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Polymer-coated spherical mesoporous silica for pH-controlled delivery of insulin

Sae Rom Choi[a,‡], Dong-jin Jang[b,‡], Sanghyun Kim[a], Sunhyung An[c], Jinwoo Lee[c], Euichaul Oh[b,*] and Jungbae Kim[a,*]

[a] *Department of Chemical and Biological Engineering, Korea University, Seoul 136-713, Republic of Korea*

[b] *College of Pharmacy, The Catholic University of Korea, Bucheon, Gyeonggi-do 420-743, Republic of Korea*

[c] *Advanced Functional Nanomaterials Laboratory, Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, Republic of Korea*

Experimental Methods

Synthesis of S-MCF and amine-S-MCF: S-MCF was synthesized by modifying the reported procedure.[1] 4 g of Pluronic P123 ($(EO)_{20}(PO)_{70}(EO)_{20}$) and 5 g of KCl were dissolved in acidic solution (10 mL of HCl (37%), 5 mL of ethanol and 65 mL of H_2O). Then, 4 g of 1,3,5-trimethylbenzene was added, and the mixture was stirred vigorously at 40 ºC for 2 h. Tetraethoxysilane (TEOS) (9.2 mL) was then added and stirred for 5 min. The resulting solution was aged at 40 ºC for 20 h under static condition. 46 mg of NH_4F was then added, and the mixture was aged at 100 ºC for 24 h. The obtained suspension was then filtered, dried at room temperature and finally calcined at 550 ºC for 4 h to remove Pluronic P123. S-MCF was further treated with 3-aminopropyltrimethoxysilane to prepare amine-S-MCF, and elemental analysis was performed to estimate the amine loading of amine-S-MCF.[2]

Stability of free HRP and adsorbed HRP in S-MCF: To investigate stability, 5 mg of S-MCF was washed with 1 mL of DI water and 0.1 M sodium phosphate buffer (pH 7.0) respectively. To prepare adsorbed HRP in S-MCF (HRP/S-MCF), S-MCF was incubated with 1 mL of 5 mg/mL HRP solution in 0.1 M sodium phosphate buffer (pH 7.0) under shaking 200 rpm for 90 min. HRP was purchased from Sigma (St. Louis, MO, USA). After incubation, the sample was centrifuged down at 10000 rpm for 5 min, and the supernatant was decanted. The sample was excessively washed with 0.1 M sodium phosphate buffer (pH 7.0) two times and was stored in 20 mL glass vial diluted with 0.1 M sodium phosphate buffer (pH 7.0). As the control sample, free HRP was prepared and also was stored in 20 mL glass vial. Both samples were stored at room temperature under continuous shaking at 200 rpm. To measure the activities of free HRP and HRP/S-MCF, 19 mL of 0.1 M sodium phosphate buffer (pH 7.0) was mixed with 1 mL of 3,3′,5,5′-tetramethylbenzidine as a

substrate dissolved in dimethyl sulfoxide (DMSO) (0.96 mg/mL). 3,3′,5,5′-tetramethylbenzidine and DMSO were purchased from Sigma (St. Louis, MO, USA). 2 µL of H_2O_2 was added to the mixture to make reaction cocktail. 50 µL of samples were reacted with 950 uL of reaction cocktail. The activity was calculated with absorbance increase at 655 nm per min (A655/min) by using UV spectrophotometer. The stabilities of the samples were observed by measuring the residual enzyme activity of each sample at the each time point and the relative activity was calculated from the ratio of residual activity to the initial activity of each sample.

Insulin loading into S-MCF and amine-S-MCF: Mesoporous silica particles, both S-MCF and Amine-S-MCF were prepared 3 mg in 2 mL EP tube and they were washed with 1 mL of DI water and 1 mL of 0.01 M phosphate buffer saline (pH 6.8) respectively. Once they were dispersed in solution, centrifuge down was used to separate silica particles from buffer solution. After washing the materials, 1 mL of 3 mg/mL insulin solution was added. Insulin from bovine pancreas was purchased from Sigma Aldrich (St. Louis, MO, USA). Insulin was dissolved in 0.01 M HCl, diluted with 0.01 M phosphate buffer saline (pH 6.8), and neutralized with 0.1 M NaOH.[3] Sample tubes were shaken at 150 rpm during 90 minutes at 4 ºC. When adsorption was over, unloaded insulin was removed by centrifuge (10000 rpm, 5 min). Those supernatant was used for bicinchoninic acid (BCA) protein assay to analyze the amount of insulin loading. BCA Protein Assay reagent kit was obtained from Pierce (Rockford, IL, USA).

pH-sensitive polymer coating: Two methacrylic acid copolymers, Eudragit® L30D-55 and Eudragit® L100 from Evonik Röhm (Kirschenallee, Germany) were employed to form pH-

responsive coating on insulin loaded mesoporous silica. The polymer coating was prepared by the following process.[4, 5] The freeze-dried insulin loaded mesoprous silica was added to 0.3 mL of 0.1 M phosphate buffer (pH 5.8), and then gently mixed with 3 mg of Eudragit® L30D-55 and 23 mg of hydrophilic fumed silica, Aerosil® 200 (Evonik Degussa, Essen, Germany). Eudragit® L100 (150 mg) was dissolved in acetone (2 mL)-methanol (1 mL) cosolvent with triethyl citrate as a plasticizer. The Eudragit® L30D-55 dispersion including the insulin loaded mesoporous silica was thoroughly mixed with the Eudragit® L100 solution to make a coating suspension. The suspension was immediately frozen in liquid nitrogen and stored in freezer (-80 ºC) for one hour. The frozen suspension was finally lyophilized for 24 hours to remove all the solvents used for coating and gently grinded.

Release studies: 45 mg of polymer coated silica powder were used for release study. At each 0.1 N HCl for pH 1.2 and 0.01 M phosphate buffer saline pH 6.8 (5 mL, respectively) by using 10 mL glass vial, release studies were conducted at 37 ºC under shaking (120 rpm). At predetermined time interval, 1 mL of solution was taken from vial and then centrifuged down (10000 rpm, 5 min) to separate supernatant and silica powder. The fresh buffer was added and sediments at the bottom were recovered together into glass vial. The concentration of released insulin was measured by using the Micro BCA protein assay kit obtained from Pierce (Rockford, IL, USA). Microplate procedure was chosen for the assay and 96-well plates were used purchased from Corning (NY, USA). Increase of absorbance at 562 nm was measured by microplate reader (Tecan, Seestrasse, Switzerland).

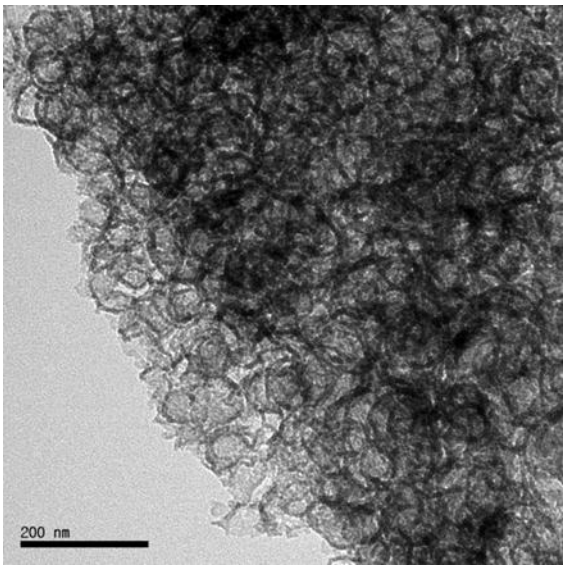

Fig. S1 TEM images of S-MCF showing cell-like pore structure inside.

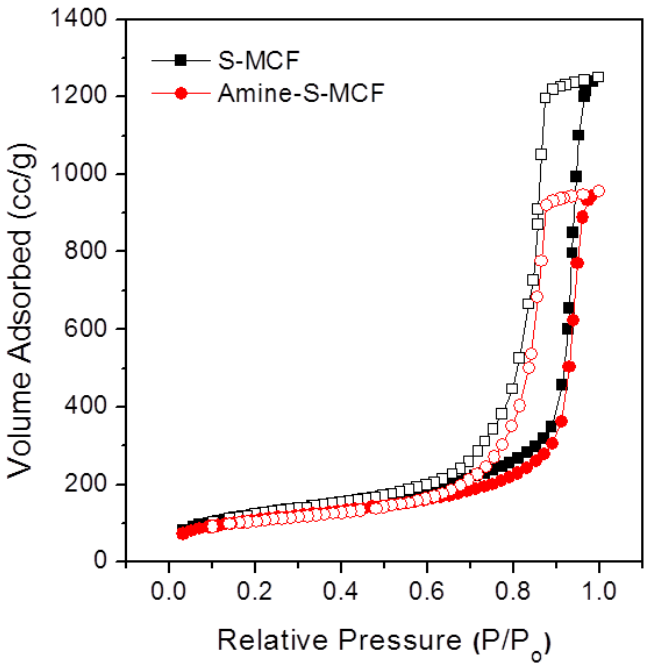

Fig. S2 BET analysis of S-MCF and Amine-S-MCF.

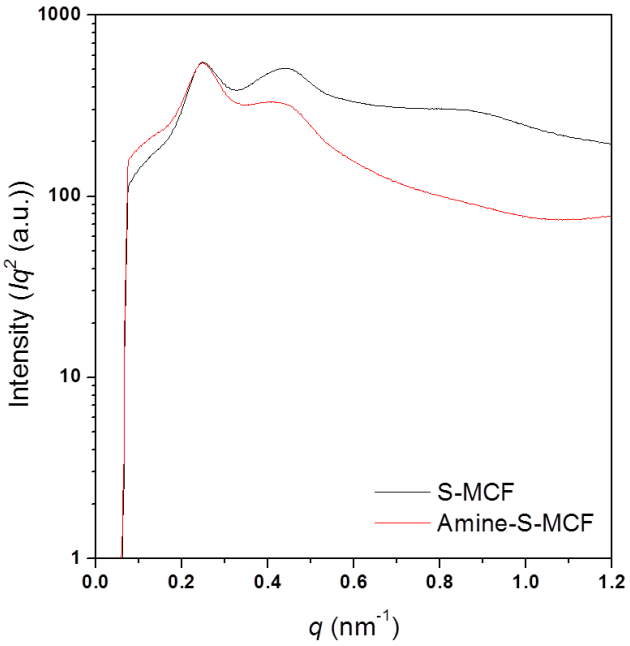

Fig. S3 Small angle X-ray scattering (SAXS) of S-MCF and Amine-S-MCF.

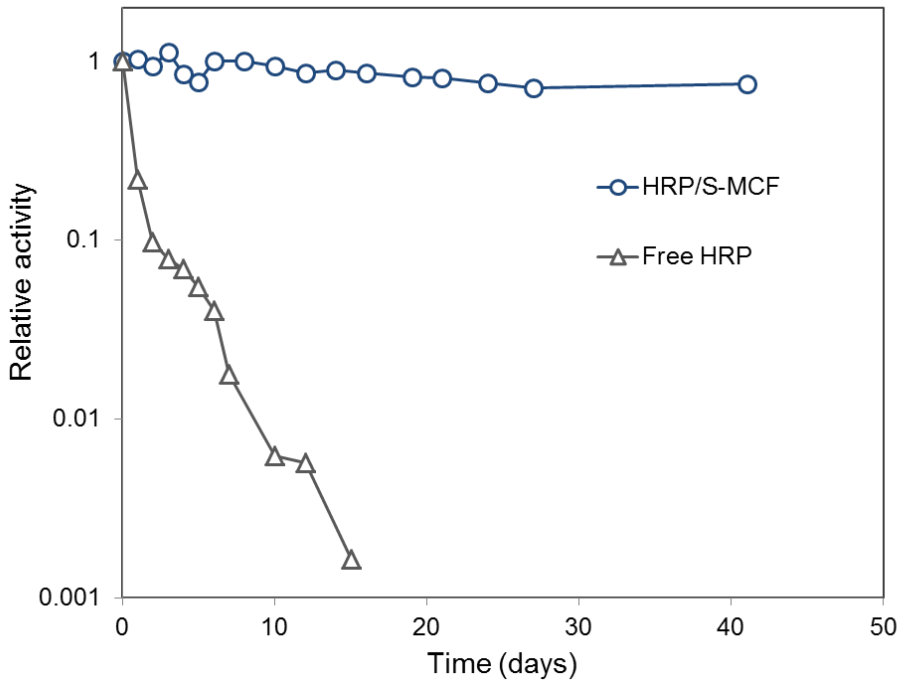

Fig. S4 Stabilities of free HRP and adsorbed HRP in S-MCF. The relative activity represents the ratio of residual activity at each time point to the initial activity of each sample. The HRP activity was measured time dependently after incubation in an aqueous buffer (0.1 M phosphate buffer, pH 7.0) at room temperature under shaking at 200 rpm.

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