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β-Cyclodextrin Functionalized Magnetic Mesoporous Silica Colloid for Cholesterol Separation

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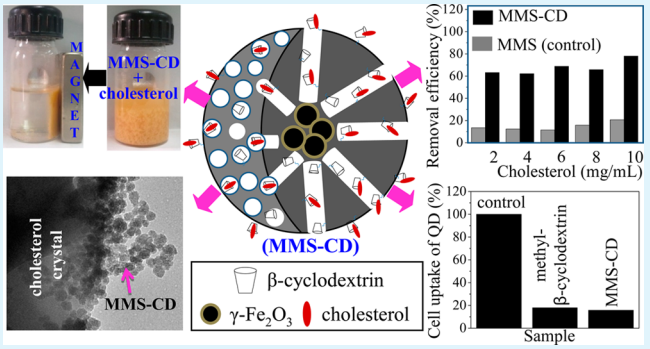
Supporting Information

ABSTRACT: Although cholesterol plays significant biochemical function in the human body, excess of it leads to various disorders, and thus, its control/separation is important in medical science and food industries. However, efficient and selective separation of cholesterol is challenging because cholesterol often exists in microheterogeneous or insoluble forms in remote organ and exists with other chemicals/biochemicals. Here, we have described a colloidal magnetic mesoporous silica (MMS)-based approach for efficient separation of cholesterol in different forms. MMS is functionalized with β-cyclodextrin for selective binding with cholesterol via host−guest interaction. The colloidal form of MMS offers effective interaction with cholesterol of any form, and magnetic property of MMS offers easier separation of bound cholesterol. Functionalized MMS is efficient in separating cholesterol crystals, water-insoluble cholesterol, and the microheterogeneous form of cholesterol from milk or a cellular environment. Developed material can be used to remove cholesterol from a complex bioenvironment and extended for large-scale cholesterol separation from food.



KEYWORDS: *cholesterol separation, nanoparticle, mesoporous silica, cyclodextrin*

INTRODUCTION

Cholesterol is an organic lipid molecule produced by the liver and other organs.[1] Cholesterol has different functions in the human body system such as building and maintenance of cell membranes, biosynthesis of hormones and vitamins, and acting as a functional component in cellular processes.[1,2] Although cholesterol plays a significant role in the human body, a high level of cholesterol can lead to the deposition of cholesterol on artery walls. These deposits can inhibit blood flow through arteries and cause complications such as chest pain, stroke, or heart attack.[3] The main external source of cholesterol for humans is food of animal origins such as eggs, fish, meat, and dairy products. Thus, separation of cholesterol is an essential aspect in most of the food processing industries.

Currently, several methods exist for cholesterol removal that include physical, chemical, and biomedical approaches. Physical methods include adsorption-based separation using saponin/digitonin,[4] supercritical fluid extraction,[5] hydrophobic adsorbant,[6] and molecular imprinted technique;[7,8] chemical methods include host−guest complexation using β-cylodextrin,[9] degradation by photocatalyst,[10] and coprecipitation by enzyme functionalized materials;[11] and biomedical approach involves controlling cholesterol synthesis via modifying fat metabolism.[12] However, most of the reported separation methods have poor selectivity, and flavor and nutritional components of foods are often compromised during the cholesterol removal step. Nevertheless, β-cylodextrin-based chemical approach is most widely used for cholesterol separation.

β-Cyclodextrin is a cyclic oligosaccharide composed of seven glucose units and has a hydrophobic cavity at the center of its molecular arrangement, which can form an inclusion complex with cholesterol via host−guest interaction[13] and has been used for the separation of cholesterol from foods.[9,14] It is desirable that β-cyclodextrin exists in soluble/dispersible form for efficient capturing of cholesterol but should be easily separable after host−guest interaction with cholesterol. Because β-cyclodextrin has good solubility and low molecular weight, it is often linked with polymer/nanoparticle support for enhanced separation performance. β-Cyclodextrin has been transformed into different forms that include cross-linked cyclodextrin,[15] polymer conjugated cyclodextrin,[16] and cyclodextrin conjugated with nanoparticle or solid supports.[17,18] These studies show that cholesterol separation performance greatly depends on material solubility/dispersibility, percentage composition of β-cyclodextrin, size/molecular weight of support and nature of cholesterol phase. Thus, research is directed to prepare material with high percent of β-cyclodextrin, which is easily accessible to cholesterol and easily separable after interaction with cholesterol.

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Mesoporous silica with high surface area and large pore volume can be an ideal support for β-cyclodextrin. Mesoporous silica has been widely used in chemical, biomedical, and environmental science that include catalysis,[19] separation of toxic metal and organic pollutants,[20,21] separation of biomolecule/cell,[22] and drug delivery.[23] Incorporation of magnetic nanoparticle into mesoporous silica offers the advantage of easier separation. In addition, mesoporous silica and nanoparticles have been functionalized with cyclodextrin and used as a drug delivery carrier,[24] in adsorption-based separation of organic molecules[25−29] and in the detection of cholesterol.[30,31] However, capability of β-cyclodextrin functionalized mesoporous materials toward cholesterol separation has not been investigated. Here, we have synthesized β-cyclodextrin conjugated magnetic mesoporous silica (MMS) colloid of 80−100 nm size and used it for efficient separation of cholesterol of different forms. Our results show that ∼10 wt % of β-cyclodextrin can be conjugated with MMS. The colloidal form of MMS offers efficient interaction of β-cyclodextrin with cholesterol of various forms, and its magnetic property enables easier separation.

EXPERIMENTAL SECTION

Materials. Tetraethylorthosilicate (TEOS), [3-(2-aminoethylamino)propyl]trimethoxysilane (AEAPS), tetramethylammonium hydroxide (25 wt %) in methanol (TMAH), octadecylamine, methyl morpholin N-oxide, octadecene, β-cyclodextrin (CD), methyl β-cyclodextrin (MBCD) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) was purchased from TCI Chemicals and used as received. Cetyltrimethylammonium bromide (CTAB) was purchased from Alfa Aesar. *N*-Hydroxysuccinimide (NHS) was purchased from Fluka. Ammonia (25 wt %) and succinic anhydride were purchased from Merck.

Instrumentation. UV−visible absorption spectra were measured using Shimadzu UV-2550 spectrophotometer in a 1 cm quartz cell. BET surface area measurement was performed using Quantachrome Autosorb-1C, and pore distributions were analyzed by Non Local Density Functional Theory (NLDFT). FEI Technai G2 transmission electron microscope (TEM) was used to obtain TEM images of samples. XRD measurements of the samples were performed using Bruker D8 Advance powder diffractometer, using Cu Kα (λ = 1.54A^0) as the incident radiation. Dynamic light scattering (DLS) and zeta-potential of the sample were measured by using a NanoZS (Malvern) instrument. The amount of cholesterol in milk was determined by HPLC (Waters 515) equipped with SunFire C18 column and UV detector (Waters 2489). Fluorescence images of cells were captured by using Olympus IX81 microscope with Image-Pro Plus Version 7.0 software. BD Accuri C6 Flow Cytometer was used for cell uptake quantification study.

Preparation of Carboxylated β-Cyclodextrin. First, 1.12 g (1 millimole) β-cyclodextrin and 0.15 g (1.5 millimole) succinic anhydride were dissolved in 8 mL dimethylformamide and taken in a three-necked flask equipped with mechanical stirrer, thermometer, and condenser. Next, 0.14 mL triethylamine was added to this solution, and the solution was purged with nitrogen. The temperature of the solution was increased to 80 °C and and held at this temperature for another 12 h. Next, temperature of the solution was cooled to room temperature, and excess chloroform was added for precipitation. Precipitated β-cyclodextrin was collected by centrifuge, washed with acetone, and dried in vacuum. Mass spectroscopic, ^{1}H NMR, ^{13}C NMR, and FTIR analyses have been used to confirm the formation of carboxylated β-cyclodextrin (Supporting Information, Scheme S1, Figures S1−S4).

Synthesis of Hydrophobic γ-Fe_2O_3 Nanoparticles. Hydrophobic γ-Fe_2O_3 nanoparticles are synthesized using our reported method.[32] Typically, 373 mg of Fe(III) stearate, 160 of mg octadecyl amine, and 160 mg of methyl morpholin N-oxide were mixed with 10 mL of octadecene solvent in a three-necked flask and degassed for 15 min under N_2 gas purging condition. Then, the temperature of the solution was increased to 300 °C and kept at that temperature for 15 min under N_2 atmosphere. Next, temperature of the solution was cooled to room temperature and stored as a stock solution for further use.

Synthesis of Magnetic Mesoporous Silica Particle (MMS). MMS has been synthesized using our previously described method.[32,33] First, 0.5 mL of as synthesized hydrophobic γ-Fe_2O_3 was transformed into water-soluble particle by silica coating[32] and 45 mL of aqueous solution was prepared. Next, 5 mL of CTAB solution (0.15 M) was added to the above silica-coated γ-Fe_2O_3 solution. After 10−15 min of stirring, 1.5 mL of NH_3 (28%) solution was added under stirring condition. Next, 0.5 mL of ethanolic solution of TEOS (300 μL of TEOS dissolved in 2.5 mL of ethanol), 2 mL of ethanolic solution of AEAPS (50 μL of AEAPS dissolved in 10 mL of ethanol), and 2 mL of DMF were added stepwise with 5 min intervals. The whole solution was stirred for 3 h, and then excess ethanol was added for the precipitation of the particles. Particles were separated by centrifuge and washed with ethanol and water three times each. To remove CTAB, we then dispersed the particles in an ethanolic solution of NH_4NO_3 and heated the solution to 70−80 °C for 1 h under stirring conditions. This process was repeated two more times, and finally, the particles were dispersed in 10 mL of water and used as stock solution.

Synthesis of β-Cyclodextrin Functionalized MMS (MMS-CD). First, 10 mL of primary amine functionalized MMS solution was prepared with the particle concentration of ∼8 mg/mL. Next, 250 mg of carboxylated β-cyclodextrin was added, and the pH was adjusted to 5.0 by adding MES [2-(*N*-morpholino)ethanesulfonic acid] buffer solution of pH 5. Next, 38 mg of EDC 38 mg of NHS were added to this solution and stirred for 24 h. The particles were separated from solution by centrifuge and washed four times by water and dried under vacuum for further use.

Preparation of Polymer Coated Quantum Dots (QD). Polymer coated amine-functionalized QD was prepared following our previously published method.[34] First, red emissive hydrophobic quantum dot (QD) was prepared by a high-temperature colloidal synthetic method. Next, hydrophobic QD was transformed to hydrophilic QD following an established polyacrylate coating method. Briefly, hydrophobic QD and acryl monomers with amine and PEG-functional groups were dissolved in Igepal-cyclohexane reverse micelle and polymerization was initiated under inert atmosphere by adding ammonium persulfate. Here, we have used polyethylene glycol-methacrylate and *N*-(3-aminopropyl) methacrylamide in 1:1 molar ratio as PEG and amine monomers, respectively. In addition, a small amount (5 mol %) of methylene-bis-acrylamide was used as cross-linker. After 1 h, the reaction was stopped by adding ethanol, and the polymer-coated QD was precipitated out from solution. Next, QD was washed with chloroform and ethanol and then dissolved in water. The aqueous solution of QD was dialyzed using a molecular weight cutoff filter (MW 12000 Da) for 24 h to make it free of any reagents.

Preparation of Cholesterol Crystal. Cholesterol crystals have been synthesized by using the reported method.[35] At first, 1 g of cholesterol powder was dissolved in 80 mL of ethanol at 60 °C. Next, the solution was slowly decreased to room temperature and maintained at 8−10 °C without disturbing the solution. After 1−2 days, cholesterol crystals precipitated. The cholesterol crystals were washed with water, and dried crystals were used for further experiments.

Cholesterol Separation Study. In the separation of cholesterol, different amounts (2−15 mg/mL) of cholesterol (solid or crystal form) were dispersed in water, and then a dispersion of MMS and MMS-CD (1 mg/mL) was added to this solution. The mixed solution was magnetically stirred for 2−4 h, particles were separated by magnet, and supernatant was discarded. Next, particles were dispersed in 2 mL of hexane to extract cholesterol and used for estimation of cholesterol after separating MMS/MMS-CD. The amount of cholesterol was determined by an HPLC system equipped with a SunFire C18 column

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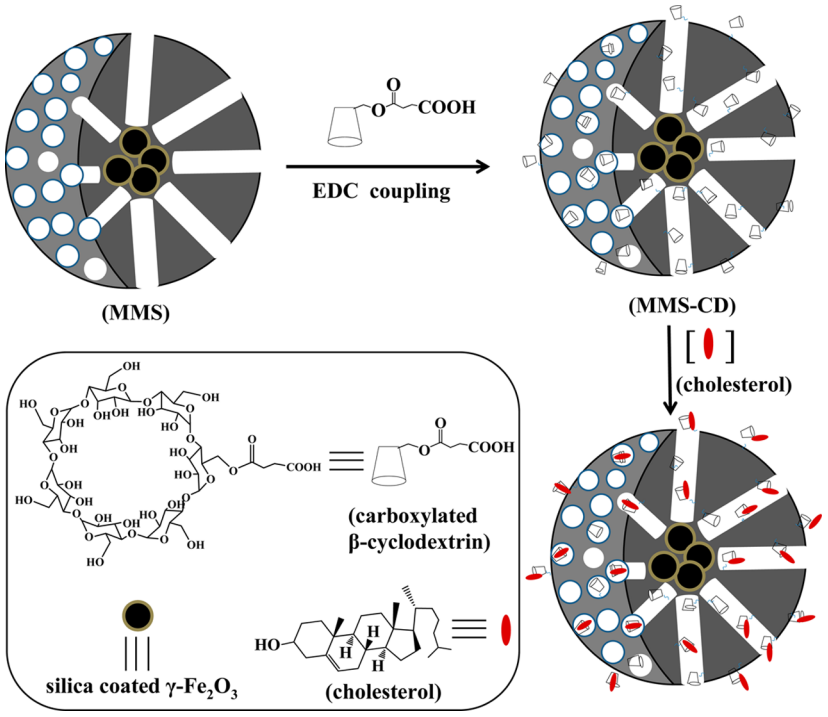
Scheme 1. Synthesis and Cholesterol Separation Strategy Using β-Cyclodextrin Functionalized MMS



and a UV detector. The mobile phase was 1:1 volume mixture of methanol−acetonitrile with a flow rate of 1 mL/min. The sample injection volume was 50 μL, and the detection wavelength was 208 nm.

Extraction of Cholesterol from Milk. At first, cholesterol present in milk was extracted following the reported method,[36] and the concentration was determined by HPLC. Typically, 5 mL of ethanolic solution of KOH (0.5 M) was mixed with 4 mL of milk and stirred for 10 min. Then, the whole mixture was heated to 80 °C for 30 min and cooled to room temperature. Next, 2 mL of water and 3 mL of hexane were added to the mixture, followed by stirring for 10 min. Next, the sample was centrifuged at 3000 rpm to achieve phase separation. The upper hexane part containing cholesterol was collected, and the hexane was evaporated to enable the collection of the cholesterol. The residue was dissolved in 2 mL of methanol and used for cholesterol analysis via HPLC.

In a separate experiment, 5 mL of milk was mixed with 10 mg of MMS/MMS-CD and stirred for 2−4 h. Next, MMS/MMS-CD particles with extracted cholesterol were isolated by magnet. Then, residual cholesterol present in supernatant milk was extracted and determined following above-described method.

Cellular Uptake Study of QD. A cellular cholesterol extraction study was performed in Chinese hamster ovary (CHO) cell. At first, CHO cells were cultured in a 12-well cell culture plate with serum-free DMEM medium. Next, 100 μL of solution of MMS-CD (2 mg/mL) or MBCD (10 mM) was added to the medium and incubated for 2 and 1 h, respectively. Next, 50 μL of QD solution was added to the culture medium and incubated for another 10 min. Then, cells were washed with PBS buffer solution (pH 7.4) three times to remove the unbound particles and mixed with cell culture medium. Finally, cells were used for imaging under fluorescence microscope. For flow cytometry study, cells were removed from the wells by using trypsin EDTA and isolated by centrifugation. Next, the cell palate was dispersed in PBS buffer (pH 7.4) and used for flow cytometry.

RESULTS AND DISCUSSION

Synthesis and Characterization of MMS-CD. The synthesis of cyclodextrin functionalized MMS and separation strategy of cholesterol is shown in Scheme 1. First, 6−8 nm size hydrophobic γ-Fe_2O_3 nanoparticles are synthesized via high temperature thermal degradation of iron stearate. Next, hydrophobic γ-Fe_2O_3 nanoparticles are transferred into water via silica coating where silica shell is ∼1−2 nm thick and composed with primary and secondary amine.[32] MMS is then synthesized through a base catalyzed condensation of mixture of TEOS and AEAPS in the presence of silica coated γ-Fe_2O_3 and CTAB. The presence of silica shell induces the growth of silica on γ-Fe_2O_3 surface as well as their incorporation into growing silica shell with the resultant formation of MMS. After synthesis, the CTAB is removed via ethanol−NH_4NO_3-based extraction. Then, MMS is covalently linked with carboxylated β-cyclodextrin via EDC coupling.

Structure and property of MMS-CD are shown in Figure 1 and Supporting Information, Figure S5. MMS-CD is spherical in shape with 80−100 nm size and incorporated with 6−8 nm γ-Fe_2O_3 nanoparticles. The porous structure of MMS-CD is also observed from high-resolution TEM. SEM image also shows the spherical shape of MMS-CD with 80−100 nm size (Supporting Information, Figure S5). The XRD pattern of MMS and the as-synthesized γ-Fe_2O_3 nanoparticles shows reflection of planes of γ-Fe_2O_3, suggesting that γ-Fe_2O_3 nanoparticles retained crystal structure inside the silica matrix (Supporting Information, Figure S6). Magnetic property has been studied earlier that shows superparamagnetic nature of MMS with saturation magnetization value of 2−3 emu/g.[21] N_2 adsorption/desorption isotherm has been conducted before and after functionalization with β-cyclodextrin. The MMS and MMS-CD show typical type-IV isotherm with BET surface area of 290 and 160 m^2/g, respectively. N_2 adsorption−desorption isotherm of MMS exhibited increased adsorption at low P/P_0 region, which indicates the presence of small micropores. The increase in adsorbed volume at the higher P/P_0 region is due to the capillary condensation, which is a secondary process that requires the preformation of an adsorbed layer on the pore walls followed by multilayer adsorption.[37,38] The pore size

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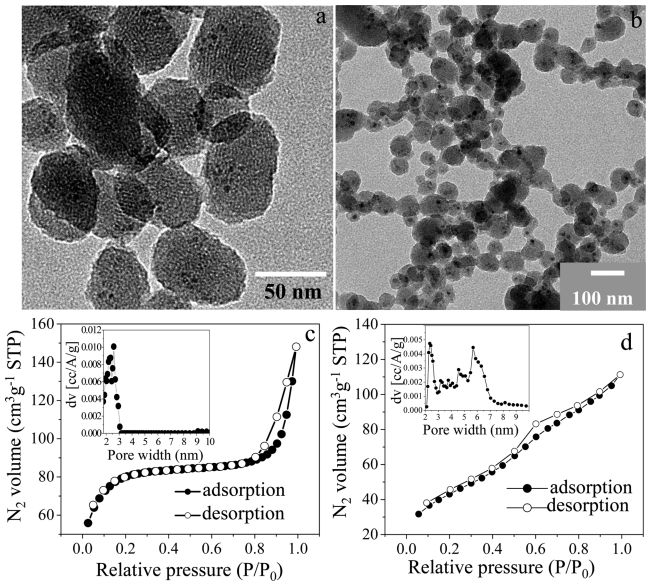

Figure 1. (a and b) TEM image of MMS-CD at two different magnification showing the porous structure. N_2 adsorption−desorption isotherm of (c) MMS and (d) MMS-CD with BET surface area of 290 and 160 m^2 g^{-1}, respectively; (inset) pore size distribution of MMS and MMS-CD.

distribution of MMS shows the presence of small micropores of 1.9 nm associated with the mesopores of 2.3 and 2.5 nm size. The surface area and pore volume of MMS-CD decrease possibly due to the partial blocking of the pores by β-cyclodextrin. In addition, the pore size distribution becomes broad after β-cyclodextrin functionalization, which may be due to inhomogeneous functionalization and partial disordering of mesoporous structure.[39] Small -angle XRD of MMS exhibited the peak at 2.4°, which is attributed due to partially order pore structure. However, this peak becomes broad in MMS-CD due to disordering of pore structure[39] (Supporting Information, Figure S7).

Functionalization of MMS with β-cyclodextrin has been confirmed by FTIR and TGA analysis (Figure 2). After functionalization with β-cyclodextrin, the FTIR spectra exhibit two new bands at 1562 and 1730 cm^{-1} due to N−H bending vibration of amide and C═O stretching vibration. This result indicates covalent conjugation of cyclodextrin with MMS. The amount of bound β-cyclodextrin was estimated from comparative TGA data of MMS and MMS-CD. TGA of MMS shows ∼8% weight loss at <100 °C due to removal of water and ∼8% weight loss at 200−600 °C due to degradation of other functional groups. In contrast, TGA of MMS-CD shows ∼5% weight loss at <100 °C for water removal and ∼18% weight loss at 200−600 °C due to functional groups. The additional weight loss of ∼10 wt % at 200−600 °C is due to cyclodextrin functionalization.[28,40] Similar findings are also observed from differential thermal analysis (DTA) data (Supporting Information, Figure S8). The number of β-cyclodextrin present in MMS-CD is estimated as 4.88×10^{19} molecules/gram, and mean density of β-cyclodextrin is estimated as 3.05×10^{17} molecules/m^2 (for details, see Supporting Information). Zeta potential measurement shows that surface charge of the MMS shifted from +14 mV to −17 mV after functionalization with β-cyclodextrin. This change in surface charge is due to the replacement of primary/secondary amines by carboxylated cyclodextrin. Dynamic light scattering measurement shows that average hydrodynamic size increases from 150 to 200 nm after cyclodextrin functionalization (Figure 2c).

MMS-CD-based Cholesterol Separation. Different forms of cholesterol have been used for their separation studies. Water-insoluble cholesterol powder, cholesterol crystals, and the emulsion form of cholesterol in milk have been used for this study. To investigate the advantage of β-cyclodextrin functionalization, we used the MMS and MMS-CD individually for cholesterol separation, and their performances have been compared. The separation procedure involves mixing MMS-CD or MMS with cholesterol of different forms followed by stirring for 4 h (Figure 3). Next, MMS-CD/MMS with bound

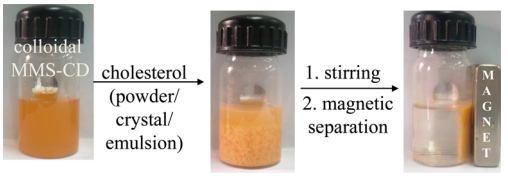

Figure 3. Strategy for MMS-CD-based cholesterol separation.

cholesterol is isolated from solution by a laboratory based bar magnet, mixed with hexane to extract cholesterol, and used for the estimation of cholesterol by UV−visible spectroscopy or HPLC. In the case of milk cholesterol separation, cholesterol-bound MMS/MMS-CD is separated from milk, and the remaining milk cholesterol is extracted and estimated by HPLC. A typical chromatogram is shown in Supporting Information, Figure S9. The results of the cholesterol separation performance are summarized in Figure 4. It shows

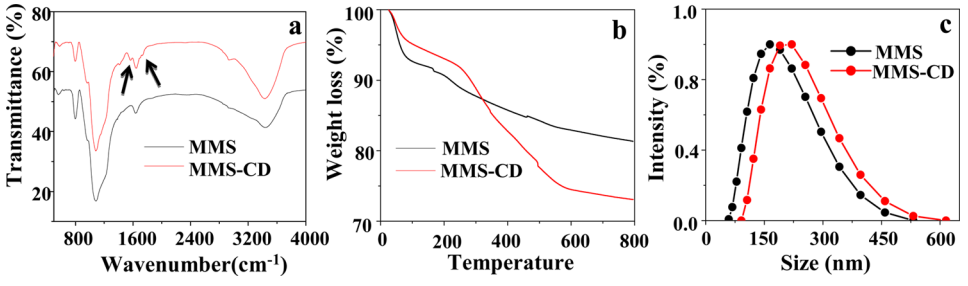

Figure 2. (a) FTIR spectra of MMS before and after β-cyclodextrin functionalization, showing that after functionalization with β-cyclodextrin two new bands arise at 1562 and 1730 cm^{-1}, respectively, for N−H bending vibration of amide and C═O stretching vibration. (b) TGA plot of MMS before and after β-cyclodextrin functionalization, showing that ∼10 wt % of β-cyclodextrin is conjugated with MMS. (c) Hydrodynamic size of MMS before and after functionalization with β-cyclodextrin.

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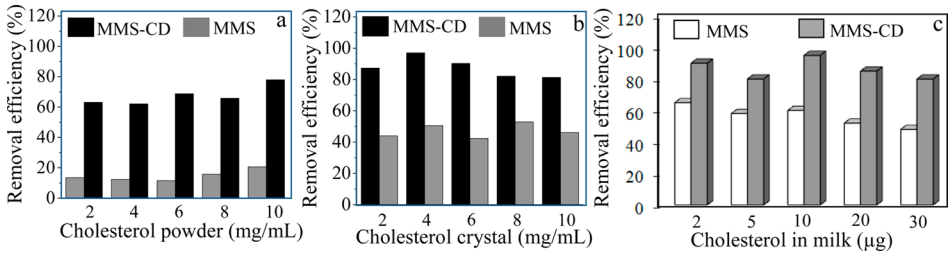

Figure 4. Comparative cholesterol separation efficiency between MMS and MMS-CD, showing the effect of β-cyclodextrin functionalization; (a) separation of insoluble cholesterol from water, (b) separation of cholesterol crystal from water and (c) separation of cholesterol from milk.

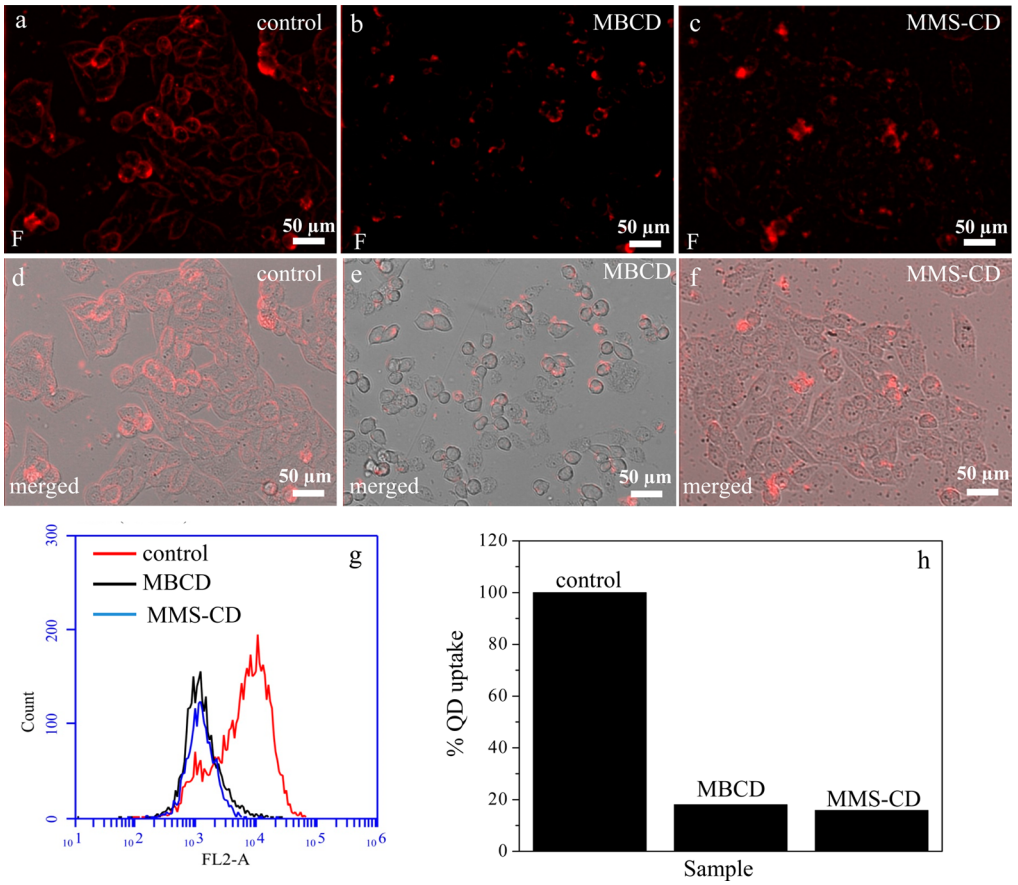

Figure 5. (a–f) Effect of cholesterol extraction on QD uptake into the CHO cells, as observed by fluorescence microscopy. Typical fluorescence images of QD labeled CHO cells shows high QD uptake (a and d). In contrast, QD uptake is lowered once the cells are incubated with MBCD or MMS-CD that extract cholesterol (b, c, e, and f). Images are captured in fluorescence mode (F) or bright field mode merged with fluorescence image (merged). Red emission is due to QDs. (g and h) Flow cytometry based quantification of QD uptake in CHO cells with or without (control) incubation MBCD or MMS-CD. QD uptake for control is considered as 100% for comparison. Mean FL2-A represents fluorescence intensity collected by standard optical filter (585/40) in flow cytometer.

that cholesterol removal efficiency by MMS-CD is generally better than MMS. For example, MMS-based cholesterol removal efficiencies are 10–20, 40–60 and 50–65% for powder, crystal and emulsion forms, respectively. In contrast, cholesterol removal efficiency using MMS-CD is much higher and in the range of 65–95% for all sample types. This result clearly indicates that β-cyclodextrin functionalization increases the cholesterol separation efficiency.

We also investigated the cellular cholesterol extraction using MMS-CD. It is well-known that cholesterol is involved in cellular endocytosis processes, and methyl-β-cyclodextin (MBCD) is commonly used for cellular cholesterol extraction and to inhibit cellular internalization.[41,42] Here, we selected a QD that has good cell uptake, and uptake is known to be inhibited by MBCD that extracts cellular cholesterol.[43] We monitored CHO cell uptake of QD after the cellular cholesterol is extracted by MMS-CD or MBCD and compared the results (Figure 5). In this study, CHO cells are first incubated with MMS-CD or MBCD for cholesterol extraction and then incubated with QD for their uptake. The fluorescence of QD has been used to follow their cellular uptake via fluorescence imaging and flow cytometry. Results show that the QD uptake in CHO cell is significantly lowered once they are treated with MMS-CD or MBCD (Figure 5). In particular, flow cytometry provides quantitative estimates of uptake inhibition by counting more than 10 000 cells, and results show that QD uptake is

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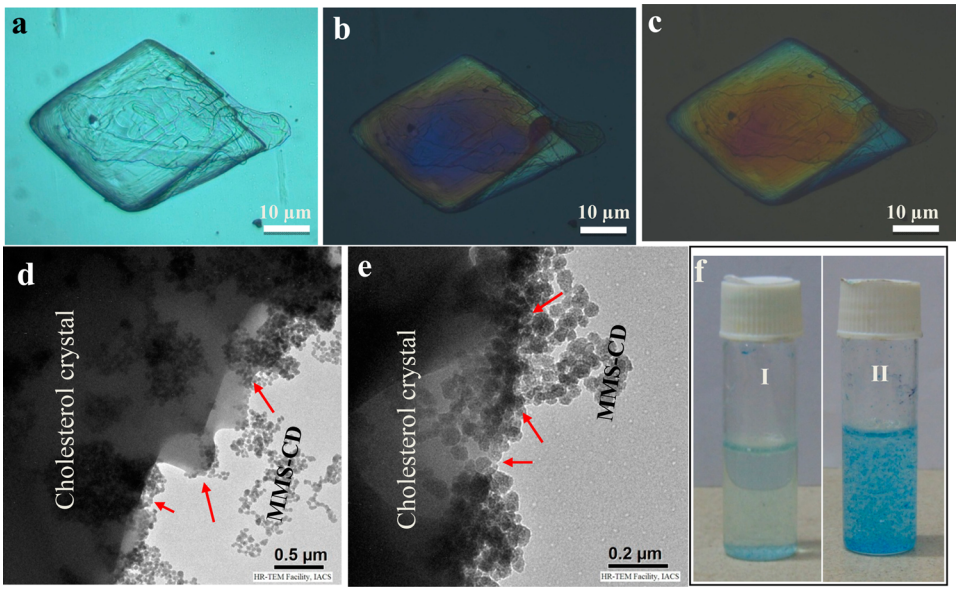

Figure 6. Images of a typical cholesterol crystal under (a) bright field and (b and c) polarized light. (d and e) Low- and high-resolution TEM images of binding of MMS-CD with cholesterol crystal (only a part of crystal is shown). Red arrows indicate interaction of MMS-CD with cholesterol crystal. (f) Prussian blue staining experiment to prove efficient binding of cholesterol crystals with MMS-CD. Cholesterol crystals are incubated with MMS or MMS-CD, and isolated cholesterol crystals are used for Prussian blue staining experiments. Appearance of strong blue color for (II) MMS-CD as compared to (I) MMS indicates binding of more magnetic particles with cholesterol crystals.

decreased to 20% for MMS-CD or MBCD, as compared to control (Figure 5). The comparable and low QD uptake for MMS-CD and MBCD treated cells indicates that MMS-CD is efficient as of MBCD.

Binding of MMS and MMS-CD with cholesterol crystals is investigated further via Prussian blue test and TEM study (Figure 6 and Supporting Information). MMS and MMS-CD are separately incubated with cholesterol crystal for 4 h. Next, cholesterol crystals labeled with MMS/MMS-CD are magnetically isolated, washed with water, and the binding of particles with cholesterol crystals is investigated by Prussian blue test and TEM study. The Prussian blue staining experiment shows that the cholesterol crystals incubated with MMS-CD give intense blue coloration, but cholesterol crystals incubated with MMS give a faint blue color. This result indicates that a greater number of MMS-CD having iron oxide binds with cholesterol crystals, which gives intense blue coloration in Prussian blue test. TEM image of cholesterol crystals shows that large a number of MMS-CD bound with crystals, which gives direct evidence that MMS-CD interact with cholesterol crystals. (Figure 6d,e). This result indicates functionalization of MMS with β-cyclodextrin offers their enhanced binding with cholesterol crystal surface.

In practical application, it is important that MMS-CD can be regeneration for repeated use. So, we have regenerated MMS-CD after they have been used for cholesterol separation and then used it again for cholesterol separation. Cholesterol adsorbed MMS-CD particles are isolated from solution by magnet and treated with hexane to dissolve the adsorbed cholesterol. Next, MMS-CD particles are isolated and washed repeatedly with ethanol and reused for the separation of cholesterol. This reuse experiment is repeated for several times. Result shows that MMS-CD can be used repeatedly for separation of cholesterol without significant loss of removal efficiency (Figure 7).

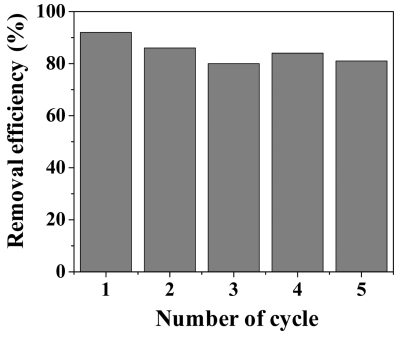

Figure 7. Cholesterol extraction efficiency of recycled MMS-CD. Water dispersed cholesterol powder has been used for this study.

MMS-CD as Unique Material for Cholesterol Separation. Several materials and methods exist for cholesterol separation; however, separation efficiency is generally low in most of the reported methods. Among them, the molecular imprinted approach is most promising due to its high selectivity.[7,8] Adsorption-based separation of cholesterol using hydrophobic adsorbents have poor selectivity.[6] Cyclodextrin immobilized glass particles have been used for separation of cholesterol from milk.[18] However, the large size of glass particles offers low surface area for the immobilization of cyclodextrin, and particles are not dispersible in solution. As a result, cholesterol removal efficiency is low.[18] Cross-linked β-cyclodextrin and molecular β-cyclodextrin are used for the separation of cholesterol. However, poor recovery efficiency of materials limits repeated use and restricts for large scale separation options.[14−16]

Compared to the existing materials/methods, presented material has several distinct advantages. First, the colloidal form of MMS-CD offers efficient interaction with cholesterol of any form. Second, the magnetic component of MMS-CD offers easier separation of cholesterol from complex environment. Third, mesoporous structure of MMS-CD offers functionaliza-

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tion of β-cyclodextrin with high−weight percent (∼10%). Fourth, MMS-CD is very specific in removing cholesterol as β-cyclodextrin cavity is selective to cholesterol. All these advantages of MMS-CD are responsible for efficient cholesterol removal, particularly from milk and cellular environments.

CONCLUSION

In conclusion, we have successfully synthesized β-cyclodextrin functionalized magnetic mesoporous silica, which shows efficient cholesterol removal performance. This approach has several advantages. The high surface area of mesoporous silica offers high loading/functionalization of β-cyclodextrin, the colloidal form of mesoporous silica offers efficient binding with different forms of cholesterol, and magnetic nanoparticles inside mesoporous silica enable magnetic separation of cholesterol. It is demonstrated that the material can be used for separation of cholesterol either in crystal, powder, or microheterogeneous form, and material can be reused.

ASSOCIATED CONTENT

Supporting Information

Mass and FTIR spectral characterization of carboxylated β-cyclodextrin, more characterization data on MMS/MMS-CD, and HPLC chromatogram of cholesterol. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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