ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Implantable Silk Composite Microneedles for Programmable Vaccine Release Kinetics and Enhanced Immunogenicity in Transcutaneous Immunization

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Supplemental Information for:

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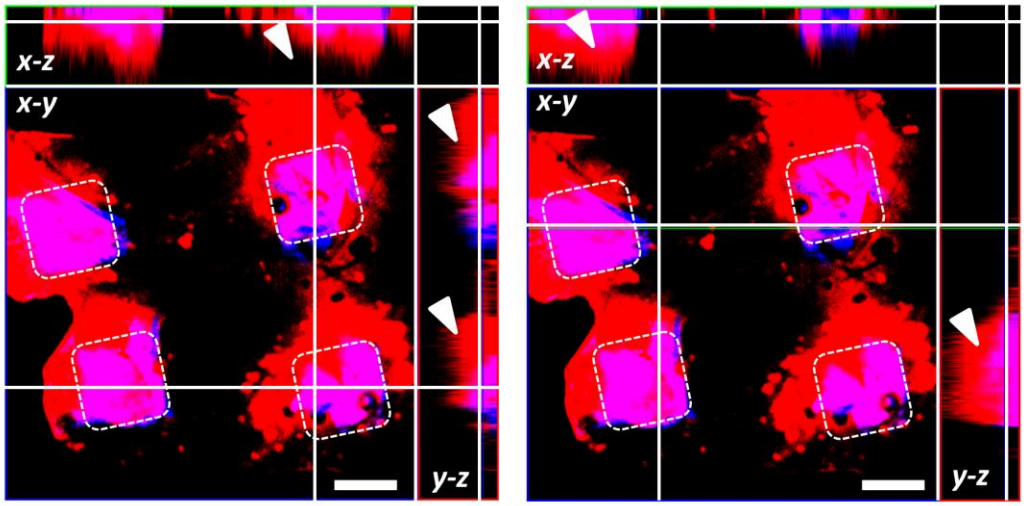
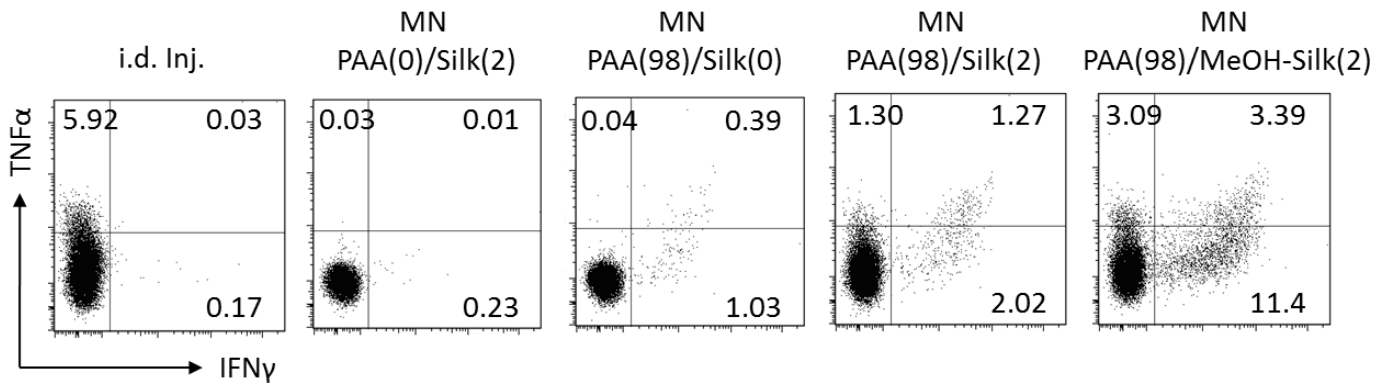
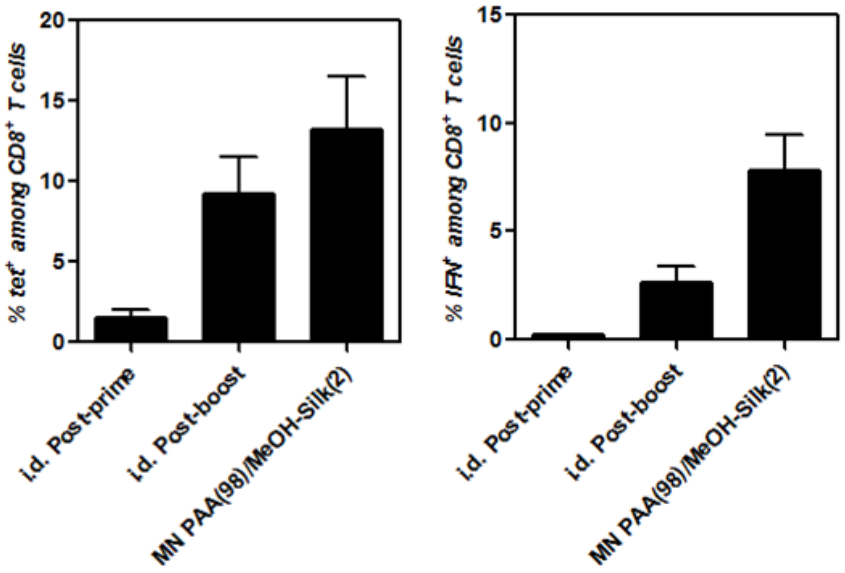


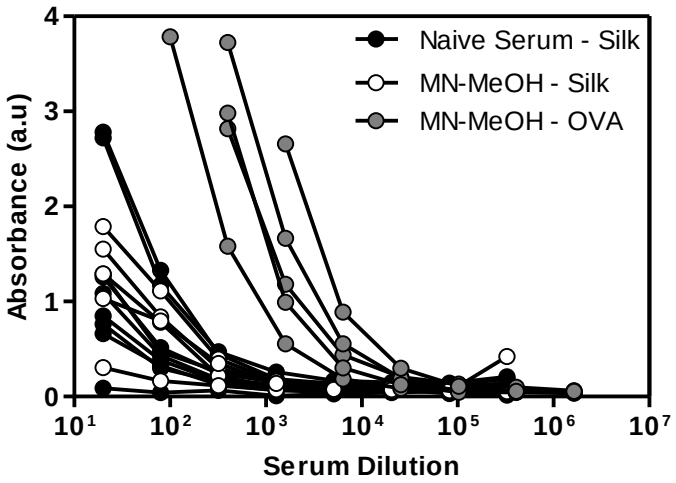
Supplemental Figure 1 | Composite microneedles give effective cutaneous delivery. Reconstructed confocal *x-y*/*x-z*/*y-z* images depicting the microneedle application site showing deposition of cargos within the cutaneous tissue (AF647-OVA – blue, AF555-OVA – red, overlay – pink, scale bar 200 μ m).



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 $IFN\gamma^+/TNF\alpha^+$ $CD8^+$ 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 μg 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 $CD8^+$ T cell proliferation and cytokine secretion in peripheral blood. Shown is quantitative analysis of peak SIINFEKL-tetramer+ $CD8^+$ T cell frequencies for 14 days following either prime or boost immunization (left) and frequencies of $IFN\gamma^+$ among $CD8^+$ 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 (±methanol to cross-link silk implants) to deliver 9 μg 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.