


## RESEARCH ARTICLE

# Hollow mesoporous silica nanoparticles as delivery vehicle of foot-and-mouth disease virus-like particles induce persistent immune responses in guinea pigs

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**Abstract**

Foot-and-mouth disease (FMD) is an acute and febrile infectious disease, which can cause great economic losses. Virus-like particles (VLPs) as an advantageous antigen can induce significant specific immune response. To improve immunity of VLPs, especially, make it induce persistent immune response, the hollow mesoporous silica nanoparticles (HMSNs) as a potential nano-adjuvant were synthesized and loaded the FMD virus (FMDV) VLPs. They were injected into guinea pigs and the specific immune response was detected. The results confirmed that HMSNs/VLPs can induce persistent humoral immunity with high-level antibody titer for more than three months. HMSNs also improve the T-lymphocyte proliferation and IFN- $\gamma$  induced by FMDV VLPs, and provides the ideal protection against FMDV challenge. These consequences indicated that HMSNs were good protein delivery vehicle and potential nano-adjuvant of vaccines.

**KEYWORDS**

foot-and-mouth disease, guinea pigs, hollow mesoporous silicananoparticles, immunization, virus-like particles

## 1 | INTRODUCTION

Foot-and-mouth disease (FMD) is a communicable disease of cloven-hoofed animals that results in major economic losses during disease outbreaks.<sup>1,2</sup> The economic losses reported for five previous FMD epidemics from 2000 to 2011 in the Republic of Korea ranged from \$23.6 million to \$1.9 billion.<sup>3</sup> The annual production losses and vaccination costs of FMD are estimated to be approximately 21 billion globally.<sup>4,5</sup> Thus, the prevention and control of FMD are crucial. Traditional vaccines for this disease have partly reduced the frequency of FMD virus (FMDV) epidemics, but limitations and problems impede proper disease elimination.

With the development of genetic engineering technology, various new vaccines, such as synthetic peptide vaccine and recombinant vaccine, have been introduced. The lack of viral genetic material is the common feature of new vaccines, which can then be used to purify the pathogens. Virus-like particles (VLPs), as a representative of new vaccines, are composed of one or more viral structural proteins and lack viral genetic material. Following the emergence of HBV-VLPs, an increasing number of VLPs vaccines, such as HPV-VLPs and HEV-VLPs vaccines, have been successively approved by the FDA. Thus, VLPs are increasingly considered as the most promising candidate vaccines.<sup>6-8</sup> However, recombinant vaccines possess some defects, such as antigenic instability and short immunization cycle. Hence, developing

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safer and more efficient adjuvants than those currently available can address the shortcomings of new vaccines.<sup>9,10</sup>

Nanoparticles can be designed for targeted antigen delivery to immune cells and stimulate the immune response to promote antigen immunogenicity. Thus, they are usually applied for developing nanovaccines and drug carriers. Nanoadjuvants are fabricated from nanosized particle materials. Nanoadjuvants exhibit strong adsorptive abilities, good slow-release function, and effective targeting and thermal stability.<sup>11</sup> At present, numerous nanoadjuvants have been developed. These materials include mesoporous silica nanoparticles, liposomes, calcium phosphate, aluminum hydroxide, polylactic-co-glycolic acid, and polylactic acid.<sup>12-14</sup> Hollow mesoporous silica nanoparticles (HMSNs), as drug delivery vehicles, possess relatively large surface areas, adjustable pore sizes, high surface paintability, controlled release capability, and good thermal stability. These properties are suitable for an ideal gene/drug delivery vehicle.<sup>15-17</sup>

In this study, specific and persistent immune responses were achieved by adsorbing FMDV VLPs onto HMSNs prepared by a sol-gel/emulsion (oil-in-water/ethanol) method. Then, guinea pigs were immunized with HMSNs/VLPs, and the resultant immune responses were evaluated. Compared with the immune responses of the animals immunized with VLPs/Freund's complete adjuvant, those of the animals immunized with HMSNs/VLPs were stronger and more persistent. The results confirmed that HMSNs should be further studied as protein delivery vehicles to develop new-generation adjuvants.

## 2 | MATERIALS AND METHODS

### 2.1 | Synthesis and characterization of HMSNs

The HMSNs were synthesized using a sol-gel/emulsion (oil-in-water/ethanol) method.<sup>15,18</sup> First, 42 mL of ethanol, 79.5 mL of H<sub>2</sub>O, 1.5 mL of tetraethoxysilane (Aladdin, Shanghai, China), and 0.24 g of hexadecyltrimethylammonium bromide (YW, Wuhan, China) were mixed and stirred continuously. Then, 1.5 mL of ammonium solution (25 wt% NH<sub>3</sub> in water) was added, and the mixture was shaken or stirred (10 000 rpm) for 6 hours and stored for 24 hours at room temperature. Afterward, the HMSNs were washed several times with deionized water and ethanol and centrifuged at 8000 to 10 000 rpm for 15 to 20 minutes. The final product was calcined in air at 200°C for 6 hours and then at 600°C for 6 hours.

The morphological characteristics of the HMSNs were detected by dynamic light scattering (DLS; Malvern Instruments Ltd, Worcestershire, UK) and transmission electron microscopy (TEM). For DLS, the incident angle was 90°, and the incident wavelength of the laser was 633 nm. Each sample was measured three times at room temperature, and the average particle diameter was expressed as mean ± standard deviation (SD). TEM samples were prepared by dipping a drop of the solution onto carbon-film-coated copper grids and staining with phosphotungstic acid. Then, a JEOL electron microscope (Tokyo, Japan) was used to observe the samples at an acceleration voltage of 100 kV. The Barrett-Joyner-Halenda (BJH) method was applied to detect the total pore volume and

pore diameter of HMSNs. The surface area of HMSNs was obtained using the Brunauer-Emmett-Teller (BET) method.<sup>19</sup>

### 2.2 | Assembly and detection of VLPs

The FMDV VLPs were produced and detected as described previously.<sup>8,20</sup> The SUMO (Smt3) gene and FMDV capsid proteins VP0, VP1, and VP3 genes were subcloned into pET-28a, and then the recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3). The transformed *E. coli* was cultivated at 37°C in Lysogeny broth medium and induced by isopropyl β-D-1-thiogalactopyranoside and then the fusion proteins were eluted with Buffer A (500 mM NaCl, 5% glycerinum, 200 mM Tris-HCl, 1% Triton-X100, and 500 mM imidazole, pH 8.0). The SUMO-tag of fusion proteins was cleaved by SUMO protease and the FMDV capsid proteins VP0, VP1, and VP3 can self-assemble into VLPs in the buffer B (400 mM Tris-HCl, 250 mM NaCl, 1 mM CaCl<sub>2</sub>, and 1 mM dithiothreitol, pH 8.0) for 24 hours at 4°C. Then, the samples were centrifuged by sucrose gradient ultracentrifugation method, and the fraction was used for dynamic light scattering instrument (DLS) and transmission electron microscope (TEM).

### 2.3 | VLP adsorption of HMSNs

VLPs were adsorbed to HMSNs as described previously.<sup>17</sup> HMSNs (0.5 mg/mL) were sonicated for 15 minutes and mixed with different concentrations of FMDV VLPs (125, 150, 175, 200, 225, and 250 μg/mL) at 4°C. After 4 and 16 hours, the solutions were centrifuged at 10 000 rpm for 5 minutes, and the proteins of the supernatant and sediments were measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, SDS-PAGE results were analyzed by the ImageJ software (National Institutes of Health, Bethesda, MD). The amount of proteins adsorbed by the HMSNs was estimated from the amount of proteins in the sediment.

### 2.4 | Size of the HMSNs/VLPs mixture

The size of the HMSNs/VLPs mixture was detected by DLS (Malvern Instruments Ltd). HMSNs (2 mg) was dispersed into PBS (1 mL) to obtain the final concentration 2 mg/mL. Then 0.25 mL HMSNs(2 mg/mL) was mixed with 0.25 mL VLPs (800 μg/mL).

### 2.5 | Release kinetics of HMSNs

HMSNs/VLPs were suspended in PBS (pH 7.0), and the solution was maintained at 37°C. At different time points, samples were obtained for centrifugation (10 000 rpm, 5 minutes). The amount of protein released by HMSNs was detected with a Bradford protein assay kit (Beyotime Biotechnology, Shanghai, China).

### 2.6 | Animal immunization and challenge protocols

Guinea pigs (300-400 g) were purchased from the Lanzhou Veterinary Research Institute and raised in isolation cages. These animals were then cared for in accordance with the regulations of the Animal Research Ethics Board of Lanzhou Veterinary Research Institute,

CAAS, China. The guinea pigs were randomized into six groups and immunized as described in Table 1. The animals were each intramuscularly immunized in the tibialis cranialis muscle of both rear legs, and serum samples were gathered every two weeks from the heart of each guinea pig.<sup>21,22</sup> Preexperiment confirmed that the challenge viral dose and 50% infectious dose for the guinea pigs were 0.2 and 10–6.5/0.2 mL, respectively. Then, 0.2 mL aliquots of homologous live virus solution diluted from 10 to 6.5 were subcutaneously and intradermally injected into the interdigital skin of each left back leg of guinea pigs at 14 weeks. The guinea pigs were observed for seven days continuously.<sup>23,24</sup> The lesion appearing only on the left back leg was considered to as an indicator of partial protection, on both back soles as an indicator of no protection and no lesion on the back as an indicator of total protection.

## 2.7 | Enzyme-linked immunosorbent assay

Serum samples collected weekly from the guinea pigs were evaluated by enzyme-linked immunosorbent assay (ELISA). Initially, a 96-well ELISA microplate (Corning Incorporated, Corning, ME) was coated with 100  $\mu$ L of FMDV 146S antigen in 0.05 M of NaHCO<sub>3</sub> coating buffer (pH 9.6) and incubated at 4°C overnight. After washing with phosphate buffered saline with Tween-20 (PBST) three times, the microplate was blocked with 120  $\mu$ L of PBST containing 1% bovine serum albumin for 1 hour at 37°C. Then, 100  $\mu$ L of diluted serum (1:32) with PBST was incubated for 1 hour at 37°C, washed three times with PBST, and then patted dry. Afterward, 100  $\mu$ L of HRP-labeled rabbit anti-guinea pig IgG (Sigma-Aldrich, St. Louis, MO) was incubated as described above, followed by incubation with 100  $\mu$ L of *O*-phenylenediamine/hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 15 minutes at 37°C. The color reaction was stopped with 100  $\mu$ L of 2 N H<sub>2</sub>SO<sub>4</sub>, and the optical density (OD) value was read using an ELISA reader (Bio-Rad, CA, USA) at 490 nm.

## 2.8 | T-lymphocyte proliferation assay

Spleens were collected from immunized guinea pigs, and then spleen cells were resuspended in RPMI 1640 medium. Lymphocyte separation medium (Sangon, China) was added, and the mixture was centrifuged at 1000 to 2000 rpm for 5 minutes. A straw was used to carefully extract the lymphocyte layer into the centrifuge tube, and then the lymphocytes were washed three times with RPMI 1640 medium. T-lymphocytes in 96-well plates (5  $\times$  10<sup>6</sup> cells per well) were added to 100  $\mu$ L of 50  $\mu$ g/mL ConA, 150  $\mu$ g/mL ConA, and RPMI 1640 and incubated at 37°C under 5% CO<sub>2</sub> for 68 hours. Next, 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT)/PMS mixture was added to each well, and the mixture was incubated at 37°C under 5% CO<sub>2</sub> for 4 hours. The OD value was measured at 490 nm, and the stimulus index was calculated to reveal the level of T-lymphocyte proliferation.

## 2.9 | Detection of IFN- $\gamma$

The guinea pig IFN- $\gamma$  levels in serum samples were determined by using a guinea pig IFN- $\gamma$  ELISA kit (Ziker, Shenzhen, China) in

accordance with the manufacturer's instructions. Initially, BSA was diluted to 9, 6, 3, 1.5, and 0.75 mmol/L and added to an ELISA plate (50  $\mu$ L per well). Then, serum samples were diluted (1:5) and added to each well (50  $\mu$ L). Measurements were performed in duplicate, and the OD450 was attained using an ELISA reader.

## 2.10 | Statistical analysis

Results among groups were presented as means and SDs;  $P < 0.05$  was regarded as statistically significant. Statistical analysis was performed with GraphPad Prism (Version 5.0). Kruskal-Wallis  $H$  test was then used to evaluate specific and neutral antibody immune responses. Significant differences in T-lymphocyte immune responses among groups were determined using the Student  $t$  test.

## 3 | RESULTS

### 3.1 | Characterization of HMSNs

HMSNs were uniformly spherical particles (Figure 1A and 1B), with an average spherical diameter of approximately 400 nm (Figure 3C). The HMSN surface area was 1035.73 m<sup>2</sup>/g, which was obtained using the BET method (Figure 1C). The pore volume and aperture size of spherical particles were 0.57 cm<sup>3</sup>/g and 22.07 Å (approximately 2.2 nm), respectively, which were obtained by the BJH method (Figure 1D). The diameters of the HMSNs/VLPs significantly increased relative to those of the HMSN control. The results also suggest that to some extent the HMSN surface can adsorb a large amount of VLPs.

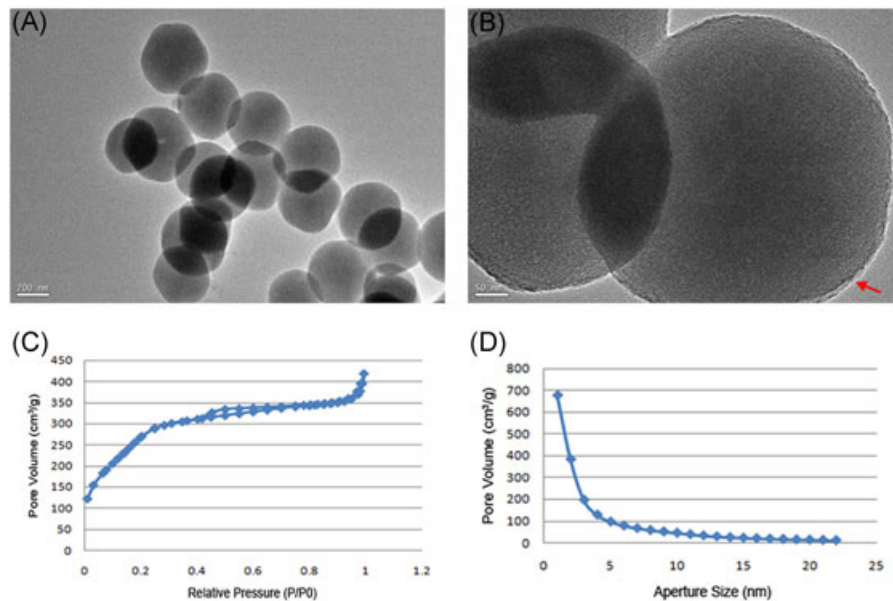
### 3.2 | Determination of FMDV VLPs

After the assembly of FMDV VLPs in the assembly buffer, the product was filtered, and the product's molecular size was determined by DLS. The products were then negatively stained and visualized through TEM. The results indicated that the FMDV VLPs were uniform hollow particles, and the average VLP diameter was approximately 20 nm (Figure 2).

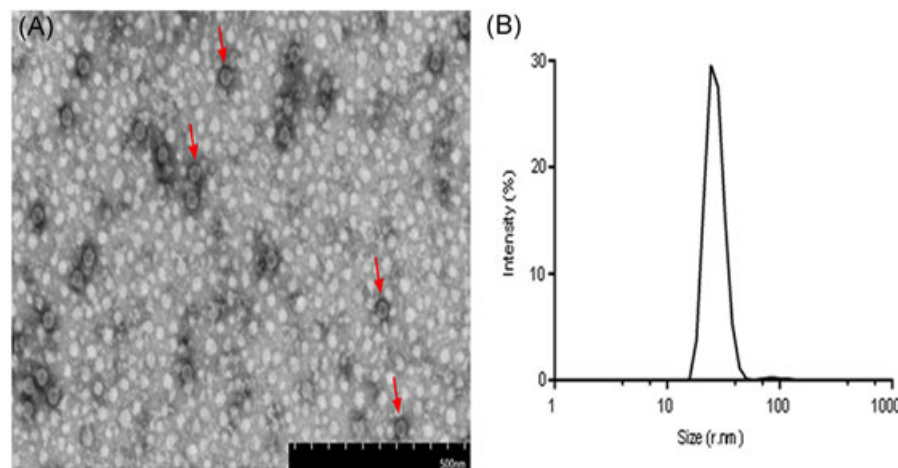
**TABLE 1** Immunization groups in guinea pigs study

Treatment group	Group description	Injected dose
1	PBS	PBS (0.25 mL)
2	HMSN	HMSN (0.5 mg)
3	VLP + Freund's complete adjuvant	VLP (100 $\mu$ g)
4	HMSN/VLP1	HMSN/VLP (0.5 mg/100 $\mu$ g)
5	HMSN/VLP2	HMSN/VLP (0.5 mg/200 $\mu$ g)
6	Unimmunized control	N/A

Abbreviations: HMSNs, hollow mesoporous silica nanoparticles; PBS, phosphate buffered saline; VLP, virus-like particles. All doses were injected intramuscularly.



**FIGURE 1** Morphologies and surface structure of HMSNs. A, B, HMSN morphologies were observed by TEM. C, The HSMN surface areas were obtained by the BET method. D, Pore volume and aperture size of the HMSNs were acquired by the BJH method. BET, Brunauer-Emmett-Teller; BJH, Barrett-Joyner-Halenda; HMSN, hollow mesoporous silica nanoparticle; TEM, transmission electron microscopy



**FIGURE 2** Characterization and size of the FMDV VLPs. A, The VLPs were distributed evenly in PBS and characterization was detected by TEM. B, The size of VLPs was tested by DLS. DLS, dynamic light scattering; FMDV, foot-and-mouth disease virus; PBS, phosphate buffered saline; TEM, transmission electron microscopy; VLP, virus-like particles

### 3.3 | Adsorption and release kinetics of HMSNs/VLPs

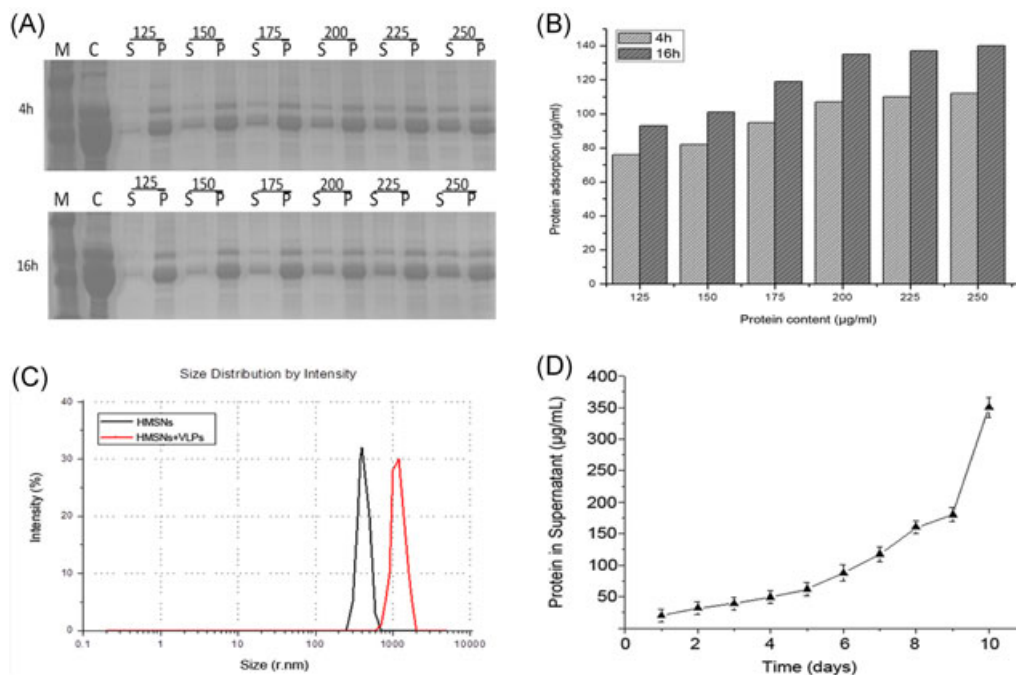
The quantity of VLPs that adsorbed onto the HMSN surfaces was measured by detecting the FMDV VLPs from the supernatant and sediment. With the increased VLPs proportion, the amount of VLPs remaining in the solution also increased at 4°C for 4 and 16 hours. But, compared with 4 hours, the amount of VLPs remaining in the solution was lower and the amount of VLPs loaded on HMSNs in the precipitation was higher for 16 hours, which means that HMSNs can load more VLPs at 4°C for 16 hours than 4 hours. Given the results, 0.5 mg/200 µg of HMSNs/VLPs was selected as the optimal proportion at 4°C incubation for 16 hours (Figure 3A and 3B). DLS results showed that the size of HMSNs/VLPs was approximately 1 µm, which indicates that numerous VLPs were adsorbed on the HMSNs (Figure 3C). Based on the results of VLPs released from HMSNs/VLPs, approximately 50% of the VLPs were released at day 9. The

HMSNs also exhibited a good ability to release proteins slowly (Figure 3D).

### 3.4 | Specific antibody in guinea pigs

To evaluate the adjuvant effect of HMSNs, the specific antibody responses were quantified by ELISA. The level of specific antibodies of guinea pigs immunized with VLPs/Freund's complete adjuvant greatly increased at the fourth week and reached the peak at the 10th week and then decreased significantly. The group immunized with HMSNs/VLPs showed the same trend as that of the former, but the antibody titers of the group vaccinated with the HMSNs/VLPs (200 µg) were significantly higher than those of the former at the 14th week. Similarly, the antibody titers of the group vaccinated with HMSNs/VLPs (200 µg) were higher than those of the group vaccinated with the HMSNs/VLPs (100 µg). Overall, the findings





**FIGURE 3** Adsorption and release kinetics results of HMSNs for FMDV VLPs. A, The SDS-PAGE of HMSNs for FMDV VLPs at different rates and times. B, The result of the ImageJ software analyzing SDS-PAGE. C, The average diameters of the HMSNs and HMSNs/VLPs were detected by DLS. D, Slow-release kinetics of the FMDV VLPs from HMSNs in PBS (pH 7.0) at 37°C. C, control; DLS, dynamic light scattering; FMDV, foot-and-mouth disease virus; HMSNs, hollow mesoporous silica nanoparticles; M, marker; P, precipitate; PBS, phosphate buffered saline; S, supernatant; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

indicated that the HMSNs exert a better slow-release effect than the Freund's complete adjuvant (Figure 4).

### 3.5 | T-lymphocyte proliferation assay

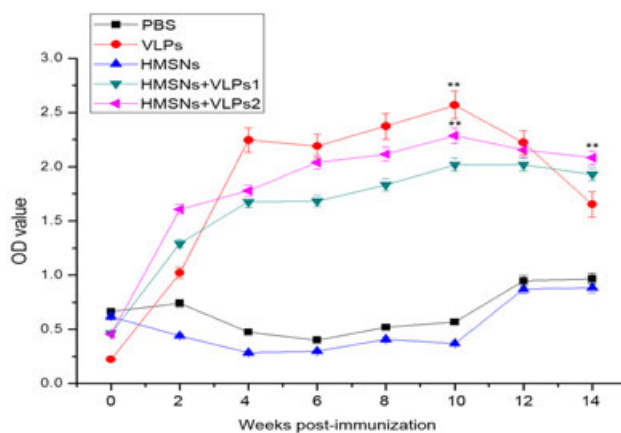
MTT was used to measure T-lymphocyte proliferation. The results showed that the proliferative capacity of the group immunized with HMSNs/VLPs at the seventh week was significant ( $P < 0.05$ ) than those in the PBS or HMSN control group. T-lymphocyte proliferation was more evident in the group immunized with HMSNs/VLPs than in the group immunized with VLPs/Freund's complete adjuvant. The results demonstrated that the HMSN as carriers and adjuvant can induce the body to produce humoral and cellular immune responses, similar to that in traditional adjuvants (Figure 5).

### 3.6 | Guinea pig serum IFN- $\gamma$ levels

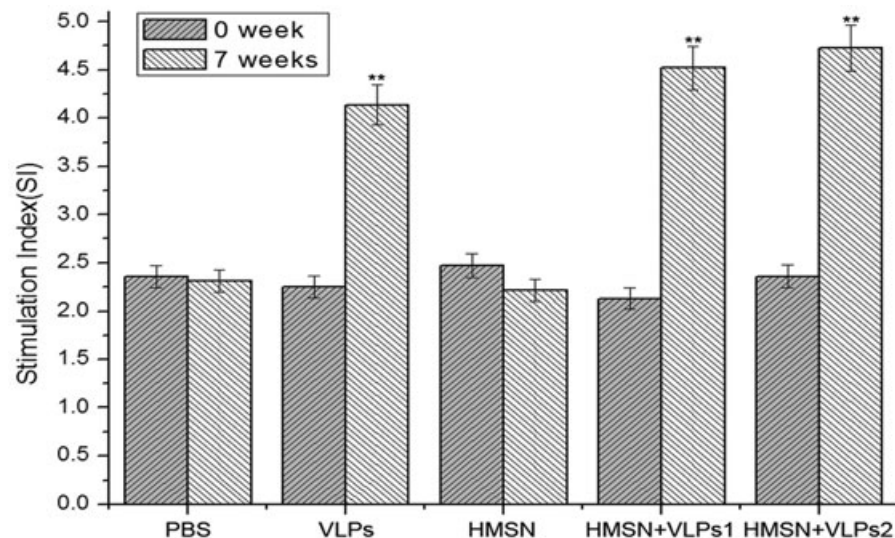
A guinea pig IFN- $\gamma$  ELISA kit was adopted to test the levels of IFN- $\gamma$  induced by VLPs. The results indicated that the levels of IFN- $\gamma$  were increased significantly in the guinea pigs vaccinated with HMSNs/VLPs at the second ( $P < 0.05$ ) to fourth ( $P < 0.05$ ) weeks compared with PBS or HMSN control group. The IFN- $\gamma$  levels in the group immunized with VLPs/Freund's complete adjuvant reached the maximum value at the second week ( $P < 0.05$ ). However, the IFN- $\gamma$  levels in the group immunized with HMSNs/VLPs (200  $\mu$ g) gradually increased and exceeded those of the group immunized with VLPs from the second to fourth weeks (Figure 6).

### 3.7 | Protective test against FMDV in guinea pigs

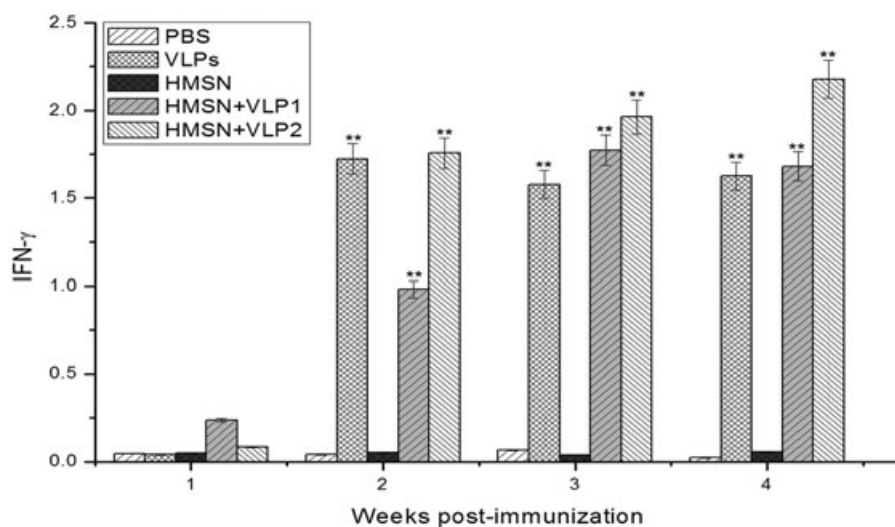
Table 2 shows that all the guinea pigs were infected in the groups immunized with PBS, HMSN, and blank control. Compared with the group immunized with HMSNs/VLPs (100  $\mu$ g), the protection rates of groups immunized with HMSNs/VLPs (200  $\mu$ g) and VLPs/Freund's complete adjuvant were higher (80%). This result indicated that the protection rate was positively correlated with the antigen immune dose.



**FIGURE 4** FMDV VLP specific serum antibody titer. Guinea pigs were immunized with PBS, VLPs, HMSNs, and HMSNs/VLPs. Serum samples were gathered every two weeks from each guinea pig heart. The bar shows the average values from five guinea pigs. FMDV, foot-and-mouth disease virus; OD, optical density; PBS, phosphate buffered saline; VLP, virus-like particles



**FIGURE 5** T-lymphocyte proliferation assay. Spleen cells were collected at 0 and seven weeks after immunization. Results represent the means  $\pm$  SDs. HMSNs, hollow mesoporous silica nanoparticles; PBS, phosphate buffered saline; VLP, virus-like particles



**FIGURE 6** IFN- $\gamma$  was detected from immunized guinea pig serum. Guinea pig serum was collected at one, two, three, and four weeks after immunization. Results represent the means  $\pm$  SDs. HMSNs, hollow mesoporous silica nanoparticles; IFN- $\gamma$ , interferon- $\gamma$ ; PBS, phosphate buffered saline; VLPs, virus-like particles

**TABLE 2** Statistical analysis of the incidence of guinea pigs

Days	Treatment Group					
	PBS	HMSN	Freund <sup>a</sup>	VLP1 <sup>b</sup>	VLP2 <sup>c</sup>	Control <sup>d</sup>
1	-----	-----	-----	-----	-----	-----
2	-----	-----	-----	-----	-----	-----
3	+++++	+++++	-----	+++--	-----	+++++
4	+++++	+++++	+----	+++--	+----	+++++
5	+++++	+++++	+----	+++--	+----	+++++
6	+++++	+++++	+----	+++--	+----	+++++
7	+++++	+++++	+----	+++--	+----	+++++

Abbreviations: HMSNs, hollow mesoporous silica nanoparticles; PBS, phosphate buffered saline; VLPs, virus-like particles.

Thirty guinea pigs were randomized into six groups of five animals each. Each “-” or “+” symbol means one of guinea pig; “-” is a guinea pig that no lesion on the back legs; “+” is a guinea pig that the lesion appearing only on the left back sole or both back soles.

Protection rates (%) = number of total protected guinea pigs per group/total number of guinea pigs per group  $\times$  100%.

<sup>a</sup>VLPs/Freund's adjuvant.

<sup>b</sup>HMSNs/VLPs (100  $\mu$ g).

<sup>c</sup>HMSNs/VLPs (200  $\mu$ g).

<sup>d</sup>Unimmunized blank control.

## 4 | DISCUSSION

Compared with the inactivated vaccines, VLPs are secure candidate vaccines that possess the following characteristics. First, these particles can be used to purify the pathogen because the material does not contain any genome. Second, fabricating VLPs is simple, cheap, and suitable for veterinary vaccine production. Previous works have confirmed that VLPs, as immunological antigens, can induce strong immune responses with the aid of traditional adjuvants.<sup>25-29</sup> In the present study, HMSNs, as new adjuvants or delivery vehicles of FMDV VLP vaccine, were further evaluated in comparison with traditional adjuvants.

In recent years, nanomaterials have been extensively applied in the biological field. They exhibit strong adsorption properties, good slow-release function, effective targeting, and thermal stability, which have attracted the attention of researchers. Nanoadjuvants have been used as protein delivery vehicles to induce efficient and persistent immune responses.<sup>12,14,30</sup> HMSNs, as new nanomaterials, were synthesized by using the sol-gel/emulsion (oil-in-water/ethanol) method. They are uniformly spherical particles with good dispersion in solution. They also possess large surface areas, which are beneficial for protein adsorption. Through the BJH method, the pore and aperture sizes of the HMSNs were found to be 0.57 cm<sup>3</sup>/g and 22.07 Å (approximately 2.2 nm), respectively. These unique structural characteristics determined the HMSNs' strong adsorption capacity.<sup>31,32</sup> Adsorption kinetic experiments indicated that the adsorption capacity of the HMSNs reached up to 300 µg/mg (VLPs/HMSNs). This result may be dependent on the large surface area of HMSNs and the high-density charge distribution on the VLPs surface. Thus, the HMSNs dosage is greatly decreased as vaccine adjuvant.

The immune response level can be reflected by specific antibody titers.<sup>33</sup> Release kinetics assay showed that VLPs can be released in a sustained manner in the solution; the continuous release of VLPs can induce a persistent immune response. Therefore, the specific antibody titers increased significantly. Statistical results of ELISA showed that the specific antibody titers of the group vaccinated with HMSNs/VLPs were significantly higher than those of the group vaccinated with VLPs/Freund's complete adjuvant at the 14th week. The antibody titers of the group vaccinated with the HMSNs/VLPs (200 µg) were also higher than those of the group vaccinated with HMSNs/VLPs (100 µg). These results indicated that HMSNs can induce a more persistent immune response than Freund's complete adjuvant. To a large extent, the sustained stimulus responses were achieved mainly depending on the low release effect of the HMSNs. In this case, the immune dose could be reduced significantly. T-cell activation and cytokines also play key roles in immune protection.<sup>20</sup> IFN-γ is a key cytokine related to the activation of macrophages and T-lymphocytes; it can efficiently inhibit early FMDV infection and replication.<sup>8</sup> IFN-γ is produced only by activated T cells and natural killer cells, which are the symbolic cytokines of Th1 cells.<sup>34,35</sup> In this study, the levels of T-lymphocyte and IFN-γ from the groups immunized with HMSNs/VLPs were significantly higher than those of the other groups. Protective tests against FMDV showed that the immune response induced by HMSNs/VLPs provided a favorable immune

protection rate. All results confirmed that the HMSNs, as carriers and adjuvant, can induce humoral and cellular immune responses.

## 5 | CONCLUSIONS

In conclusion, HMSNs can be used as delivery vehicle and adjuvant in VLP vaccine development. These particles not only help the antigen to be engulfed by phagocytosis but also retard antigen release to achieve a sustained immune response. However, although the amount of HMSNs used as vaccine adjuvant was significantly reduced, the biological residue requires further study.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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