nature materials

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT3550

Polymer multilayer tattooing for enhanced DNA vaccination

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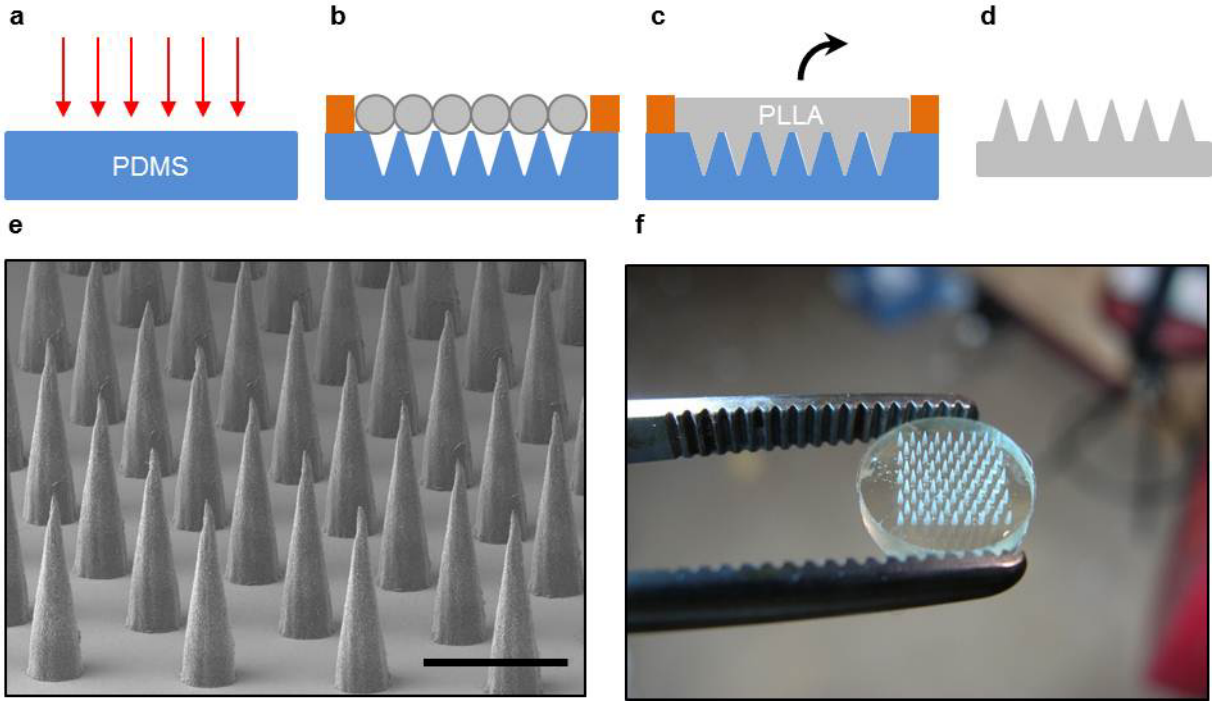
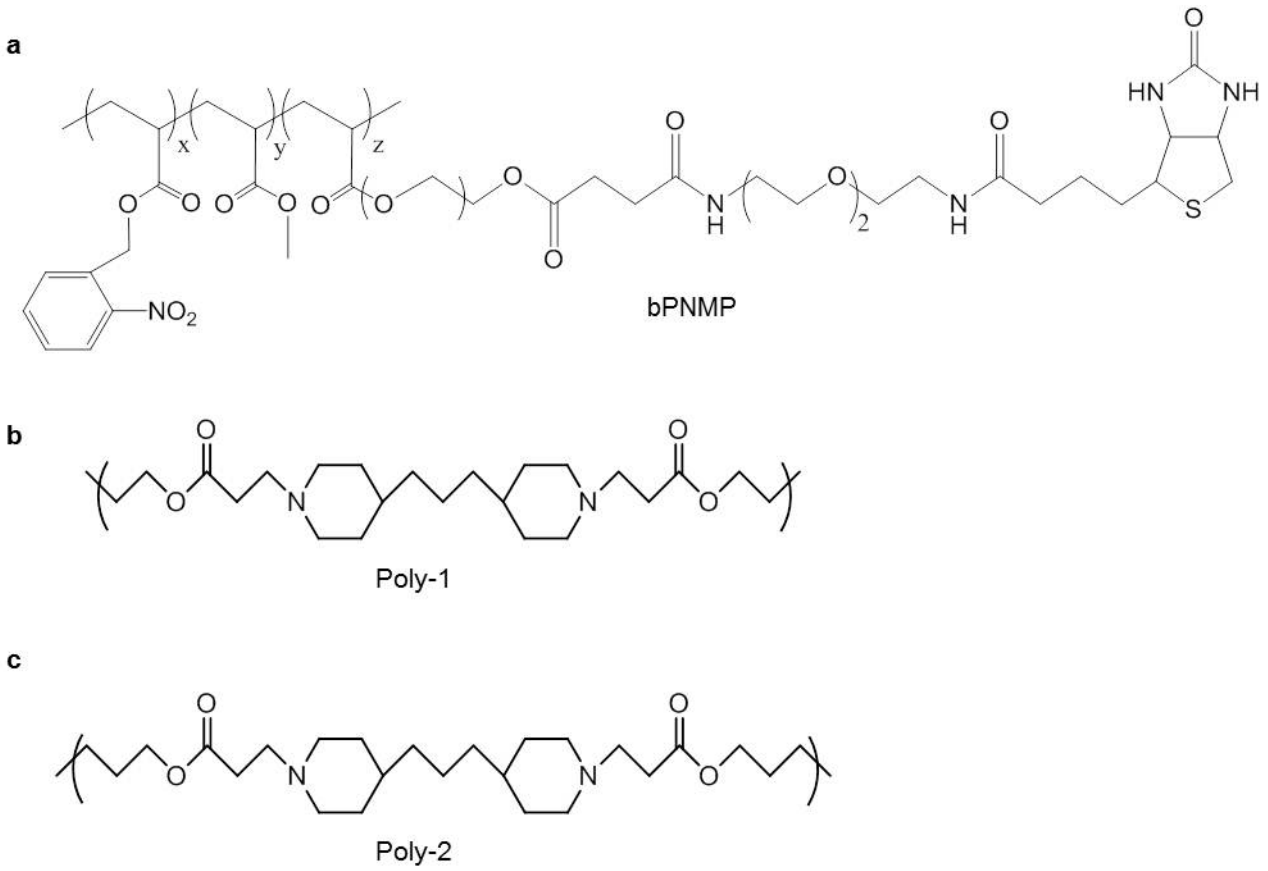


Supplementary Figure 1 Microneedle Fabrication. (a) PDMS is laser ablated to form micron-scale cavities. **(b)** PLLA is added to the surface of the PDMS mold. **(c)** PLLA is melted under vacuum and then cooled before **(d)** removal of PLLA microneedle arrays. **(e)** SEM and **(f)** optical micrograph of PLLA microneedle arrays produced through PDMS melt-casting (scale bar - 500μm).



Supplementary Figure 2 Chemical structure of polymers. (a) Structure of biotinylated-PNMP (bPNMP, MW~17,000 Da, x:y:z – 31:59:10) in which a pendant biotin is conjugated to the free hydroxyl terminus of the PEG-methacrylate monomer unit. **(b)** Chemical structure of poly-1 (MW~15,000 Da) and **(c)** poly-2 (MW~21,000 Da) used in this study.

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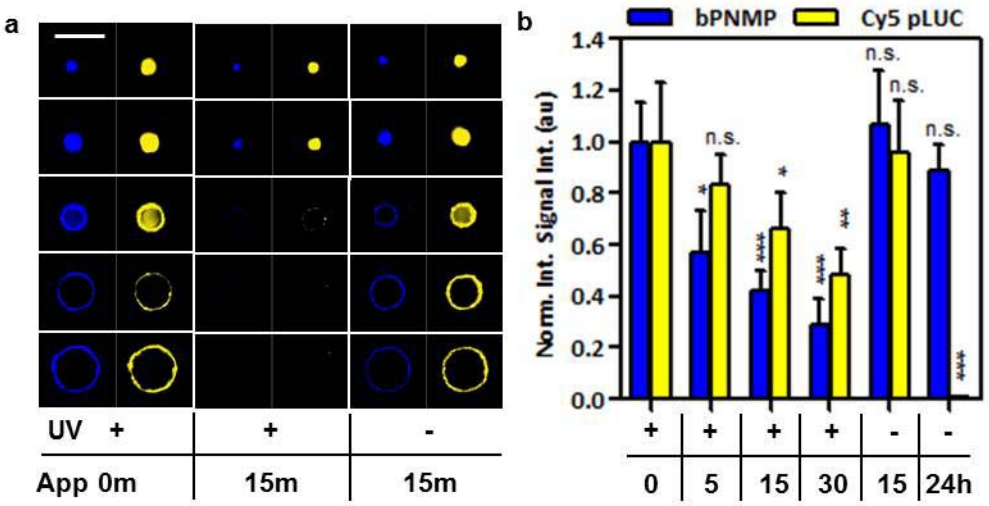
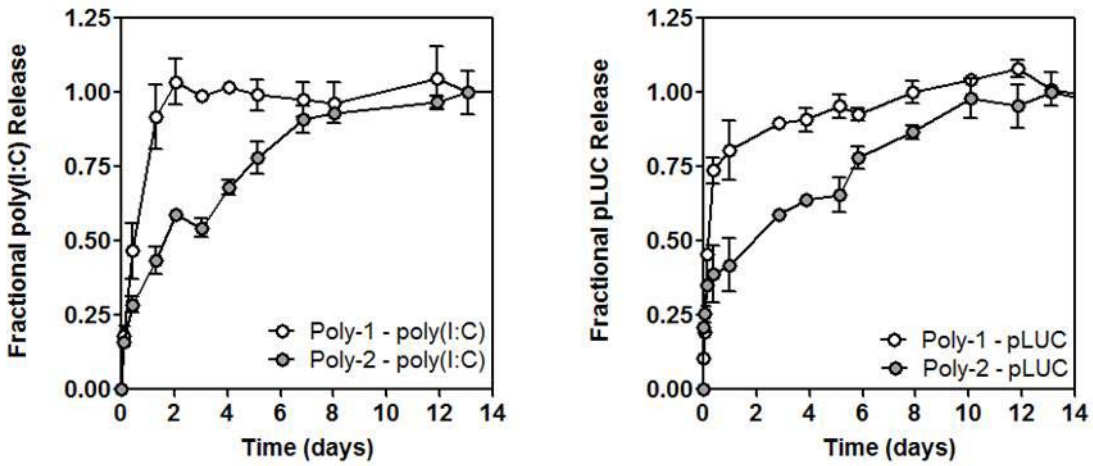


Supplementary Figure 3 Microneedle-based films rapidly delaminate *in vitro* due to release layer dissolution. (a) Representative confocal images of a (SAv488-bPNMP)(PS/SPS)$_{20}$(poly-1/Cy5-pLUC)$_{35}$-coated PLLA microneedle prepared with UV treatment of the PNMP release layer, before immersion in pH 7.4 PBS (left; lateral sections - 100µm interval; scale 200µm; blue - Sav488-bPNMP; yellow - Cy5-pLUC) and after 15 min in PBS (middle); this is compared to an identical multilayer-coated needle immersed in PBS for 15 min, where the PNMP release layer was not primed with UV prior to multilayer assembly (right). **(b)** Quantitation of confocal imaging (n = 15 microneedles) showing UV-dependent loss of SAv488-bPNMP and Cy5-pLUC signal following PBS exposure.



Supplementary Figure 4 Multilayer films control release of pLUC and poly(I:C) *in vitro.* *In vitro* release of pLUC and poly(I:C) from $(PS/SPS)_{20}(PBAE/pLUC)_{35}$ and $(PS/SPS)_{20}(PBAE/poly(I:C))_{35}$ films on silicon.

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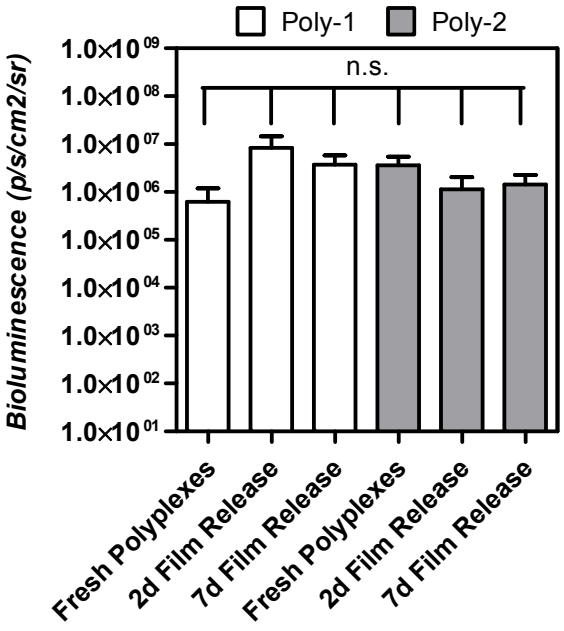
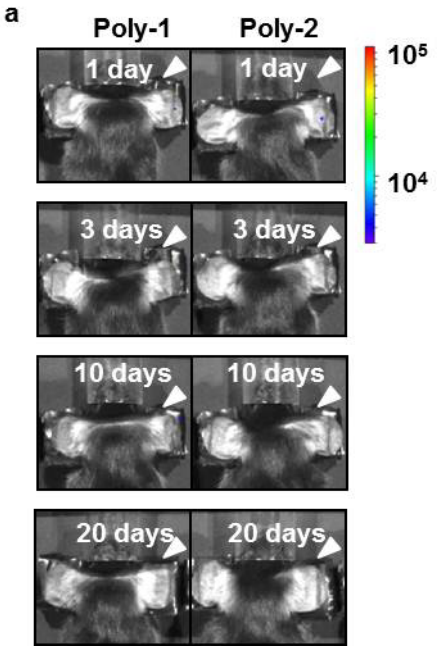


Supplementary Figure 5 pLUC released from degrading $(PS/SPS)_{20}(PBAE/pLUC)_{25}$ films produces effective luciferase expression *in vivo*. $(PS/SPS)_{20}(PBAE/pLUC)_{25}$ films were constructed using either poly-1 or poly-2 as the PBAE component. Films were then incubated in PBS at 37°C for 7 days and release fractions were collected after 2 or 7 days. Release fractions were normalized for concentration and injected intradermally (1μg) at the auricular skin. Fresh PBAE/pLUC polyplexes were formed by mixing poly-1 and poly-2 with fresh pLUC and these were similarly injected for comparison with polyplexes released from degrading $(PS/SPS)_{20}(PBAE/pLUC)_{25}$ films.



Supplementary Figure 6 (PBAE/pLUC) multilayers are not released from microneedles and give no transfection without dissolution of the PNMP release layer. Whole animal bioluminescence images of pLUC expression at application site 1, 3, 10, or 20 days following a 15 min application of (SAv488-bPNMP)$((PS/SPS)_{20}(poly\text{-}1/pLUC)_{35}$ coated microneedle array without UV pretreatment of the PNMP release layer. No bioluminescence signal was detected in the treated ears (marked by white arrows).