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Magnetic field activated drug delivery using thermodegradable azo-functionalised PEG-coated core-shell mesoporous silica nanoparticles

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Abstract

Core-shell Fe₃O₄@SiO₂ mesoporous silica nanoparticles coated with a new thermodegradable polymer allowed the release of a model drug through heating caused by a high frequency oscillating magnetic field. The thermodegradable polymer was made of poly(ethylene glycol) (PEG) functionalised with azo bonds that break with an elevation of temperature.

Mesoporous silica nanoparticles (MSN) have been intensively studied over the past decade as they represent a potential carrier material for targeted drug delivery and controlled drug release.¹ Indeed their high porosity and surface modification allow one to encapsulate a high amount of drug as well as to target harmful species such as infected or cancerous cells. One of the key elements in the battle against disease is the ability to release a therapeutic cargo on demand in the desire location. To do so, scientists have employed different mechanisms to trigger this release, making use of internal cellular stimuli (pH, redox, enzymatic) or external stimuli (light, ultrasound, magnet).² Magnetic heating is a promising external stimulus because the magnetic field can penetrate through the body in comparison to other stimuli such as light.³ Iron oxide nanoparticles have been successfully embedded in mesoporous silica in which the pores were blocked with a thermosensitive valve.⁴ The magnetic nanoparticles were then able to release the cargo when heated by an oscillating magnetic field (OMF). In these examples, the increase in temperature induced either melting of double-stranded DNA to open the pores, or through thermoreversible dissociation of pseudorotaxane caps on the pores.

Although hyperthermia is the most common application of using an OMF and appears to be efficient against tumor cells,⁵ it is critical to control this phenomenon and prevent it from killing the surrounding healthy cells or creating new metastasis. It is possible to finely control the heating induced by the superparamagnetic iron oxide nanoparticles (SPION) core

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of core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$ mesoporous nanoparticles. The concept of nanothermometer encapsulated in a silica matrix was developed by Dong *et al.* who determined that the temperature inside a core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$ mesoporous nanoparticle under an oscillating magnetic field increased significantly above the ambient temperature while little concurrent heating of the bulk solution was observed.⁶ These results demonstrate it is therefore feasible to design a thermosensitive cap able to release the cargo from the pore of a mesoporous carrier through heating localised to nanoscopic volumes by means of an oscillating magnetic field without inducing significant heating in the surrounding environment.

Polymers have been used as stimuli-responsive caps for the nanochannels within the silica matrix of mesoporous silica nanoparticles.⁷ Generally, thermally responsive polymers used in drug delivery applications undergo a physical change (phase transition, solubility, swelling, shrinkage), which can limit the efficacy and the control over the drug release. To the best of our knowledge, there are no examples of thermally *degradable* polymers showing the breaking of a covalent bond within a physiologically relevant temperature range to trigger the drug release.⁸ Azo compounds are well known to polymer chemists. Their ability to thermally decompose makes them useful as initiators for radical polymerizations. Despite their widespread utility as thermal initiators, very few reports have exploited their degradation for uses other than generating radicals. A notable exception is a report by Berkowski *et al.* who functionalised PEG with azo moieties and demonstrated that the azo-modified polymer was cleaved site-specifically upon exposure to ultrasound.⁹ Upon thermal decomposition, covalent C-N bonds break, resulting in the generation of lower molecular weight fragments. Herein we have designed a new thermoresponsive polymeric cap for magnetic core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$ mesoporous nanoparticles by incorporating azo bonds into the backbone of poly(ethylene glycol) (Azo-PEG). The structure of the polymer and the general modification of the nanoparticles are shown in Scheme 1. To our knowledge, this is the first example of a thermodegradable and biocompatible polymer that demonstrates the breaking of a covalent bond (azo bond) at a physiologically relevant temperature.

As proof-of-concept a fluorescent dye, rhodamine 6G, was used as the cargo and was physically sequestered inside the nanochannels of the silica matrix. The nanoparticles were then coated with Azo-PEG that blocks the pores and prevents dye leakage at body temperature. An elevation of temperature induces the cleavage of the C-N bond of the azo moieties resulting in the release of N_2 gas and, most importantly, the release of the entrapped cargo molecules. This is the first example of a cargo release from nanoparticles induced by the chemical degradation of a polymeric coating under a variation of temperature. Two external heat sources were employed to trigger the release of the dye, either through conventional bulk heating (using a hot plate) or through high frequency oscillating magnetic field (375 kHz, 20 kA m^{-1}).

The thermodegradable polymer (Azo-PEG) was synthesised by coupling 4,4'-azobis(4-cyanovaleric acid) (ACA) at both ends of a diamine-terminated PEG ($M_n = 1500 \text{ g mol}^{-1}$) to form hydrolytically stable amide bonds. Azo-PEG was characterized by $^1\text{H-NMR}$, gel permeation chromatography (GPC), MALDI-TOF, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (see $\text{ESI}^\dagger$). MALDI-TOF spectra show an increase of mass in the Azo-PEG polymer compared to the initial polymer ($\text{NH}_2\text{-PEG-NH}_2$), that

matches the added mass of two ACA molecules (Fig. S1C). This result constitutes strong proof of conjugation.

The degradation of Azo-PEG was studied by GPC and $^1\text{H-NMR}$ at two relevant temperatures; 37°C (body temperature) and 65°C (the half-life of ACA in water at 65°C is 10 h) (Fig. 1). While no change in GPC chromatograms was observed at 37°C over 24 h, the signal intensity of the Azo-PEG peak significantly decreases over 24 h at 65°C suggesting a notable degradation of the polymer (Fig. 1A). It is important to note that no significant shift in the retention time is expected, as the change in molecular weight upon thermal degradation is very small. These results were confirmed by $^1\text{H-NMR}$ (Fig. 1B). The multiplet observed at 1.6 ppm assigned to the two methyl groups adjacent to the azo bond gradually disappears over 24 h at 65°C indicating cleavage of the C–N bond, while this multiplet does not change at 37°C , indicating the azo group remains intact (Fig. 1B).

The core-shell nanoparticles were synthesised according to modified literature protocol.¹⁰ Arginine was used as a base instead of the commonly used sodium hydroxide to synthesise mesoporous silica nanoparticles. Arginine is basic enough to catalyse the sol-gel reaction and form silica nanosphere or mesoporous silica nanoparticles.¹¹ Moreover, we have previously demonstrated that Ag@SiO_2 mesoporous core-shell nanoparticles can be synthesised in presence of arginine.¹² The obtained particles were approximately 70 nm in diameter and possessed a 20 nm Fe_3O_4 core as well as a mesoporous spherical silica structure with a radial orientation and open pores about 3.2 nm in diameter. The BET surface area and the total pore volume were $894\text{ m}^2\text{ g}^{-1}$ and $1.24\text{ cm}^3\text{ g}^{-1}$ respectively (Fig. 2).

The surface of the core-shell $\text{Fe}_3\text{O}_4\text{@SiO}_2$ mesoporous nanoparticles was functionalised with amine groups using (3-aminopropyl)triethoxysilane (APTES) for further coupling with carboxylic acid-terminated polymers. Next, the nanoparticles were impregnated with a fluorescent dye, rhodamine 6G (R6G). R6G was chosen as a model therapeutic due to its good thermal and optical stability, and similar size ($\sim 2\text{ nm}$) to many anticancer drugs. Either azo-PEG or PEG-COOH, (thermodegradable and nonthermodegradable, respectively) were covalently conjugated to the surface of the particles by a standard coupling reaction between the carboxylic groups of the polymers and the amines at the surface of the particles (Scheme 1). The nanoparticles grafted with PEGCOOH and Azo-PEG are designated MSN-PEG and MSN-Azo-PEG respectively. The grafting of the polymers was confirmed by FTIR analysis (Fig. 3). The absorption bands at 3450 , 1100 , and 965 cm^{-1} were assigned to the O–H stretching, Si–O–Si asymmetric stretching and Si–O stretching, respectively.¹³ After coupling with either Azo-PEG or PEG-COOH, the FTIR spectra show new bands at 2880 and 2930 cm^{-1} characteristic of C–H stretching vibration of the polymer chains and a new sharp band at 1554 cm^{-1} corresponding to the secondary amide. The band of primary amide groups overlapped with the bending vibration of the remaining aliphatic amine (N–H) groups at 1634 cm^{-1} . The expected spectroscopic evidence of an amide bond may indicate a

[†]Electronic Supplementary Information (ESI) available: [detailed synthesis of the polymers, core-shell nanoparticles and their functionalisation; $^1\text{H-NMR}$ spectrum of Azo-PEG; GPC, MALDI-TOF, DSC, TGA of the polymers; real time release profile; cell viability assay; release experiment set-up]. See DOI: 10.1039/c000000x/

successful coupling reaction, however both (unconjugated) polymers contain an amide group. Nevertheless, the band present at 1730 cm^{-1} in the unconjugated polymer spectra, assigned to the C=O stretching vibration of the carboxylic acid, disappears after conjugation to the amino-modified nanoparticles and gives strong evidence of the coupling. Additionally, a new small band appears at 1756 cm^{-1} for the Azo-PEG conjugation (Fig. 3B) that it is not observed for PEG-COOH conjugation (Fig. 3A). This band is assigned to the C–O stretch of the bond formed during the activation of the carboxylic group with one of the coupling agents (HOBt and/or EDC), and suggests that a few carboxylic groups are not conjugated to the nanoparticles and are instead pendant from the surface. Because no dye leakage was observed at room temperature post conjugation, the pores were capped and the pendant carboxylate terminated chains did not inhibit the drug sequestration.

The polymer coating was also confirmed by zeta potential measurement at pH 7. Initially, the surface of nanoparticles was positively charged (+23 mV) due to the presence of the alkylammonium groups. In contrast, after coupling with the carboxylate-terminated polymers, the charges were screened due to the amide bond formation, inducing a drop of zeta potential (−5 mV). The grafting density of polymer was determined by TGA (Fig. S2), and found to be 7 wt% and 13 wt% for MSN-PEG and MSN-Azo-PEG respectively.

Drug loading was monitored by UV-visible spectroscopy of the supernatant solution during the washing process after conjugating the polymer to the surface. The loading capacity is defined as:

$$\text{loading capacity (\%)} = (\text{mass of dye}) / (\text{mass of the particles}) \times 100$$

and is calculated by subtracting the total amount of dye in the initial loading solution and the dye removed from the surface of the particles during the several washing steps. The calculated loading capacity was 18 wt%.

The release study was performed according to the following process: 250 μL of nanoparticles suspended in water (10 mg mL^{-1}) were poured into a homemade cassette sealed with a cellulose membrane (see ESI[†] for details). The cassette was then placed in a 4 mL cuvette. The fluorescence of the released dye was monitored either continuously throughout the experiment (Fig. S3) or from aliquots of the solution taken at different time points (Fig. 4). The cuvette was placed inside a 5-turn coil and an oscillating magnetic field (375 kHz, 20 kA m^{-1}) was applied. The first three points in the graph correspond to equilibration before magnetic activation, showing no dye leakage. After 1 h, a series of five cycles of 90 seconds heating on and 110 seconds off were applied; the cooling cycle was used to minimize overheating the bulk solution, which may occur with continuous heating. After 30 min an aliquot was taken and the fluorescence was measured. The fluorescence signal from the solution MSN-Azo-PEG increases, whereas no significant difference is seen in the fluorescence of the MSN-PEG solution. A total of four series of five cycles were applied, followed by fluorescence quantification (after 30 minutes of solution equilibration). Two additional fluorescence measurements were taken after 60 and 75 minutes. After 75 minutes the fluorescence signal plateaus, indicating that the release from the pores had ended. A slight increase in fluorescence is observed for the MSN-PEG samples, which

might correspond to detachment of a small amount of residual dye that was adsorbed on the surface of the particles (but not released from within the channels). This experiment clearly demonstrates that the dye was efficiently trapped inside the core-shell nanoparticles by the polymeric caps, and is released from MSN-Azo-PEG particles under an oscillating magnetic field, indicating that the heating effect of the SPION core degrades the Azo-PEG cap. The temperature of the bulk solution was measured during the experiment and never exceeded 30 °C, indicating that the generated heating was localised to nanoscale regions affecting only the material and could not induce hyperthermia in those conditions. This result is in agreement with our previous study showing that the nanoparticle temperature was much higher than that of the surrounding solution during the exposure.⁶ The release of the dye from azo-PEG coated nanoparticles was also successfully triggered by heating the bulk solution at 55 °C using a standard hotplate (data not shown).

The cytotoxicities of both the polymer-modified nanoparticles and of the degradation products caused by the magnetic field exposure were evaluated using the LIVE/DEAD cell viability assay. This assay simultaneously determines intracellular esterase activity and plasma membrane integrity using two distinct dyes: 1) calcein, a membrane-permeable polyanionic dye that must be hydrolyzed by intracellular esterases (dead cells lack active esterases) to produce a green fluorescence, and 2) ethidium homodimer, a membrane-impermeable red fluorescent dye that can penetrate only cells with compromised membranes. NIH 3T3 fibroblast cells were treated with different concentrations (up to 200 µg mL, Fig. 5) of MSNAzo-PEG and MSN-PEG and exposed to a magnetic field for 15 cycles of 90 seconds heating and 110 seconds cooling (cumulative time of exposure = 22 min 30 sec) mimicking the exposure profile used for the drug release experiment. The assay showed high cell viability for all tested conditions (Fig. S4), suggesting that the polymer-coated nanoparticles are not cytotoxic in that concentration range. Additionally, the exposure of the cells to a magnetic field does not induce any toxicity. Finally, the degradation products of Azo-PEG generated under a magnetic field do not affect the cell viability.

Conclusions

This work presents a new thermally degradable drug delivery nanocarrier in which the drug release can be activated on demand upon exposure to a magnetic field. This approach is advantageous in several aspects. First, we used an azo-functionalised polymer (Azo-PEG) as a coating to block the mesopores of the silica nanoparticles, preventing drug leakage at body temperature. Second, Azo-PEG is very thermoresponsive allowing the drug to release within a narrow temperature range that is biologically relevant. Azo-PEG responds to temperature by breaking of covalent bonds, which makes it the first example of a thermodegradable polymer to trigger the drug release.

Third, the heat is generated by an external magnetic field and produced locally within a nanoscopic volume, which should prevent damage to surrounding tissues. Finally, this approach exhibits no cytotoxicity towards fibroblasts, demonstrating its safety. These new azo-functionalised PEG-coated silica nanoparticles represent highly attractive and promising

candidates to deliver active therapeutics and may be of great interest for nanomedicine applications such as cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

(A) Surface modification of amino-modified $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles (MSN) with Azo-PEG (MSN-Azo-PEG), and drug release after magnetic heating. (B) Chemical structures of the polymers: Azo-PEG (thermodegradable polymer) and PEG-COOH (non-thermodegradable polymer used as a control). (C) Reaction scheme of the coupling of Azo-PEG to amino-modified silica surface under the following conditions: i) DIEA/HOBt/EDC.HCl, 12 h, RT; and the degraded products after thermodegradation. (DIEA = N,N-diisopropylethylamine; HOBt = Hydroxybenzotriazole; EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide).

Fig. 1. Temperature-dependant degradation of Azo-PEG monitored by GPC (A) and ¹H-NMR in D₂O (B) at 37 °C and 65 °C.

Fig. 2. Nitrogen adsorption/desorption (left) and TEM image (right) of the core-shell particles. Inset: NLDFT pore size distribution curve.

Fig. 3. FTIR spectra of core-shell Fe₃O₄@SiO₂ mesoporous nanoparticles functionalised with amine group (MSN), grafted with PEG-COOH (MSN-PEG) (A) and Azo-PEG (MSN-Azo-PEG) (B).

Fig. 4.
Release profile of the core-shell particles in presence of an oscillating magnetic field, starting after 1 h equilibration (arrow).

Fig. 5. Cytotoxicity assays of core-shell particles coated with PEG-COOH and Azo-PEG polymers with and without an oscillating magnetic field against fibroblasts at a concentration of $200 \mu\text{g mL}^{-1}$ of particles.