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Vaccine delivery with microneedle skin patches in nonhuman primates

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To the Editor

Transcutaneous drug delivery from planar skin patches is effective for small-molecule drugs and skin-permeable vaccine adjuvants.¹ However, to achieve efficient delivery of vaccines and other macromolecular therapeutics into the skin, new technologies based on skin patches bearing arrays of micron-scale projections (‘microneedles’) are under intense development.² Topically applied microneedles penetrate the stratum corneum, enabling vaccines coated on or encapsulated within the microneedles to be dispersed into the skin. Though millimeter-scale syringes have shown promise for vaccine delivery in humans³ and technologies such

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as the Derma-roller exist for creating microscale punctures in skin for delivery of solutions of therapeutics,² solid microprojection microneedles coated with dry vaccine formulations offer a number of valuable features for vaccination, including (i) reduced risk of blood-borne pathogen transmission or needle-stick injury, (ii) potential for vaccine administration by minimally-trained personnel or even self-administration, and (iii) use of solid-state vaccine formulations that may reduce or eliminate cold-chain requirements in vaccine distribution.² Recent studies have demonstrated the ability of microneedles to effectively deliver vaccines to the skin, eliciting protective immunity in mouse models of influenza, hepatitis C, and West Nile virus.² However, this approach has not yet been tested for efficacy in non-human primates, an important large-animal model thought to be more predictive of human immune responses than rodents.^{4, 5} Replication-incompetent adenoviral serotype 5 (Ad5), Ad26, and Ad35 vectors have been extensively explored in preclinical and clinical vaccine studies.⁶ Given the anatomical and immunological differences between mice and primates or humans⁷ and the common failure of small-animal models to predict vaccine success in humans,⁸ we set out to test the immunogenicity of a prototypical microneedle patch delivering an adenovirus vaccine vector in Rhesus macaques.

We employed microneedles made from poly(L-lactic acid) (PLA), a bio-resorbable polymer used in resorbable sutures, as these polymer microneedles insert effectively into skin,⁹ and would be amenable to low-cost mass production. PLA microneedle patches were fabricated through melt-molding,⁹ yielding skin patches 1 cm in diameter bearing 78 conical microprojections, each 650 μ m in height and 250 μ m in diameter at the base. Prior work has demonstrated that entrapment of vaccines in a solid sucrose or trehalose matrix imparts temperature and dehydration stability to the cargo.¹⁰⁻¹² Thus, microneedle patches were coated with Ad5 vectors encapsulated in a sucrose sugar-glass matrix. Adenovirus was thus coated by applying a 5% aqueous sucrose solution containing Ad5 vector (2.5×10^9 – 2.5×10^{11} viral particles/mL, vp/mL) and 0.01% Tween-20 surfactant to individual patches, followed by drying at 25°C under vacuum (Fig. 1a). The resulting microneedles were coated with a conformal sugar-glass layer and retained the sharp tips of the original polymer array (Fig. 1b). Preliminary testing of adenovirus-microneedle (Ad-MN) vaccines demonstrated that sucrose-coated patches effectively delivered functional virus into the skin of mice (Supplementary Figure 1), enabled storage of adenoviral vectors at room temperature for several months without loss of bioactivity (Supplementary Figure 2), and elicited systemic and mucosal immune responses in mice largely equivalent to traditional syringe injections, though modestly increased frequencies of peripheral antigen-specific central memory T-cells and increased vaginal wash IgG titers were observed in mice receiving Ad-MN compared to i.m. vaccines (Supplementary Figures 3-4).

To our knowledge the effectiveness of microneedle vaccines has not been tested in non-human primates, an important step guiding advancement to human clinical trials. We thus assessed vector delivery and immunogenicity of Ad-MN patches in rhesus macaques, an accepted preclinical model for HIV and many other infectious diseases. We first tested the efficiency of Ad5 delivery into macaque skin from microneedles: Patches were applied manually to the shaved deltoid skin of recently euthanized macaques and secured in place for 5 min. Trypan blue staining and histology of treated sites showed reliable microneedle

insertion into the epidermis (Fig. 1c-d). To assess the local bioactivity of Ad5 vectors delivered into macaque skin, we applied Ad-MN delivering 1×10^8 vp luciferase-encoding Ad5 (Ad5-LUC) to freshly explanted macaque skin *ex vivo*. Whole-tissue bioluminescent imaging of the cultured skin samples revealed equivalent strong luciferase expression for both microneedle-treated skin and skin treated by i.d. injection of a dose-matched aqueous solution, with peak expression observed on day 2 after administration (Fig. 1e-f).

To evaluate the functional immunogenicity of adenovirus delivery from microneedles, we formulated patches coated with sucrose-encapsulated Ad5 vectors encoding the model HIV antigens, SIV-gag or -env. We then applied four patches for each vector to the shaved deltoid skin of anesthetized macaques for 5 min to deliver the vaccines; animals were boosted by the same patch administration regimen at 12 weeks. No adverse reactions were noted at the application sites in any animals. As shown in Fig. 2a-c microneedle delivery of Ad vectors elicited robust ELISPOT responses against gag and env peptide pools. Gag responses to Ad-MN vaccination were comparable to dose-matched intramuscular injections following priming, and slightly exceeded syringe injection responses after boosting at week 16 (Fig. 2b). T-cell responses to env epitopes were initially weaker than those against gag but were clearly boosted following the Ad-MN vaccination at week 12 (Fig. 2c).

Intracellular cytokine staining on restimulated T-cells from blood or colorectal biopsies at week 16 showed readily detectable SIV-gag-reactive CD8⁺ and CD4⁺ T-cells, suggesting the induction of both systemic and mucosal immune responses by the microneedle vaccines (Fig. 2d). Analysis of env-specific antibody titers in serum showed the induction of gp120-specific humoral responses following priming which was further increased >10-fold following boosting (Fig 2e). Anti-env IgG was also detected in rectal mucosal secretions at week 16 (Fig. 2f). Skin patch vaccines also induced high neutralizing antibody titers against a neutralization-sensitive clone of SIVmac251 virus (Fig. 2g). We did not have ICS or humoral response data for comparison, but gag ELISPOT data from an earlier study using the same lot of Ad5-SIV-gag administered by traditional i.m. syringe injection at the same dose showed gag responses to Ad-MN vaccination were comparable to intramuscular injections following priming, and slightly exceeded syringe injection responses after boosting at week 16 (Fig. 2b).

Thus, microneedle delivery of adenovirus vaccines induces strong cellular and humoral immunity in macaques, eliciting systemic cellular responses equivalent to traditional intramuscular injection of an adenoviral vaccine and promoting both systemic and mucosal immune responses, as expected from this vector. Together with the many known practical advantages of microneedles, these results indicate the significant promise of microneedle patch vaccination to improve immunization, particularly in the developing world. Finally, the flexibility of recombinant adenoviral vectors should allow this technique to be easily translated to deliver vaccines targeting a variety of diverse pathogenic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Glenn GM, et al. Transcutaneous immunization with heat-labile enterotoxin: development of a needle-free vaccine patch. *Expert Rev Vaccines*. 2007; 6:809–819. [PubMed: 17931160]
2. Kim YC, Park JH, Prausnitz MR. Microneedles for drug and vaccine delivery. *Adv Drug Deliv Rev*. 2012; 64:1547–1568. [PubMed: 22575858]
3. Laurent PE, Bourhy H, Fantino M, Alchas P, Mikszta JA. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. *Vaccine*. 2010; 28:5850–5856. [PubMed: 20600481]
4. Langhorne J, et al. The relevance of non-human primate and rodent malaria models for humans. *Malar J*. 2011; 10
5. Morgan C, et al. The use of nonhuman primate models in HIV vaccine development. *PLoS Med*. 2008; 5:1200–1204.
6. Barouch DH, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature*. 2012; 482:89–U115. [PubMed: 22217938]
7. Godin B, Touitou E. Transdermal skin delivery: Predictions for humans from in vivo, ex vivo and animal models. *Adv Drug Deliv Rev*. 2007; 59:1152–1161. [PubMed: 17889400]
8. Shedlock DJ, Silvestri G, Weiner DB. Monkeying around with HIV vaccines: using rhesus macaques to define ‘gatekeepers’ for clinical trials. *Nat Rev Immunol*. 2009; 9:717–728. [PubMed: 19859066]
9. DeMuth PC, Su XF, Samuel RE, Hammond PT, Irvine DJ. Nano-Layered Microneedles for Transcutaneous Delivery of Polymer Nanoparticles and Plasmid DNA. *Adv Mater*. 2010; 22:4851–+. [PubMed: 20859938]
10. Alcock R, et al. Long-Term Thermostabilization of Live Poxviral and Adenoviral Vaccine Vectors at Supraphysiological Temperatures in Carbohydrate Glass. *Sci Transl Med*. 2010; 2
11. Choi HJ, et al. Stability of influenza vaccine coated onto microneedles. *Biomaterials*. 2012; 33:3756–3769. [PubMed: 22361098]
12. Martin CJ, Allender CJ, Brain KR, Morrissey A, Birchall JC. Low temperature fabrication of biodegradable sugar glass microneedles for transdermal drug delivery applications. *J Control Release*. 2012; 158:93–101. [PubMed: 22063007]

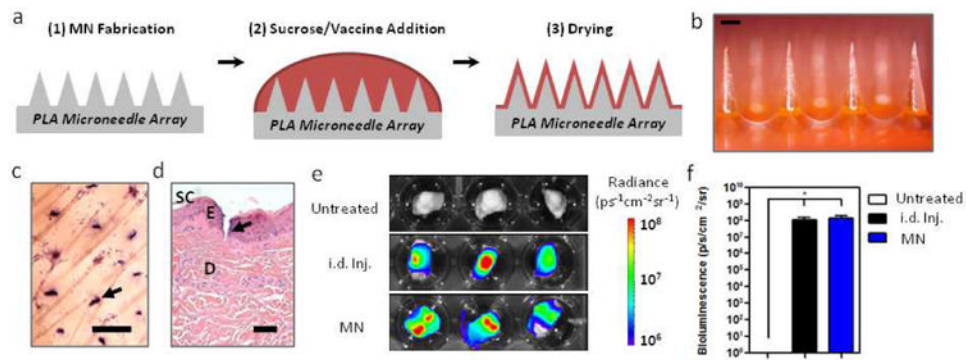


Figure 1. Fabrication and delivery of microneedle vaccines

a, Schematic of microneedle (MN) coating: PLA microneedles were fabricated by poly(dimethylsiloxane) (PDMS) molding (1), followed by application of an aqueous sucrose/Ad5 solution (2), and drying under vacuum to solidify a conformal sucrose/Ad5 coating over the MN array (3). **b**, Optical micrograph of sucrose-coated microneedles (scale, 100 μm). **c**, Optical micrograph of macaque deltoid skin surface stained with trypan blue following microneedle application (scale bar 500 μm , arrow denoting a single microneedle insertion site). **d**, Histology of microneedle-treated macaque skin showing microneedle insertion site (arrow) (SC, stratum corneum; E, epidermis; D, dermis; scale bar 200 μm). **e**, Representative whole-tissue bioluminescent images of full-thickness macaque deltoid skin (plan view), treated with 1×10^8 vp Ad5-LUC by i.d. injection or microneedle delivery. **f**, Total bioluminescent signal measured on day 2 following delivery of equivalent total doses of Ad5-LUC by i.d. syringe injection or from a MN array.

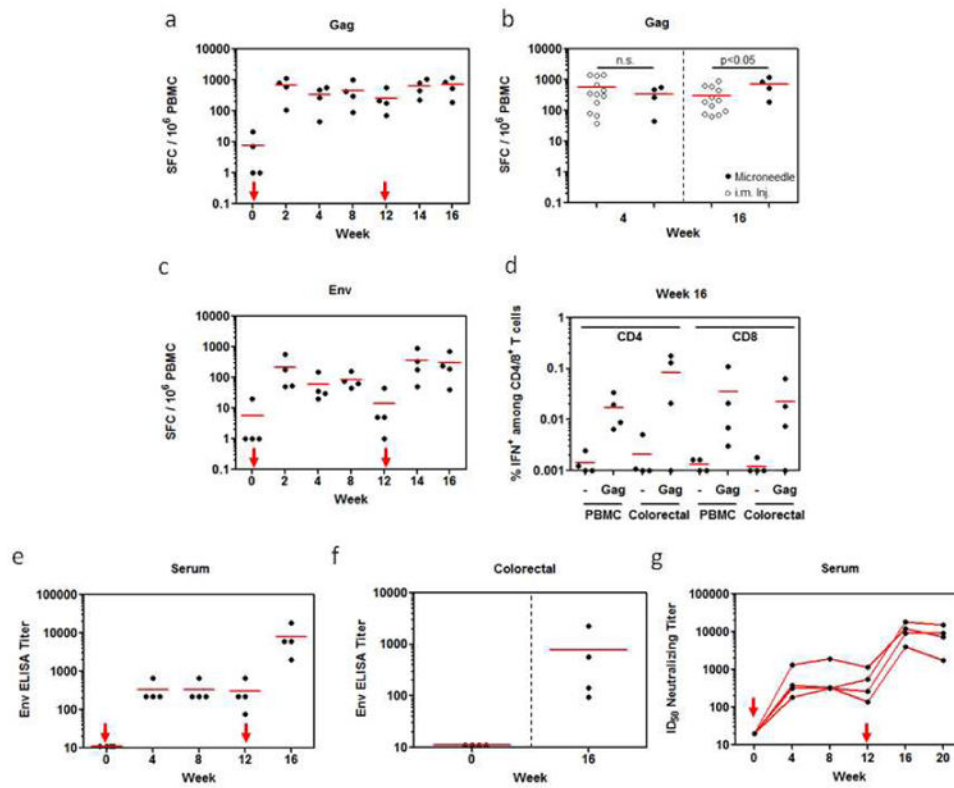


Figure 2. Microneedle patch vaccination is immunogenic in rhesus macaques

Macaques ($n = 4$) were immunized with 4×10^9 Ad5-SIV-gag vp and 7×10^9 vp Ad5-SIV-env vp by microneedle patch application for 5 min at week 0 and week 12. Results for individual animals are shown together with the arithmetic means (red lines). **a-c**, ELISPOT analysis of IFN- γ spot-forming cells (SFCs) among peripheral blood mononuclear cells (PBMCs) following *ex vivo* restimulation with SIV-gag (**a-b**) or -env (**c**) peptide pools. Results for macaques immunized in an earlier study by standard i.m. injection with the same lot and dose of Ad5-SIV-gag are compared to microneedle responses in (**b**). **d**, Week 16 flow cytometric analysis of CD8⁺/CD4⁺ IFN γ ⁺ cells isolated from peripheral blood or colorectal biopsy following *ex vivo* stimulation with media or SIV-gag peptide. **e-f**, Anti-SIV-env IgG titers in **e**, serum and **f**, colorectal secretions at week 16. **g**, Serum neutralizing antibody titer analysis from 4 individual animals for SIVmac251 homologous virus. Shown are ID₅₀ neutralizing titers.