Research Article

Fabrication of Heparinized Mesoporous Silica Nanoparticles as Multifunctional Drug Carriers

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In this study, heparinized multifunctional mesoporous silica nanoparticles were successfully synthesized and characterized. The new material not only maintains intrinsic functions of bare magnetic and fluorescent mesoporous silica materials such as targeting, imaging, and sustained release of drugs, but also generates several novel activities such as the enhancement of biocompatibility, selective loading drugs, and dual loading of anticancer drug and bFGF, rendering it a promising candidate to be used as a multifunctional carrier. The strategy of combination of multifunctional mesoporous silica materials with bioactive molecules could be a new effective approach to improve their capabilities in the drug delivery.

1. Introduction

The design of mesoporous silica nanoparticles (denoted as MSNs) as a multifunctional platform for targeted drug delivery has attracted tremendous interests recently. The unique properties of MSNs—large surface area, large pore volume, tunable pore sizes, and versatile chemistry for surface functionalization—make them serve not only as reservoir for drugs payload but also as motifs for functional groups assembly, thus yielding various MSNs-based multifunctional drug delivery systems (DDSs). For example, to endow MSNs-based DDSs with image tracking and magnetic targeting properties, several composite systems have been employed, such as Fe_3O_4 -quantum dots (QDs) [1, 2], Fe_3O_4 -organic dye [3], and Fe_3O_4 - $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}/\text{Tm}^{3+}$ [4]. To improve the dispersibility and inherently low biocompatibility of MSNs-based DDSs under physiological condition, various functional groups (amino, carboxyl, thiol, and phosphonate groups), block copolymers, poly (ethylene glycol) (PEG), and phospholipids have been incorporated [5–13]. To achieve controlled release of drugs in a stimuli-responsive manner, such systems as quantum dots, gold nanoparticles, iron oxide nanoparticles, and dendrimer were utilized as gates to cap the pores of the MSNs and prevent guest drugs from leaking [14–17]. To target drug delivery to specific cell populations such

as tumoral cells, antibodies or folic acid ligands were grafted on the MSNs [18, 19], thus allowing them to selectively bind to the cell surface to trigger receptor-mediated endocytosis. For a satisfactory nanoparticulate delivery device, it would be desirable to successfully combine targeting, imaging, therapeutics, biocompatibility, and other functions into one system. However, even for the magnetic and fluorescent MSNs-based one (denoted as MFMSNs), which are close to the requirements of desired DDSs due to the combination of therapeutics, targeting, and imaging functions, such activities as specific recognition of cell populations are still lacking. Moreover, in many cases, it is not easy to incorporate simultaneously several functional groups into one nanoparticle in a sufficient concentration due to the limitation of the number of attachment sites on the particle surface. Accordingly, how to further enhance the bioactivities of these systems is still a big challenge. Recently, the combination of nanoparticles and biological molecules is of intense interest owing to the synergistic properties offered by such newly synthesized composites [20], thus creating possibility for these multifunctional MSNs to further strengthen their capabilities in drug delivery.

Heparin, polyanionic glycosaminoglycans (GAGs) that naturally cover the surface of all eukaryotic cells, is mainly consisted of alternating units of sulfated glucuronic acid and

structurally diverse glucosamine derivatives [21]. Heparin is predominantly used as an anticoagulant and is also being investigated as a possible agent to regulate complement activity and inflammation [22–24]. Heparin is also able to inhibit cancer cell angiogenesis, tumor growth, and metastasis [25]. Recently, a variety of research has begun to combine the biological activities of heparin with the useful properties of nanomaterials, thus providing novel and enhanced activities with potential applications ranging from improving anticoagulant activity for anticancer therapy to tissue engineering and biosensors [20]. For example, the presence of heparin on polymeric nanoparticles has been reported to increase their circulation time [26]. The surface modifications of heparin to the magnetite nanoparticles are effective in delaying phagocytosis and preventing blood clotting in vitro [27]. A doxorubicin-(DOX-) loaded heparin-DOCA (deoxycholic acid) conjugate was found to exhibit high suppression of tumor growth compared to free DOX [28]. Though heparin has been conjugated to the surface of the nanoparticles, such as magnetic and metallic nanoparticles, or biodegradable and nondegradable synthetic polymers [20], unfortunately, the conjugate of heparin with MSNs is still lacking. In this study, heparin was covalently bonded to amino-modified MFMSNs (denoted as MFMSN-NH₂) via amide formation, and then the bioactivity of heparinized MFMSNs (denoted as MFMSN-HP) was assessed via plasma recalcification time (PRT), uptake and release of drugs, binding with growth factors that are capable of targeting recognition of tumor cells, and cytotoxicity assay against tumor cells. The aim of the study is to construct a multifunctional anticancer drug carrier by combining the merits of the two substances.

2. Experimental Section

2.1. Materials. Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), 3-aminopropyltriethoxysilane (APTES), heparin sodium (Mw 12,000), doxorubicin, and indomethacin were purchased from Shanghai Chemical Corp. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 4-morpholineethanesulfonic acid (MES), and fluorescein isothiocyanate (FITC) were obtained from Sigma-Aldrich (St. Louis, MO). Basic fibroblast growth factor (bFGF) was purchased from Pharmacia. All other chemicals are of analytical grade.

2.2. Synthesis of FITC-APTES. A solution of FITC (8.6 mg) in EtOH (2 mL) was mixed with APTES (0.197 mL) and then the mixture was stirred at room temperature for 24 h. The prepared FITC-APTES stock solution was kept at 4 °C.

2.3. Synthesis of MFMSNs-NH₂. The spherical magnetic Fe₃O₄ particles were prepared according to the literature [29]. The core-shell structured MFMSNs were prepared according to the literature [3, 30]. In a typical procedure, as-prepared Fe₃O₄ nanoparticles (0.1 g) were treated with a mixed solution of ethanol (20 mL) and HCl (0.1 M, 40 μ L) by ultrasonication for 15 min. Subsequently, the treated nanoparticles

were separated by a magnet, washed with ethanol and water several times, and then dispersed in a mixture of CTAB (0.32 g), deionized water (50 mL), ammonia solution and ethanol (70 mL). At last, 0.5 mL of TEOS, and 50 μ L of FITC-APTES solution was added to the above reaction solution in sequence. After 10 min, 50 μ L of APTES were added and the solution was stirred for 6 h. The obtained products were collected and separated with a magnet, washed several times with ethanol and water, and dried at 80 °C for 12 h. The structure-directing agent CTAB was subsequently removed by ultrasonication in acidic (HCl) ethanol (about 0.12 w/w) three times [31]. Thus, the amine-functionalized MFMSNs were obtained.

2.4. Heparinization of MFMSNs. Heparin was immobilized onto MFMSNs using a previously reported protocol. MFMSNs-NH₂ (100 mg) were suspended in 0.1 M MES buffer (pH 5.5) and hydrated for 2 h, to which EDC (40.0 mg) and NHS (24.0 mg) were added, and then 1.9 mg of heparin was slowly added. The reaction mixture was incubated by rotating overnight. Samples were collected by centrifugation and then dialyzed against deionized water to get rid of the residual EDC, NHS, and heparin. The obtained products denoted as MFMSNs-HP were lyophilized and kept at 4 °C. The contents of heparin incorporated in the MFMSNs were examined by a toluidine blue assay [32].

2.5. PRT Assays. The PRT assays were performed according to a previously reported method [27]. Fresh blood collected from a healthy human was immediately mixed with 3.8 wt% sodium citrate solution at a dilution ratio of 9:1. It was then centrifuged at 3000 rpm and 8 °C for 20 min to obtain the platelet-poor plasma (ppp). 0.1 mL of ppp was transferred to a clotting tube at 37 °C, and 0.1 mL saline solution (0.9% NaCl) was added to the ppp. 0.1 mL of 0.025 M CaCl₂ solution was then added, and the stop watch started. The clotting tube was tilted at 15 s intervals until a firm clot was formed. For investigating the effect of the as-synthesized and heparin-functionalized nanoparticles on the plasma clotting time, 0.1 mL of different concentrations of MFMSNs-NH₂ or MFMSNs-HP solution was added in place of the saline solution. At least three experiments were carried out for each sample and the mean clotting time was reported. The differences between the PRT obtained with saline, MFMSNs-NH₂, and MFMSNs-HP were analyzed statistically using the two-sample *t*-test. The differences observed between these samples were considered significant at *P* < 0.05.

2.6. The Loading/Release of Drugs into/from MFMSNs-HP. MFMSNs-HP (25 mg) was dispersed in DOX PBS solution (1 mL, 1 mg mL⁻¹) or IDM ethanol solution (1 mL, 12 mg mL⁻¹). After stirring for 24 h under dark conditions, the DOX- or IDM-encapsulated MFMSNs-HP was centrifuged and washed with PBS or ethanol solution. To evaluate the DOX or IDM loading amount and efficiency, the supernatant solutions were collected and the residual DOX or IDM was measured by UV-vis spectroscopy at 480 nm or 250 nm, respectively. Drug loading efficiencies were expressed as

FIGURE 1: SEM images of pure Fe_3O_4 nanoparticles and MFMSNs ((a), (b)). HRTEM images of MFMSNs ((c), (d)). The magnetic hysteresis loops (e) of pure Fe_3O_4 nanoparticles (A), MFMSNs (B), and MFMSNs-HP (C). Confocal laser scanning microscopy image of MFMSNs (f).

percentages of drugs in MFMSNs-HP nanoparticles with respect to the initial amount of drugs used to prepare the drugs-loaded MFMSNs-HP nanoparticles.

The *in vitro* release test was performed by immersing the DOX-loaded samples (25 mg) in 4 mL of PBS solution (pH 7.4) and ultrasonication at 40 W for 1 min. The dispersed DOX-loaded MFMSNs-HP nanoparticles in PBS solution were gently shaken at 37°C in a water bath at 180 rpm. At given time, the medium was replaced with fresh medium. The amount of the released DOX was determined by UV-vis spectrophotometer.

2.7. The Binding of bFGF to MFMSNs-HP. The binding of bFGF to MFMSNs-HP was monitored by fluorescence titra-

tion. Fluorescence spectra were measured with RF-5301PC Shimadzu spectrofluorometer. bFGF and DOX were excited at 278 nm and 450 nm, respectively. All spectra were recorded at room temperature in 20 mM pH 7.4 PBS buffer solutions, MFMSNs-HP titrations were conducted by adding aliquots of either a stock (10 mg mL^{-1}) into 1 mL of $2\text{ }\mu\text{M}$ bFGF solutions or a stock $100\text{ }\mu\text{M}$ bFGF solution into 0.5 mg mL^{-1} MFMSNs-HP solutions. After gentle shaking for 5 min, MFMSNs-HP was separated with a magnet, and the supernatant was sent to fluorescence measurement.

2.8. Characterization. Scanning electron microscopy (SEM) images were obtained on a field emission JEOL JSM-6700F

FIGURE 2: N₂ adsorption-desorption isotherms (a) and the corresponding pore size distributions (b) of MFMSNs (A), MFMSNs-HP (B), IDM-loaded MFMSNs-HP (C), and DOX-loaded MFMSNs-HP (D).

FIGURE 3: X-ray diffraction patterns of pure Fe₃O₄ nanoparticles (A), MFMSNs (B), and MFMSNs-HP (C).

microscope. Transmission electron microscopy (TEM) analyses were conducted on a JEM-2100F electron microscope operating at 200 kV. UV-vis spectra were recorded on a UV-3101PC Shimadzu UV-vis spectroscope. For photoluminescence (PL) spectroscopy, an RF-5301PC Shimadzu spectrofluorometer was used. X-ray diffraction (XRD) pat-

SCHEME 1: The formation process of heparinized mesoporous silica nanoparticles embedded by magnetic and fluorescent groups.

tern was collected using a Rigaku D/Max-2200 PC X-ray diffractometer with Cu target (40 kV, 40 mA). Nitrogen adsorption-desorption isotherms at 77 K were measured on a Micromeritics Tristar 3000 system. X-ray photoelectron spectroscopic (XPS) analysis was performed on an Escalab MKII X-ray photoelectron spectrometer with Al K α X-ray source. To compensate for the surface-charging effect, all core-level spectra were referenced to the C 1s hydrocarbon peak at 284.6 eV. Zeta potential of the nanocomposites was measured by Zetasizer (Nano ZS-90, Malvern Instruments). Confocal images were recorded on Olympus FV1000 confocal laser scanning microscope.

TABLE 1: Structural parameters of MFMSNs, MFMSNs-HP, IDM-loaded MFMSNs-HP, and DOX-loaded MFMSNs-HP.

Sample	V_p (cm^3/g)	S_{BET} (m^2/g)	D_p (nm)	Heparin loading (wt%)	Indomethacin loading (wt%)	DOX loading (wt%)
MFMSNs	0.33	502	2.65			0.1787
MFMSNs-HP	0.35	395	2.14	2		
IDM-loaded MFMSNs-HP	0.35	383	2.13			
DOX-loaded MFMSNs-HP	0.36	296	2.10			2.5

^aThe specific surface area (S) was calculated by the BET equation. ^bThe pore volume (V) was obtained according to BJH method. ^cThe pore size (D) was calculated by the BJH method from N_2 desorption isotherms.

TABLE 2: Comparison of PRT obtained in the absence and presence of MFMSNs-HP.

Sample	PRT [s]
Control with saline solution	205 ± 20
Control with 0.1 mg mL^{-1} heparin solution	>1386
With 0.1 mg mL^{-1} as-synthesized MFMSNs	200 ± 16
With 0.1 mg mL^{-1} MFMSNs-HP	631 ± 29
With 0.3 mg mL^{-1} MMSNS- NH_2 -HP	1386 ± 25

3. Result and Discussion

The fabrication of MFMSN-HP is presented in Scheme 1. Typically, the as-prepared Fe_3O_4 particle was treated by a modified Stöber procedure, to be coated by a silica layer on its surface. Subsequently, cetyltrimethylammonium bromide (CTAB) was selected as the organic template for the formation of the outer mesoporous silica layer on Fe_3O_4 particle. To yield more uniform surface coverage of amino and fluorescent groups on mesoporous silica, APTES (3-aminopropyltriethoxysilane) and fluorescein-isothiocyanate-(FITC) derived APTES were embedded into the silica walls by the cocondensation method. After removal of the organic templates, heparin was finally covalently immobilized onto the outer mesoporous silica layer by employing standard carbodiimide chemistry.

SEM images and HRTEM images indicated that pure Fe_3O_4 particles prepared by a solvothermal process consist of monodisperse spherical nanoparticles with an average diameter around 100 nm (Figure 1(a)). The uniform coating of magnetic particles by the inorganic silica with thicknesses of about 50 nm not only retained the morphological features, but also led to the formation of the core-shell-structured silica spheres (Figures 1(b), 1(c), and 1(d)), which can be clearly distinguished from different electron penetrability between the magnetic cores and silica shells. As shown in Figure 1(d), HRTEM images also indicated that the silica spheres possessed randomly distributed mesopores. The mesoporous characteristics were further demonstrated by N_2 adsorption-desorption isotherms, whereas type IV isotherm with a hysteresis loop was exhibited (Figure 2). The pore size calculated using BJH method was 2.65 nm, and the BET surface area and the total pore volume were $502 \text{ m}^2 \text{ g}^{-1}$ and $0.33 \text{ cm}^3 \text{ g}^{-1}$ respectively (Table 1). The uniform mesopores

along with large surface area are advantageous for drug loading. The X-ray diffraction results indicated that the composites still retained the original crystallinity of magnetic particles (JCPDS number 19-0629), but had a relatively low intensity due to the shielding effect of the silica shell (Figure 3). Field-dependent magnetism at room temperature showed no hysteresis (Figure 1(e)), exhibiting the superparamagnetic features, which is desirable for their applications to targeted drug delivery. In addition, the nanoparticles exhibited strong green fluorescence emission visualized by the confocal microscope images due to the embedding of fluorescent dyes (Figure 1(f)), which allows the drug release system to be easily tracked by fluorescent images. Although these designed nanoparticles have possessed mesoporous, magnetic, and fluorescent features, hopefully the introduction of bioactive heparin into nanoparticles could afford new functions for drug delivery.

XPS was firstly employed to confirm the grafting of heparin onto MSNs. Figures 4(a) and 4(b) show the XPS survey scan spectra of amino-modified MSNs and heparin-modified MSNs. After heparin immobilization, the appearance of S 2p peak indicates the presence of heparin. The S 2p core-level spectrum of heparin-modified MSNs is asymmetrical and can be curve-fitted with two peak components at binding energies at 168.8 and 170 eV, respectively, owing to the $-\text{OSO}_3$ and $-\text{NSO}_3$ moieties in heparin (Figure 4(c)) [27]. Herein, it should be mentioned that MSNs without containing FITC was used for the XPS measurement in order to rule out S disturbance of FITC since APTES-or FITC-derived APTES was cocondensed into silica layer of $\text{Fe}_3\text{O}_4@n\text{SiO}_2$. The N 1s core-level peaks of heparin-modified MSNs at 398.5 eV and 399.8 eV are attributed to amine nitrogen and amide nitrogen respectively (Figure 4(d)). The peak area of amide nitrogen, is much larger than that of amine nitrogen, suggesting that most of the amine groups have been converted to amide groups after heparin immobilization. Moreover, after heparin loading, the BET surface area of the nanoparticles was decreased to $395 \text{ m}^2 \text{ g}^{-1}$ (Table 1), and the zeta potential of the nanoparticles at pH 7.4 PBS buffer solution was reduced from -16 mV to -30 mV as well. Therefore, all these results indicated that heparin had been successfully grafted to the nanoparticles, and the loading amount was approximately 2.0% (w/w) determined by toluidine blue assay [29].

It is well known that heparin has a variety of bioactivities such as anticoagulant activity. After the immobilization of heparin on the backbone of MFMSNs, its bioactivities

FIGURE 4: Survey XPS spectra of amino-modified MSNs (a); heparin-modified MSNs (b); XPS S 2p spectrum of heparin-modified MSNs (c); XPS N 1s spectrum of heparin-modified MSNs (d).

are expected to be remained for the following biomedical application. Thus, the assay of plasma recalcification time (PRT) was performed. As heparin is a known anticoagulant, the PRT of MFMSNs-HP dispersion would be expected to be longer than the normal PRT. As can be seen from Table 2, the PRT of 0.1 mg mL^{-1} of MFMSNs- NH_2 did not show a clear difference compared to the blank control (0.9% NaCl). However, the PRT of 0.1 mg mL^{-1} of MFMSNs-HP was larger than that of the blank control (200 s versus 630 s, $P < 0.05$), and furthermore, the PRT of MFMSNs-HP can be prolonged to 1380 s at the concentration of

0.3 mg mL^{-1} , suggesting that the immobilization of heparin on the MFMSNs increases their anticlotting activity in a dose-dependence style. In a recent report, the anticoagulation activities of multifunctional nanoellipsoids (MFNEs) were evaluated via prothrombin time (PT) and activated partial thromboplastin time (APTT) assays [4]. The result showed that the PT and APTT values of the blood plasma after the exposure to MFNEs did not show a clear difference compared to the blank control (PBS solution), indicating that MFNEs do not cause coagulation. In this regard, the anticoagulation effects of MFMSNs-HP are better than that

FIGURE 5: (a) Release profile of DOX from MFMSNs-HP (pH = 7.4). (b) Fluorescence spectra of bFGF in the absence/presence of MFMSNs-HP: free bFGF (2 μ M, 1 mL) (A), bFGF (2 μ M) + 10, 20, and 30 μ L of MFMSNs-HP (10 mg mL⁻¹) (B, C, D). (c) Fluorescence spectra of DOX in the absence/presence of bFGF: free DOX (1 nmol, 1 mL) (A), 2 nmol, 1 mL (D), DOX-loaded MFMSNs-HP (0.5 mg mL⁻¹, 1 mL) + 10 and 20 μ L of bFGF (100 μ M) (B, C).

of MFNEs but lower than that of heparinized magnetic nanoparticles [27]. The main reason is possibly the low-density surface coating of heparin on the mesoporous silica. For heparinized magnetic nanoparticles, the modification of poly(NIPAAm) to the magnetic nanoparticles allowed a large amount of heparin molecules to be immobilized on their surface. Therefore, the strategy of the immobilization of heparin on MFMSNs-NH₂ needs to be adjusted to further improve their blood compatibility. The layer-by-layer coating

of the MFMSNs-NH₂ with negatively charged heparin and positively charged polyelectrolytes might be a better choice.

To explore MFMSNs-HP as a candidate of drug carriers, we selected both doxorubicin (DOX) and indomethacin (IDM) as the model drugs. Positively charged DOX is a typical anticancer drug and negatively charged IDM is a nonsteroidal anti-inflammatory agent. The loaded amount of drugs was quantified with UV-vis spectroscopy by subtracting the supernatant amount from the feeding

amount. Approximately 0.06 mg of DOX was loaded per milligram of MFMSNs-HP (6.0% by mass) and the loading of DOX in MFMSNs-HP further decreased the BET surface area of MFMSNs-NH₂ to 296 m² g⁻¹ (Table 1). While for IDM, the loading value is very low and even could be neglected. N₂ adsorption/desorption parameters of IDM-loaded MFMSNs-HP do not exhibit notable differences compared to those of free MFMSNs-HP (Table 1), suggesting that nearly no IDM penetrates into the MFMSNs-HP. These results clearly indicated that electrostatic attraction played an important role in the drugs loading. But for the resistance of MFMSNs-HP to hydrophobic IDM, ionic interaction alone is not enough because lots of hydrophobic drugs had been reported to be encapsulated in mesoporous silica for the enhancement of drug dissolution [33]. In the case of MFMSNs-HP, the heparin molecules are probably arranged as a brush-like shell on the surface of nanoparticles, thus allowing their surface more hydrophilic and their zeta potential much lower than that of original materials. The failure of IDM to be attached in the MFMSNs-HP could result from both hydrophilic and electrostatic repellences. Therefore, MFMSNs-HP could selectively bind with those of positively charged and hydrophilic drugs. Subsequently, the release of DOX from MFMSNs-HP was performed at pH 7.4 of PBS solution. As shown in Figure 5(a), after the burst release of DOX within the initial 8 h, the release process could be sustained to more than 72 h. This is rather promising for the application of MFMSNs-HP as drug carriers.

Ligand-directed delivery of drugs to tumors by binding to cancer cell-surface receptors has found success in current DDSs [34]. Growth factors such as basic fibroblast factor (bFGF) or epidermal growth factor (EGF) as one of the represented targeting ligands for tumor cells have been incorporated into several drug carriers [35, 36]. The mechanism of growth-factors-derived DDSs is mainly involved in specific affinity of these growth factors with their cognate cell-surface receptors that are found to be overexpressed in numerous tumor cells in comparison to normal cells. Heparin has high affinity with bFGF and the binding of heparin stabilizes the proteins against proteolysis and maintains their conformation. Such advantage along with the brushlike arrangement of heparin at the exterior of MFMSNs-HP gives us a hint to further modify DOX-loaded MFMSNs-HP with bFGF. The binding of bFGF to DOX-loaded MFMSNs-HP was verified by fluorescence titration, where the endogenous fluorescence of protein was quenched little by little with an increase in the amount of DOX-loaded materials (Figure 5(b)). Herein, it is necessary to investigate whether the binding reaction brought about the leakage of drugs from materials. To study this problem, 0.5 mg equal of materials containing 50 nmol of DOX was mixed with varied concentration bFGF in 1 mL of PBS solution. After incubation at the same time intervals (5 min) as those of the binding reaction, the fluorescence of DOX was measured. As shown in Figure 5(c), the fluorescence intensity of all these samples was in the middle of that of 1 μ M and 2 μ M of free DOX, indicating that the immobilization of bFGF on the MFMSNs-HP nearly has no influence on the loaded drugs. Thus, it is feasible for MFMSNs-HP to load anticancer drugs and

growth factors simultaneously, suggesting the potential of the obtained nanoparticles in tumor targeted drug delivery.

4. Conclusion

A number of drug delivery systems based on functionalized mesoporous silica nanoparticles had been developed recently [37–41]. In the present work, a novel system based on heparinized multifunctional mesoporous silica nanoparticles was also described. The system not only maintains intrinsic functions of bare magnetic and fluorescent mesoporous silica materials such as targeting, imaging, and sustained release of drugs, but also generates several novel activities such as the enhancement of biocompatibility, selective loading drugs, and dual loading of anticancer drug and bFGF. The strategy of combination of multifunctional mesoporous silica materials with bioactive molecules could be a new effective approach to improve their capabilities in the drug delivery. Besides drug delivery, the properties of MFMSNs-HPs may allow it to be useful in other biomedical applications, such as magnetic resonance (MR) and fluorescence imaging, and affinity purification of heparin-binding growth factors. Additionally, there is a problem that should be mentioned. The incorporation of anticoagulant heparin into mesoporous silica nanoparticles not only endows this drug delivery system with biocompatibility but may also introduce the risk of inducing hemorrhage at the time of intravenous administration. Therefore, further biochemical evaluation for this system by in vivo/vitro assays is needed, but the aggregates of the obtained nanoparticles will make some investigations not to be carried out, such as cells uptake and cells toxicity. The optimization of reaction conditions to improve the structure of this material is underway.

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