Supplementary information

Flexible deformation and special interface structure in nanoparticle-stabilized Pickering bubbles strengthen the immunological response as adjuvant

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Supplementary Tables

Supplementary Table 1. The particle size of PNP under each parameter condition

Parameter		Size (nm)	PDI
Oli-water ratio	1:2	767.6±15.4	0.43±0.01
	1:4	431.8±2.4	0.24±0.06
	1:6	559.9±3.3	0.30±0.03
	1:8	560.7±5.5	0.07±0.05
	1:10	549.5±1.6	0.30±0.06
PVA concentration (%)	0.2	330.2±1.8	0.39±0.01
	0.5	256.7±3.3	0.22±0.06
	1.5	278.9±0.6	0.30±0.03
	2.5	316.5±6.2	0.24±0.06
	3.5	418.7±5.6	0.35±0.02

PLGA with different monomer ratios and molecular weight were selected to prepare PNP. Each number represents the monomer ratio and molecular weight as follows: 1 - (50:50, 1W), 2 - (50:50, 2.9 W), 3 - (50:50, 6.1 W), 4 - (75:25, 1.3 W), 5 - (75:25, 5 W).

Supplementary Table 2. The particle size of PNP under different specifications of PLGA

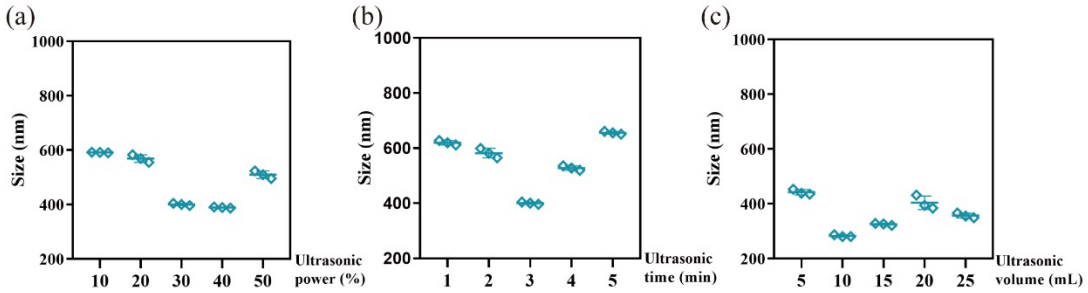
Specification	Size (nm)	PDI
1	280.5±1.8	0.30±0.04
2	304.3±5.7	0.29±0.02
3	322.6±4.4	0.23±0.08
4	223.3±3.6	0.17±0.06
5	280.4±2.8	0.24±0.02

Mice (n = 6 mice per group) were immunized intramuscularly with 100 μL suspension of various adjuvants containing ovalbumin on day 0 and day 14. The components and contents of each immunization formulation were shown in Table 3.

Supplementary Table 3. Components of each vaccine formulation

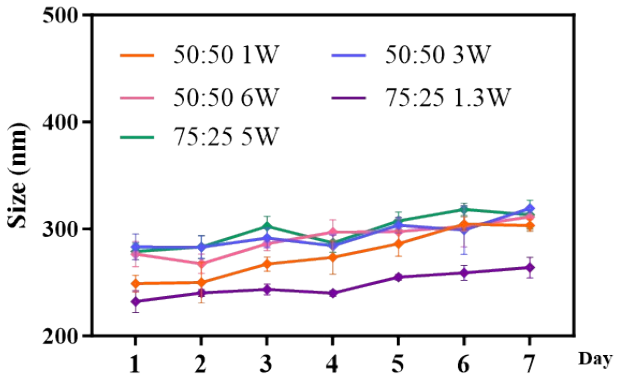
Group	Antigen ($\mu g \cdot mL^{-1}$)	Particles ($\mu g \cdot mL^{-1}$)	Immune volume (μL/per)
OVA	100	/	100
Al	100	2500	100
PNPs	100	2500	100
PPBs	100	2500	100

Supplementary Figures



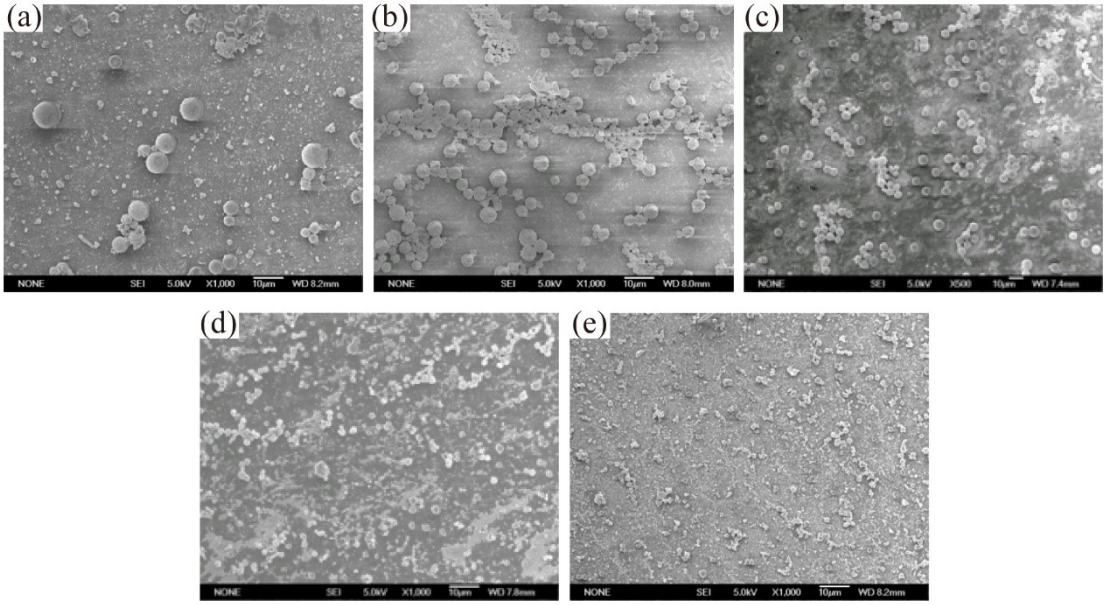
Supplementary Figure 1. The final ultrasonic power, time and volume conditions were selected as 30%, 3 min and 10 mL, respectively.

To evaluate the stability of PNPs, the solution containing PNPs was maintained at 4 °C for 7 days, during which there was no significant change in the particle size of PNPs.

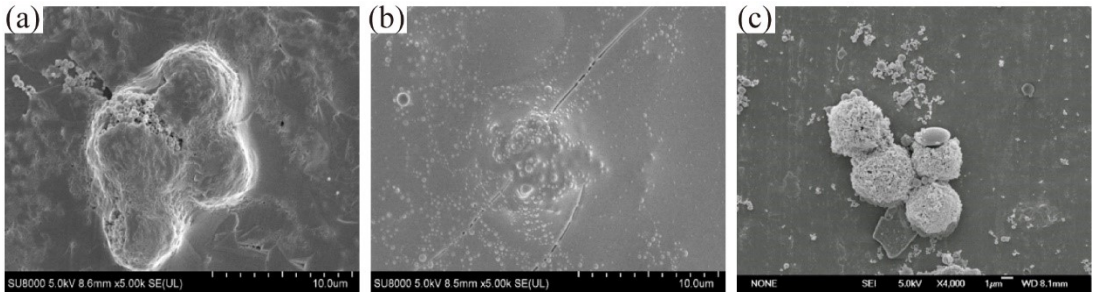


Supplementary Figure 2. The stability of particle size storage at room temperature was investigated. PNPs could be stably stored for 7 days after optimizing the parameters. The final preparation conditions were selected as the oil-water ratio of 1:4, the PVA concentration of 0.5 % and the molecular weight of PLGA was 1,3000 with a monomer ratio was 75:25.

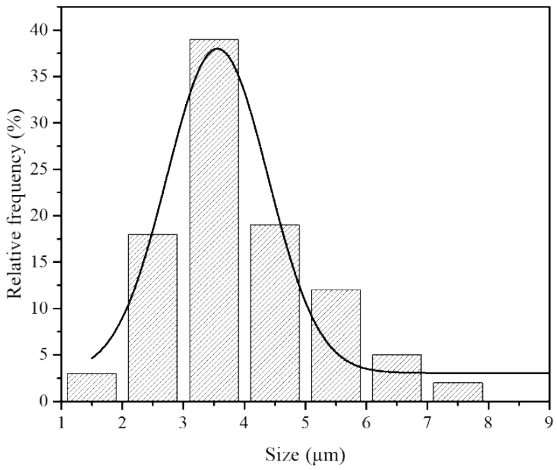
The preparation conditions of the PPBs were optimized by screening the homogeneous power and outer water phase.



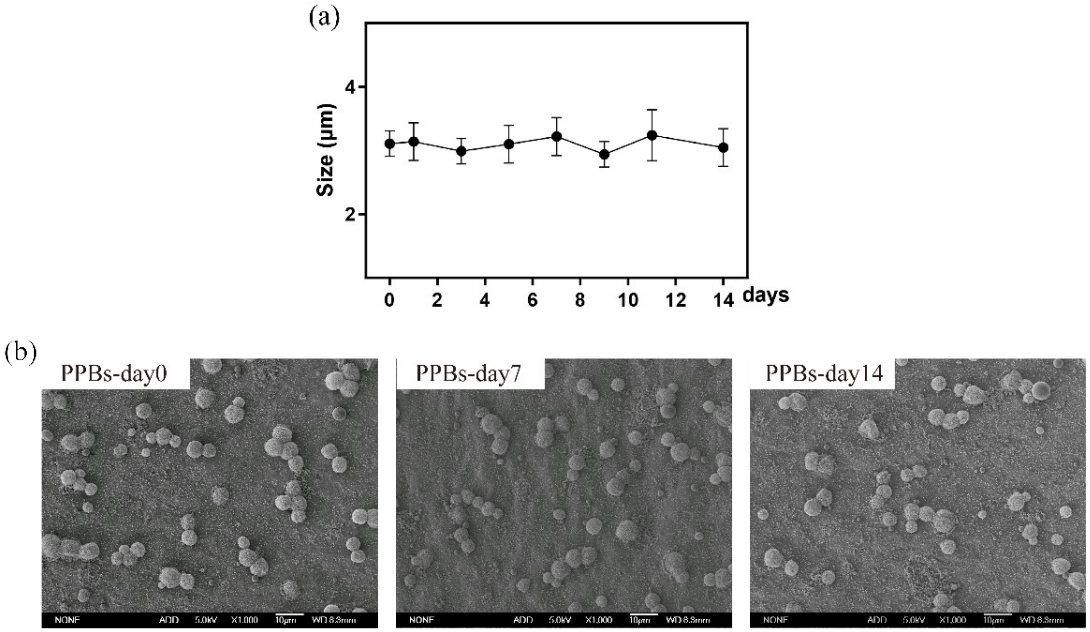
Supplementary Figure 3. The homogenous power screening for PPBs preparation was a) 7,000 rpm, b) 11,000 rpm, c) 15,500 rpm, d) 20,000 and e) 24,000 rpm, respectively. PLGA nanoparticles (PNP) with uniform particle size can be obtained when the homogeneous power was 15500 rpm.



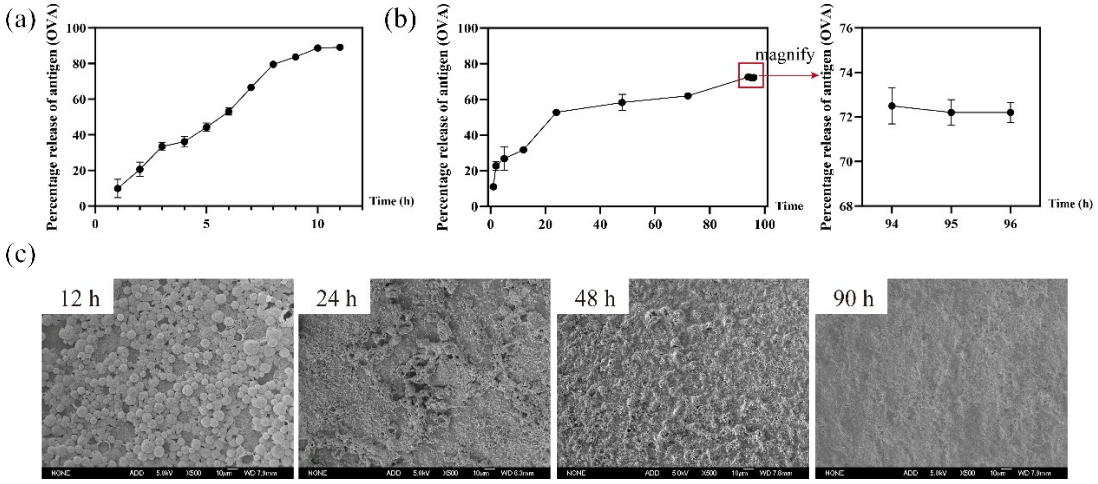
Supplementary Figure 4. The external aqueous phases for PPBs preparation were a) phosphate buffered saline, b) citrate buffer, pH=5.8, and c) deionized water, respectively. Pickering bubbles stabilized by particles can be prepared by using deionized water as the external water phase. It can be seen from the figure that particles were stabilized at the gas-liquid interface.



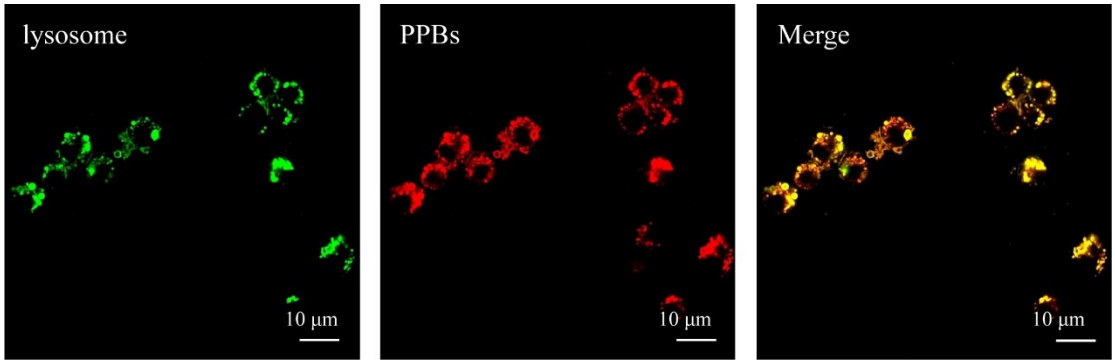
Supplementary Figure 5. The average size of PPBs. The size of PPBs in SEM images was calculated by specialized software (nano measurer). After optimizing the preparation conditions, the average particle size of the PPBs obtained was about 3.5 μm.



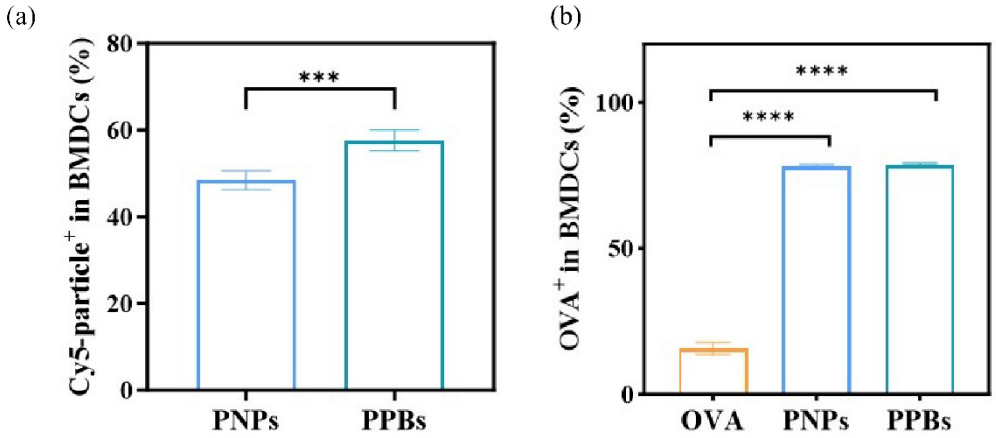
Supplementary Figure 6. (a) Particle size change of PPBs stored at 4° for different times. (b) The particle size variation of PPBs fluctuated around 3.3 μm. Meanwhile, the morphologies of PPBs were observed by SEM.



Supplementary Figure 7. The antigen release in (a) PNPs and (b) PPBs was detected *in vitro* in a simulated *in vivo* environment. (c) SEM image of PPBs degradation.

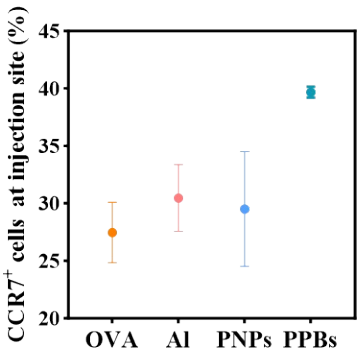


Supplementary Figure 8. PPBs was co-localized with lysosomes after uptake by cells. The fluorescence channels of 638 nm (PPBs was labeled by Cy5, red) and 561 nm (lysosome was labeled by Lyso-Tracker Red, green) were selected for observation by CLSM. Scale bar = 10 μm.

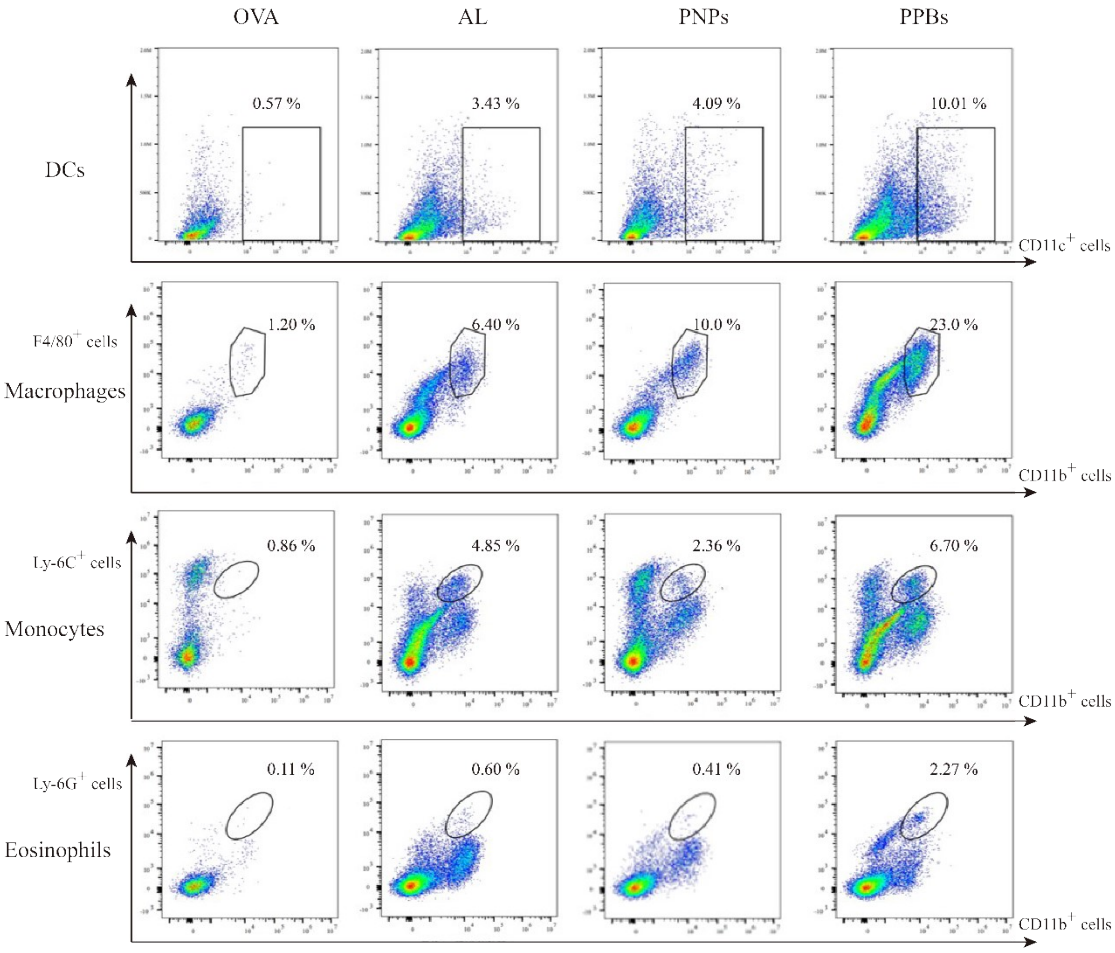


Supplementary Figure 9. (a) The quantitative analysis data of PPBs uptake by cells. (b) The antigen uptake by J774A.1 cells after PPBs loaded was quantitatively analyzed. Data were expressed as mean ± standard error of the mean (s.e.m.) (n = 6). **** $p < 0.0001$, analyzed by t-test. * Indicated statistically significant differences in load antigen between PPBs and control

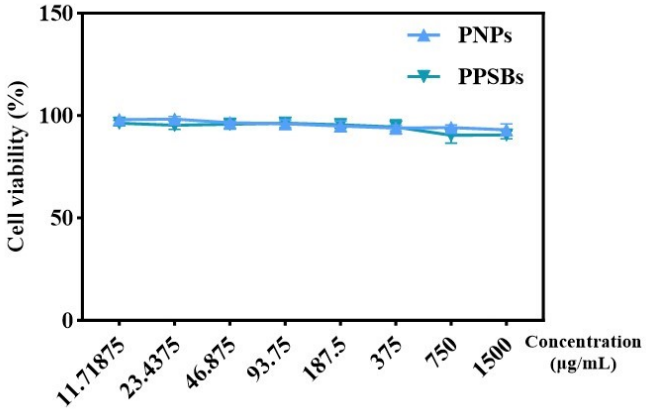
groups.



Supplementary Figure 10. The expression of CCR7 receptor at the injection site after immunization.



Supplementary Figure 11. Flow cytometry of APCs recruitment experiment on day 7.



Supplementary Figure 12. Biocompatibility evaluation via CCK-8 cytotoxicity experiment. Data were expressed as mean ± standard error of the mean (s.e.m.) (n = 3).