ABSTRACT SUMMARY

We introduce the Nanopatch – a practical needle-free device that targets a narrow layer just below the skin surface rich in antigen presenting cells for improved vaccination over the needle and syringe (which instead primarily targets the muscle). Case studies of vaccination are presented, including Nanopatch delivery of influenza vaccines inducing immune responses comparable to needle and syringe delivered vaccine but at a much lower delivered dose (x150).

INTRODUCTION

Efficiently and safely delivering biomolecules to the skin’s immunologically sensitive cells holds the promise of advancing radical new drugs, vaccines and immunotherapies for major diseases. The application of practical, commercially-viable physical methods to the achievement of this goal presents unique engineering challenges in the physical transport of genes and drugs to skin sites.

In this presentation, we outline research into physical targeting methods to meet this goal, using arrays of micro-nanoprojections on patches (termed “Nanopatches”; prototype shown in Figure 1a and 1b).

We begin by analysing the skin target, choosing the delivery to Langerhans cells and dermal dendritic cells (and associated cells) of dermis/epidermis for immunotherapeutics as a pertinent case study. The location of Langerhans cells are presented together with an analysis of key skin mechanical properties. Briefly, current physical approaches for targeting these cells are introduced, together with a discussion of their effectiveness and limitations as practical delivery devices in the immunotherapy of major diseases.

This presentation then focuses on the Nanopatch technology. The of arrays of projections—on a patch—accurately, efficiently and safely deliver biomolecules not just to specific skin cells, but also to organelles within them. Conceptually, the delivery device is a set of needles (of microscale length with nanoscale tips), coated with drug substance and applied to the skin as a small patch. The patch is pain-free and needle-free. By eliminating the cold-chain, it is applicable to developing world vaccinations. We will present Nanopatch configurations, coating approaches and key immunology in the skin generated by delivery.

Finally, we will provide an overview of resultant vaccinology progress of Nanopatch delivery for both conventional and DNA vaccines. Examples will include the delivery of Ovalbumin and also a commercial influenza vaccine (Fluvax 2007) – achieving equivalent immune responses as the needle and syringe, but with orders of magnitude of dose reduction. The dose reduction achieved, as well as the possibility of self-applicability of the influenza vaccine coated Nanopatch may be of great utility in a pandemic situation.

EXPERIMENTAL METHODS

Nanopatches were synthesised from silicon using a process of Deep Reactive Ion Etching in Rutherford Appleton Laboratory, Oxford, UK according to patent¹. The Nanopatches are solid silicon, sputter coated with a thin layer of gold (400-1500 nm in thickness). An individual patch is 5x5 mm in size and the central 4x4 mm area contains 3364 densely packed MNPs. The distance between the centres of adjacent MNPs is 70 µm. The morphology of MNP patches and coating were characterized by a JEOL scanning electron microscope 6400.

The Nanopatches were coated with a generic approach applied to all the tested vaccines. Here, we describe briefly the coating procedure applied influenza vaccines as just one of the test cases. Briefly, 6-8 microliters of coating solution, containing methylcellulose and influenza vaccine (Fluvax 2007®), was applied onto each individual patch. Then a gas-jet (6-8 m/s) was used to evenly distribute the coating solution on the patches and quickly dry coating excipients on micro-nanoprojections.

We describe here the vaccinology approach – again selecting influenza as just one example of the vaccines described in the full paper. Four groups of four C57BL/6J female mice aged 6 to 8 weeks were vaccinated with influenza vaccine either intramuscularly using the conventional needle and syringe at different doses, or onto the ventral side of the ear skin using coated patches. One patch per ear was used in the vaccinations (i.e. a total of 2 patches per mouse). Two vaccine coated Nanopatches (one on each inner ear lobe) were applied to mouse with a spring device which ejects the Nanopatch to the skin with a velocity of 2 m/s. The patch was kept for 15 min for the vaccine to release in the dermis/epidermis. The vaccine amount delivered by coated Nanopatches was determined by a Dot-blot assay². After 63 days, mice were bled and sera collected. The procedures described herein were approved by the University of Queensland Animal Ethics Committee.

To measure the resultant systemic immune response to vaccination, we assayed the sera from vaccinated mice (by Nanopatch, and separately, control mice vaccinated with the needle and syringe) for hemagglutinin inhibitory activity against the individual vaccine component strains as a measure of functional anti-HA antibody, which correlates with protective effectiveness³.
RESULTS AND DISCUSSION

In Figure 1, we introduce the Nanopatch at different magnifications – showing both the uncoated and coated projections – and also the penetration profiles in skin following Nanopatch application.

Nanopatches are arrays of densely-packed silicon projections (>10000 /cm²) invisible to the human eye (~100 µm in length, tapering to tips < 1000 nm; Fig. 1b) and they are coated with vaccine in dry form and applied to the skin as a small patch (Fig. 1c). The coated patches can pierce skin for subsequent vaccine release (Fig. 1d).

Figure 1 Prototype of Nanopatch (a), scanning electron microscopy image of Nanopatch before (b) and after (c) vaccine coating; the coated Nanopatch was applied to the skin and Cryo-SEM was used to visualize the skin during Nanopatch application (d). In fig. 1d, SC: stratum corneum, E: viable epidermis, D: dermis.

We will also present data: quantifying the consistency of projection penetration into the skin; and three-dimensional multi-photon microscopy images of the co-localized delivery of vaccine with the skin’s population of antigen presenting cells. We will then discuss the resultant quantification of the direct targeting (e.g. influenza vaccine is delivered to 40% of Langerhans cells within the target site).

With our established highly-effective targeting of skin immune cells, we then tested the hypothesis that Nanopatch delivery of influenza vaccine will allow significant dose reductions compared to conventional needle-syringe vaccinations. Figure 2 summaries the results – obtained with one of the three influenza vaccine strains (Wisconsin A). Clearly, Nanopatch delivery of 0.04 µg HA induced hemagglutination inhibition (HI) levels significantly greater than those generated by 0.8 µg HA delivered by intra-muscular injection (p=0.024), and equivalent to 6.0 µg delivered by intra-muscular injection (p=0.357). We will present data for all three strains – all with the same findings.

These data show the Nanopatch achieves a surrogate for vaccination protection against the influenza vaccine, when only 150th of the dose delivered with the conventional needle and syringe is deposited in the skin.

To our knowledge, this delivered dose-sparing gain for a conventional vaccine is an order of magnitude greater than achieved by any other delivery method (without the reliance of an added adjuvant). The next step is testing if these Nanopatch dose-sparing benefits are successfully achieved in humans (with a range of diseases). If we do achieve this success, then the Nanopatch will potentially transform vaccines, superseding the needle and syringe with a new practical technology that achieves protection with only a fraction of the dose and is expected to be easily transported (without refrigeration) to the patient to self-administer.

Figure 2. Hemagglutinin Inhibition assay (HI) was performed using the sera at different dilutions against Wisconsin A.

CONCLUSION

In summary, we introduce the Nanopatch as a new technology designed “from the ground up” to target the skin’s immune system for better vaccines. We present key data from vaccination test-cases to show this is achieved in practice – in the case of influenza, inducing immune responses comparable to needle and syringe delivered vaccine but at a much lower delivered dose (x150).

REFERENCES


ACKNOWLEDGMENTS

This work was supported by Australian Research Council, National Health and Medical Research Council and the Queensland Government (Smart State scheme), Australia.