Electronic Supplementary Material

Optimising the oil phases of aluminium hydrogel-stabilised emulsions for stable, safe and efficient vaccine adjuvant

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S - I Optimizations of ASEs

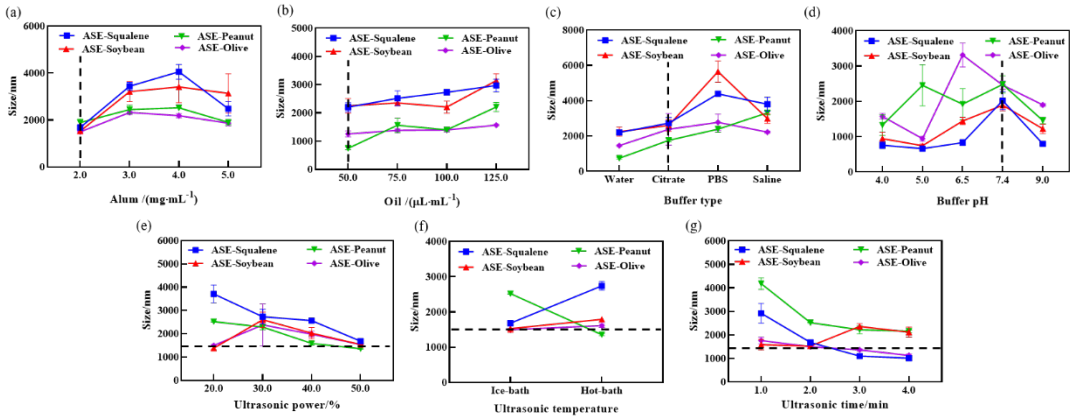

Figure S1. Optimizations of ASEs on (a) alum concentration and (b) oil phase volume for smallest sizes, (c) buffer type, (d) buffer pH, (e) ultrasonic power, (f) ultrasonic temperature (Ice-bath was in ice-water mixture, Hot-bath was at 60 ℃ hot water), and (g) ultrasonic time for the similar size. Data were shown as mean ± s.e.m. (n = 3).

S – II Zeta potentials of ASEs in different buffer type

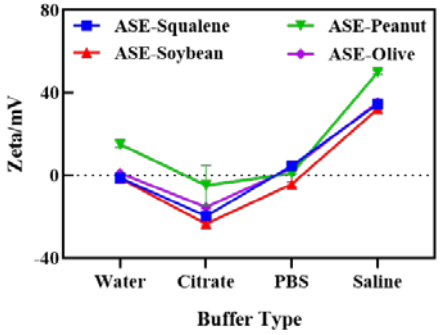

Figure S2. Zeta potentials of ASEs in different buffer type. Data were shown as mean ± s.e.m. (n = 3).

S – III Fluidic alum/antigen complex proportions of ASEs

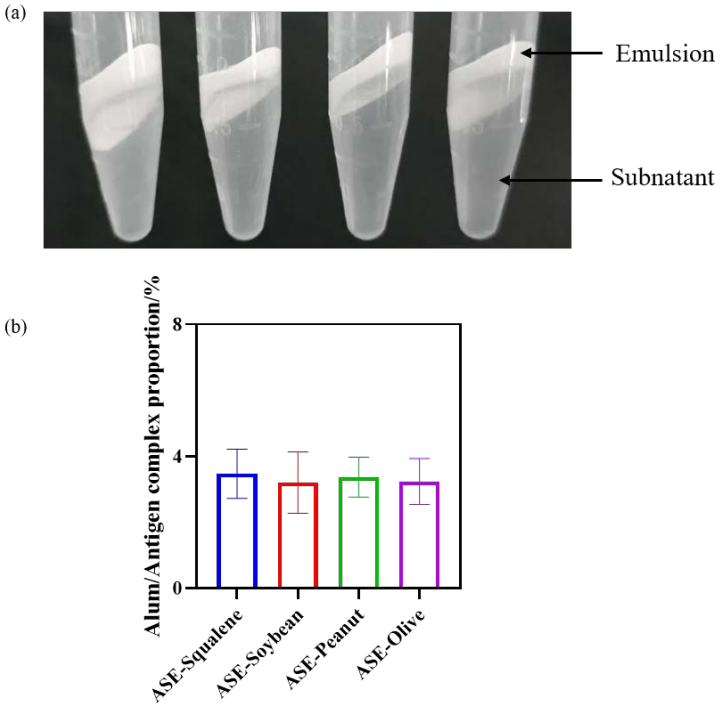

Figure S3. (a) Pictures of ASEs after centrifugation and (b) Alum/Antigen complex (antigen concentration in the subnatant). Data were shown as mean ± s.e.m. (n = 3).

S – IV Endotoxin level of ASEs and alum

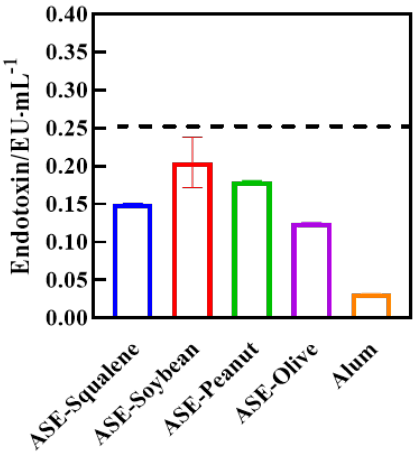

Figure S4. Endotoxin level of ASEs and alum. FDA guidelines indicate that sterile water for injection may contain 0.25 EU·mL^{-1} of endotoxin (dashed line). Data were shown as mean ± s.e.m. (n = 6).

S - V Tissue distribution analysis

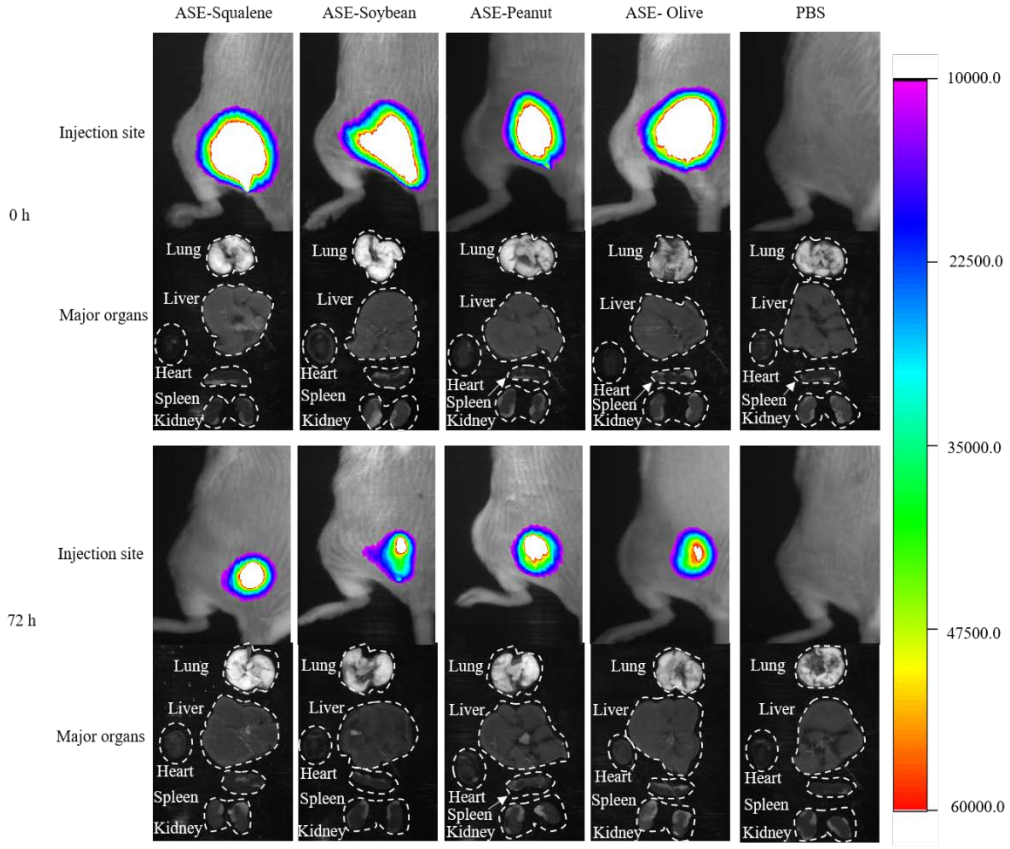

Figure S5. Tissue distribution analysis on the emulsion droplets. The local retention at the injection sites was illustrated at the up of the images.

Antigens were labeled by Cy5 and co-loaded on ASEs, which were subsequently injected intramuscularly and traced by *in vivo* fluorescence imaging system. As the result, no datable fluorescent signals were observed in lungs, livers, hearts, spleens or kidneys, indicating that the droplets were hardly distributed in the major organs.

S - VI Zeta potentials of ASEs

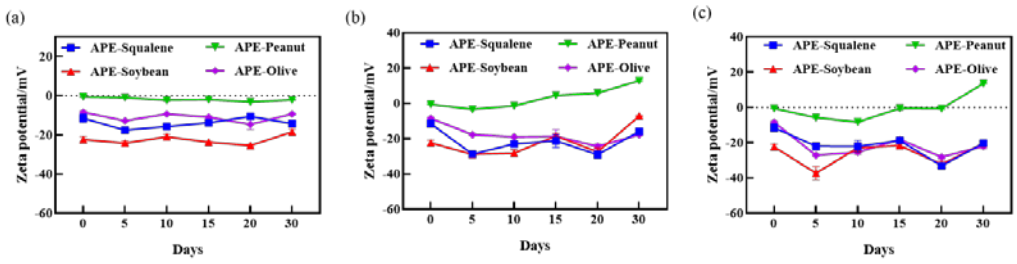

Figure S6. Zeta potentials of ASEs from Day 0 to Day 30 of storage at (a) 4 ℃, (b) 25 ℃ and (c) 37 ℃. Data were shown as mean ± s.e.m. (n = 6).

Table S1 Aluminum concentration ratio in organ homogenate for 3 days after intramuscularly injected with ASEs

	Heart/%	Liver/%	Spleen/%	Lung/%	Kidney/%
ASE-Squalene	0.13 ± 0.02	0.022 ± 0.34	0.11 ± 0.013	0.08 ± 0.012	0.08 ± 0.028
ASE-Soybean	0.03 ± 0.007	0.026 ± 0.014	0.05 ± 0.011	0.06 ± 0.017	0.14 ± 0.009
ASE-Peanut	0.02 ± 0.029	0.15 ± 0.11	0.026 ± 0.016	0.22 ± 0.08	0.093 ± 0.016
ASE-Olive	0.01 ± 0.02	0.009 ± 0.004	0.04 ± 0.029	0.08 ± 0.054	0.07 ± 0.065
PBS	0.02 ± 0.004	0.03 ± 0.028	0.05 ± 0.013	0.057 ± 0.01	0.014 ± 0.01

Aluminum concentration ratio = Aluminum concentration in organ homogenate / Total aluminum of ASEs × 100%