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Light- and pH-activated intracellular drug release from polymeric mesoporous silica nanoparticles

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ABSTRACT

Surface modified mesoporous silica nanoparticles (MSNs) with reduced toxicity were prepared for light and pH dual triggerable drug delivery system. Both 413 nm light and acidic environment can activate the drug release process, improving the pharmacological action. By applying rhodamine B (RhB) as a model drug, the accumulative RhB release is as high as 95% in pH 5.0 and in irradiation of 413 nm light, compared to only 55% in pH 7.4 and in dark. The anti-cancer drug camptothecin (CPT) loaded nanoparticles can kill cancer cells with IC_{50} value of 0.02 $\mu g\,mL^{-1}$ in exposure of 413 nm light, which is much lower than free CPT (about 0.1 $\mu g\,mL^{-1}$). Multimodal nonlinear optical imaging microscopy (NLOM) was employed to acquire *in vitro* coherent anti-Stokes Raman (CARS) and two-photon excited fluorescence (TPEF) images of live MCF-7 cells and showed that the nanoparticles can be taken up by breast tumor cell MCF-7 with high efficiency, indicating its great potential for anti-cancer drug delivery system.

1. Introduction

Stimuli-responsive (especially dual stimuli-responsive) drug delivery systems have been widely used for the purpose of reducing premature release and targeted release of the toxic pharmaceutical cargo [1–3]. Stimuli include exogenous stimuli such as temperature, light, electric fields, magnetic fields, and internal stimuli such as pH, redox, biological ions *etc.* [4–6]. Among the exogenous stimuli, light is receiving increasing attention, since light can induce instantaneous activation and have high focal control with precise wavelength and intensity specifications. With the superiority of remote control, low toxicity, non-invasion, light has been considered as a safe and specific trigger for nanomedicines [7–11]. The drug release process could be controlled by tuning the light wavelength or energy, and entrapped drugs could be released at the desired time and location [12,13]. Azobenzene presents a UV-induced trans-to-cis isomerization property, and it has been widely exploited for drug release due to its photo-switchable host–guest interaction with α-cyclodextrin [14,15]. Moreover, the self-assembly of its trans-conformation through π conjugation gives it more applications as light-triggered hydrogel formation, peptide structure changing and guest molecule release [16–18]. When azobenzene is irradiated at 413 nm, both cis and trans azobenzene isomers have almost the same extinction coefficient, and the constant trans–cis photoisomerization of the N=N bond will cause dynamic wagging motion of the azobenzene derivatives, triggering the entrapped molecules out [19]. So it provides a good candidate for the light-sensitive valve. pH-responsiveness has always been a hot topic in the controlled drug release field [20–22], since the pH value in tumor intra/intercellular milieu (5.0–6.8) is lower than that of normal extracellular matrices and blood

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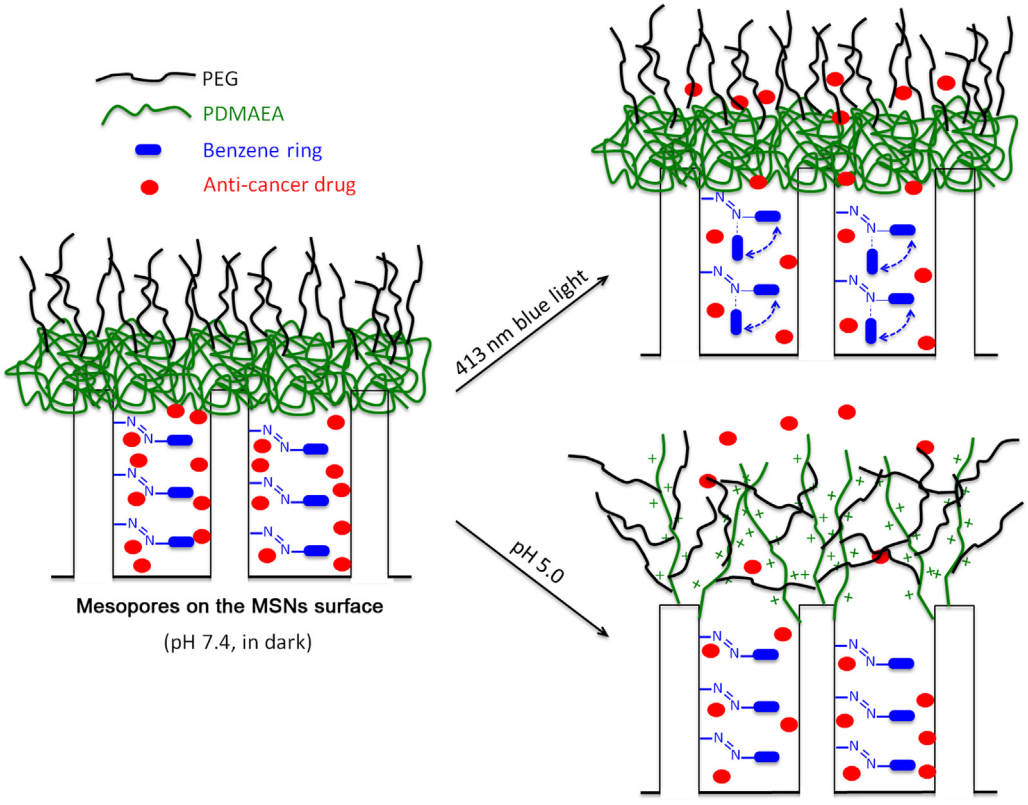

Fig. 1. Schematic illustration for the light and pH dual triggered drug release from the mesopores of MSN-Azo/PDMAEA-PEG.

(≈7.4) [23]. Biocompatible poly[2-(dimethylamino)ethyl acrylate] (PDMAEA) exhibits pH-sensitive behavior, attributed to the protonation of the tertiary amine groups [24,25]. It is hydrophilic in a low-pH environment and will become hydrophobic at alkaline conditions [26]. This polymer has attracted interests for pH-sensitive release system.

Mesoporous silica nanoparticles (MSNs) provide ideal matrix for drug carrier, due to its thermal stability, extremely high specific surface area (>1000 m^2/g), large pore volume (>1.0 cm^3/g), highly homogeneous porosity, regular and tunable mesopore diameters (2–10 nm) [27–29]. These properties provide MSNs high capacity to host different guest molecules and make them quite easy to be functionalized on their external or internal surface. Functional groups, polymers or nanoparticles could act as the "gatekeeper" for the mesopores, such as iron oxide nanoparticles, gold nanoparticles, stumili-responsive polymers, protein, dentrimers and cyclodextrin [30–35]. These "gatekeepers" can open or close the door to release entrapped molecules at will.

Coherent anti-Stokes Raman (CARS) microscopy is an emerging imaging modality for fast visualization of extracellular and intracellular structures with dense C—H bonds such as intracellular lipids droplets, proteins in nuclei, cell membrane and extracellular lipid-laden structures in tissue [36]. CARS exhibits high spatial resolution, enabling the detection of single molecules, and is free from auto-fluorescence backgrounds because its high frequency. Thus CARS provided label-free visualization of cell structures with sub-micron resolution *in situ* [37–39]. Two-photon excited fluorescence (TPEF) is a standard technique in modern microscopy. Due to lower scattering, enhanced skin penetration, and reduced photodamage, TPEF offered an ideal tool for examining the intracellular localization of nanoparticles [40,41].

In this study, we report the synthesis of a light and pH dual triggered drug delivery system based on MSNs, coded as MSN-Azo/PDMAEA-PEG (Fig. 1). Firstly, we prepared periodic MSNs which have mono-dispersed diameters of 30 nm, following the method by Yokoi [42,43]. Then we functionalized the silica surface with light-sensitive azobenzene group and pH-sensitive polymer PDMAEA. At last, polyethylene glycol (PEG) was conjugated to the material surface to increase the biocompatibility. As shown in Fig. 1, at neutral pH and in dark, azobenzene displays trans-conformation and self assembles through π conjugation [18], and PDMAEA collapses onto the mesopores of the silica nanoparticles. So the valves for drug releasing are locked. When the azobenzene exhibits both trans and cis conformation in the same extinction coefficient at 413 nm irradiation [19], or the PDMAEA is complete soluble at mild acidic conditions, the entrapped drugs starts to be triggered out. The highest release efficiency should happen when both acidic environment and 413 nm light applied. CARS and TPEF will also be used to investigate intracellular delivery of nanoparticles.

2. Results and discussions

2.1. Synthesis and characterization of MSN-Azo/PDMAEA-PEG

To prepare functionalized MSNs with light and pH dual-responsiveness, we firstly synthesized amino- and acrylic-derivatized MSNs, then conjugated azobenzene onto amino groups, and polymerized DMAEA onto the acrylic groups. As shown in Fig. 2a, the spherical particle shape and the polymer coating were confirmed by transmission electron microscope (TEM). The diameter of the silica nanoparticle was 30 nm. In the comparative Fourier transform infrared spectrometer (FTIR) spectra (Fig. 2b), peaks at 1090 cm^{-1}, 800 cm^{-1} and 461 cm^{-1} were all characteristic absorbance bands of silica [44], corresponding to Si—O—Si asymmetric stretching, Si—O—Si symmetric stretching and Si—O—Si bending, respectively. On the FTIR spectrum of MSN-Azo/PDMAEA-PEG, the appearance of peak at 1731 cm^{-1} belonging to —COO— and peaks around 2900 cm^{-1} belonging to $—CH_2—$, $—CH_3$ confirmed