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Supporting Information for

Uniform dendrimer-like mesoporous silica nanoparticles as nano-adjuvant for foot-and-mouth disease virus-like particles vaccine

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Chemicals.

Tetraethoxysilane (TEOS, ≥99.0%), Cetyltrimethylammonium chloride (CTAC, ≥99.0%) were purchased from Sigma-Aldrich. Triethanolamine (TEA), chlorobeneze, 3-aminopropyltriethoxysilane (APTES, ≥80.0%), fluorescein isothiocyanate (FITC, ≥90.0%) were obtained from J&K Scientific Ltd. Ultrapure water (18 MΩ cm^{-1}) was generated with a Merck millipore Direct-Q-8UV integral pure and ultrapure water purification system and used throughout the experiment. All other chemicals were of A.R. grade and were used as received without further purification.

Characterizations and measurements.

Transmission electron microscopy (TEM) images were obtained on a Hitachi HT7700 microscope operated at 80 kV. For TEM measurements, the as-prepared samples were dispersed in ethanol and then dropped on the surface of carbon film on a copper grid. Image J software was used to calculate the number-average diameter and coefficient of variance by measuring about 150 particles in the TEM images. Scanning electron microscopy (SEM) images were taken on a JEOL JSM-6701F scanning electron microscope at 10 kV. N_2 adsorption-desorption isotherms were measured at 77 K using a Micromeritics ASAP 2020 system. Before measurements, the samples were degassed at 100 °C overnight on a vacuum line. The pore size distribution curve was derived from the adsorption branch of the isotherms using the Barrett-Joyner-Halanda (BJH) method. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface areas. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure (P/P_0) of 0.99. Hydrodynamic diameter and zeta potential was characterized by dynamic light scattering using the Malvern Zetasizer Nano-ZS at 25°C.

Synthesis of MSNs and SSNs.

The monodisperse MSNs were synthesized following the previous method by Xu et al. with some modifications[1]. 4 mL of 25 wt% CTAC solution and 1mL TEA solution (3.5 wt%) and 5 mL ultrapure water were mixed and stirred (400 rpm) at 60 °C for 1h, Then premixed 10 mL of oil phase (containing 1.25 mL TEOS and 8.75 mL chlorobenzene) was added to the bottom of water phase. The mixture was stirred at 60 °C under a stirring speed of 150 rpm for 12 h. The solid samples were centrifuged at 10000 rpm for 30 min and washed with ethanol for three times. The final product was obtained after calcination in air at 600 °C for 8 h at a heating rate of 3 °C min^{-1}.

The solid silica nanoparticles (SSNs) were synthesized by using a slightly modified well-known Stöber process[2]. Absolute ethanol (20 mL) was mixed with deionized water (12 mL) and ammonium hydroxide solution (3 mL, 28%), then 10 mL (20 v/v %) TEOS in ethanol was rapidly added to the water-ethanol-$NH_3 \cdot H_2O$ solution under vigorous stirring. After 6 h, the as-synthesized nanoparticles were separated by centrifugation, and washed with ethanol and deionized water. The final product was obtained by drying at 100°C overnight.

Amino functionalisation and FITC modification of MSNs

In a typical amino functionalisation process, 0.1 g of calcined MSNs was dispersed in 30 mL toluene, followed by addition of 0.1 mL APTES and the mixture was heated to 110 °C for 20 h. Afterwards, precipitates were isolated by centrifugation at 10000 rpm for 10 min and washed with absolute ethanol for three times, and then dried.

In FITC modification, 100 mg of amino modified HMSNs were suspended in 50 mL of absolute ethanol with sonication for 10 min. Afterwards, 10 mg of FITC was added and stirred the suspension for overnight in dark. The products were centrifuged and washed with ethanol extensively until the supernatant was colorless.

Cell culture and cell uptake.

Raw 264.7 cells were provided by the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS, China). Raw 264.7 cells were grown routinely in DMEM medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), and culture was at 37 °C in a 5% CO_2 humidity incubator.

For confocal imaging of the cellular uptake of the MSNs, Raw 264.7 cells growing in log phase were plated in 3.5 cm confocal petri dish and allowed to adhere overnight. The cells were subsequently treated with 50 μg mL^{-1} of MSN-FITC for 4 h. Then the cells rinsed with PBS three times and imaged immediately. Confocal fluorescence imaging was obtained using a Leica TCS SP8 laser scanning confocal microscope with a 60× oil-immersion objective lens.

Cell viability assay.

The cytotoxicity of MSNs was determined by MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega). Briefly, Raw 264.7 and BHK-21 cells were seeded into 96-well culture plates (1×10^4 cells per well), respectively. After cell attachment, the MSNs with different concentrations

(0, 25, 50, 100, 200, 400 μg mL^{-1}) were added into cells and incubated for 24 h at 37 °C under 5% CO_2. Then 10 μL MTS stock solution was added to each well for another 3 h under the same conditions. Finally, the absorbance at 490 nm was measured by a microplate reader (ELX800 UV, BIO-TEK, USA).

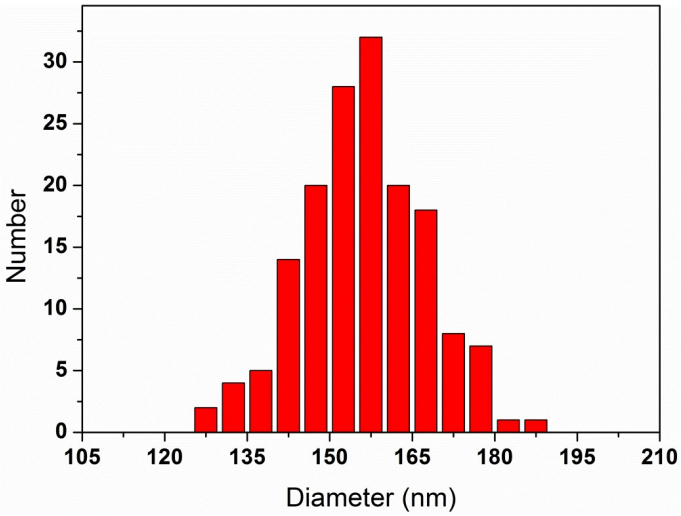

Fig. S1. The corresponding diameters of the MSNs by measuring about 150 particles in the TEM image. The number-average diameter and coefficient of variance was calculated by Image J software.

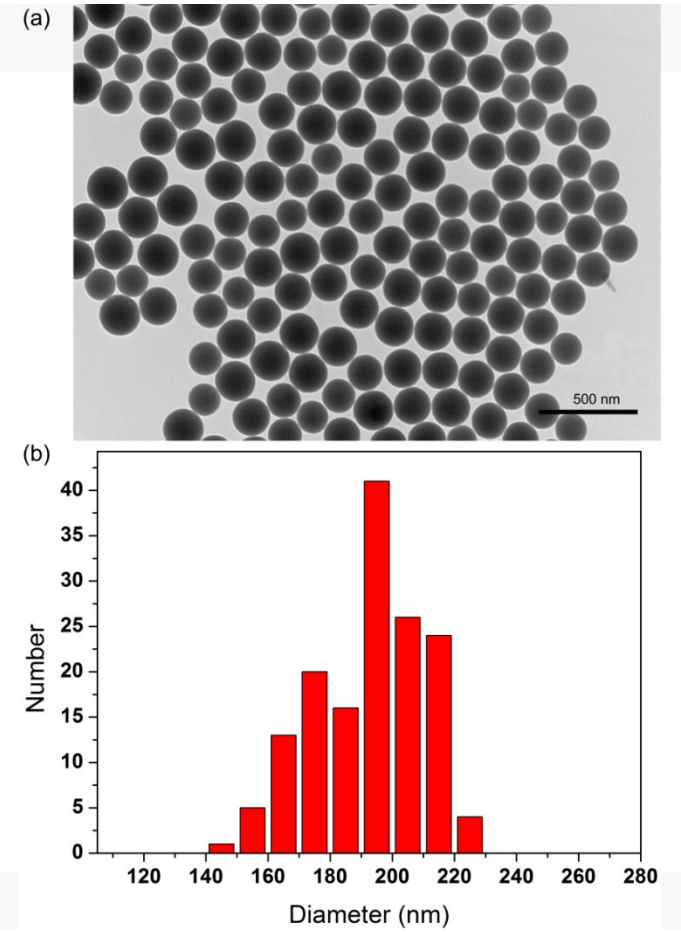

Fig. S2. The TEM images and corresponding diameters of the SSNs.

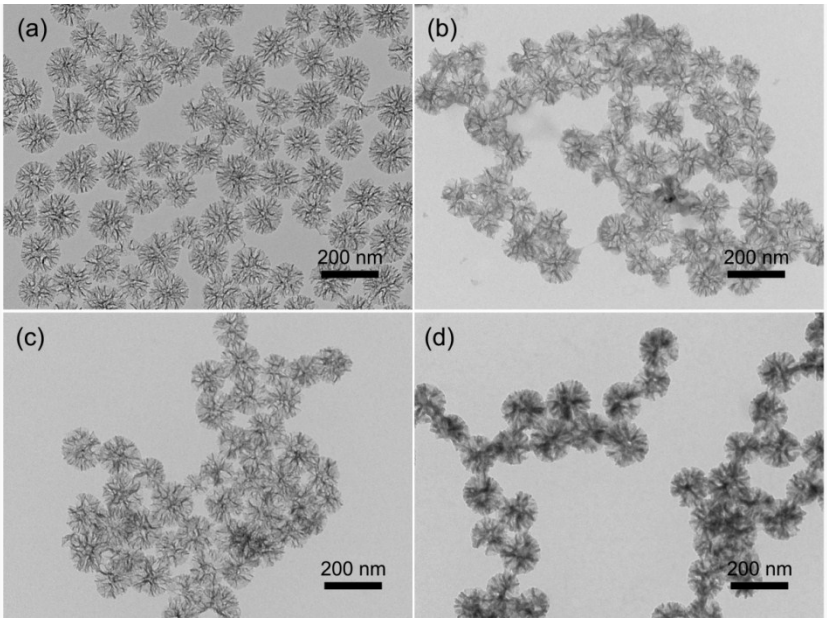

Fig. S3. TEM images of MSNs (a,c) and FMD VLPs / MSNs (b,d) before (a,b) and after (c,d) negatively stained with 2% (w/v) phosphotungstic acid for 1 min.

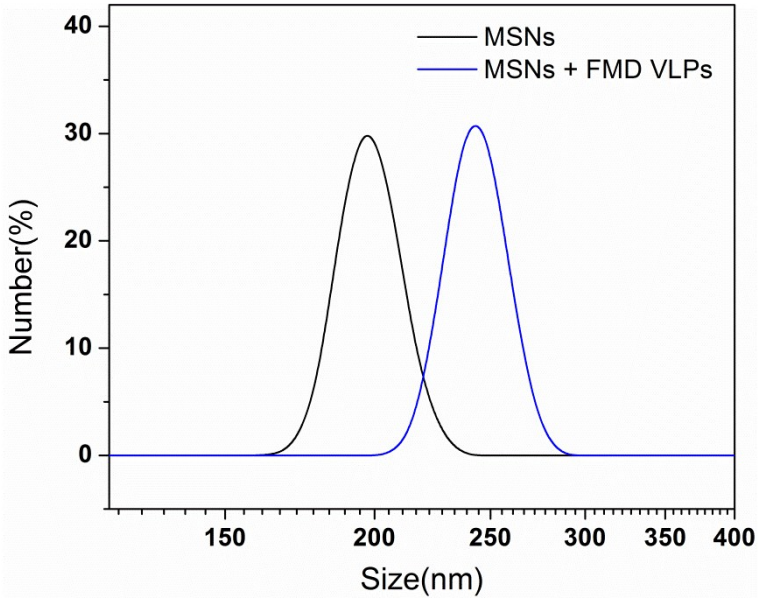

Fig. S4. Size distribution of MSNs after loaded FMD VLPs was detected by dynamic light scattering (DLS). The average particle size of MSNs, MSNs+VLPs are 197, 243 nm, in addition the values of PDI of MSNs, MSNs+VLPs are 0.294, 0.525, respectively.

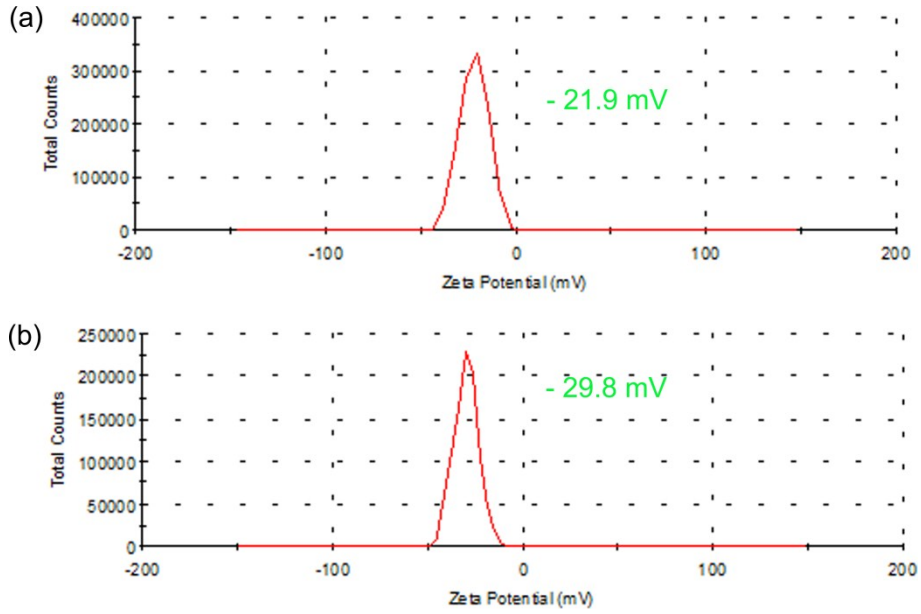

Fig. S5. Zeta potential distribution of MSN (a) and MSNs+VLPs (b) in PBS solution.

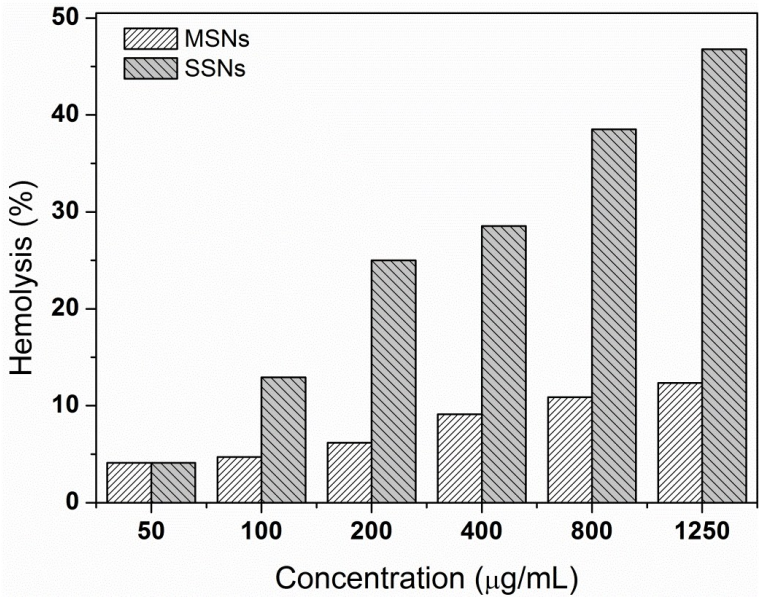

Fig. S6. The hemolytic ratio of the MSNs and SSNs. Guinea pigs RBCs incubated different concentrations (50 - 1250 μg mL^{-1}) of SSNs and MSNs, respectively. Deionized water was served as positive control and PBS was served as negative control.

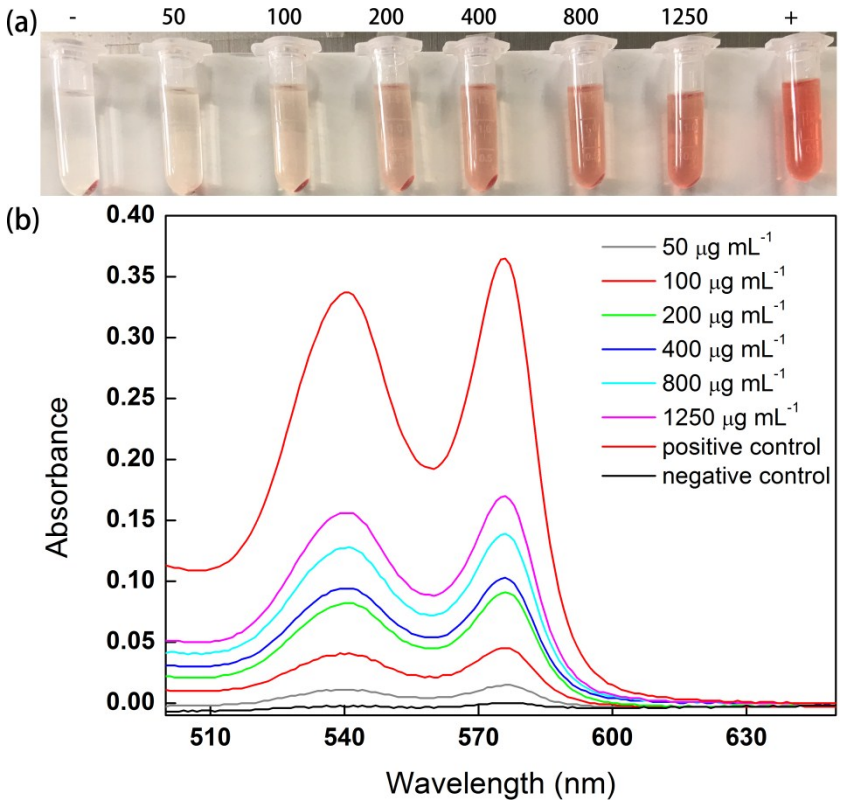

Fig. S7. Hemolysis assay of the SSNs at different concentrations. (a) Optical images and (b) UV–vis absorption spectroscopy of the hemoglobin released from guinea pigs RBCs. (-) and (+) controls are the RBCs in PBS and water, respectively.

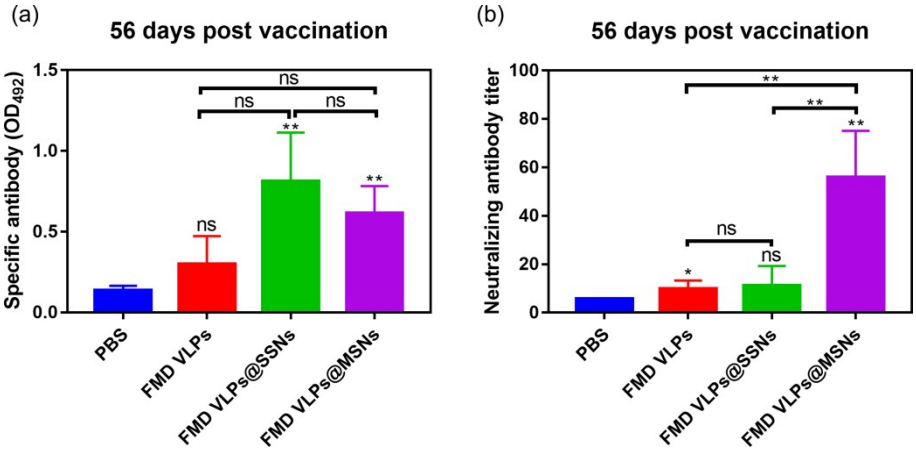

Fig. S8. The specific antibody levels and neutralizing antibody titer of control group and experimental group at 56 dpv. The statistical significance in difference were analysed using a Student's *T*-test: *P < 0.05, **P < 0.001.

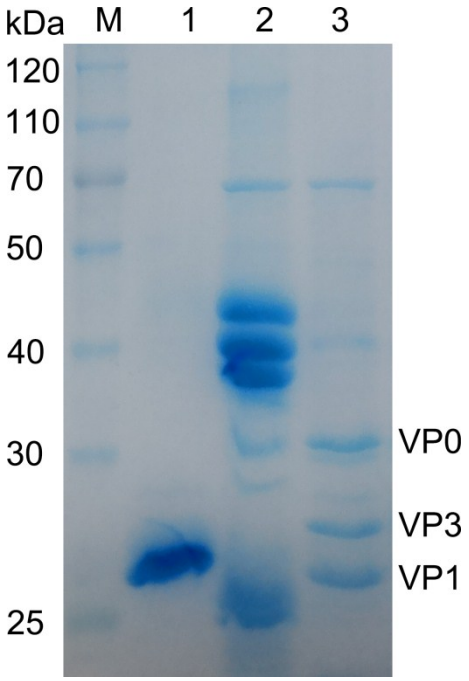

Fig. S9. SDS–PAGE stained with Coomassie Blue R250. M, protein marker; Lane 1, enzyme; Lane 2, purified SUMO-tagged FMD VLPs proteins; Lane 3, FMD VLPs proteins by enzyme cleavage.

Table S1. Experimental procedure for immunization of guinea pigs and the results of protection against FMDV challenge in guinea pigs.

Group	Injection dose 200 μL/Guinea pigs	No. of guinea pigs	No. of guinea pigs survived after 56 dpv	affected	unaffected	Rate of protection (%)
PBS	PBS	8	6	6	0	0
FMD VLPs	50 μg FMD VLPs	8	6	6	0	0
FMD VLPs@SSNs	300 μg SSNs+50 μg FMD VLPs	8	5	4	1	20
FMD VLPs@MSNs	500 μg MSN+50 μg FMD VLP	8	7	3	4	57

Rferences

1 C. Xu, M. Yu, O. Noonan, J. Zhang, H. Song, H. Zhang, C. Lei, Y. Niu, X. Huang, Y. Yang and C. Yu, *Small*, 2015, **11**, 5949-5955.
2 W. Stöber, A. Fink, *J Colloid Interface Sci*, 1968, **26**, 62-69