**INTRODUCING THE NANOPATCH: A SKIN-BASED, NEEDLE-FREE VACCINE DELIVERY SYSTEM**

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**Introduction**

Over 13 million people die from infectious diseases every year (1). Whilst public and private research initiatives continue to develop novel vaccines for many diseases, the issue of how best to formulate, package, distribute and administer these vaccines across the world remains a significant unsolved problem. Most vaccines have been delivered by the needle and syringe, however this technology has several important disadvantages including needlestick injuries, disease transmission through needle reuse (2), limited thermostability, the need for training/expertise for administration, lack of targeting to immune-rich regions of the body, and the issues of pain/phobia that result in avoidance of medical care in ~10% of the population.

MicroProjection Arrays (MPAs) can overcome some of these challenges and have thus far shown promising results in terms of immunogenicity and protection both in preclinical and Phase 1 human trials. As described below, we have recently extended the field with an ultra-high density projection array – the Nanopatch – to deliver vaccine into the epidermis and dermis, the skin layers rich in antigen-presenting cells (APCs). Key advantages of its design result in improved immune responses (with 100th of the dose required by the needle and syringe into muscle) and greatly improved thermostability in comparison to needle/syringe delivery, enabling better suitability for applications in developing countries and broad applicability across a range of different vaccine types.

**The Problem**

Approximately 95% of people who die from infectious diseases reside in resource-limited areas (1). Many of these diseases could be prevented through widespread distribution of currently available vaccines. Effective and widespread distribution of vaccines in these areas is limited by breakdowns in refrigeration (‘cold chain’), lack of trained personnel for safe vaccine administration, needlestick injuries resulting from cross-contamination or needle sharing, and the phobia associated with needle pain. Indeed, even in developed countries, approximately 10% of people actively avoid medical care due to needle phobia. While development of new and improved vaccines will continue into the future for a range of diseases, the only way to meet the resource challenges worldwide is to engineer better ways of administering vaccines in a simple and effective way to more people than is currently the case (3).

Hypodermic needles were first introduced in the 1850s and since then, intramuscular injection of vaccines has been the most popular delivery method due to its ease of administration (4). However, this technology has several disadvantages. Firstly, muscle contains relatively few APCs, which are important in generating protective immune responses. Secondly, the stability of vaccine formulations is heavily dependent upon environmental factors such as temperature and humidity, with costs in maintaining the cold chain potentially sufficient to vaccinate a further 10 million children if refrigeration were not required (5).

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**Fig. 1. Comparison between three key modes of vaccine delivery via the skin.**

A. Intramuscular injection, in which payload is delivered intramuscularly below the skin’s epidermis/dermis.
B. Intradermal injection, in which payload is delivered into the dermal cavity via the Mantoux method.
C. Nanopatch, which rapidly releases dry-coated vaccine payload in the vicinity of APCs in the epidermis/dermis.

LC = Langerhans cells; dDC = dermal dendritic cells; APC = antigen-presenting cell.
Finally, needle-based vaccine delivery in low-resource regions requires qualified medical practitioners to reduce needlestick injuries, unsafe injections and biological waste exposure (2). Clearly, there is a pressing need for new vaccine delivery tools that are needle-free, pain-free, simple and inexpensive to distribute and use, with improved efficacy or reduced dose per person, in order to vaccinate more people per dollar.

Skin as an Alternative Target Organ for Vaccine Delivery

The skin is the largest organ in the human body and the first line of defence against most opportunistic pathogens. It is composed primarily of three layers (Fig. 1) (6): the stratum corneum (SC; 9–13 µm deep), viable epidermis (VE; 50–135 µm) and the dermis (>50–135 µm). The SC is composed of corneocytes encased in a lipid-rich matrix, resembling a ‘bricks-and-mortar’ physical barrier. The VE is largely composed of keratinocytes (95%), along with melanocytes (8%) and Langerhans cells (2–5%, LCs). The LCs are a type of APC, presenting foreign materials to the adaptive immune system to generate appropriate antibody and cellular immune responses. The dermis is highly vascularised, containing dermal dendritic cells (dDCs), which are also capable of initiating antigen-specific immune responses, and hosting nerve fibres, glands and hair follicles, originating deep within the tissue. The combination of physical barrier and potent immune responses provides the basis of the front-line defence functions of the skin. Both of these aspects also present a unique challenge for vaccination: the need for shallow and precise targeting of vaccine antigens to the APC-rich regions while accounting for the complex mechanical properties of the individual skin layers.

Physical Methods for Targeting Vaccine to the Skin

There are several emerging approaches using physical methods to deliver vaccines through the skin, taking advantage of the abundance of resident APC populations. Passive methods including topical application of creams/lotions are effective for small, lipophilic compounds that traverse the SC. However, delivery of vaccines (typically > 0.1 MDa) is ineffective via this route (7). Intradermal injections (e.g., ‘Mantoux method’; Fig. 1B) are known to elicit improved antibody responses in comparison to the intramuscular route. Difficult to administer accurately, even for highly trained clinicians, this is not in widespread use in vaccine administration (8). Needle-free techniques include liquid- or gas-powered jet injectors that ballistically deliver vaccine (either in liquid or solid particle form) at > 100m/s into the skin, achieving comparable immune responses (and in some cases, improved responses) to the intramuscular route (9). While these techniques have taken important steps in using the skin as a site for vaccination, issues including pain, targeting variability due to different skin type and other complexities remain as challenges.

MicroProjection Array Technology

MicroProjection Arrays (MPAs) form part of the push for physical targeting of vaccines to the skin. The key concept is to fabricate arrays of microprojections to pierce the tough SC layer and rapidly deliver vaccine payloads to the epithelia. The first reports on the fabrication and testing of silicon arrays for transdermal delivery appeared in the late 1990s (10), and since then rapid expansion of fabrication methods has occurred. Low density (1–100 projections/cm²) and medium density (100–5000/cm²) arrays have now been fabricated from silicon, metal, polymer and ceramic materials, generally in the size range of 0.3–3 mm in length. Key design parameters include the shape, density, length, and tip sharpness of the projections, along with the application velocity, as these directly affect the skin penetration depth achieved by the array. Furthermore, coating strategies aim to optimise formulations for long-term thermostability (e.g., incorporating ‘glassy’ sugars to stabilise biomolecules when dried), rapid release of vaccine payload following skin insertion, high release efficiency, and the potential for process scale-up. The methods, along with their comparative advantages and disadvantages, are reviewed thoroughly elsewhere (11).

Compelling data have been published showing that MPAs can elicit protective immune responses in comparison to standard intramuscular or subcutaneous injection in animal models (11). While pre-treatment of skin with MPAs followed by topical vaccine application produced inconsistent results, vaccine-coated MPAs invoke consistent immune responses in mice, often exceeding standard delivery routes. Influenza vaccine administration has been the most investigated test-case using coated projections (i.e., MPAs), showing protective immunity in comparison with the needle, on the basis of total IgG antibody levels, hemagglutination inhibition titres and neutralising antibody activity. Results include enhanced immune responses in comparison to intramuscular injections and dose reduction in which only 1/10th of the dose was required for equivalent IgG responses (as measured by ELISA) and long-term immune responses even six months after vaccination. To date, clinical trials have shown early promise both in terms of pain reduction and immunogenicity in comparison to standard intramuscular injections for a range of approved and emerging vaccines.

The Nanopatch

While early results in pre-clinical and clinical trials investigating MPA technology are encouraging, key challenges of achieving significant dose reduction (leading to significant improvements in vaccine distribution and availability) and significantly improved thermostability remain. Our group designed the Nanopatch (Fig. 2), an ultra-high density MPA with dry-coated vaccine, to target the dendritic cell populations within the epithelia with the aim to meet this need. Using multi-photon microscopy to determine APC density in mice (e.g., LCs; 500/mm², located in a layer ~14.9 µm beneath SC (12)), combined with a custom-designed applicator and biomechanical
analyses of the skin (13), it was hypothesised that targeted delivery of vaccine to thousands of APCs in both the viable epidermis and dermis – without widespread cell death – would improve immunogenicity in comparison to the needle and syringe (14). Deep Reactive Ion Etching (DRIE) of silicon chips allowed us to overcome previous limitations and produce very high density (>20,000/cm²) arrays with short (30–300 µm) and sharp projections (15). In a key proof-of-concept study, Nanopatches indeed targeted ~50% of available APCs in mice (both in the viable epidermis and dermis), requiring less than 100th of the standard intramuscular dose required for equivalent protective immune responses using an influenza-based mouse model (14).

We then sought to explore broader applications of the Nanopatch by investigating a range of different vaccines, developing novel coating technologies and formulations to support long-term thermostability. Using a novel jet-coating approach, the Nanopatch technology has been used to deliver a range of different vaccines, including inactivated whole virus vaccines (e.g., FluVax® – commercially available seasonal influenza vaccine (14)), virus-like particles (e.g., Gardasil® – commercially available tetravalent human papilloma virus vaccine (16)), DNA plasmids (e.g., pre-clinical herpes simplex virus 2 vaccine (17)) and other compositions, using the same jet-coating procedure. In the case of FluVax, only ~100th of a standard intramuscular dose delivered via the Nanopatch was required to produce equivalent protective immune responses as determined by total IgG measured by ELISA and hemagglutination inhibition assays. More recently, we demonstrated that co-delivering FluVax® with the adjuvant Quil-A in a mouse model improved this level of dose reduction from 100-fold to 900-fold (18). We also demonstrated long-term thermostability of our dry-coated Nanopatch vaccines, showing comparative immunogenicity with freshly coated devices or those coated and stored for over six months at 23°C prior to skin application (19).

**Novel Applications for the Future**

Delivery of drugs to the skin is only one potential application of Nanopatch technology. The collection and processing of whole blood prior to immunoassays contributes to the high cost, complexity, long turnaround time and expertise required to perform diagnostic tests for diseases. Rapid screening in infectious diseases is a key concern, and in the future, rapid screening to aid personalised treatment of complex diseases (e.g., cancer, diabetes, cardiovascular disease) is likely to benefit from advances in this area. The skin is highly vascularised, with a blood vessel density ranging from 20–40 vessels/mm² at a depth of 58-65 µm (6). In our group, we have recently introduced the ‘Micropatch’, which, based on a similar MPA concept, is designed to selectively capture blood-borne disease biomarkers from the skin for applications in rapid diagnostics (20,21).

**Conclusions**

In conclusion, new technologies are urgently required to overcome challenges in distributing vaccines to those who need them, especially in the developing world. Needle-free vaccination via the skin offers key advantages in comparison to standard needle and syringe methods. These include targeting of APCs in the skin dermis and epidermis and enhanced thermostability through dry coating technology and reduced doses per application, enabling more people to be vaccinated. The Nanopatch has shown ~100-fold dose reduction for FluVax® vaccinations in comparison to standard intramuscular injections in pre-clinical animal studies. We are now focussed on progressing this technology through clinical trials.

**References**


![Fig. 2. The Nanopatch, a needle-free vaccine delivery system, magnified under scanning electron microscopy at A. 200-fold, B. 500-fold and C. 1200-fold magnification. These images show the individual projections of the Nanopatch, arranged in an ultra-high density array (>20,000 projections/cm²), with high aspect ratio and tips with sub-µm sharpness (15).](image-url)