Electronic supplementary information

A fast-dissolving microneedle array loaded with chitosan nanoparticles to evoke systemic immune responses in mice

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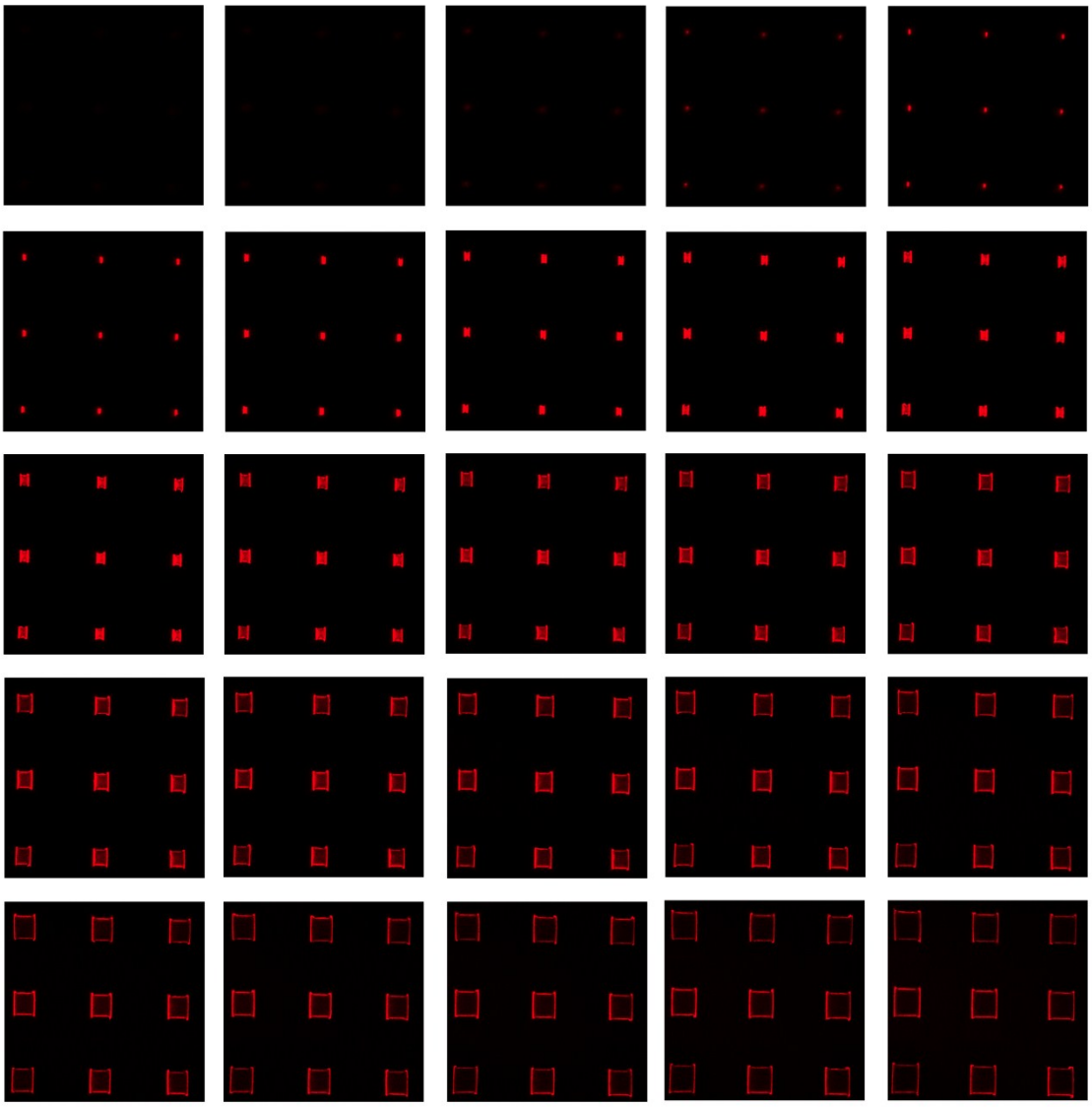

Fig. S1 The Confocal scanning photos of the microneedle loading CS-OVA-CpG NPs in tips. The MN array was placed on a microscope slide and observed using confocal laser scanning microscope LSM800 (Carl Zeiss AG, Germany) at an excitation wavelength of 676 nm and emission wavelength of 696 nm. Images were obtained in the xy-plane (parallel to the base of the MN). The initially scanned the tips of MN (z = 0 μm) was defined as the imaging plane of the first photo. Scanning was conducted once at the interval of 22 μm from the tips through the z-

axis perpendicular to the xy-plane, and each needle was photographed via layer-by-layer scanning, and a total of 25 images were taken.

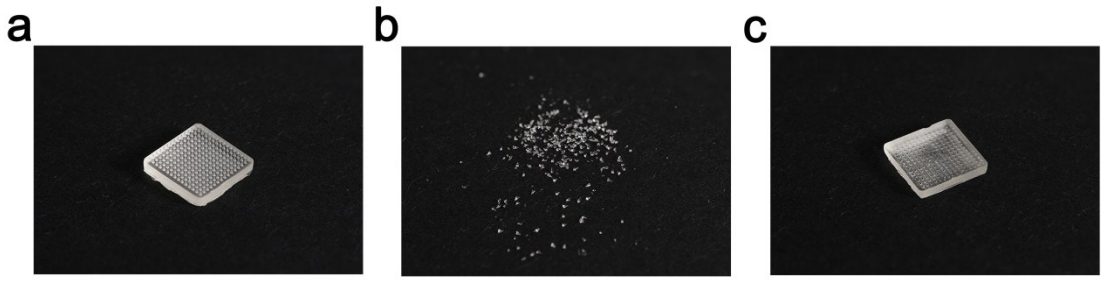

Fig. S2 The images of intact MN (a), needles cut from MN (b) and the MN base without needles (c). Then these collected needles were dissolved in 350 μL of ultrapure water to determine the amount of OVA and CpG.

Table 1 The recovery of OVA and CpG in MN loading CS-OVA-CpG NPs and MN loading free OVA and CpG.

	Recovery (%)			Average (%)	RSD (%)
OVA in MN free OVA +CpG	98.57	97.14	100.36	98.69	1.32
CpG in MN free OVA+ CpG	99.52	98.68	101.28	99.83	1.08
OVA in MN NPs	99.82	102.16	100.14	100.71	1.04
CpG in MN NPs	98.17	98.33	100.56	99.02	1.09

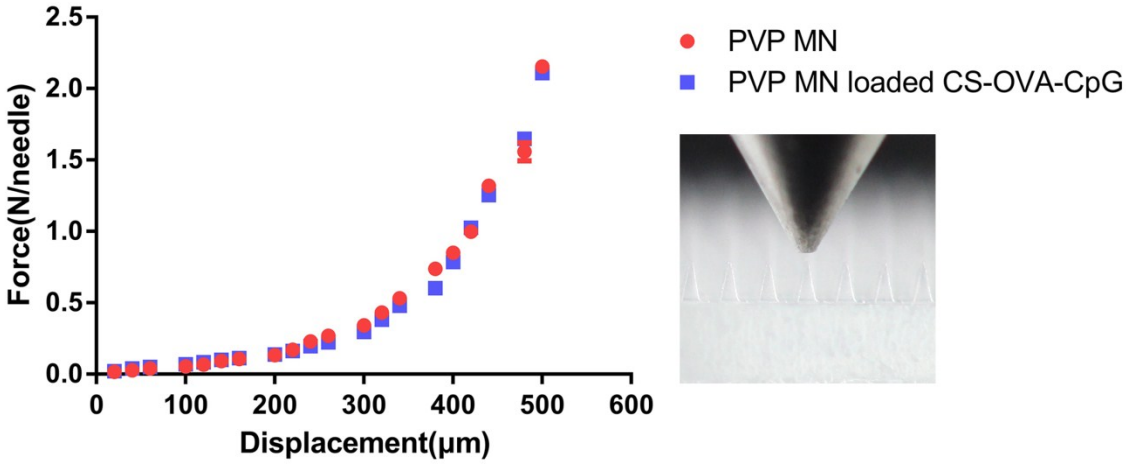

Fig. S3 The mechanical property test results of PVP MN and PVP MN loaded CS-OVA-CpG NPs. Mechanical compression tests were performed using a universal testing machine (MARK-10, Copiague, USA). The MN array was fixed on the surface of flat stainless-steel plate, and then an axial force perpendicular to the axis of the MN array was applied by a moving sensor. The testing machine recorded the needle force when the moving sensor touched the uppermost point of the MN needle with required displacement move of the MN.

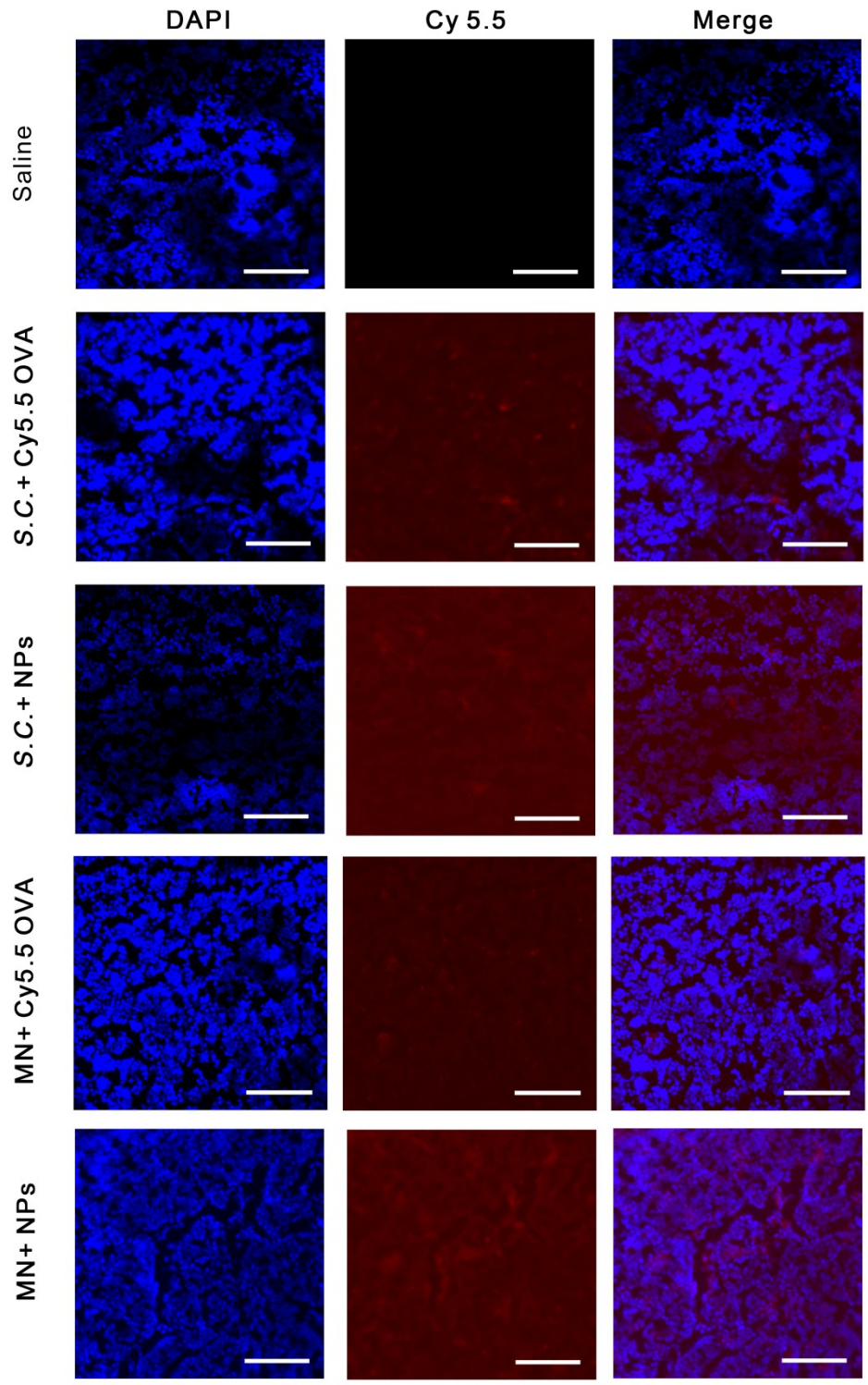

Fig. S4 Frozen sections of LNs after subcutaneous injection or MN administration with saline, free OVA and CS-OVA-CpG NPs. Cell nuclei (DAPI, blue), Cy5.5-OVA (red) were observed by

inverted fluorescence scanning microscope (×200). Scale bars represent 50 μm.

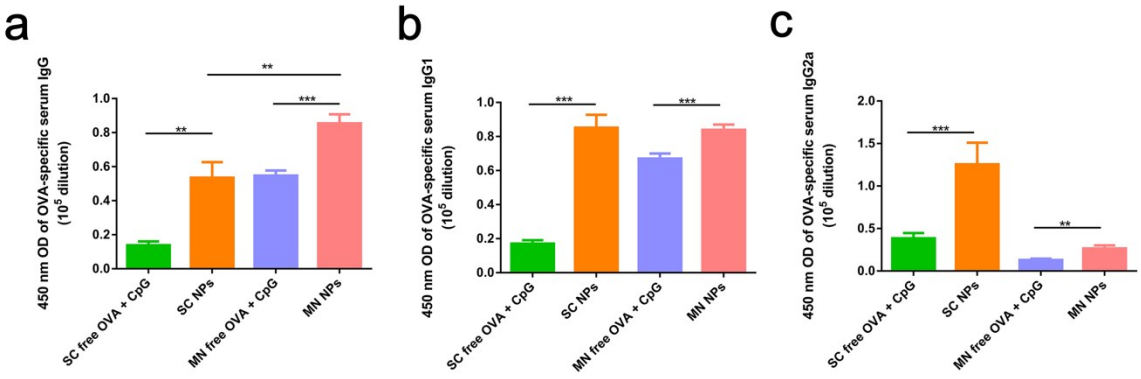

Fig. S5 The influence of free OVA and CS-OVA-CpG NPs on OVA-specific antibody levels. OD_{450} of OVA-specific IgG (a), IgG1 (b), and IgG2a (c) serum antibody levels three weeks post immunization (n = 5) were assayed using ELISA kits. Serum samples were diluted at 1:100000 for IgG and IgG1, and 1:10000 for IgG2a. Data represent mean ± SD. *p< 0.05, **p< 0.01, *** p<0.001

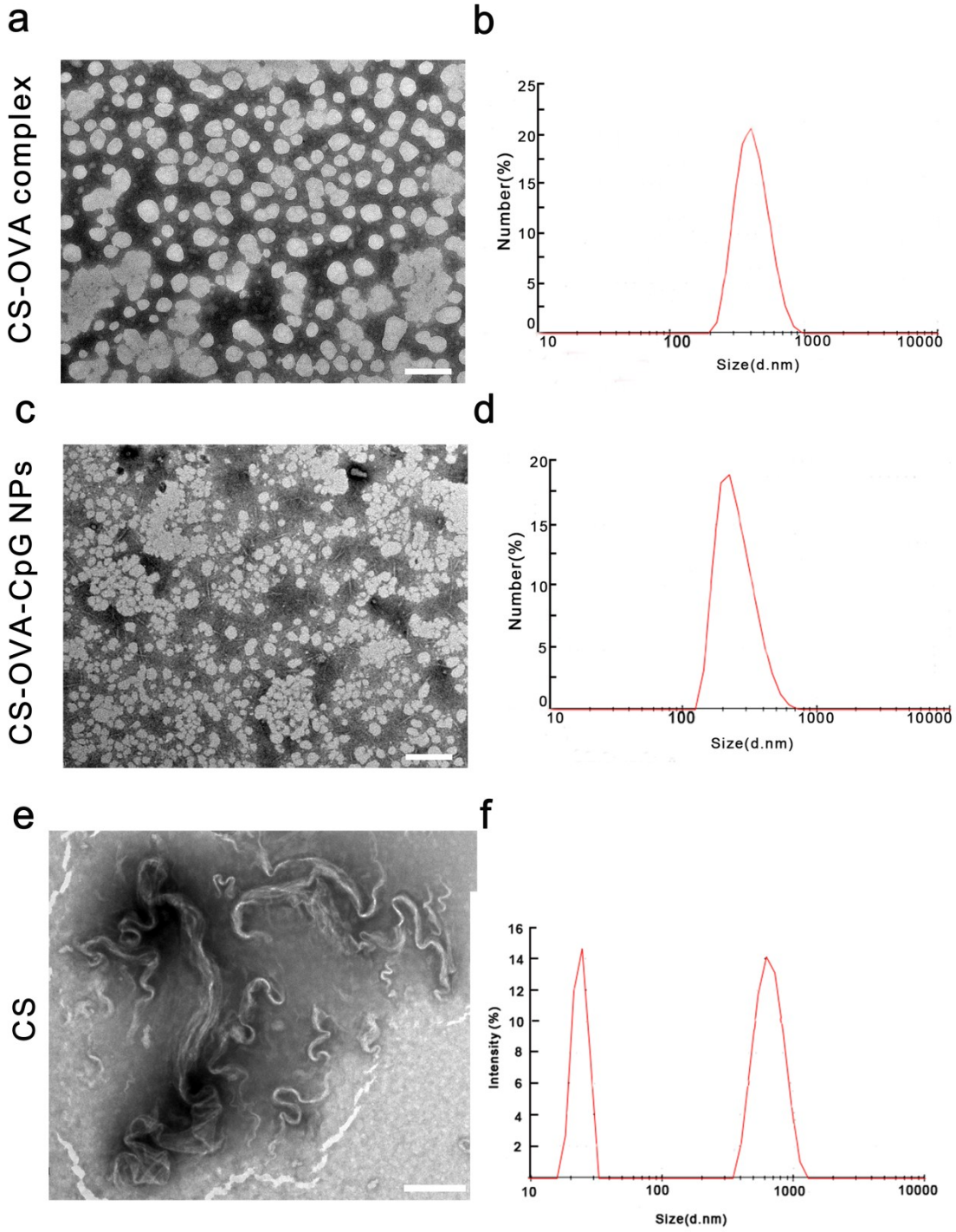

Fig. S6 The aggregation morphology of CS-OVA complex (a) and CS-OVA-CpG NPs (c), Scale bars represent 200 nm. The dynamic light scattering (DLS) diameters of CS-OVA complex (b) and CS-

OVA-CpG NPs (d). (e) Chitoan is a linear glycopolymer forming entanglements under TEM. Scale bar represents 500 nm. (f) Size distribution profiles of CS solution by DLS measurement.