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The Dual-Stimulated Release of Size-Selected Cargos from Cyclodextrin-Covered Mesoporous Silica Nanoparticles

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ABSTRACT A drug delivery system of dual-stimulated release of size-selected cargos from β-cyclodextrin-covered mesoporous silica nanoparticles was prepared. Calcein loaded mesoporous silica nanoparticles (MSN) were capped by β-cyclodextrin (β–CD) through photocleavable moiety to control its release. Then the small size cargo p-coumaric acid (CA) was loaded. The cavity of β-cyclodextrin was blocked by ferrocene through the host-guest interaction between the ferrocene and β-cyclodextrin. The small cargo can be released by the escape of ferrocene under +1.5 v electro-stimuli. Calcein molecules could not pass through the cavity of the β-cyclodextrin due to its bigger size. The calcein was released from the MSN after the detachment of β-cyclodextrin cap from the MSN surface with the UV irradiation. Fluorescence spectra demonstrate that the different size cargoes were released successfully step by step under external UV-light and electro stimuli respectively.

Key Words: mesoporous silica particles; photo-response; electro-response; size-selected release

Introduction

Controlled drug delivery systems have attracted considerable interest in biomedical materials. In stimuli-responsive systems, the biologic molecule or medical-related drugs can be released precisely under specified conditions. [1-4] Drug delivery system (DDS) based on polymers usually face the challenges of aggregation, burst release and rapid clearance during the circulation.[5] As an alternative, biocompatible inorganic nanomaterials used for nanocarrier as drug delivery have attracted much attention. [6-8] Inorganic nanomaterials such gold,[9] mesoporous silica[10] and magnetic nanoparticles[11, 12] have attracted much attention in recent year. Among them, Surface functionalized mesoporous silica as DDS has excellent biocompatibility, stable structure, tunable pore size, large surface area, and well-defined surface, which make it an ideal material for drug delivery.[13] The research of capping system based on MSN has been proved an excellent method for blocking the drugs in the pore channel. The capping agents tethered to the MSN surface include inorganic nanoparticles,[14, 15] polymer,[16] biomacromolecules,[17] and supramolecule assemblies.[18] These "smart cap" can be removed with different external stimuli, such as temperature,[19] pH,[20] light,[21, 22] enzymes,[23, 24] glucose,[25] and redox.[26]

On the other hand, combination drug therapy has been considered as another approach to overcome drug resistance and enhance treatment effect.[27] For instance the paclitaxel and beavacuzymab was successful to improve the metastatic breast cancer antitumor efficacy.[28] The monotherapy efficacy of VEGFR-tyrosine kinase inhibitor or VEGF antibodies was proved to be limited while the combinational treatment showed excellent advancement.[29] However, in most cases, multidrug delivery systems usually face the problem of uncontrolled release, such as dosage, rate, timing, and release different drugs at desired step. [30] In order to release cargoes

controllably, it is necessary to design a proper dual-stimuli delivery system. Novel dual-stimuli responsive nano-carriers that response to a combination of two signals such as pH/redox,[26] enzyme/pH, [31] temperature/redox,[32] and glucose/pH[33] have been developed. Dual-stimuli DDS is the important issue in multifunctional medical application, and it also provides the possibility to deliver the drugs at the specific pathological site and desired time. As for multidrug delivery, different drugs can be released either simultaneously or in sequential method.

Thus, it is necessary to design a proper dual-loaded delivery system and release the cargos respectively in succession. Some reports have shown this type of system in recent years. Wang *et al* prepared the dual-stimuli drug delivery system based on MSN, which is capable of releasing two different size drugs step by step. In the condition of redox activation and low pH levels, the small and large drug can be released in succession.[34] Du *et al* designed a novel multidrug delivery system based on DNA-gated MSN. The loaded two drugs can be released by exerting basic pH, DNase I and NIR light controllably.[35]

Herein, we report the dual-stimulated release system of size-selected cargos from cyclodextrin-covered mesoporous silica nanoparticles. The prepared MSN was modified with photocleavable o-nitrobenzyl moiety, [21] The top and bottom diameter of β-CD is 6 and 6.5 Å respectively,[34] which was functionalized on the surface of MSN. Its cavity is small enough to stop calcein (17 Å, measured using software Chem3D Ultra 8.0, CambridgeSoft) from escaping out the pore channel of MSN. However, the smaller p-coumaric acid (CA, 4.5 Å) [34] is able to load into the pore though the cavity of β-CD. The opening of β-CD was blocked by FcA-PEG. CA and calcein can be released step by step from the nanoparticles. Firstly, by applying 1.5 v external voltage, the smaller CA escaped from the pore because of the separation of FcA-PEG.

Subsequently, upon the exposure of UV light, the large Calcein was released on cleavage of o-nitrobenzyl bond, as shown in Fig. 1.

Experimental Section

Chemicals and Materials. 3-Aminopropyltriethoxysilane (99 %, Acros), 5-hydroxy-2-nitrobenzyl alcohol (97 %, Aldrich), .succinic anhydride (Aladdin chemistry Co. Ltd), propargyl bromide (99 %, Aldrich), N, N'-diisopropylcarbodiimide (DIC) (99 %, Aladdin chemistry Co. Ltd), 4-(dimethylamino)pyridine (DMAP) (99 %, Aladdin chemistry Co. Ltd), ferrocenecarboxylic acid (FcA) (98 %, Aladdin chemistry Co. Ltd), methoxy poly(ethylene glycol) (PEG, Mn, GPC =550) (99 %, Alfa Aesar) were used as received. Calcein, p-coumaric acid (CA), triethylamine, triethylamine, Calcein, p-coumaric acid (CA), $CuSO_4 \cdot 5H_2O$, sodium ascorbate, sodium hydroxide, n-hexane, β-cyclodextrin (β-CD), sodium azide (99 %, NaN_3) and triphenylphosphine (PPh_3) were obtained from Sinopharm Chemical Reagent Co. Ltd. All solvents were treated with calcium hydride and distilled before use. β-CD was dried at 100 ℃ for 24 h in vacuum oven before use.

Characterizations. FT-IR spectra were obtained by a Perkin–Elmer spectrometer. TEM images were collected on transmission electron microscopy (HRTEM, JEOL, JEM-2010FEF, Japan) with an accelerating voltage of 100 kV. The samples were prepared by placing a drop of the particle suspension in MeOH onto a 200-mesh copper grid with carbon film and dried at room temperature. Powder X-ray diffraction (Bruker D8 ADVANCE, Germany) using Cu Kα radiation (λ= 1.54056 Å). Fluorescence spectra of calcein and p-coumaric acid were recorded on a LS55 luminescence spectrometry (Perkin-Elmer) with excitation at 490 nm and 286 nm, and emission data range between 300 and 600 nm. The solid-state spectra were collected on a Varian

Infinity plus-400 solid-state NMR spectrometer. A 4 mm CP / MAS probe at room temperature was used. The spinning speed was 10 kHz. The thermal gravimetric analysis (TGA) was performed by A TGS-2 thermogravimetric analyzer (PerkinElmer). The electrochemical experiments (cyclic voltammetry) studies were operated at CHI610C electrochemical working station. The particles covered the glassy carbon working electrode. Ag / AgCl worked as reference electrode and Pt as counter electrode. The electrolyte was PBS buffer solution.

Synthesis of NH_2-Functionalized MSN (MSN-NH_2). A NaOH solution (0.875 mL, 2 M) was added to the aqueous solution (120 mL) of cetyltrimethylammonium bromide (CTAB, 0.25 g) and heated to 80 ℃. After 10min, tetraethylorthosilicate (TEOS, 1.25 mL) was added to the solution. The reaction was stirred at 80 ℃ for 2 h. The result particles was filtered and washed with amount of H_2O and methanol. The prepared MCM-41 was dried under vacuum for 24 h.[36] 270 mg MCM-41 was suspended in 35 mL anhydrous toluene, and then 2 mL APTES was added subsequently. The reaction mixture was heated at 116 ℃ under reflux for 24 h. The particles were separated by centrifugation (8000 rpm, 20 min) and washed with ethanol for three times. The particles were dried in vacuum.

Synthesis of COOH-Functionalized MSN (MSN-COOH). MSN-NH_2 (270 mg) was suspended in anhydrous toluene (25 mL), and then the succinic anhydride (0.12 g) and triethylamine (0.186 mL) were added into the solution. The reaction solution was stirred at 60 ℃ for 48 h. The particles were separated by centrifugation (8000 rpm, 20 min), and then washed several times with ethanol. CTAB was removed by stirring in methanol (48 mL) and concentrated hydrochloric (2.7 mL) at 60 ℃ for 24 h, washed with ethanol, filtered the particles and dried in vacuum.

Synthesis of Alkyne-functionalized MSN (MSN-alkyne). MSN-COOH (33 mg), DMAP (0.01 g) and DIC (0.09 mL) were added to anhydrous DMF (30 mL) and stirred in 0 ℃ for 30 min. Subsequently, the 5-propargylether-2-nitrobenzyl alcohol (40 mg) was added and reacted at room temperature under argon for 24 h. The particles were then separated by centrifugation (8000 rpm, 20 min), washed with DMF / H_2O and dried in vacuum.

Synthesis of Calcein-loaded MSN-1. MSN-alkyne (16 mg) and calcein (15 mg) were stirred in a mixture solution of PBS (5 mL, pH=7) and DMF (2 mL) at room temperature for 24 h. Followed by per-6-azido-β-cyclodextrin (18 mg), $CuSO_4 \cdot 5H_2O$ (16 mg) and sodium ascorbate (2 mg) was added to the solution ,the mixture was stirred at room temperature for 72 h under argon atmosphere after which the particles were filtered , washed with methanol / H_2O and dried under vacuum. The drug loading content of MSN-1 was calculated as 2.3 wt% by fluorescence spectrophotometer. Release studies were carried out subsequently.

Synthesis of CA loaded MSN-2. Unloaded MSN-1 (7 mg) were stirred in an aqoeous solution (1.5 mL, 5 mM) of CA at room temperature for 24 h. Followed by PEG-FcA (10 mg) suspended in H_2O (1.5 mL) was added and stirred for another 24 h, the mixture was filtered, washed with DI H_2O and dried under vacuum. The drug loading content of MSN-2 was calculated as 3.1 wt%. Release studies were carried out subsequently.

Synthesis of dual-loaded MSN-3. Calcein-loaded MSN (7 mg) were handled according to MSN-2. After capping by PEG-FcA, the CA loading content of MSN-3 was calculated as 0.6 wt%.

Elecotro-responsive release. 1.5 mg CA-loaded MSN-2 were dispersed into 1 mL ethanol and dropped to a polished glass carbon electrode. The sample on the electrode was dried under

infrared lamp. The electrochemical experiments (cyclic voltammetry) studies were performed using CHI610C electrochemical working station. The nanoparticles covered glassy carbon was working electrode, Ag / AgCl worked as reference electrode and Pt as counter electrode. The CV experiment was carried out in 3 mL PBS buffer (pH=7.4) at room temperature. Then, we gave a +1.5 v electro-stimuli to the solution for 60 min. The release of CA was measured by UV-vis spectroscopy (λ_{ex}=351 nm, λ_{em}=441 nm). In control, another example of 1.5 mg CA-loaded MSN-2 was also measured fluorescence intensity in the same situation without electro-stimuli.

Light-responsive release. 3 mg MSN-1 was added into 3 mL PBS buffer (Ph=7.4). The solution was vigorously stirred to disperse the Nanoparticles fully. The solution was placed under UV (350 nm) light irradiation. Subsequently, the supernatant of solution was taken periodically from the suspension. The release of calcein was measured by fluorescence spectrophotometer (λ_{ex}=490 nm) in the dark. In control, the fluorescence intensity of another same example was measured without UV irradiation.

Dual cargos release. 5 mg sample (MSN-3) was stimulated by +1.5 v voltage for 2 h until no more release was observed. Subsequently, the particles were washed with DI H_2O and then placed the sample under UV irradiation for 5 h. The release process was monitored by fluorescence spectrometer.

Results and discussion

For the design of the externally triggered dual-stimulated release system, three components were chosen, inorganic nanoparticles supporter, UV and electric sensitive gatekeepers. In this work, MCM-41 was selected as a drug carrier because of its excellent properties. The releasing mechanism was described in Figure 1. β-CD and ferrocene served as

the gatekeepers for large calcein molecules and the smaller CA. The smaller cargoes are released after positive electro-stimuli, and the large ones can be released by UV irradiation. Because of difference in size of the calcein and CA, it is suitable for them to load into the carrier as a model system respectively. Different fluorescent spectra make them easy to trace the release process. Transmission electron microscopy (TEM) images show the distinct feature of multifunctional MSNs. The diameter of the as synthesized MSNs was about 100 nm. (Figure 2a, b, c). The uniform feature of the MSN was confirmed by SEM. (Figure S8) The shape of the nanoparticles did not change when the MSN coated by β-CD and FcA-PEG, which indicated the stability structure of MSN after chemical modification. Small-angle X-ray diffraction (XRD) analysis was employed to character the well-ordered porous structure of the MSNs, which could be indexed as (100), (110) and (200) Bragg peaks. The decreased peaks of MSN-1 and MSN-2 were attributed to the introduction of β-CD and the Fc-PEG, which covered the pore. (Figure 2d) The size of mesopore was 2.5 nm obtained by N_2 adsorption / desorption isotherms. After the modifications, the surface area and pore volume were reduced, and the pore diameter cannot be obtained because of the blocking of pore. (Figure 3)

FT-IR spectra were used to detect the process of the surface functionalization of MSNs (As shown in Figure 4). The FT-IR of MSN-COOH showed the absorption band at 1560 cm^{-1} and 1740 cm^{-1},[37] which attributed to amide and carboxyl stretching vibration. MSN-Alkynyl was obtained by the esterification with 5-propargylether-2-nitrobenzyl alcohol in the presence of DIC and DMAP, which produced the photo cleavable o-nitrobenzyl ester moiety. The absorption band at 2132 cm^{-1} was arised from the Alkyne stretching vibration.[38] Thereafter, the calcein was loaded into the MSN-Alkynyl by stirring in PBS (pH 7.4) and DMF solution. The per-6-azido-β-cyclodextrin was then added to the mixture to cap the mesopore via the “click chemistry”

reaction between β-CD and the Alkyne groups of MSN. The disappearance of 2132 cm^{-1} peak indicated the alkyne groups on the surface had been completely reacted. The smaller cargo CA was then loaded into the pore through the β-CD cavity. To further confirm the successfully connected β-CD, MSN-1 was characterized by magic-angle-spinning (CP-MAS) solid-state NMR spectroscopy. The signals between 10 and 64 ppm are attributed to the aliphatic carbons. The signal at 173 ppm is assigned to characteristic peak of the amide carbonyl carbon. The signals resonating of aromatic ring and triazole carbons can be found between 110 and 165 ppm. The signals resonating between 50 and 105 ppm belong to the carbon peaks of β-CD. (Figure 5). After stirring for 24 h, the opening of β-CD was plugged with FcA-PEG. The enhanced absorption band at 2885 cm^{-1} and 2925 cm^{-1} (Figure 4) indicated the C-H stretching vibration of PEG.

In order to estimate the weight percentages of β-CD and FcA-PEG modified on MSNs, the thermal gravimetric analysis (TGA) curves of different MSNs were displayed in Figure 6. The weight losses of MSN-alkyne, MSN-1 and MSN-2 from 60 ℃ to 800 ℃ were 74.5%, 67.2% and 63.5% respectively. Thus, the weight percentages of β-CD $(N_3)_7$ and PEG-FcA on the nanoparticles were estimated to be 6.6% and 3.6%. The β-CD $(N_3)_7$: PEG-FcA molar ratio was calculated to be 1.07:1, which indicated that most of the β-CD modified on MSNs were combined by PEG-FcA.

Calcein loaded MSN-1, in which the β-CD rings were connected to the surface of MSN by using photocleavable o-nitrobenzyl moiety was prepared. The release process of the Calcein loaded MSN-1 was show in Figure 7. After the cleavage of o-nitrobenzyl ester bond under UV irradiation (λ =350 nm), the Calcein cargo can be released from the pore because of the removal

of the CD gatekeeper. A sample of MSN-1 was dispersed in the PBS buffer (pH=7.4). The mixture was stirring upon exposure to UV light. As a function of time, the supernatant was taken out to monitor the release of Calcein using fluorescence measurements at 520 nm. In control, the fluorescence intensity of another example was kept in the dark. As shown in Figure 7, the calcein loaded in the pores of MSN-1 were not released, which is attributed to the blocking of CD moiety on the mesoporous surface. In contrary, Fluorescent intensity of Calcein from MSN-1 under UV irradiation show remarkable increase over irradiation time, which suggests that the CD unit could be removed by the breakdown of o-nitrobenzyl ester by UV light exposure. Also we monitored the release process with the UV irradiation discontinuously. The solution was stirred under UV irradiation for 20 min, and we took the supernatant to measure its fluorescence intensity. Subsequently, placed the sample in the dark for 20 min and monitored the fluorescence intensity. This process was periodically repeated for 8 times. The result showed that the release of Calcein is well controllable. (Figure 8). Based on the result of fluorescence measurement, 0.09 μmol Calcein was released from 3 mg MSN-1 after around 5 h photo stimuli. The release capacity was calculated as 1.8 wt %.

To investigate the electro-triggered release of smaller cargo CA, the MSN-2 system was designed. Ferrocenecarboxylic acid (FcA) can exactly form a 1:1 inclusion complex with β-CD. However, the charged species (Fc^+) will dissociate from the β-CD cavity immediately. The formation constant between FcA and β-CD is 2200 M^{-1}. [39] The FcA can be oxidized and dissociates from β-CD after applying a positive voltage. We modified the FcA with PEG to elevate the biocompatibility and water-solubility of FcA. After the loading of CA into MSN-2, the FcA-PEG was used to combine with β-CD and entrap the CA. We gave a +1.5 v electro-stimuli to the solution for 60 min. As shown in Figure 9, with no external electric field, the CV

curve exhibited a +0.2 v half wave potential, indicating the formation between β-CD and FcA. After applying +1.5 v voltages for 1 h, Fc was oxidized to Fc+ and released from β-CD, which can be indicated by decreasing the half-wave potential to +1.5 v.[40] The release of CA was measured by the absorption intensity of UV-vis spectroscopy as a function of time. The CA was released from MNS-2 in around 1 hour as shown in the Figure 10. In control, another MSN-2 sample with no external electro-stimuli showed little release. By using the working line, we obtained the concentration of CA released from the MSN-2 in the supernatant, indicating that the 0.14 μmol CA was released from 1.5 mg MSN-1 after 1 h electro-stimuli. The release capacity was calculated as 1.5 wt %. (Figure 10)

We did show that the calcein and CA can be released with the UV radiation and electro stimuli respectively. We prepared the MSN-3 with two loaded cargos, and control the release of two cargos in succession from the nanoparticles. Firstly, the release of CA from the pore was triggered by applying +1.5 v voltage to MSN-3 for 1 h. The fluorescence intensity around 440 nm was observed. And yet we did not found the fluorescence intensity of calcein at 520 nm, which indicated the single release of CA. Another sample of MSN-3 with no external electro-stimuli, no intensity was found around 440 nm. (Figure 11a). The fluorescence spectra results indicated that about 0.12 μmol released from 5 mg MSN-3. The release capacity was calculated as 0.39 wt %. (Figure 11c). (We did not observe the FL band at 520 nm). After the centrifugation of the solution, the supernatant was removed. The MSN-3 was washed by PBS to remove the CA completely. The release process of calcein was operated under UV irradiation for 280 min. The fluorescence intensity around 520 nm confirmed the release of calcein. In control, no obvious fluorescence intensity was observed when sample placed in the dark. (Figure 11b). The fluorescence spectra result indicated that about 0.153 μmol released from 5 mg MSN-3,

corresponding to a 1.9 wt% release capacity. (Figure 11c). The result showed that the calcein and CA can be released in succession by different stimulation. The releasing process of two cargoes in succession was visually showed in Figure 11d.

Conclusions

A novel drug delivery system of dual-stimulated release of size-selected cargos from β-cyclodextrin-covered mesoporous silica nanoparticles was prepared. Calcein loaded mesoporous silica nanoparticles (MSN) was capped by β-cyclodextrin through photocleavable moiety to control its release. Then the small size cargo p-coumaric acid was loaded. The cavity of β-cyclodextrin was blocked by ferrocene through the host-guest interaction between the ferrocene and the β-cyclodextrin. The small cargo can be released by the escape of ferrocene under +1.5V electro-stimuli. Calcein molecules could not pass through the cavity of the β-cyclodextrin due to its bigger size. The calcein was released from the MSN after with the detachment of β-cyclodextrin cap from the MSN surface with the UV irradiation. The dual-Stimulated Release of Size-Selected delivery system provides valuable information for the treatment of combination drug therapies.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China (21204070), the National Key Basic Research Program of China (2011CB606202), and Research Fund for the Doctoral Program of Higher Education (20110141120009).

Figures

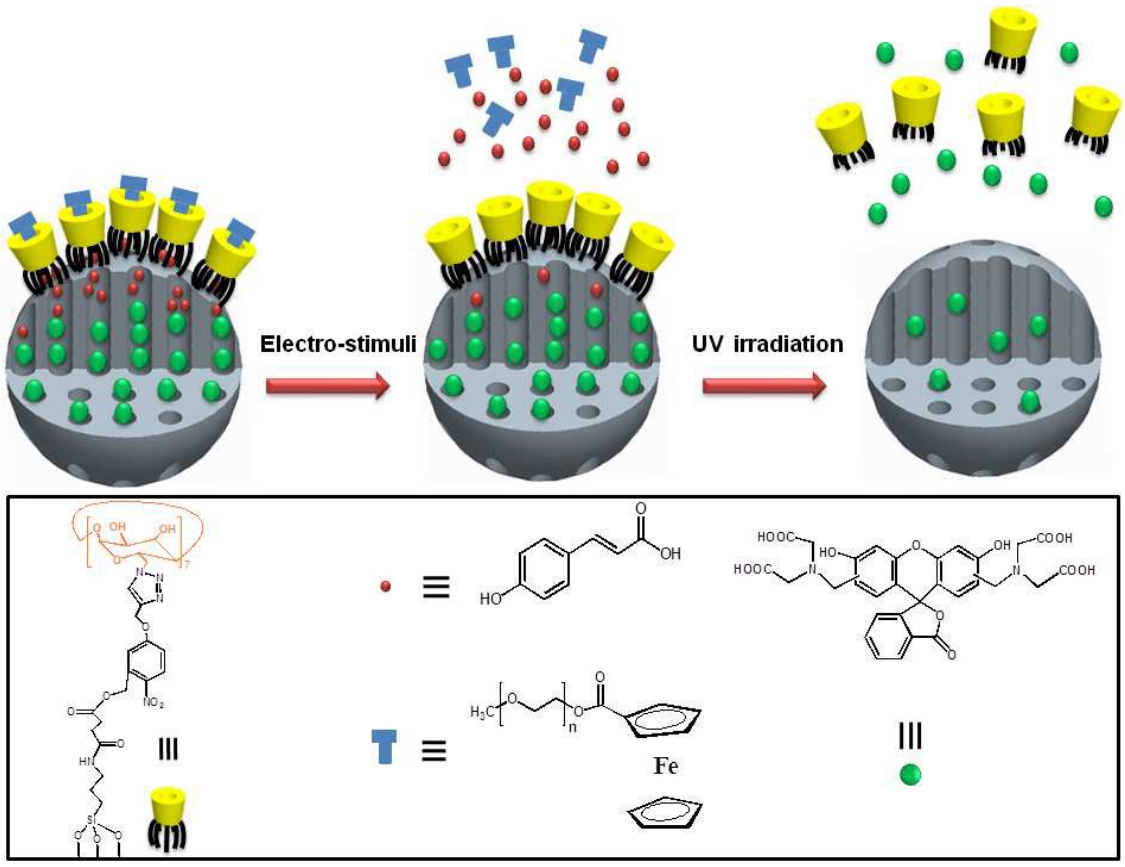

Figure 1. Schematic illustration of the release of guest molecules from the MSNs.

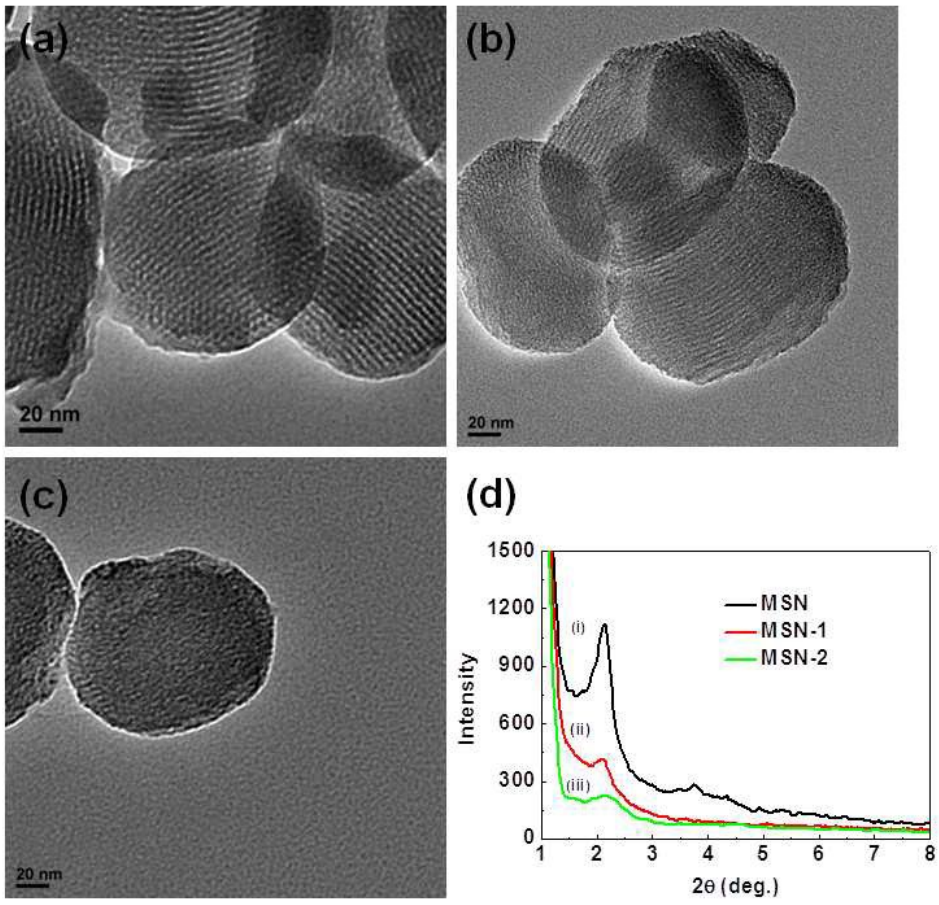

Figure 2. (a) TEM images of MSN, (b) MSN-1, (c) MSN-2, (d) Low-angle XRD pattern

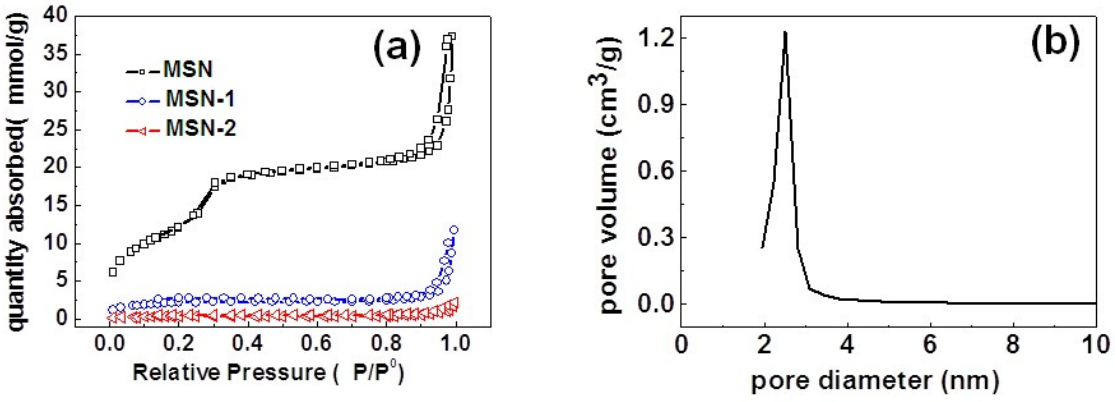

Figure 3. (a) BET N_2 adsorption / desorption isotherms of Different nanoparticles. (b) Pore size distribution plots of MSN.

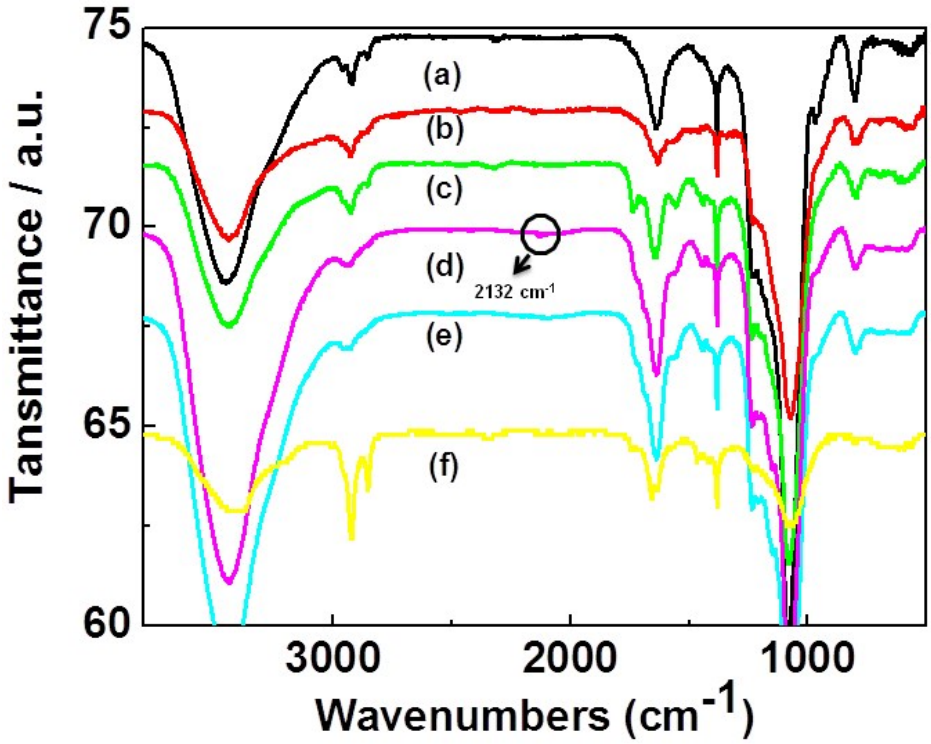

Figure 4. FTIR spectra of MSNs with a series of surface functionalization. (a) MSN, (b) MSN-NH_2, (c) MSN-COOH, (d) MSN-Alkyne, (c) MSN-1, (d) MSN-2.

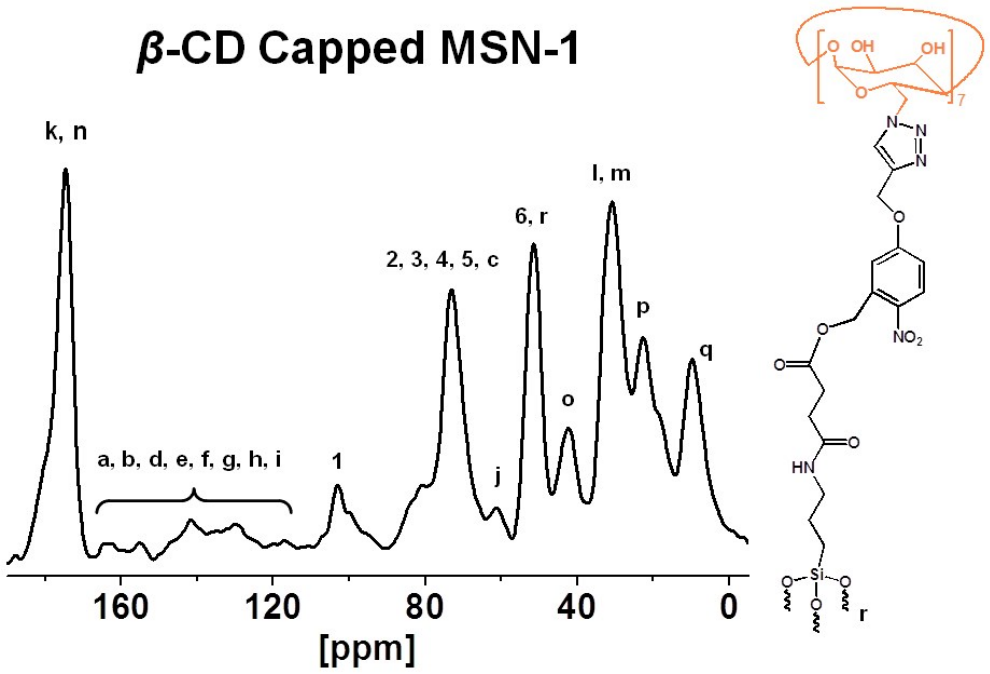

Figure 5. ^{13}C CP-MAS solid-state NMR spectrum of MSN-1**.**

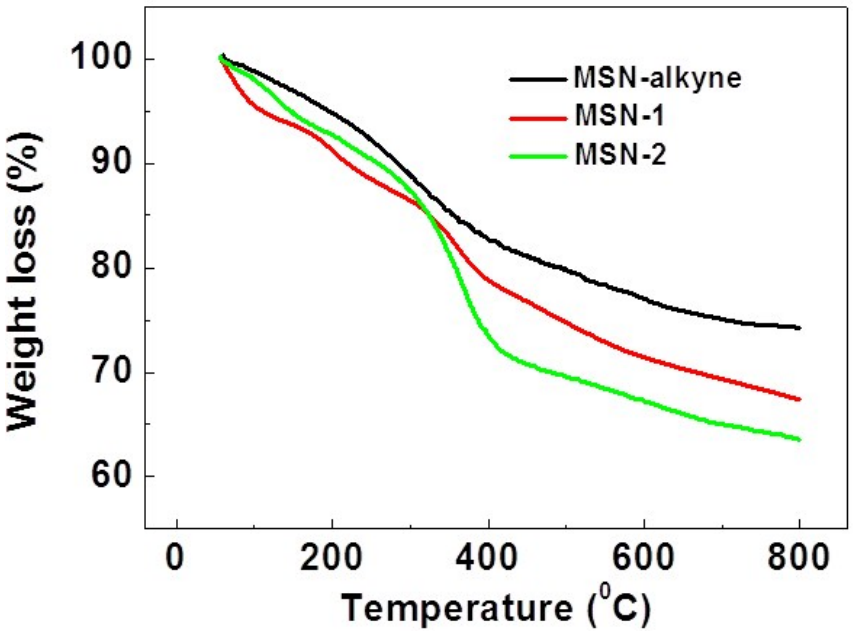

Figure 6. TGA curves of MSN-alkyne, MSN-1 and MSN-2.

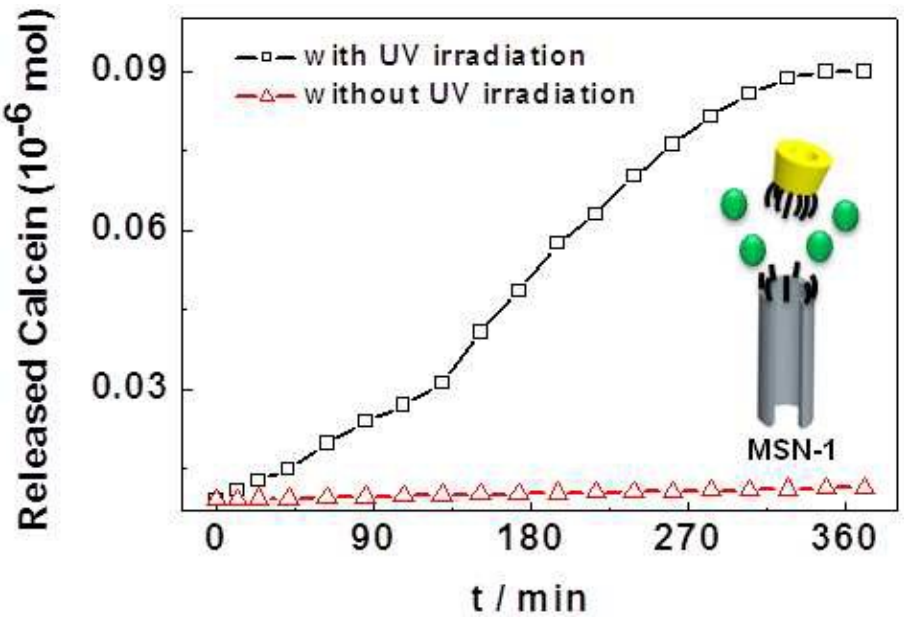

Figure 7. Release profiles of calcein-loaded MSN-1 in PBS (pH 7.4) triggered by UV irradiation.

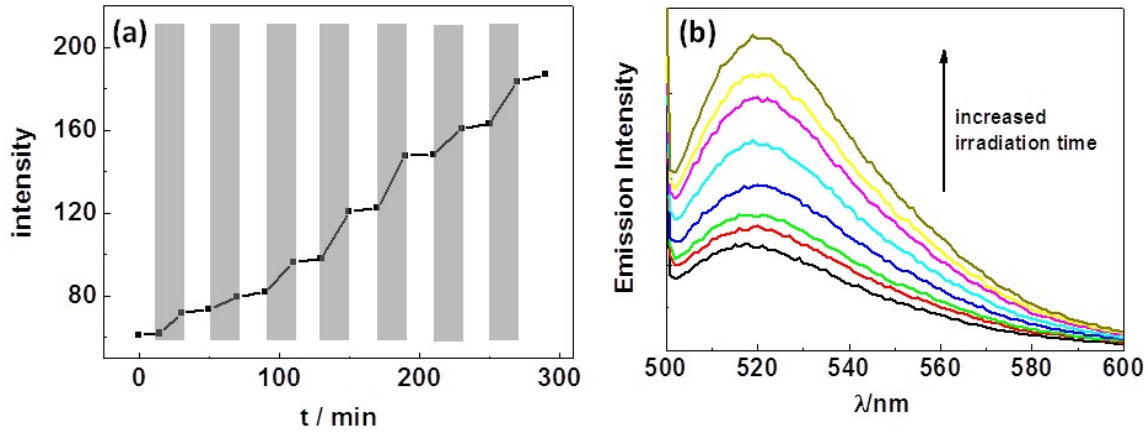

Figure 8 (a) Cargo release of MSN-1 as a function of time (λex= 490 nm). Shaded regions indicate the UV exposure. (b) The fluorescence curve of released calcein with increased irradiation time.

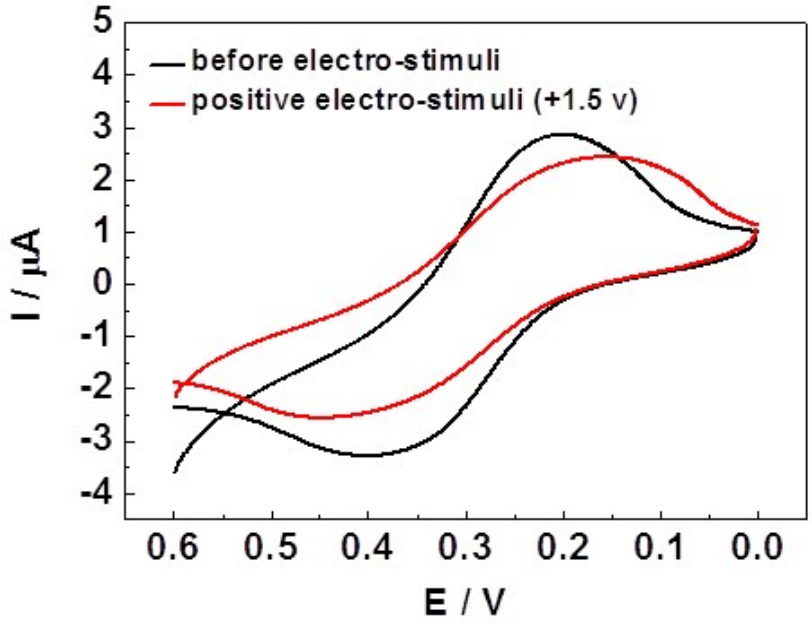

Figure 9. Cyclic voltammetry response on a MSN-2 decorated gold electrode before and after +1.5 v electro-stimuli. The scan rate was set to 50 mv / s. The scan voltage parameters were set as: High v=0.6 v, Low v=0 v, Initial v=0.6v. The supporting electrolyte is PBS buffer (pH=7.4).

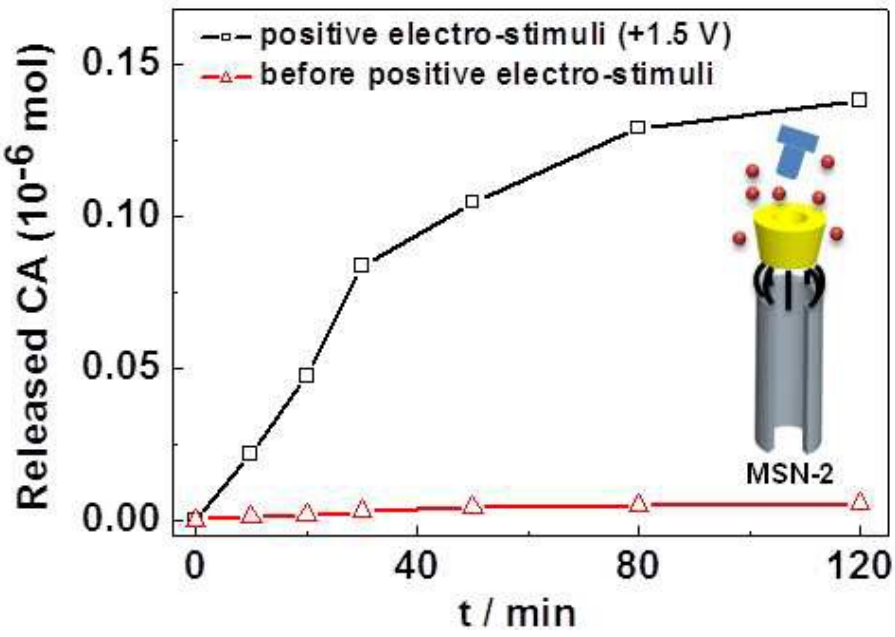

Figure 10. Release profiles of CA-loaded MSN-2 in PBS (pH= 7.4) triggered by +1.5 v voltages.

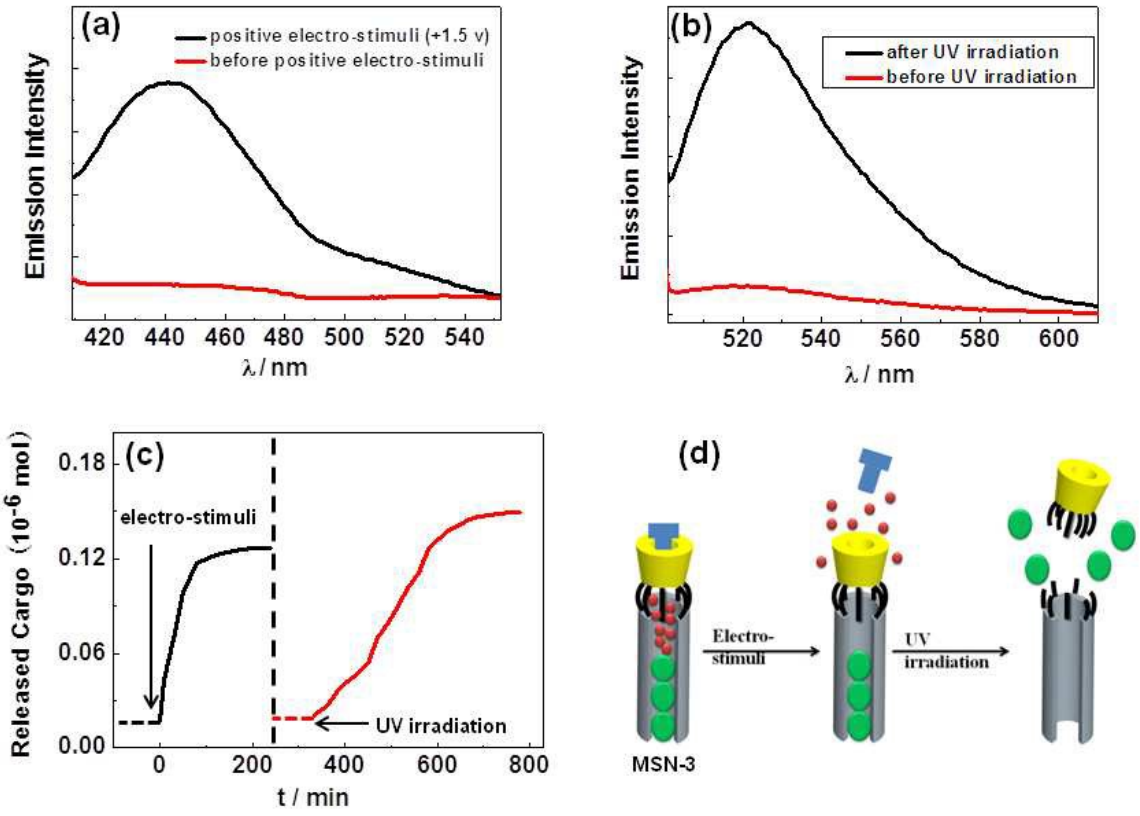

Figure 11. Fluorescence spectra of dual-loaded MSN-3 a) before and after applying +1.5 v electro-stimuli, b) before and after the irradiation of UV light. c) Step-by-step release profile of dual-loaded MSN-3 in PBS (pH=7.4) triggered by applying external +1.5 v voltage and then placing under UV light.

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