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**Supplementary Information for**

# Transdermal Vaccination via 3D Printed Microneedles Induces Potent Humoral and Cellular Immunity

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**This PDF file includes:**

Figures S1 to S7

**A**

| Coating formulations | Components | Weight Percentage (wt%) |
|---|---|---|
| OVA | Methylcellulose | 3.4 |
| | Sucrose | 21 |
| | OVA | 7 |
| CpG | Methylcellulose | 3.4 |
| | Sucrose | 21 |
| | CpG | 1.2 |

**B**

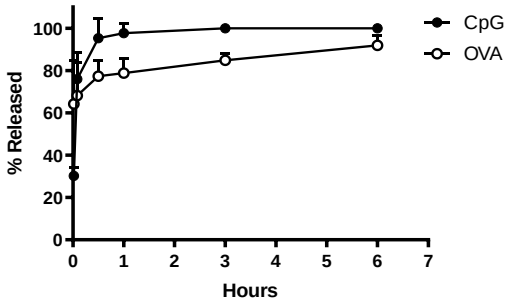


**Fig. S1.** Cargo coating formulations (A) and release profiles from coated square pyramidal MNs (B). Data are presented as mean ± standard deviation (n=4).

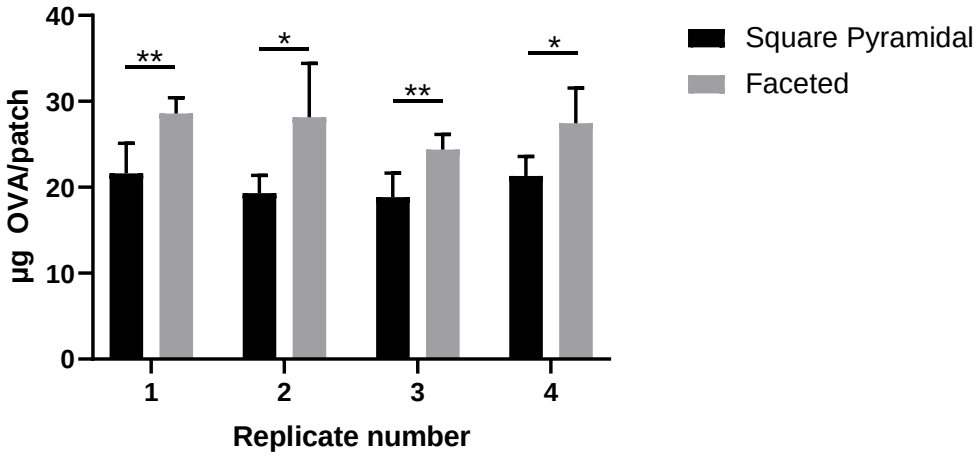


**Fig. S2.** Reproducibility of cargo coating on square pyramidal and faceted microneedle patches. Microneedles were coated with OVA utilizing 4 individually prepared coating solutions, 4-5 patches each. Cargo loading was evaluated after release in water for 2 hours at room temperature. OVA concentration was determined using a BCA assay. Data are presented as mean ± standard deviation with unpaired *t*-tests, * $p<0.05$, ** $p<0.01$.

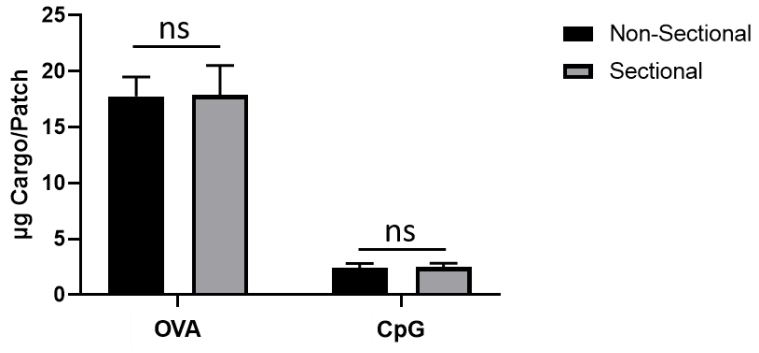


**Fig. S3.** Cargo coating on faceted microneedles. OVA and CpG were loaded on microneedles utilizing either a non-sectioned or sectioned coating mask, resulting in microneedles co-loaded with both OVA and CpG on the same needles, or OVA and CpG loaded on separate needles (70% needles for CpG and 30% for OVA). Data are presented as mean ± standard deviation (n=5), with unpaired *t*-test analysis. “ns” = not significant.

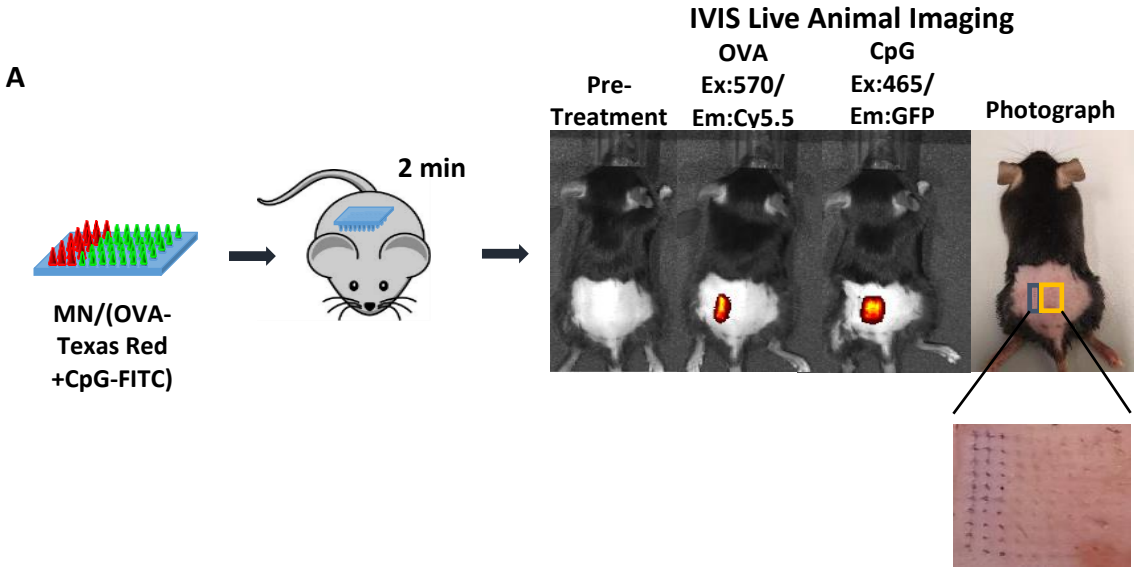


**Fig. S4.** Delivery of cargos in mouse skin via MN patches. C57BL/6 mice were shaved and nair-treated in the lower back, then applied with a MN patch co-coated with OVA-Texas Red and CpG-fluorescein for 2 min and removed. The mice were imaged with IVIS Lumina at two fluorescence channels as labeled. A representative fluorescence image is presented to show OVA and CpG fluorescence. In addition, a photograph of mouse after application of MN shows the skin indentations from needles and cargos left in the pores.

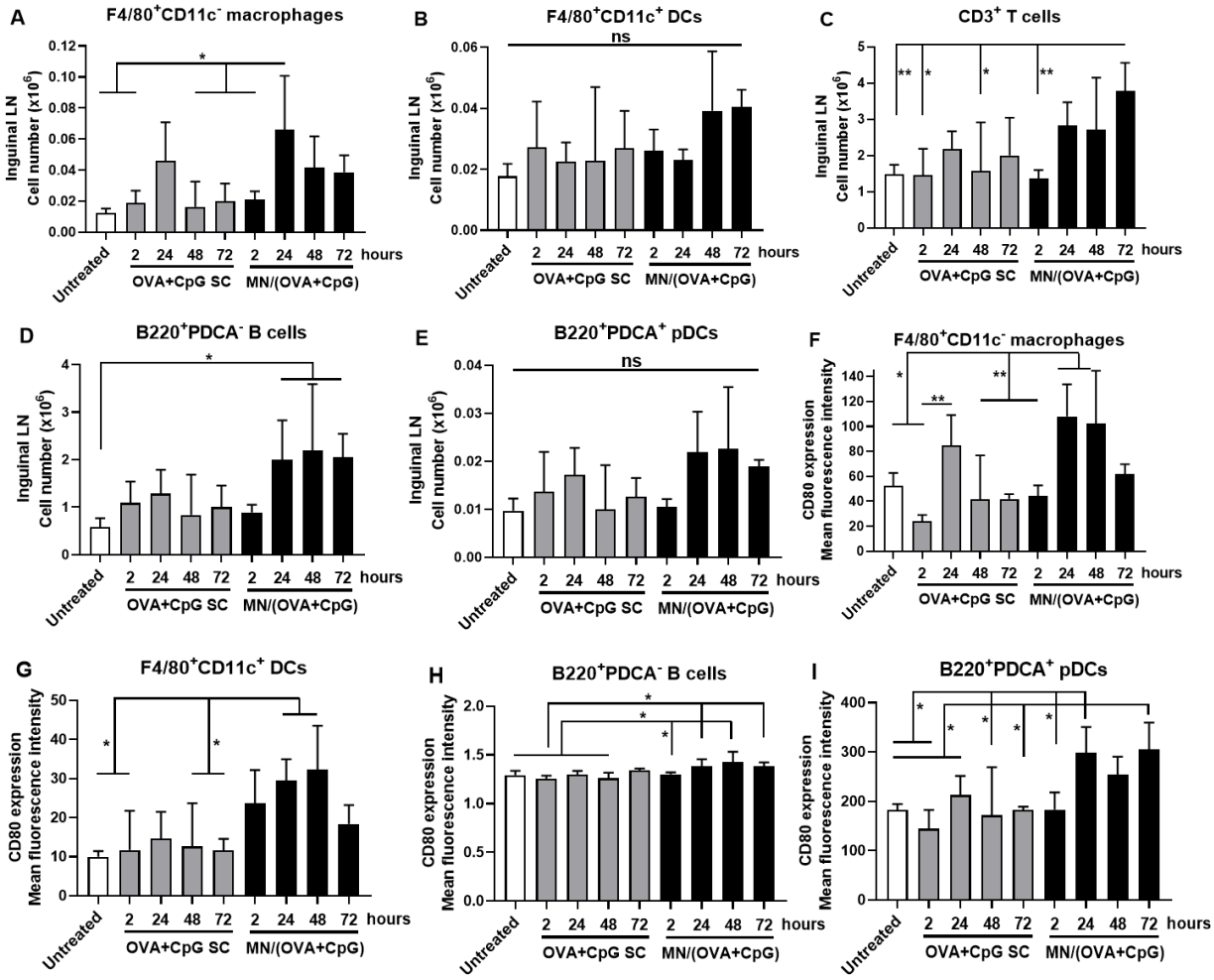


**Fig. S5.** Immune cell population (A-E) and CD80 expression in mean fluorescence intensity (F-I) in the draining inguinal LNs, after application of MNs or injections of soluble formulations. Data are presented as mean ± standard deviation of samples from individual animals, n=4. Statistical analysis was done by One-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. "ns" = not significant.

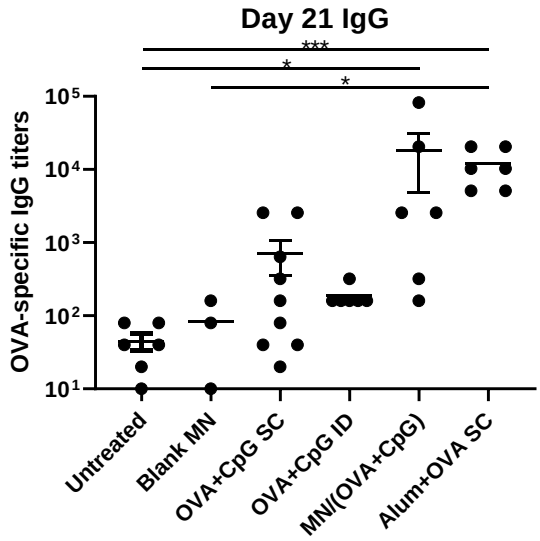


**Fig. S6.** Vaccine induced humoral immune responses on day 21 post immunization. Data are presented as mean ± standard deviation of samples from individual animals, n=6-9. Statistical analysis was done by One-way ANOVA. * $p<0.05$, *** $p<0.001$.

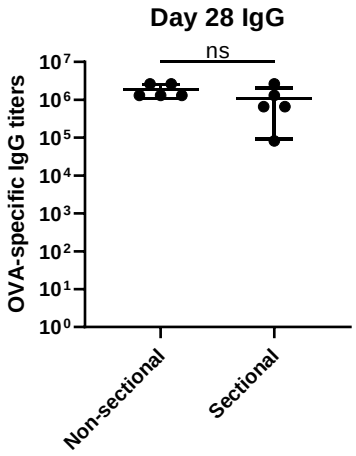


**Fig. S7.** Humoral immune responses to faceted microneedles co-loaded with OVA and CpG via non-un-sectioned or sectioned coating mask. Female C57BL/6 mice received two immunizations on days 0 and 21, with OVA and CpG levels shown in Figure S3. Data are presented as mean ± standard deviation of samples from individual animals (n=5), with unpaired student's *t*-test analysis. "ns" = not significant.