Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Polyethyleneimine-complexed charge-reversed yeast cell walls for enhanced oral delivery of pseudovirus-based antigens

I. Cell culture

HEK293T and MODEK cells were cultured in Dulbecco's modified eagle medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. For RAW 264.7 cells, the medium was changed to Roswell Park Memorial Insti-tute (RPMI) 1640 medium. To obtain bone marrow-derived dendritic cells (BMDCs), 6-8 weeks C57BL/6 mice were euthanatized and the femur bones were obtained. Cells derived from bone marrow were cultured in RPMI 1640 containing 10% FBS, 1% penicillin-streptomycin, and 20 ng/ml GM-CSF. On day 3, half of the culture medium was replaced with a fresh one, and cells were obtained for fur-ther treatment on day 7. All cells were cultured at 37 °C and 5% CO2.

II. Production of pseudoviruses

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pseudoviruses were prepared by using a len-tivirus-based four plasmid system. In brief, the spike protein with 19 amino acids truncated at its C terminal was inserted into PCDNA3.1 plasmid downstream of a cytomegalovirus promoter. PCDNA3.1, PLP1, PLP2 and PLVX-ACGFP plasmids were used together for HEK293T cells transfection. After 72 hours culture, the supernatant of the culture me-dium containing free virus particles was collected. To pellet the viruses, the supernatant was ultracentrifuged at 100,000 g for 3 hours. The virus particles were washed three times with phosphate buffer saline (PBS), and resuspended to 100 µl PBS before further use. To determine the virus concen-tration, p24 protein was used as a quantitative marker and measured using an enzyme-linked immunosorbent assay (ELISA) kit (Sino Biological SEK11695) following the in-structions.

III. Western blot

To detect the spike protein on pseudoviruses, the protein concentration of virus particles was determined using a bi-cinchoninic acid (BCA) assay. An amount of 2 µg viral protein was introduced into SDS-PAGE after mixing with SDS-PAGE loading buffer and boiling for 10 minutes at 95 °C in metal bath. Rabbit anti-spike polyclonal antibody was used to detect the existence of the spike protein. Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG antibody and HRP substrate were used for chemilumi-nescence analysis.

IV. Extraction of yeast cell walls

Baker's yeast was obtained from Lesaffre. To prepare yeast cell walls, 20 g yeast was first incubated in 300 ml 1 M hy-droxide at 80 °C for 90 minutes, then transferred into a pH 4.0 hydrochloric acid solution at 60 °C for another 90 minutes. Subsequently, the sample was washed twice with deionized water, four times with isopropyl alcohol, and twice with acetone. Finally, the residual organic solvent was removed at 60 °C in an oven. The dried product was stored at -20 °C for further use.

V. PEI modification

Before modification, the dried yeast cell walls were resus-pended to 10 mg/ml in deionized water and an ultrasonic dispersion was carried out in water bath for 30 minutes at room temperature. The yeast cell walls were then washed three times with deionized water, and transferred into an 8 mg/ml PEI (Sigma 408727, branched, average Mw ~25,000 by LS) solution for 1 hour incubation under shaking at 250 rpm. The final product was washed with deionized wa-ter for three times and P-YC were collected by centrifuging at 5,000 g for 5 minutes.

VI. Encapsulation of SARS-CoV-2 pseudoviruses

The loading of virus particles into P-YC was carried out by a simple incubation procedure. An amount of 100 μg P-YC was resuspended with 1 ml PBS containing virus particle with 100 ng/ml p24 protein. After incubation for 1 hour, pseudovirus-loaded YC (V@P-YC) were collected by cen-trifuging at 5,000 g for 5 minutes and washed three times with PBS to remove free virus particles. The encapsulation efficiency was determined by measuring the residual p24 at supernatant after incubation using an ELISA kit.

VII. Characterizations

The diameter and zeta potential of particles in PBS were measured using dynamic light scattering (DLS, Malvern Zetasizer Nano S90). For laser scanning confocal micro-scope (LSCM) imaging, yeast cell walls were stained by calcofluor-white or fluorescein isothiocyanate (FITC), and virus particles were stained with 1,1'-dioctadecyl-3,3,3',3' -tetramethylindocarbocyanine perchlorate (Dil). To visual-ize PEI, 20 mg FITC was mixed with 100 mg PEI in dime-thyl sulfoxide (DMSO) containing 4-dimethylaminopyridine equimolar to FITC as a deacid reagent. The liquid mixture was reacted overnight and FITC-conjugated PEI was puri-fied by dialysis. For transmission electron microscopy (TEM) observation, a drop of 10 μl sample was loaded onto a Formvar/carbon 300-mesh grid for 10 min and rinsed three times with distilled water. After air drying, the samples were observed by TEM.

VIII. Cell uptake and immune activation in vitro

RAW 264.7 cells and BMDCs were incubated with 100 μg/ml V@P-YC or equivalent virus particles in culture me-dium. For LSCM and flow cytometric analysis, virus parti-cles were stained with 1 μM membrane fluorescent probe 4-chlorobenzenesulfonate

salt (DID) for 30 minutes. To evaluate the cell uptake efficiency, cells were collected after 4 hours incubation for further tests. To determine the im-mune activation ability, 100 μg/ml V@P-YC or equivalent virus particles were added into culture medium of BMDCs and incubated for 24 hours. Afterward, cells were harvested, washed with PBS, and incubated with 1% bovine serum albumin dissolved in PBS for 30 minutes on ice to block Fc receptor. Subsequently, samples were incubated with 1:1000 diluted antimouse-CD86-APC, antimouse-CD80-PE, antimouse-MHC II-Pe/Cy7 and antimouse-CD11c-Pe/Cy 5.5 antibodies for another 60 minutes in dark. Cells washed with PBS for 3 times after incubation were used for flow cytometric analysis.

IX. Stability assay

Simulated gastric fluid (SGF) was prepared by adding 20 mg NaCl and 32 mg pepsin in 10 ml deionized water and pH was adjusted to 2.0 by adding HCl solution. Simulated intestinal fluid (SIF) was prepared by adding 68 mg KH_2PO_4 in 5 ml deionized water and the pH was adjusted to 6.8 by adding NaOH solution, and further mixing with 100 mg trypsin in 5 ml deionized water. The membrane structure integrity of virus particles was quantified by staining with 1 μM membrane fluorescent probe Dil. An amount of 100 μg V@P-YC, equivalent virus particles, and virus particles plus YC were incubated in SGF or SIF for 20 minutes. The total fluorescence intensity was recorded by a multidetection microplate reader.

X. Cell viability assay

10,000 MODEK cells each well in a 96 well plate were treated with virus particles, P-YC, V@P-YC and PBS in an euqal amout of P-YC concentration for 24 hours. Afterwards the culture supernatant was removed, and 100 μl fresh culture medium containing 0.5 mg/ml MTT was added into each well. 4 hours later, the supernatant was aspirated away and 100 ul DMSO was added into each well to fully resuspend sediment. The abosorbance at 590 nm of medium in each well was determined using a microplate reader

XI. Animals

Male ICR mice aged 6 to 8 weeks were purchased from Jiesijie Laboratory Animal Technology. The animal research was approved by the Institutional Animal Care and Use Committee of the Shanghai Jiao Tong University School of Medicine and followed the guidelines of the Shanghai Medical Experimental Animal Care.

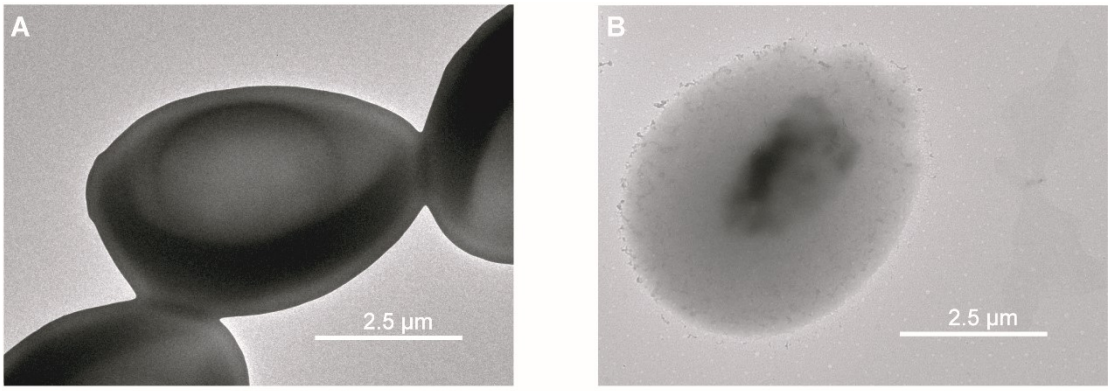

Fig. S1 Typical TEM images of (A) live yeast cells and (B) yeast cell wall

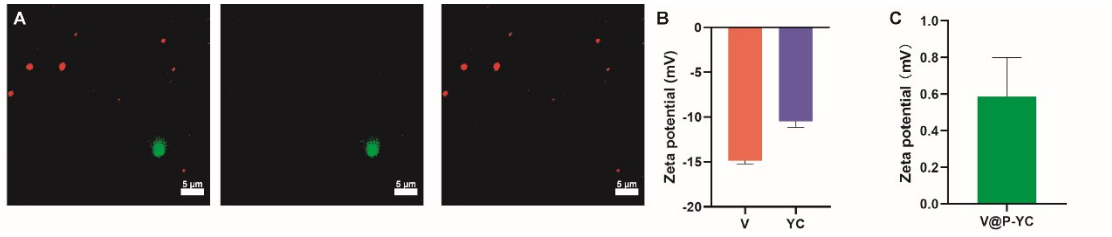

Fig. S2 (A) Representative confocal images of YC after incubation with virus particles. Green and red fluorescence signals refer to FITC-labelled YC and Dil-stained virus particles. (B) Zeta potentials of YC and virus particles. (C) Zeta potential of V@P-YC.

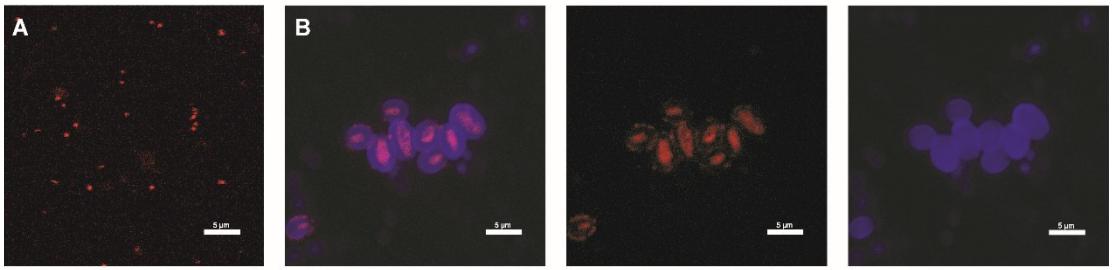

Fig. S3 (A) A typical LSCM image of p24-mCherry virus particles. (B) Representative LSCM images of V@P-YC encapsulated with p24-mCherry virus particles. Blue and red fluorescence signals indicate calcofluor-white stained YC and p24-mCherry virus particles.

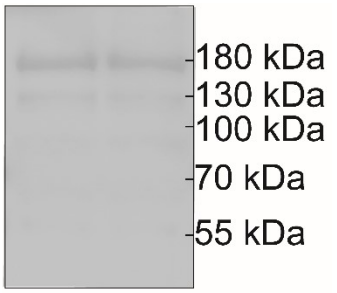

Fig. S4 Detection of the spike protein on pseudoviruses by western blot.

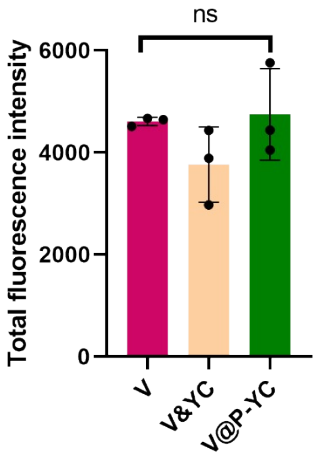

Fig. S5 Stability of virus particles, V&YC, and V@P-YC after treatment with SIF for 20 mins by detecting the fluorescence intensity of lipid membrane on the surface of viral envelope.

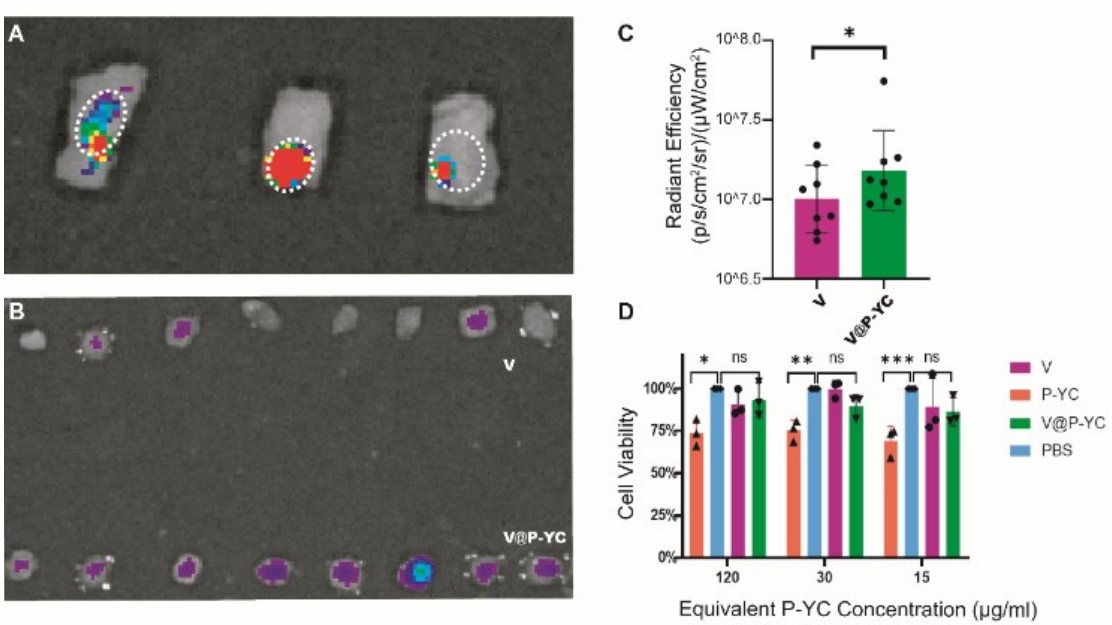

Fig. S6 (A) Accumulation of V@P-YC in Peyer's patches (circled with white dots) located in the gut loop. Tissues were sampled at 4 hours post-injection of V@P-YC containing Dil-stained virus particles. (B) Fluorescence images of isolated Peyer's patches captured by IVIS. (C) Quantification of the accumulation of V@P-YC or free viruses in Peyer's patches by calculating the radiant efficiency using IVIS. Data are mean ± SEM (n = 8). Statistical analysis was performed using one-tailed paired t test. (D) Viabilities of MODEK cells after incubation with free viruses, P-YC, and V@P-YC for 24 hours. PBS was used as a control and cell viability was evaluated by cck-8 assay. Data are mean ± SEM (*n* = 3). Statistical analysis was performed using one-way ANOVA; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.