Tuning the growth, crosslinking, and gating effect of disulfide-containing PGMAs on the surfaces of mesoporous silica nanoparticles for redox/pH dual-controlled cargo release†

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The surface modification of mesoporous silica nanoparticles (MSNs) with poly(glycidyl methacrylate) (PGMA) was conducted through surface-initiated atom transfer radical polymerization (SI-ATRP). The pendant epoxide of PGMA was ring-opened by cystamine dihydrochloride, a disulfide bond-based bifunctional primary amine. The grafted PGMAs on MSN surfaces could then be cross-linked by changing the stoichiometric ratio of the epoxide to cystamine or through a KI/H2O2 (30%) assisted disulfide bond exchange after ring opening by cystamine dihydrochloride. The MSN–PGMA hybrid nanocarriers show excellent cargo release behaviour in response to the changes of external pH. Significantly, these disulfide linkages on MSNs can be cleaved in the presence of physiological concentrations of glutathione (GSH) in cancer cells, so that the surface PGMA network degrades thus opening the pore entrances for cargo release. These disulfide cross-linked PGMA network-coated MSNs are of great interest for on-demand anticancer drug delivery applications.

Introduction

Polymer-based stimuli-responsive materials are becoming more and more attractive in nanomedicine and materials science, owing to their great potential in therapeutic cargo delivery, diagnostics or tissue engineering, biosensors, coatings, and so on.1–3 Among them, stimuli-responsive polymeric nanoparticles have received great attention for applications in bio-related areas,4–6 because they could limit the undesired premature release of drug molecules, prolong the circulation of therapeutic molecules, and realize controlled drug release in targeted disease areas in response to specific internal or external stimuli including redox,7–13 pH,14,15 temperature,15–18 light,19 enzyme,20,21 and the competitive agent.15,22 Meanwhile, as an important cleavable linker, the disulfide bond has been widely explored in drug delivery systems based on different platforms such as nanogels,5 capsules,6,23–25 micelles11,26 and inorganic–organic hybrid nanoparticles.17–29 It can be efficiently cleaved by glutathione (GSH) or thioredoxin (Trx) in a cellular reduction environment through the thiol–disulfide exchange reaction, so as to better serve controlled drug delivery for cancer therapy.5,7,29,30 Poly(glycidyl methacrylate) (PGMA) is a typical type of functional polymer with plenty of pendant epoxy groups, which could readily undergo ring-opening reactions with –OH, –SH, –NH2 and –COOH to result in a variety of functional PGMA derivatives. With an inexpensive monomer source and excellent structural tunability by controlled radical polymerization such as atom transfer radical polymerization (ATRP),31,32 and reversible addition–fragmentation chain transfer (RAFT),33–35 PGMA has become a new kind of useful surface linker or brush for materials science and biochemistry.36–42 PGMA and their abundant derivatives have shown promising applications in drug and gene delivery, enzyme immobilization, immunological assay, and antibacterial agents.43–45 Our research group has reported on the construction of biohybrid nanomaterials based on PGMA derivatives and mesoporous silica nanoparticles (MSNs) for stimuli-responsive drug delivery through the layer-by-layer (LbL) supramolecular assembly technique, which could efficiently respond to pH changes, UV-light, temperature, competitive binding agents, etc.42,46–49 However, until now, no report has shown an efficient method of growing PGMA-type of polymers on MSN surfaces directly to build stimuli-responsive gating systems for controlled drug release.

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Herein, we explore a new kind of stimuli-responsive polymer–MSN biohybrid material via surface-initiated ATRP (SI-ATRP) of glycidyl methacrylate (GMA) on MSNs. Considering the degradable advantages of the disulfide bond, we introduce disulfide bonds into PGMA side chains via cystamine dihydrochloride. The Rhodamine 6G (Rh6G) loaded, cystamine cross-linked PGMA brush-functionalized MSNs show a GSH/pH dual-responsive behaviour for cargo release. We envision that this covalent method could be used to construct various polymer–inorganic biohybrid nanoplatforms for nanomedicine and can pave a new avenue for stimuli-responsive drug delivery employing organic–inorganic hybrids.

Results and discussion

Immobilization of the initiator on the hydroxy-terminated MSNs

The PGMA-functionalized MSNs were fabricated according to the procedure in Schemes 1 and 2. Firstly, hydroxy groups of MSN-OH were converted to 2-bromoisobutyric acid esters in the presence of triethylamine (TEA).41,56 The resulting MSN-Br can then act as a functional initiator for the subsequent SI-ATRP of GMA. X-ray photoelectron spectroscopy (XPS) (Fig. 1) shows the C 1s of MSNs ([a] MSN-OH, [b] MSN-Br). For

Fig. 1 XPS C 1s core-level spectra of (a) MSN-OH (BE at about 284.6, 286.2 eV attributed to the C–H, C–O), (b) MSN-Br (BE at about 284.6 and 286.2 eV, and 288.7 eV attributed to the C–H, C–O/C–Br, and O=C=O), (c) XPS Br 3d core-level spectrum of MSN-Br, and (d) MSN–PGMA (BE at about 284.6, 286.4 eV and 288.7 eV, attributing to the C–H, C–O, and O=C=O).

MSN-Br, the presence of the Br 3d signal at a binding energy (BE) of around 69 eV (Fig. 1c) and the increase of C–O/C–Br and O=C=O components suggested by the C 1s peak of MSN-Br (Fig. 1b) indicate that the 2-bromoisoctyrate group has been successfully immobilized on the MSN surface. The peak at 1729 cm$^{-1}$ in the Fourier transform infrared (FT-IR) spectrum of MSN-Br is attributed to the carbonyl (C=O) stretching vibrations of the ester groups on the initiator (Fig. S1a and b, ESI†), confirming that the ATRP initiators were successfully anchored onto the surfaces of MSNs. Changes in chemical components of MSNs after the modification by ATRP initiators were also investigated by thermogravimetric analysis (TGA, Fig. 2), further demonstrating the successful immobilization of 2-bromoisoctyrate acid esters onto MSN-OH. Compared to MSN-OH, about 6.7% more weight loss between 200 and 900 °C for MSN-Br was found, which is consistent with
the elemental analysis (Table S1, ESI†). About 0.32 mmol g\(^{-1}\) of 2-bromoiso-}

**Synthesis and characterization of PGMA grafted MSNs**

PGMA grafted MSNs were synthesized from initiator-immobilized MSN by SI-ATRP. The resulting MSN–PGMA was first ascertained by FT-IR spectroscopy (Fig. S1c, ESI†). The appearance of the peaks \(\nu_{C=O} 1729 \text{ cm}^{-1}, 1223 \text{ cm}^{-1}\) and \(1168 \text{ cm}^{-1}\) compared with that in the spectrum of MSN–Br (Fig. S1b, ESI†) indicated that PGMA has been grafted onto the surface of MSNs. XPS analysis also revealed that PGMAs were successfully grafted onto the MSN surface (Fig. 1d). Three curve-fitted peaks with BEs of about 284.6 eV, 286.4 eV, and 288.7 eV, were attributed to the C–H, C–O and O–C–O components of PGMA, respectively. Since MSNs are thermally stable, the weight loss of its surface groups of MSN–PGMA was evaluated using the TGA method (Fig. 2). A larger weight loss (39.1\%) of MSN–PGMA than MSN–Br (14.9\%) between 200 and 900 °C further indicated that PGMA polymers were grafted onto MSNs. Compared with the elemental analysis of MSN–PGMA, the weight loss is at the same level (Table S1, ESI†), which also confirmed the presence of PGMA on MSNs. The morphology of MSN–OH was examined by using a scanning electron microscope (SEM, Fig. 3a), which showed that MSNs are mainly monodisperse with diameters of about 78.6 ± 8.4 nm by particle size statistic analysis. After surface modification, the MSN–PGMA surface became less smooth with diameters of ca. 84.6 ± 10.2 nm and its pores became blurry (Fig. 3b). This morphology change illustrated that the PGMA grafted MSNs were successfully fabricated. The microcrystalline structure of MSN–OH was also confirmed by a small-angle powder X-ray diffraction (XRD, Fig. S4, ESI†). MSN–OH showed clear standard Bragg peaks (100, 110, 200) and a faint peak (210), which indicated a highly ordered mesoporous structure in MSN–OH, which was also proven directly by transmission electron microscopy (TEM) (Fig. 3c and S12b, ESI†). After PGMA modification of MSNs, the material, MSN–PGMA, only reserves a Bragg peak (100), which may be attributed to the facts that (a) MSN pores are blocked by PGMA polymers and (b) although the channel array is reserved, the regularity degree is reduced. The 2D hexagonal array of MSN–OH was also invisible in the TEM image of MSN–PGMA (Fig. 3d and S12b, ESI†). Changes in the specific surface areas and pore diameters (Fig. S5, ESI†) of MSN–OH and MSN–PGMA were monitored by N\(_2\) adsorption–desorption isotherms. The Brunauer–Emmett–Teller (BET) surface area of MSNs decreases from 829 m\(^2\) g\(^{-1}\) to 302 m\(^2\) g\(^{-1}\) after PGMA grafting. MSN–OH exhibited the typical type IV isotherms of mesoporous materials while the PGMA grafted one, MSN–PGMA, reveals an isotherm characteristic of the materials.\(^{13}\) Sorption type change, together with a decrease in the surface area and pore size distribution (Table S2, ESI†), indicated that MSNs were capped with PGMAs.

**Cross-linking of PGMA on MSN–PGMA surfaces**

The functionalization of MSN–PGMA was done by cross-linking PGMAs via the reaction of epoxide groups of PGMA with cystamine dihydrochloride (the molar ratio is about 2 : 1 in theory, see Scheme 2), which has been used to construct cross-linked PGMA based materials.\(^{36,50}\) Under these conditions, epoxide groups are opened by cystamine molecules and form disulfide bonds between different epoxide groups. The resulting cross-linked polymer shell on the MSN surface could effectively hold the cargo from an undesired premature leakage. The XPS S 2p spectrum was used to confirm cystamine on the MSN–PGMA surface (Fig. S3a, ESI†). The above reaction needs a higher temperature which may lead to the denaturation of the loaded drug molecule, and thus a mild and faster reaction was developed. We first performed ring-opening of the MSN–PGMA surface grafted PGMA by excessive cystamine (MSN–PSGMA), and then used a reductant to break...
the disulfide to get thiol-pendanted PGMA derivatives, followed by connecting these thiols by H2O2 oxidation. The comb-polymer PSGMA was successfully cross-linked in water at room temperature. The resulting material shows good GSH stimulus-responsive behaviours (data not shown). But in a further experiment, we found that, even when the disulfide bonds were not reduced before H2O2 catalysis, the resulting material also show good GSH-responsive behaviours. According to the report that the disulfide bonds could exchange with each other intermolecularly by simple heating,51 we deduce that the disulfide–disulfide interchange occurred in this system.

**Disulfide bond exchange of PSGMAs**

The construction of disulfide-containing materials is important in biomedical applications. It was reported that the construction of disulfide can be realized by thiol direct cross-linking in the presence of a catalytic amount of DTT or H2O2.6,23,52,53 Meanwhile, researchers also utilized the thiol–disulfide bond exchange approach to construct disulfide-containing cross-linking polymers. For example, the lipoic ring containing polymer structure has been proven to undergo ring-opening to form a disulfide cross-linked network upon applying a catalytic amount of DTT;5,24 and, pyridyl disulfide (PDS) was used as a versatile self-crosslinkable component to construct disulfide linkages in polymers, under a DTT catalyst.4,20 Moreover, Li et al. reported that a disulfide-containing OEGylated polypeptide could undergo a disulfide bond exchange to lead to intermolecular cross-linking upon heating.51 Therefore, we aim to explore whether the addition of catalysts could facilitate the cross-linking of the cystamine-modified comb-shaped PGMA derivative, i.e., PSGMA, via the disulfide bond exchange mechanism.

PSGMA was obtained by ATRP, and its polymeric structure was confirmed by 1H NMR (Fig. S6, ESIT) and gel permeation chromatography (GPC, Mn = 4840, PDI = 1.24) (Fig. S7, ESIT). The PGMA structure was also characterized by FT-IR spectroscopy (Fig. S2a, ESIT). After the ring-opening of epoxy groups of PGMA by cystamine, the epoxy peak at 890 cm−1 totally disappeared in the resulting polymer, PSGMA (Fig. S2b, ESIT), which confirmed that epoxy groups of PGMA underwent ring opening reactions.

To independently ascertain the disulfide exchange-based cross-linking, we performed the catalytic reaction in water using the homopolymer PSGMA. Briefly, a DMF solution of PSGMA was added dropwise into distilled water. The clear and transparent water solution can be kept at room temperature for more than 7 days, and does not become turbid. Once a catalytic amount of KI and H2O2 was added, the solution became cloudy in 2 hours and the suspension increased with increasing reaction time. Under certain reaction times, the turbid solution could quickly turn back to a transparent solution in response to the addition of GSH (30 mM) (Fig. 4). These results indicated that disulfide based cross-linking occurs. Dynamic light scattering (DLS) was used to determine the hydrodynamic diameter changes of PSGMA during the catalysis process. As PSGMA is not fully soluble in water, an aggregation with a diameter of ca. 70 nm was formed whose size did not increase with increasing reaction time (Fig. 5a). After the catalytic reaction, the particle size greatly increased from 70 nm to more than 1000 nm, revealing the occurrence of cross-linking (Fig. 5b).

To further confirm that the cross-linking is based on S–S bonds, the degradation of this aggregate was studied by adding GSH as a reducing agent. The results showed that the diameter of this cross-linked large aggregate decreased to ca. 70 nm in 1 min in the case of less than 8 h of catalytic reactions (Fig. 5c). But if the catalytic reaction was more than 24 h, 1 mL of the aggregate could not be fully reduced by GSH (30 mM) in 0.5 h (see Fig. 5d). This may be attributed to the fact that more S–S cross-linkers were formed in this PSSGMA cross-linked polymer system. If a large excess amount of GSH was added into the 24 h cross-linked PSSGMA, a decrease in the diameter of the aggregate was observed within 0.5 h, and a size decrease of the original one needs about 5 h. As DMF is a good solvent for PSGMA, if the aggregate had resulted from physical interactions, this interaction could be disrupted followed by the re-dissolution of the aggregate upon the addition of more DMF solvent. But even if 10-fold DMF was added to the precipitation-contained system, the aggregate was still visible. This control experiment also confirms that covalent-crosslinking was involved in the aggregation process. In the control experiment, without H2O2 or KI catalysis under otherwise identical conditions, no visible precipitation was observed even with a reaction time of more than 7 days. In order to further confirm that the reaction originated from the disulfide bond exchange, 1,2-ethanediamine (EDA)-modified PGMA (PEGMA) was added instead of PSGMA under otherwise identical catalytic conditions. No visible product was yielded with a long reaction time (more than 7 days). Then, FT-IR spectroscopy was used to analyse the PSSGMA polymer (Fig. S2c†), but no significant change was observed. In the XPS S 2p spectrum, a typical S–S band at a BE of ca. 164 eV was observed in 2 h aggregates (Fig. S3c, ESIT). As over oxidation may occur during this process, XPS was also used to further evalu-
ate the S–S change in this system. After 24 hours of reaction, a BE of ca. 169 eV (attributed to $\ce{-SO3^-}$) was observed, indicating that over oxidation did occur (Fig. S3d, ESI†). However, a lot of S–S bonds were still reserved in the aggregates, and the aggregation could not be dissolved in DMF and still preserved its redox responsive ability. This means that the over oxidation could be controlled by tuning the reaction time. Zeta potential was also recorded when the aggregate formed in 2 h (Table S4, ESI†), which was decreased from +23.8 mV to +15.4 mV as compared with the control experiment. This may be because that part of the pendant cystamines which had positively charged amine groups was cut off by a disulfide intermolecular reconnection. Although over oxidation occurred, the aggregation still showed a positive charge (+13.9 mV) even after 24 hours of reaction. In order to further confirm our conclusion, we repeated the reaction by mixing a disulfide containing molecule, i.e., 2-2((2-hydroxyethyl)disulfanyl)ethyl 2-bromo-2-methylpropanoate (HO-SS-Br) in the presence of KI and H$_2$O$_2$. After stirring for 12 h at room temperature (Scheme S1†), a mixture of bis[2-(2-bromoisobutyryloxy)ethyl]-disulfide (Br-SS-Br) and HO-SS-Br was collected (Fig. S8–S10, ESI†), in which almost 92% was Br-SS-Br. Control experiments were performed by only adding KI or H$_2$O$_2$ under otherwise identical conditions; no changes in HO-SS-Br were observed, demonstrating that the disulfide bond exchange reaction only occurs in the presence of both KI and H$_2$O$_2$. This KI/H$_2$O$_2$ catalyzed disulfide bond exchange process easily proceeded in water at room temperature, which can be seen as an efficient and environmentally benign method to construct cleavable S–S bonds by disulfide bond exchanges. Meanwhile, a catalytic amount of DTT can also work as an efficient catalyst for this kind of reaction in aqueous solutions. It could be anticipated that many other disulfide-containing polymer structures may also be cross-linked by the disulfide bond exchange mechanism through this kind of catalysis process.

Redox-controlled drug release

Based on the above results, we constructed cross-linked disulfide-containing polymers to form stimuli-responsive coatings on MSNs. The probe molecule, Rh6G, was loaded into the MSN–PGMA hybrid material to give Rh6G@MSN–PSSGMA. Both FT-IR (Fig. S1, ESI†) and XPS S 2p spectra (Fig. S3, ESI†) indicated that the surface cross-linked polymer networks were successfully constructed. Their zeta potentials were recorded after cargo loading (Table S4, ESI†), where the potentials of the final materials changed from $-20.4 \text{ mV}$ to $+5.5 \text{ mV}$ (for Rh6G@MSN–PSSGMA-1) and $+12.6 \text{ mV}$ (for Rh6G@MSN–PSSGMA-2). There was about 6.7 wt% of Rh6G loaded into Rh6G@MSN–PSSGMA-1 and 6.1 wt% in the case of Rh6G@MSN–PSSGMA-2. No severe leakage of Rh6G was found in the KI/H$_2$O$_2$-catalyzed cross-linking process.

To confirm whether these cross-linked MSNs possess a stimuli-responsive cargo release capability, the in vitro release profiles of Rh6G were first recorded in distilled water. UV-vis absorption spectroscopy was used to measure the cumulative release of Rh6G from Rh6G@MSN–PSSGMA-1 and

**Fig. 5** Characteristics of diameter changes in particle sizes as monitored by dynamic light scattering (DLS) (a) without the addition of KI and H$_2$O$_2$; (b) with a catalyst in an aqueous solution of PSGMA at room temperature, (c) the particle sizes change in response to the addition of GSH (30 mM); (d) diameter changes after 48 h catalysis and its response to GSH.
Rh6G@MSN–PSSGMA-2. A flat baseline indicates a negligible leakage of dye molecules without external stimuli (Fig. S11a, ESI†). But, the fast release of Rh6G from Rh6G@MSN–PSSGMA-2 or Rh6G@MSN–PSSGMA-1 was observed upon the addition of GSH (10 mM) for 5 h or for 20 h (Fig. S11a and c, ESI†). This indicates that the PGMA polymers on MSNs are firmly cross-linked by degradable disulfide bonds.

Materials fabricated by direct cross-linking in water have the same stimulus-response behaviour (Fig. S11c, ESI†). Next, we investigated the release profile of Rh6G from Rh6G–MSN–PSSGMA-2 in phosphate buffers (PBS) with different pH values, i.e., pH 7.0, pH 5.0 or pH 2.0. As shown in Fig. 6a, the addition of GSH (10 mM) resulted in an accelerated release of Rh6G in any of the above PBS buffers. Meanwhile, this kind of material also showed a pH responsive cargo release (Fig. S11b and d, ESI†). This may be attributed to the partial cross-linking of PSGMAs on MSN surfaces and the unreacted free or secondary amines that could be protonated at a lower pH. The electrostatic repulsion between the positively charged polymer backbones results in the expansion of polymer coatings, which is responsible for the “gate-opening” state at a lower pH for Rh6G transportation out of the mesopores of MSNs. This phenomenon is consistent with other pH-responsive systems built from the amine-containing polymer complexes.47,55–59 As a control experiment, the release of Rh6G from cystamine ring-opened MSN–PGMA (MSN–PSGMA) was also studied, and the cumulative release of Rh6G was found to be much higher than that from the cross-linked ones (Fig. 6b). The different release rates of Rh6G from Rh6G–MSN–PSSGMA-2 and MSN–PSGMA suggest that the model cargo had been confined inside the MSNs more tightly in the case of the disulfide cross-linked MSN–PSSGMA nanocarrier system.

Conclusions

In summary, PGMA–MSN hybrid nanocarriers were fabricated for drug delivery by introducing disulfide bonds as redox-responsive cross-linkers. First, epoxy-pendant-functionalized PGMA polymers were successfully grafted on the surfaces of MSNs by SI-ATRP. Then, a new, facile approach to build disulfide-containing cross-linking structures, which can undergo intermolecular disulfide exchanges, was successfully developed through epoxy ring opening reactions of PGMAs by cystamine dihydrochloride. The KI/H2O2-assisted disulfide bond exchange of PGMAs on MSNs generated polymer-network coatings on MSNs through the cross-linking of PGMA brushes, which in turn can tune the tightness of the polymer gating effect on MSN surfaces. The fabricated organic–inorganic hybrid nanoparticles are stable at physiological pH and could release the encapsulated cargo molecule, be they dyes or drugs, in response to external dual-stimuli, i.e., pH and GSH. We envision that this new approach could have potential applications in constructing a variety of stimuli-responsive biocompatible materials with degradable S–S bonds for various biomedical applications.

Experimental section

Materials

Most of the reagents were of analytical grade and used without further purification unless otherwise noted. 2-Hydroxyethyl disulfide, 2-bromoisobutryl bromide and ethyl 2-bromoisobutyrate (EBiB) were purchased from Sigma-Aldrich and used as received. GMA was purchased from Sigma-Aldrich and passed through a column of basic alumina to remove inhibitors before use. Toluene was dried by refluxing in the presence of Na/benzophenone. Hydroxy-terminated MSN (MSN-OH) and homopolymer PGMA were prepared according to the methods we previously reported.49

Instruments

The chemical composition of MSN-OH and the functionalized MSN–PGMA materials were determined by XPS, performed on
a thermo ESCALAB 250 spectrometer. The elemental composition and contents of MSN-OH and functionalized MSN-Br were analyzed by a Vario EL cube elemental. FT-IR spectra were recorded on a Shimadzu FTIR 8400S spectrometer. TGA was carried out on TA Q500 with a heating rate of 10 K min\(^{-1}\) under a N\(_2\) atmosphere. The morphology of different materials was observed via SEM on a HITACHI SU4800 instrument and TEM on JEM-2100F with an accelerating voltage of 200 kV. Powder XRD patterns were collected on a PANalytical B.V. Empyrean powder diffractometer. The hydrodynamic diameter and zeta potentials of MSN–PGMA hybrids were measured by DLS on a Zetasizer Nano ZS90 instrument (materials were dispersed in deionized water). N\(_2\) adsorption–desorption isotherms were determined by a Micromeritics ASAP 2020 system. The surface area was obtained through the BET method. The Barrett–Joyner–Halenda (BJH) model was used to calculate the pore diameter. The sample was outgassed at 120 °C for 3 h before measurement. Controlled release processes were monitored on a Shimadzu UV-2550 spectrophotometer. GPC was performed on a Waters instrument equipped with a Waters e2695 HPLC pump and a Waters 2414 detector. The mobile phase was THF at a flow rate of 1 mL min\(^{-1}\).

**Immobilization of initiators on MSNs (synthesis of MSN-Br)**

Hydroxy-terminated MSNs (MSN-OH, 0.5 g) were dispersed in anhydrous toluene (150 mL) and sonicated for 1 hour, then the mixture was cooled in an ice-water bath. Triethylamine (TEA, 6.8 mL) was added to the above system, followed by the dropwise addition of 2-bromoisobutyl bromide (3 mL). The reaction mixture was kept at 0 °C for 2 h and then stirred at room temperature for 12 h. Finally, the solvent was removed by centrifugation (13 000 rpm, 30 min) and washed with ethanol (3 × 30 mL), distilled water (3 × 30 mL), ethanol (1 × 30 mL) and chloroform (3 × 30 mL). The resulting sample was dried under vacuum at room temperature overnight and characterized by FT-IR, TGA, elemental analysis and XPS.

**Surface-initiated ATRP from MSN-Br (synthesis of MSN–PGMA)**

MSN-Br (200 mg) was dissolved in DMF (20 mL) in a 25 mL branch-neck, round-bottomed flask, followed by the addition of CuBr\(_2\) (5 mg, 0.022 mM), and N\(_2\),N\(_3\),N\(_4\),N\(_5\)-pentamethyl-diethylenetriamine (PMDETA, 9 μL, 0.044 mM). The reaction mixture was maintained at 50 °C and purged with high purity nitrogen for 0.5 h. After the addition of L-ascorbic acid solution (0.2 mL, 2.2 mM in water), the reaction system was sealed under a nitrogen atmosphere, then monomer GMA (0.5 mL, 3.65 mM) was added to start the reaction. After stirring the reaction mixture for 12 h, the crude product was separated by centrifugation (13 000 rpm, 30 min), washed with chloroform (3 × 30 mL), ethanol (1 × 30 mL), distilled water (3 × 30 mL), and ethanol (1 × 30 mL). The final product was dried under vacuum at room temperature for 12 h and the yielded white solid was characterized by FT-IR, TGA, XPS, etc.

**Ring-open of epoxy in PGMA by a primary amine**

The PGMA material (50 mg, M\(_n\) = 4840, PDI = 1.24) was dissolved in DMF (5 mL) in a 10 mL round-bottomed flask and heated to 80 °C with stirring. About four stoichiometric amounts of the primary amine (cystamine or ethylenediamine) were transferred into the system to start the reaction. After the reaction mixture was stirred for 12 h, cystamine-modified PGMA (PSGMA) or ethylenediamine-contained PGMA (PEGMA) were obtained. The products were separated by precipitation with excess diethyl ether (in the case of PSGMA) or by dialysis against deionized water (in the case of PEGMA). The final products were then dried in a vacuum oven for 24 h for further use.

**Rh6G loading and cross-linking of MSN–PGMA hybrid nanoparticles via cystamine (Rh6G@MSN–PSGMA-1 or Rh6G@MSN–PSGMA)**

The model compound, Rh6G, was loaded into the MSN–PGMA nanocarriers by soaking the MSN–PGMA samples (20 mg) in an aqueous solution of Rh6G (3 mM) at room temperature for 24 h. The unloaded dye was removed by centrifugation and the solid was further washed with distilled water until the supernatant became colourless. The resulting Rh6G@MSN–PGMA was dried under vacuum for 24 h. Rh6G@MSN–PGMA (10 mg) was dispersed in water (10 mL) and cystamine hydrochloride (2 mg) was transferred into the mixture which was heated at 80 °C under stirring for another 12 h to obtain cystamine cross-linked Rh6G@MSN–PSGMA-1. Upon raising the amount of the added cystamine hydrochloride (about 4 times more), we can obtain MSN–PGMA hybrid nanoparticles, where all epoxy groups are opened by cystamines, namely, Rh6G@MSN–PSGMA. The product was separated by centrifugation (13 000 rpm, 30 min) and washed with distilled water (5 × 30 mL). The supernatants were collected for the determination of the amount of unloaded Rh6G by UV-vis. The loading capacity of Rh6G was calculated as we reported before.49 The final material was dried under vacuum overnight.

**KI, H\(_2\)O\(_2\) assisted disulfide bond exchange in water for the construction of PSSGMA or Rh6G@MSN–PSGMA-2**

Typically, PSGMA (400 μL, 10 mg mL\(^{-1}\)) was added to distilled water (5 mL) dropwise and mixed by magnetic stirring (for cross-linking of PSGMA on the surface of Rh6G@MSN–PSGMA, 20 mg sample was dispersed in 20 mL distilled water). The cross-linking of PSGMA was carried out in the presence of KI (2.0 mg) and H\(_2\)O\(_2\) (10 μL, 30%) at room temperature (the ratio of reactants in this reaction mixture was about 2.4 : 1 : 9.2 for each disulfide bond on PSGMA: KI : H\(_2\)O\(_2\)). The stability and degradation ability of the produced PSSGMA was monitored by taking 1 mL of the colloidal suspensions (PSSGMA) at specified times followed by the addition of GSH directly to observe the disassembling behaviour of the aggregation. Control experiments were performed by only adding one of the catalysts or without the addition of any catalyst (for conditions see the ESI, Table S3†).
Release of the Rh6G cargo from Rh6G@MSN–PSSGMA nanoparticles controlled by GSH addition

The Rh6G@MSN–PSSGMA sample (1.5 mg) was dispersed in PBS buffer (1 mL, pH 7.4 and pH 5.0) or phosphoric acid buffer (1 mL, pH 2.0), and loaded into dialysis bags (cut off \( M_w = 8000–14000 \) Da), and immersed into a release medium (10 mL). The activation of the MSN–PSSGMA hybrid material was accomplished by adding GSH (10 mM) into one parallel release medium. The release medium (3 mL) was withdrawn was accomplished by adding GSH (10 mM) into one parallel release medium. The release medium (3 mL) was withdrawn and replenished with an equal volume of fresh buffer at certain time intervals. The concentration of the released Rh6G was determined by UV-vis spectroscopy at 526 nm and the cumulative release of Rh6G was calculated according to the Rh6G calibration curve that was obtained by measuring its standard solutions with different dye concentrations.

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Notes and references