Supporting Inforamtion

Mesoporous silica nanoparticles with redox-responsive surface linkers for charge-reversible loading and release of short oligonucleotides

Jixi Zhang,[a] Minna Niemelä,[b] Jukka Westermarck,[b, c] and Jessica M. Rosenholm [a]*

[a] *Centre for Functional Materials Laboratory for Physical Chemistry Department of Natural Sciences Åbo Akademi University Porthansgatan 3–5, Turku 20500, Finland. Fax: +358 22330228; Tel: +358 2215 3255; E-mail: jerosenh@abo.fi*

[b] *Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Tykistökatu 6A, Turku 20520, Finland. Fax: +35822518808; Tel: +358407423007; E-mail: jukwes@utu.fi*

[c] *Department of Pathology, University of Turku, Kiinamyllynkatu 10, Turku 20520, Finland*

* *Address correspondence to jerosenh@abo.fi*

1. Particle characterizations

Transmission electron microscope (TEM) images were obtained by a JEM-1400 Plus (JEOL, Japan) instrument with 120 KV acceleration voltages in order to investigate the size, morphology and structure of the nanoparticles. Samples were dried on holey carbon-coated Cu grids.

Scanning electron microscopy (SEM) measurements were performed with a Zeiss DSM 962 microscope operated at an accelerating voltage of 10.0 kV, in order to investigate the morphological properties of the particles. Before scanning, the samples were coated with gold using a vacuum evaporator.

X-ray diffraction (XRD) measurements were performed using a Kratky compact small-angle system (Hecus Braun, Austria). The system is equipped with a position-sensitive detector consisting of 1024 channels of 55.5 μm width each. A Seifert ID-300 X-ray generator, operating at a maximum intensity of 50 kV and 40 mA provided the Cu KR radiation at λ=1.542 Å. Detailed experimental procedures were described earlier in our previously published article.[1]

Nitrogen sorption isotherms were measured with a ASAP2010 analyzer (Micromeritcs, USA). Before measurements, the samples were dried in a vacuum oven at room temperature for 24 h, and outgassed in the instrument at 60 °C for 24 h. The specific surface areas were calculated by the Brunauer-Emmett-Teller (BET) method[2] in a linear relative pressure range between 0.05 and 0.25. The pore size distributions were derived from the desorption branches of the isotherms by the NLDFT method[3] using NLDFT kernel file developed for silica exhibiting a cylindrical pore geometry. The totoal pore volumes were derived by the nitrogen sorption amount at the relative presure values where the capillary condensation of the primary mesopores finished, in order to exclude the contribution from the textual porosity of the nanoparticles.[4]

The FT–IR spectra were collected over the range of 4000–400 cm^{-1} on a Spectrum 100 infrared spectrophotometer (Perkin Elmer, USA) using KBr technique.

The zeta potentials of the samples were measured at a particle concentration of 0.25 mg mL^{-1} using dynamic light scattering (DLS) techniques by a Zetasizer Nano instrument (Malvern, UK) at 25°C.

Circular dichroism spectra were collected by a Jasco J-810 spectropolarimeter equipped with a Peltier temperature control device. The samples were located in 1.00 cm path length cuvettes, and measurements were carried out at 25°C. The spectral resolution is ±0.1 nm.

2. Quantitative determination of amino groups on the particle surface

In order to determine the surface density of accessible primary amines functionalized on MSN-NH2 particles, we employed a simple, fast and relatively harmless ninhydrin-based method. Ninhydrin (2, 2-dihydroxyindane-1, 3-Dione) destroys each primary amine to form a deep purple chromogen referred to as Ruhemann's Purple, which has a maximum absorption at about 580 nm. For particle samples, the colored product formed has the "sequestered" nitrogen atom of primary amine which was tested, and thus the nitrogen atom "immobilized" on the solid substrate is extracted by the ninhydrin molecule and becomes part of the colored species in the test solution.[5] The standard curve for the determination was made by using 3-aminopropyltrimethoxysilane (APTMS) as a standard material. Typically, 1 mL of ninhydrin solution in ethanol (3.5 mg mL^{-1}) was mixed with 0.2 mL ethanol solutions of APTMS with different amounts (1–20 μmol) in a vial. Subsequently, the vials were capped to avoid the loss of solvent and then transferred to a heating bath at 100 °C. After the solutions were stirred at the same temperature for 8 min, the vials were taken out and cooled to room temperature. Finally, the absorption values of the solutions were measured at 580 nm using a UV-Vis Spectrophotometer (NanoDrop 2000c, Thermo). The measurements for MSN-NH2 samples followed the same procedure except that 5 mg of the particles were used instead of APTMS and the sample solutions were sonicated for 10 min prior to the heat treatment.

3. Measurement of surface linker density by disulfide bond quantification

To determine the grafting density of disulfide-containing surface linkers after the modification steps, the as-prepared MSN-Linker-Cys particles were treated with DTT to get thiol groups terminated organic functions. The grafting density can be indicated by the surface thiol groups density measured by the Ellman's reagent method, in which thiol groups react with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to give UV detectable 5-thionitrobenzoic acid.[6] Typically, 0.5 mg MSN-Linker-Cys particles were treated with 2 mL DTT solution (10 mM in PB buffer) at room termperature for 5 h. Afterwards, the reduced product were centrifuged from the solution, and then washed with water three times to remove reduced species. Subsequently, the particles were redispersed in 0.2 mL PB buffer and 1 mL of DTNB solution (25 mM in PB buffer) was added. After 10 min of reaction, the particles were separated by centrifugation and the absorption value was measured at 412 nm using a UV-Vis Spectrophotometer (NanoDrop 2000c, Thermo). For the preparation of a standard curve, 2-aminoethanethiol was used instead of DTT treated particle samples. The same procedures were followed and 0.2 mL of 2-aminoethanethiol solutions at different concentrations (0.5–5 mM) were mixed with 1 mL of DTNB solution for UV measruements.

4. Measurement of disulfide cleavage/reduction ratio in response to reducing agents

To determine the changing of disulfide cleavage ratio of MSN-Linker-Cys in response to reducing agents, the as-prepared MSN-Linker-Cys particles were treated with 10 mM DTT/GSH in HEPES buffer (pH 7.4) for different time periods. Typically, 0.5 mg MSN-Linker-Cys particles were suspended in 2 mL DTT/GSH solution (10 mM in PB buffer) at room termperature. At different time intervals after incubation, the reduced product were centrifuged from the solution, and then washed with water three times to remove reduced species. Subsequently, the particles were redispersed in 0.2 mL PB buffer and 1 mL of DTNB solution (25 mM in PB buffer) was added. After 10 min of reaction, the particles were separated by centrifugation and the absorption value was measured at 412 nm using a UV-Vis Spectrophotometer (NanoDrop 2000c, Thermo). The thiol groups density of the reduced particles was then calculated, and the disulfide cleavage ratio was obtained by comparing with the total disulfide bond density as determined from the procedures above.

5. Supporting figures

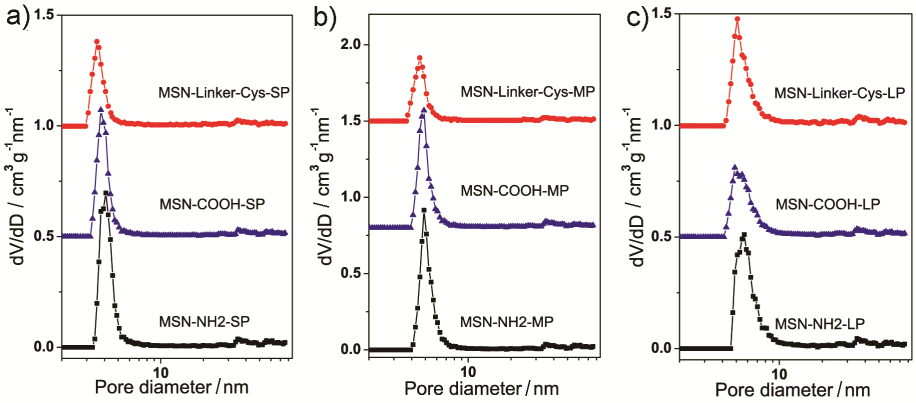

Fig. S1. Pore size distributions of the as-prepared amino-co-condensed MSNs with small (MSN-NH_2-SP, a), medium (MSN-NH_2-MP, b), and large (MSN-NH_2-LP, c) pore sizes and their corresponding products after succinylation (MSN-COOH) and conjugation with cystamine (MSN-Linker-Cys), derived from their desorption branch of the nitrogen sorption isotherms by the NLDFT method.

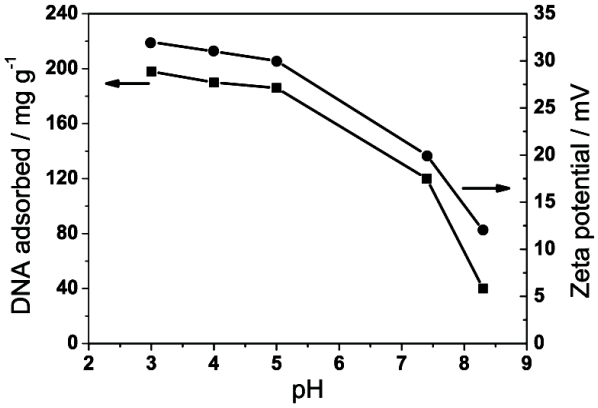

Fig. S2. Comparison of DNA adsorption amount and zeta potential for MSN-Linker-Cys-MP particles at varying pH values. An initial DNA concentration of 0.3 mg mL^{-1} and a particle concentration of 1 mg mL^{-1}, at which a saturated DNA loading was realized in the adsorption kinetics, were used for all experiments. A particle concentration of 0.25 mg mL^{-1} was used for zeta potential measurements. Solution pH values were tuned by using 0.1 M NaOH/HCl.

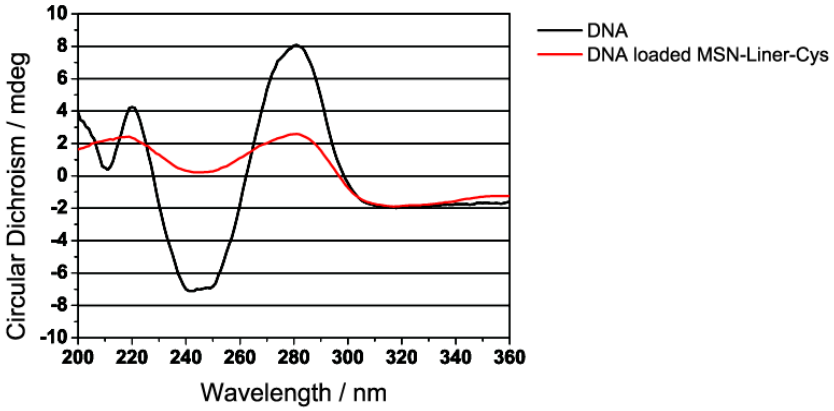

Fig. S3. Circular dichroism spectra of DNA and DNA loaded MSN-Linker-Cys in MES buffer (pH 5). DNA loaded MSN-Linker-Cys particles for the measurement was prepared from a saturated adsorption of DNA onto 0.3 mg MSN-Linker-Cys-MP particles. A DNA concentration of 0.05 mg mL^{-1} in the measurement solution was used for both free DNA and DNA loaded MSN-Linker-Cys-MP.

CD spectrum of free DNA in solution presents a positive peak at around 280 nm corresponding to base-pair packing and a negative peak at around 244 nm corresponding to helicity, indicating a typical B form of DNA. In comparison, the positive maxima of the DNA CD spectrum at 280 nm and at 220 nm decreased after they were loaded in MSN-Linker-Cys particles by adsorption. It can be also observed that the intensity of the negative band centered around 244 nm is enhanced and shifted to positive values.This anomalous CD spectrum is quite silimar to that of a +Ψ spectrum reported for DNA in systems where charge neutralization of DNA phosphate groups takes place at certain degrees.[7] However, the origin of the anomalous CD spectra might be attributed to condensed A-form DNA which was also found in systems of polycations and lipid bilayers.[8] In our MSN system, the conformation change and the condensation should be resulted from the adsorption of DNA onto MSN surfaces.

References

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