resulting polymeric–organic–inorganic hybrid nanomaterials were responsive to UV-light, competitive binding agents and temperature, and showed better performance on encapsulation and release of cargos compared with the traditional-type of nanovalve system utilizing β-CD itself instead of PGMA–CD polymers as demonstrated by the controlled release experiments. Their extremely low cytotoxicity to cells also proves the suitability for their further use in drug release. More importantly, polymeric supramolecular assembly and disassembly on the surface of MSNs to build and regulate the gating machines were realized in this work, providing a novel and feasible method in the field of on command drug release.

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Notes and references
