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Research paper

Controlled drug release with surface-capped mesoporous silica nanoparticles and its label-free *in situ* Raman monitoring

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ABSTRACT

Mesoporous silica nanoparticles (MSNs) have drawn attention as efficient nanocarriers for drug delivery systems owing to their unique physiochemical properties. However, systemically controlling the kinetics of drug release from the nanocarriers and *in situ* monitoring of the drug release are still challenging. Here, we report surface-capped MSNs used for controlled drug release and demonstrate label-free *in situ* Raman monitoring of released drugs based on the molecule-specific spectral fingerprints. By capping the surface of MSNs with amine moieties, gold nanoparticles, and albumin, we achieved high loading efficiencies (up to 97%) of doxorubicin and precisely controlled drug release stimulated by changing pH value. Moreover, we monitored in real-time drug release profile and visualized cellular distribution of the delivered drug at nanoscale based on its intrinsic Raman peak. Finally, we evaluated drug responses in cancer cells and normal cells to investigate whether capped-dMSNs exhibit selective drug release. Our findings would be beneficial for designing smart drug carriers and directly monitoring the release behavior of drugs in actual cellular environments.

1. Introduction

Mesoporous silica nanoparticles (MSNs) have been extensively studied as nanocarriers for delivering various molecules such as drugs, proteins, and nucleic acids [1–3]. Physicochemical properties of the MSNs, including large surface area attributed to cylindrical pores, easy surface functionalization, and degradability, are beneficial for their potential use in controlled drug delivery systems [4–6]. Several surface modification strategies of the MSNs have been adopted for stimulating the release of loaded drugs, such as decorating their surface with various capping materials (*e.g.*, metal nanoparticles, macromolecules, polymers, and proteins) [7–10]. To trigger drug release, internal or external stimuli such as pH, temperature, enzymatic reaction, and/or light are usually applied to MSNs [11–15]. In this regard, lots of attempts have been made to sophisticatedly design MSNs as drug carriers and to effectively stimulate drug release from these carriers on demand.

However, comparably less effort has been undertaken for developing monitoring techniques for drug release. Many researchers used model drugs with absorbance, such as doxorubicin or chemical dyes, to characterize their release behaviors by measuring the absorbance or fluorescence [16–19]. However, these methods were usually conducted after a series of sampling procedures to investigate the drug release kinetics. As an indirect monitoring method of the drug release, fluorescence resonance energy transfer (FRET)-based technique was also reported [20]. These previous methods were usually carried out *ex situ* monitoring of the drug release. In order to trace the drug release in real-time without sampling procedure, fluorophores have been employed to tag drug molecules [21,22]. Although fluorescence imaging has been most frequently used in biological fields, it has another limitation in achieving high-resolution imaging. To overcome this limitation, fluorescence-based super-resolution microscopy has been recently developed to achieve high-spatial resolution [23–25]. However, fluorescence-based methods are generally not efficient for monitoring drug release over a long-time period due to photodegradation of the fluorophore [26].

For this reason, Raman spectroscopy, which allows for the direct collection of molecule-specific vibrational spectra in real-time without labelling them with tags [27–29] is especially useful for *in situ* monitoring of drug release. On the basis of intrinsic Raman spectra, drugs even not having a specific absorbance can also be directly monitored. Moreover, it is a reliable analytical technique that can distinguish the chemical composition of complex samples from the fingerprint spectra of individual molecules. Additionally, intrinsic molecular specific peaks allow for mapping the spatial distribution of specific molecule at a

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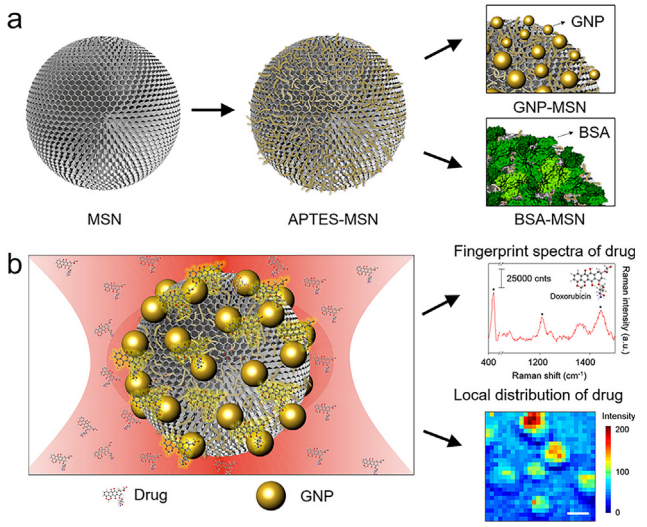

Fig. 1. Schematic illustration of the surface-capping procedures of mesoporous silica nanoparticles (MSNs) and *in situ* Raman monitoring of drug release and distribution. (a) Surface-capping procedures of MSNs including 3-aminopropyl triethoxysilane (APTES) grafting and subsequential attachment of gold nanoparticles (GNPs) or bovine serum albumin (BSA). (b) Conceptual demonstration of *in situ* monitoring and mapping of drug release behaviors with the fingerprint Raman spectrum of doxorubicin dissolved in pH 7 condition and cellular Raman mapping. Raman intensity map was collected with an indicator peak of DOX (454 cm^{-1}). Scan area is 100 μm × 100 μm, and pixel size is 4 μm. Scale bar represents 20 μm.

cellular level. Therefore, we envision that Raman spectroscopy-based *in situ* monitoring of drug release would be achievable without labelling them with tags and high-spatial drug distribution can be visualized via Raman intensity mapping.

In this study, we synthesized MSNs and attached a couple of capping materials onto their surface to systemically control the drug release characteristics. As illustrated in Fig. 1a, different capping materials including amine moieties, gold nanoparticles (GNPs), and bovine serum albumin (BSA) were tested for regulating drug release. Furthermore, we attempted to directly monitor the release profile of drugs in real-time and visualize their actual distribution in cellular environments based on Raman spectra, as described in Fig. 1b.

2. Materials and methods

2.1. Materials

Tetraethylorthosilicate (TEOS), hexadecyltrimethylammonium bromide (CTAB), 3-aminopropyl triethoxysilane (APTES), bovine serum albumin (BSA), poly-L-lysine (PLL), silibinin, resveratrol, and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Carbenicillin disodium and, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder were purchased from Duchefa biochemie (Haarlem, Netherlands). Doxorubicin and paclitaxel were purchased from LC laboratories (Woburn, MA, USA). Gold nanoparticles were purchased from BBI solutions (Cardiff, UK). A polydimethylsiloxane (PDMS) elastomer kit (Sylgard 184) was purchased from Dow Corning (Midland, MI, USA).

2.2. Synthesis of mesoporous silica nanoparticles (MSNs)

MSNs were synthesized using a modified synthetic protocol based on previously reported methods [30]. Briefly, 1 mL TEOS was mixed with 40 mL deionized (DI) water, 20 mL ethanol, 0.6 mL aqueous ammonia, and 6 mL CTAB solution. After stirring overnight, the precipitated particles were centrifuged, washed 3 times with ethanol, and dried under vacuum. Finally, the particles were calcined in air at 550 °C for 3 h. Nitrogen adsorption isotherms were obtained at 77 K using a nitrogen sorption instrument, (ASAP 2000; Micromeritics, GA, USA). The surface area and pore size distributions were determined according to the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) procedures from the adsorption branches of isotherms.

2.3. Doxorubicin (DOX) loading onto MSNs

Before surface modification, DOX was loaded onto MSNs. Equivalent concentration of DOX (500 μg/mL) was slowly added into the MSN suspension, and stirred overnight in dark to avoid photodegradation. DOX-loaded MSNs (dMSNs) were centrifuged, and the supernatant was collected to evaluate the loading efficiency. Absorbance of DOX was measured at a wavelength of 480 nm using the plate reader, SpectraMax M2e microplate spectrophotometer (Molecular Devices, CA, USA). A calibration curve for DOX (concentration range 0–100 μg/mL; prepared using serial dilution) was obtained. Loading efficiency (LE) of DOX was evaluated using Eq. (1).

$$\mathrm{LE(wt\%)} = (W_i - W_{sup})/W_i \times 100 \quad (1)$$

where W_i is the initial concentration (μg/mL) of for loading DOX and, W_{sup} is the concentration of free DOX in supernatant calculated from the calibration curve.

2.4. Surface modifications of MSNs

The surface of MSNs was functionalized with amine groups by APTES using the previously reported method [31]. Briefly, 5 mg MSNs were dissolved in 10 mL DI water and transferred to a glass vial. Thereafter, 30 μL APTES aqueous solution was added to 5 mL MSN suspension, and stirred vigorously for 6 h. The suspension was centrifuged and washed to remove the unreacted APTES. The obtained precipitates were referred to as APTES-MSNs. As a result, the surface charge of the MSNs became positive owing to the amine-moieties, which utilized to attach the negatively charged GNPs or BSA on APTES-MSN by electrostatic interaction. Next, an equal volume of 10 nm GNPs (OD = 1, c.a., 5.70×10^{12} particles/ml) or 1% w/v of BSA was added into the aqueous solution of APTES-MSNs and incubated for 1 h at room temperature to further cap the pores of MSNs. Finally, suspensions were centrifuged and washed to remove the excessive capping material. Thereafter the precipitates obtained were named GNP-MSNs or BSA-MSNs and re-dispersed in DI water or phosphate-buffered saline (PBS) before further analysis.

2.5. Characterization of capped MSNs

TEM experiments were performed using the LIBRA 120 (Carl Zeiss, Germany) instrument at an acceleration voltage of 120 kV. Samples for TEM analysis were prepared by transferring the suspensions onto a 300-mesh copper grid. Samples were blotted away by incubation for 1 h at room temperature and rinsed out by DI water. Next, the hydrodynamic diameter (dynamic light scattering, DLS) and surface charge (ζ-potential) of MSNs and their derivatives were measured using the ELS-Z2 (Otsuka Electronics, Japan) at room temperature. All samples are analysed in triplicates after dispersing them in DI water.

2.6. Drug release experiments

The uncapped and capped (APTES, GNP, and BSA)-dMSN were immersed in 1 mL of PBS (pH 5, pH 7, and pH 9) at room temperature. At each time point, a 200 μL aliquot of supernatant separated by