

**Supplemental Information for:**

**Implantable silk composite microneedles for programmable vaccine release kinetics and enhanced immunogenicity in transcutaneous immunization**

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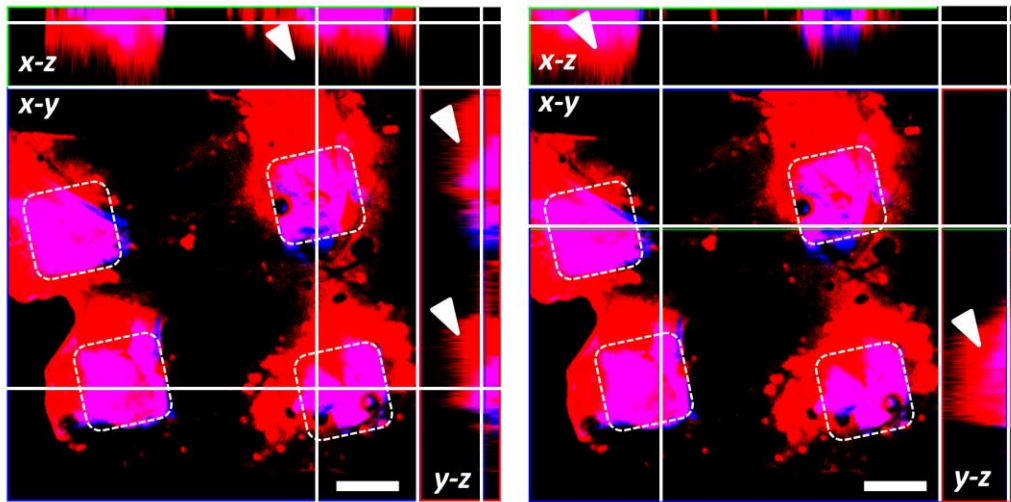
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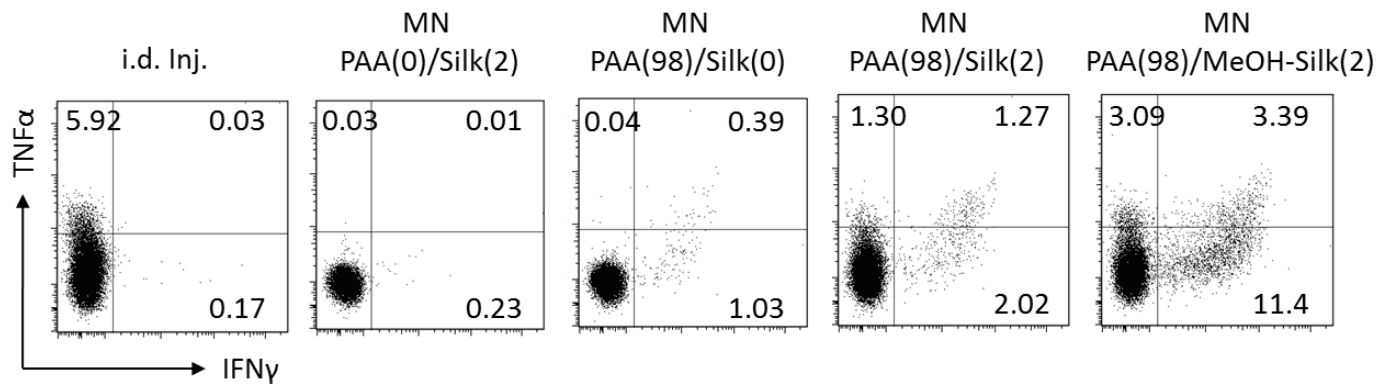
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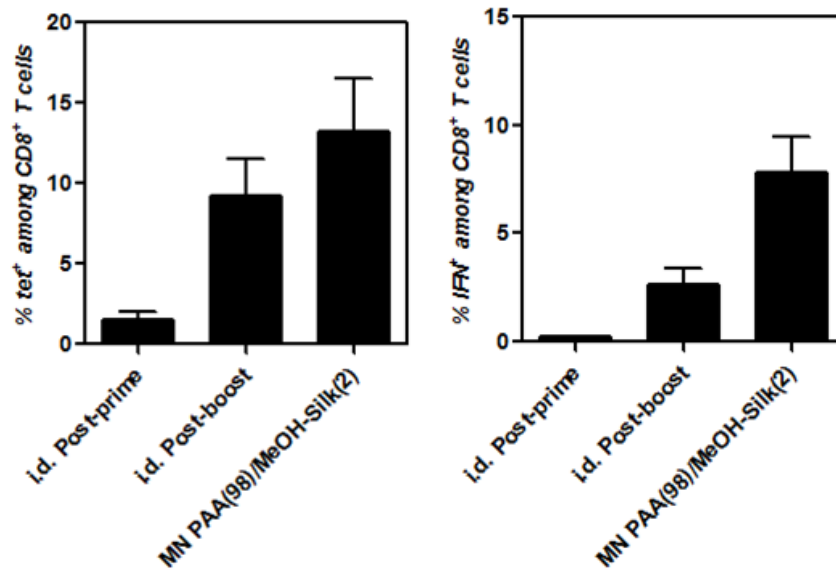
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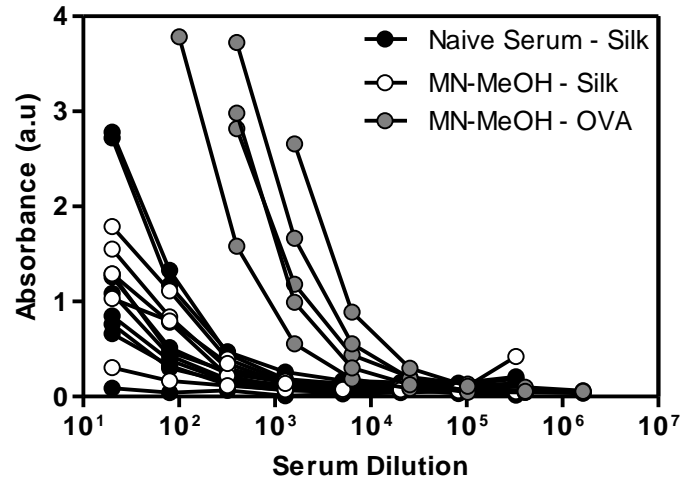
**Supplemental Figure 1** | Composite microneedles give effective cutaneous delivery. Reconstructed confocal  $x$ - $y$ / $x$ - $z$ / $y$ - $z$  images depicting the microneedle application site showing deposition of cargos within the cutaneous tissue (AF647-OVA – blue, AF555-OVA – red, overlay – pink, scale bar 200  $\mu$  m).



**Supplemental Figure 2** | Microneedle vaccination gives enhanced effector function. Flow cytometry analysis of inflammatory cytokine expression following *ex vivo* antigen stimulation of PBMCs. Shown are representative cytometry plots of IFN $\gamma$ <sup>+</sup>/TNF $\alpha$ <sup>+</sup> CD8<sup>+</sup> T cells measured on day 14.



**Supplemental Figure 3** | Single microneedle vaccination gives comparable cellular immunity relative to prime-boost injection. Mice were vaccinated on day 0 and 35 by i.d. injection, or on day 0 by microneedle treatment (+methanol to cross-link silk implants) to deliver 9  $\mu$ g OVA and 150 ng polyI:C. . Microneedles were fabricated with 98% of the total vaccine dose in the PAA fraction, with the remaining 2% in the silk implant (MN PAA(98)/MeOH-Silk(2)). Flow cytometry analysis of antigen-specific CD8<sup>+</sup> T cell proliferation and cytokine secretion in peripheral blood. Shown is quantitative analysis of peak SIINFEKL-tetramer<sup>+</sup> CD8<sup>+</sup> T cell frequencies for 14 days following either prime or boost immunization (left) and frequencies of IFN $\gamma$ <sup>+</sup> among CD8<sup>+</sup> T cells on day 14 post prime/boost following *in vitro* restimulation with SIINFEKL (right).



**Supplemental Figure 4** | Silk is non-immunogenic. Mice were vaccinated on day 0 by microneedle treatment ( $\pm$ methanol to cross-link silk implants) to deliver 9  $\mu$ g OVA and 150 ng polyI:C. Microneedles were fabricated with 98% of the total vaccine dose in the PAA fraction, with the remaining 2% in the silk implant (MN PAA(98)/Silk(2) and MN PAA(98)/MeOH-Silk(2)). Shown are anti-Silk or anti-OVA (representative positive responses) serum dilution curves for naïve serum and MN PAA(98)/MeOH-Silk(2) immunized animals.