Supporting Information

Antigen uptake and immunoadjuvant activity of the pathogen-mimetic hollow silica particles conjugating with β-glucan

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Materials and Methods

1.1. Materials

Styrene (analytical grade), polyvinylpyrrolidone (PVP, K29-K32), 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AIBA, 97 %), *N,N'*-Carbonyldiimidazole (CDI) were purchased from Aladdin (Shanghai, China). 3-Aminopropyltriethoxysilane (APTES, 99 %), ovalbumin (OVA, grade V), bovine serum albumin (BSA), lipopolysaccharides (LPS) from Escherichia coli O111:B4, Tween 20, fluorescein isothiocyanate isomer I (FITC), 3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyl tetrazolium bromide (MTT) and 5,5'-tetramethylbenzidine (TMB) were obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA). β-Glucan (derived from yeast Succhuromyces cerevisiae) was supplied by Zhuhai TXY Biotech Holding Co., Ltd. (Zhuhai, China). Complete Freund's adjuvant, Incomplete Freund's adjuvant and Alhydrogel® adjuvant 2 % were purchased from InvivoGen (San Diego, CA, USA). DQ™ ovalbumin (DQ-OVA), Hoechst 33342 and LysoTracker™ Red DND-99 were purchased from Thermo Fisher Scientific (Waltham, MA USA). Goat anti-mouse IgG/IgG1/IgG2a/IgG2b/IgG3/IgE-HRP conjugates were purchased from Southern Biotech (Birmingham, AL, USA). PE-labeled anti-mouse MHC Class I, PE-labeled anti-mouse MHC Class II, PE-labeled anti-mouse CD40, PE-labeled anti-mouse CD80, PE-labeled anti-mouse CD86, anti-mouse CD282 (TLR2) functional grade purified and the corresponding isotypes were purchased from eBioscience (San Diego, CA, USA). Mouse dectin-1/CLEC7A antibody was purchased from R&D Systems (Minneapolis, MN, USA). Anti-mouse CD16/CD32 was purchased from BD Biosciences (San Diego, CA, USA). Ammonium hydroxide solution (NH_4OH, 25-30 %), tetraethylorthosilicate (TEOS, analytical grade) and all other chemicals or solvents used were analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

1.2. Preparation of PS (polystyrene) Particles and Biological Templates

The PS particles were synthesized via a modified emulsifier-free emulsion polymerization method [S1]. Briefly, 10 g of styrene, 6 g of polyvinylpyrrolidone

(PVP) and 90 g of H_2O were added into a 250-ml three-neck flask. The mixture was stirred under nitrogen atmosphere at 300 rpm for 30 min, then the temperature of the oil bath was gradually increased to 60 ºC and 10 ml of 2,2'-azobis (2-methylpropionamidine) dihydrochloride solution (AIBA, 0.026 g/ml) was added dropwise to the mixture for at least 30 min. Lastly, the temperature increased to 70 ºC and the mixture was stirred under nitrogen atmosphere at 300 rpm for 24 h.

Escherichia coli and *Staphylococcus aureus* cells were used as biological templates. The bacteria were cultured in LB (Luria–Bertani) medium at 37 ºC under shaking. Cells were harvested by centrifugation (4000 rpm, 10 min) during their late exponential growth phase and washed three times with PBS (phosphate buffer solution). The washed cells were fixed for 12 h with 4 % (v/v) glutaraldehyde solution in PBS. Then, the cells were washed for five times with PBS and resuspend in deionized water.

1.3. Preparation of HSPs

PS particles emulsion (10 ml), anhydrous ethanol (25 ml) and deionized water (10 ml) were added to a flask. The mixture was treated with ultrasonication for 10 min, and then magnetically stirred at 70 ºC. 5 ml of tetraethylorthosilicate (TEOS, 0.2 g/ml) ethanol solution was added dropwise to the mixture for at least 30 min. Then 3 ml of ammonium hydroxide solution was added to the mixture. The reaction was carried out for 8 h and the silica-coated PS particles could be directly obtained. The silica-coated PS particles were separated by centrifugation at 10000 rpm for 30 min, washed with

deionized water and ethanol, and dried at 60 ºC in a vacuum oven. Finally, the dried particles were calcinated at 600 ºC for 5 h to remove the PS templates (PS HSPs).

The silica-coated bacterial particles were synthesized via a modified Stöber method [S2]. Briefly, 1.8 ml of bacterial suspension (*Escherichia coli* or *Staphylococcus aureus,* 8×10^{10} cells/ml), 5 ml of ammonium hydroxide solution (NH_4OH, 25-30 %) and 73.2 ml of anhydrous ethanol were added to a flask. The mixture was treated with ultrasonication for 5 min to complete the cell dispersion. 20 ml of TEOS (0.2 g/ml) ethanol solution was added dropwise to the mixture under stirring condition and the reaction continued for 72 h at room temperature. The silica-coated bacterial particles were harvested by centrifugation (4000 rpm, 10 min) and washed with deionized water and ethanol. The resulted samples were dried at 60 ºC in a vacuum oven. Finally, the dried particles were calcinated at 600 ºC for 5 h to remove the biological templates (E. coli HSPs or S. aureus HSPs).

1.4. Preparation of Fluorescein Isothiocyanate(FITC) Conjugated OVA (FITC-OVA)

FITC-OVA was prepared by a method reported by Li et al. [S3]. Twenty milligrams of FITC were dissolved in 10 ml of carbonate buffer (220 mM, pH 9.5) and 100 mg OVA was added. The mixture was gently stirred in the dark at 4 °C for 18 h. Unbound FITC was removed by dialysis (MWCO 10000). The resultant FITC-OVA solution was freeze-dried.

1.5. In Vitro Cytotoxicity Assay

The cytotoxicity of HSPs was tested on RAW264.7 cells by MTT assay. Briefly, RAW264.7 cells cultured in RPMI 1640 medium with 10 % FBS and 1 % penicillin-streptomycin were seeded in 96-well plates at a density of 5000 cells/well. After incubating for 24 h at 37 ºC in CO_2 (5 %), the medium was replaced by fresh medium containing various amounts of HSPs (PS HSPs, PS HSPs@glucan, E. coli HSPs, E. coli HSPs@glucan, S. aureus HSPs or S. aureus HSPs@glucan). After incubating for another 24 h, the cells were washed with PBS, and then incubated with fresh medium containing MTT (0.5 mg/ml) for 4 h. Subsequently, 200 μl of DMSO was added to each well, and the optical density was recorded at 550 nm by using a Multiskan FC microplate reader (Thermo Fisher Scientific, USA). The cell viability was calculated from: cell viability = (OD sample/OD control) × 100%, where the sample represents the cells treated with HSPs and the control means non-treated cells.

Results

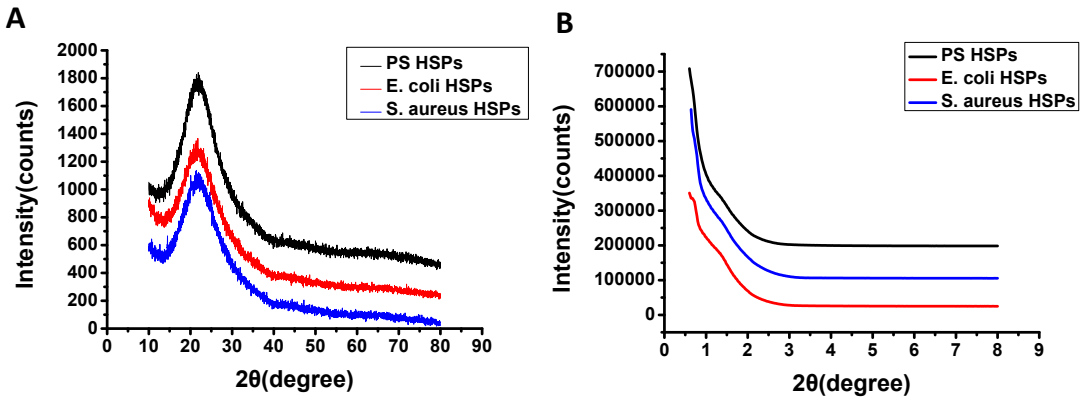

Figure S1. (A) Wide-angle and (B) small-angle XRD patterns of HSPs.

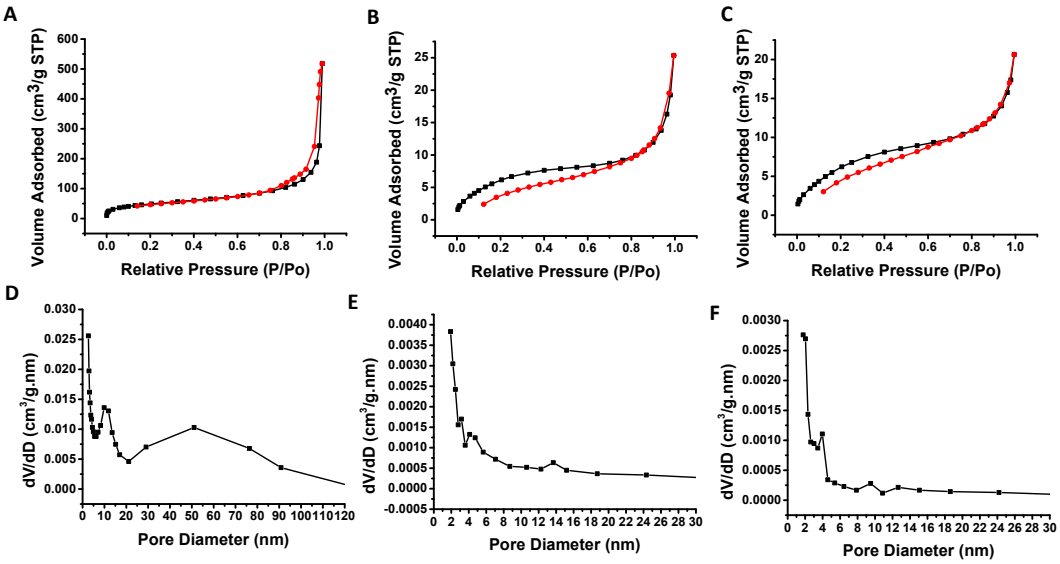

Figure S2. N_2 adsorption/desorption isotherms of (A) PS HSPs, (B) E. coli HSPs and (C) S. aureus HSPs. BJH pore size distributions of (D) PS HSPs, (E) E. coli HSPs and (F) S. aureus HSPs.

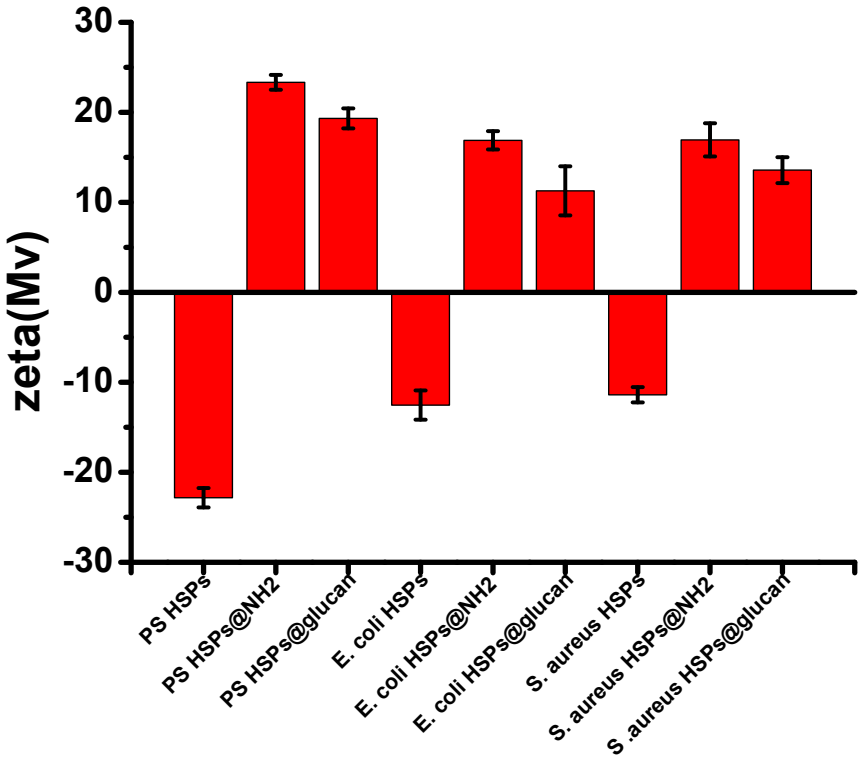

Figure S3. Zeta potentials of PS HSPs, PS HSPs@NH_2, PS HSPs@glucan, E. coli HSPs, E. coli HSPs@NH_2, E. coli HSPs@glucan, S. aureus HSPs, S. aureus @NH_2 and S. aureus HSPs @glucan. The results are expressed as mean ± SD (n = 5).

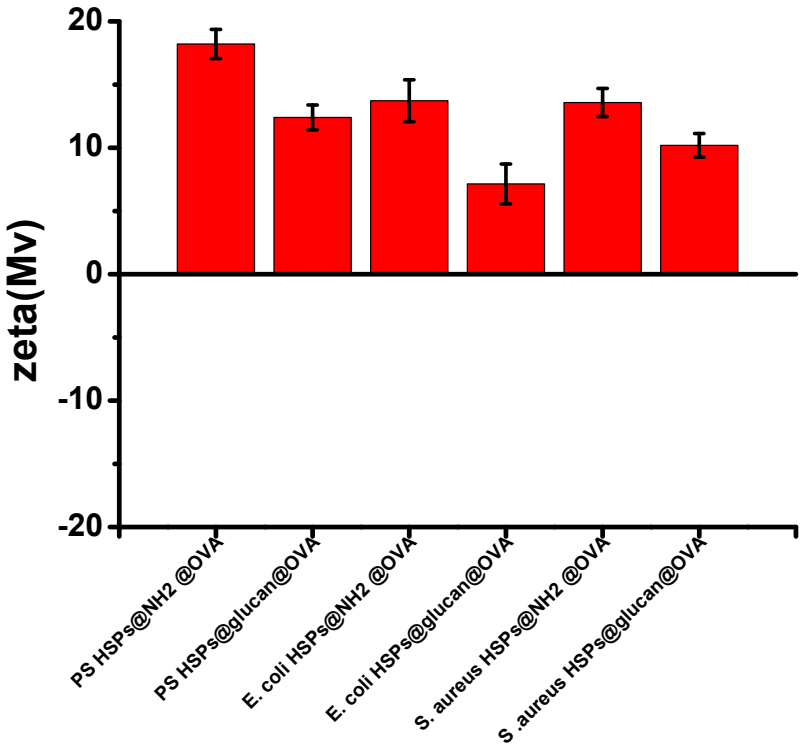

Figure S4. Zeta potentials of PS HSPs@NH_2@OVA, PS HSPs@glucan@OVA, E. coli HSPs@NH_2@OVA, E. coli HSPs@glucan@OVA, S. aureus @NH_2@OVA and S. aureus HSPs @glucan@OVA. The results are expressed as mean ± SD (n = 5).

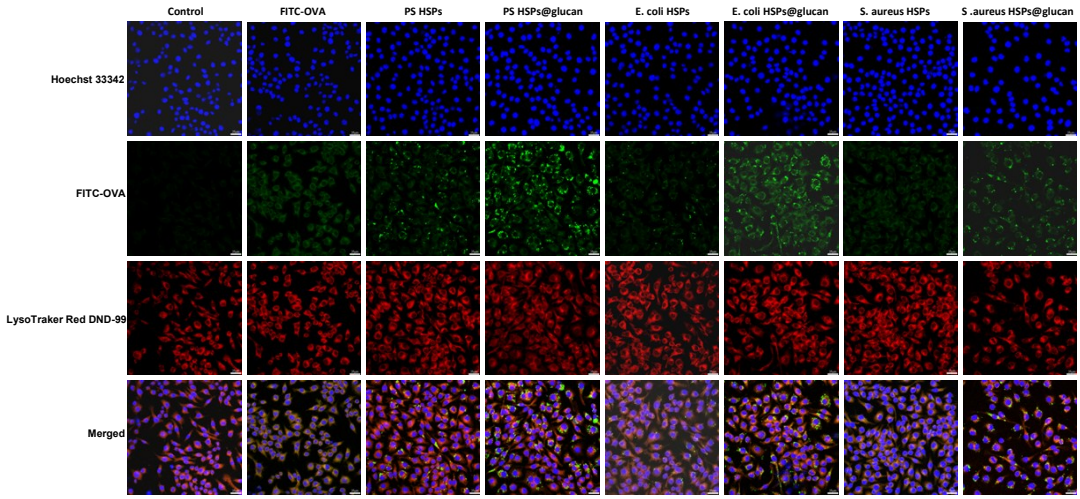

Figure S10. Confocal laser scanning microscopic images of cellular uptake and intracellular localization of FITC-OVA in RAW 264.7 cells. The scale bars represent 20 μm in length.

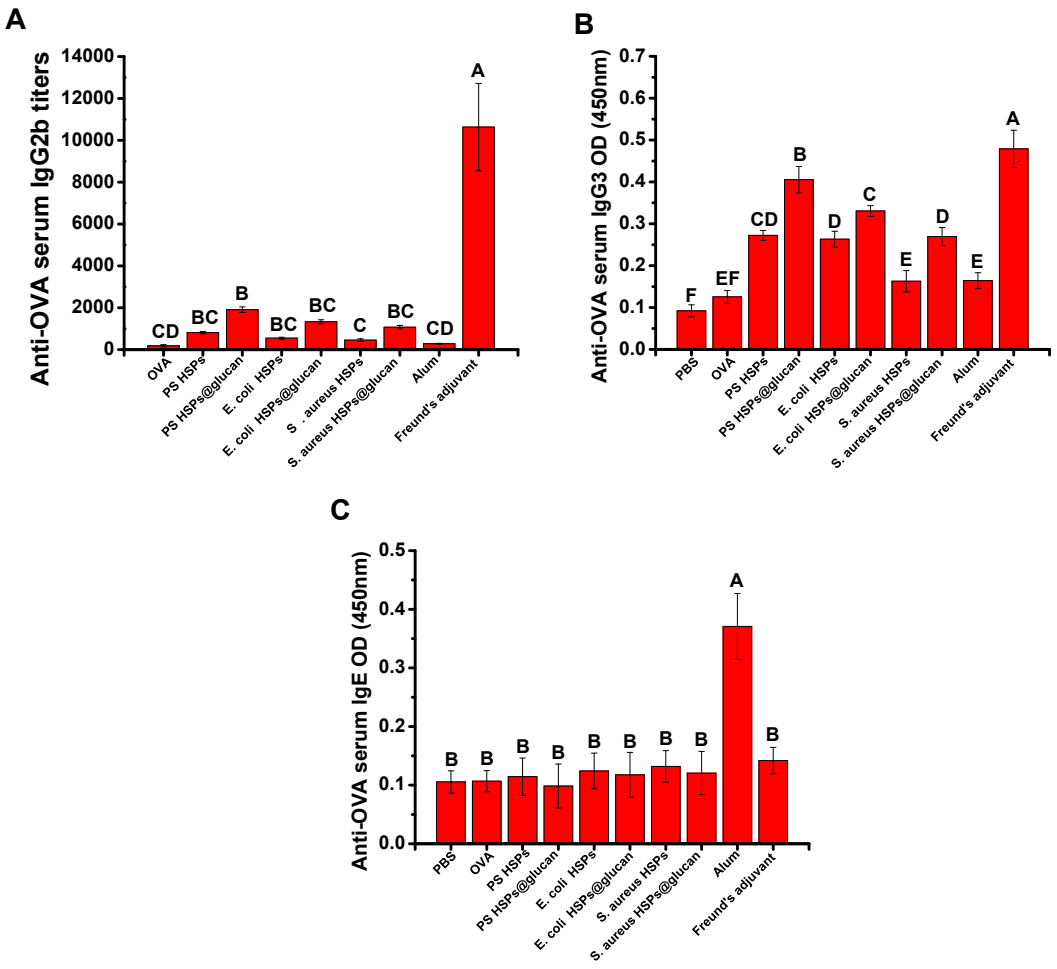

Figure S13. OVA specific antibody titers in serum of BALB/c mice subcutaneously immunized with different formulations. The detailed immunization groups and immunization schedule were described in Section 2. Materials and methods of the main text. IgG2b (A) titers in serum after the last booster immunization. (B, C) OD values of OVA specific IgG3 and IgE in serum after the last booster immunization. The results are presented as mean ± SD (n = 6), and the marks with different letters are significantly different from one another at $p < 0.05$.

Supporting References

[S1] Deng, Z.; Chen, M.; Zhou, S.; You, B.; Wu, L. A novel method for the fabrication of monodisperse hollow silica spheres. Langmuir 2006, 22, 6403-6407.

[S2] Nomura, T.; Morimoto, Y.; Tokumoto, H.; Konishi, Y. Fabrication of silica hollow particles using Escherichia coli as a template. Mater. Lett. 2008, 62, 3727-3729.

[S3] Li, Z.; Dong, K.; Zhang, Y.; Ju, E.; Chen, Z.; Ren, J.; Qu, X. Biomimetic nanoassembly for targeted antigen delivery and enhanced Th1-type immune response. Chem. Commun. 2015, 51, 15975-15978.