ABSTRACT SUMMARY

Our skin holds great promise as a site for improving vaccines and the detection of infectious disease. Key to this is both its immunological function (e.g. an abundance of immune cells), and its external proximity – providing ease of access for practical devices.

Here I present approaches based upon an ultra-high density array of projections on a patch (called a Nanopatch) designed to meet both the vaccine delivery and diagnostics challenges. These approaches are assessed against standard needle-based methods using disease test cases in the live animal model. The pathway to clinical translation is also discussed.

INTRODUCTION

Efficiently targeting the skin’s immunologically sensitive cells holds the promise of advancing (1) immunogenicity of vaccines (through delivery); (2) diagnosis of disease (through extraction of disease biomarkers). The application of practical, commercially-viable physical methods to the achievement of these goals presents unique challenges.

This paper focuses on the Nanopatch concept to meet both of these challenges.

First, the vaccine delivery application: targeting antigen directly to several thousand skin immune cells for improved immunogenicity – compared to existing delivery methods. Then, I will explain how this targeting is achieved in practice, with a delivery device that is a set of projections (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. I will present Nanopatch configurations, coating approaches and demonstrate that Nanopatches deliver antigen directly to thousands of skin APCs. The following outcome results will be reported:

(a) resultant vaccinology progress of Nanopatch delivery (e.g. influenza vaccination and 100 fold dose-reduction compared to intramuscular injection; and improved immunogenicity of HPV vaccines).

(b) Stability data confirming Nanopatches do not require refrigeration during storage and transportation.

Then, my focus will switch to using similar geometry devices to extract biomarkers from skin for non-invasive and rapid detection of disease. I will introduce the idea and then present key findings achieved by extracting antibodies (influenza) and, separately, disease proteins (dengue NS1).

EXPERIMENTAL METHODS

Vaccination: The Nanopatches (4x4 mm bearing 3364 projections, 110 µm in length; Fig 1) are fabricated from silicon by a well-established technique pioneered by the semiconductor industry – called Deep Reactive Ion Etching (DRIE): offering large scale manufacture at a low relative cost.

Figure 1. The Nanopatch projection array.

One of the key challenges for vaccine delivery to skin by these microprojections is coating the surface with vaccine — because of their extremely high packing density. We set about meeting this need by developing a gas-jet drying coating method to coat vaccines on the very small and densely packed microprojections. The patches are applied to the ears of live mice at a velocity of 2 ms⁻¹, and held in place for 2
minutes, in which the vaccine dissolves and diffuses in the skin.

**Diagnostic extraction:** The disease diagnostic biomarker extraction device makes use of the same silicon patch geometry and applicator – with the key difference being the silicon projections are covalently attached with capture antibody, using EDC/NHS chemistry. Patches were held on the skin for 5 minutes, and then placed into wells for ELISA processing.

In this study, we targeted dengue fever detection (NS1 protein) as a test case. BALB/c mice were then injected intravenously with NS1 as a pseudomodel of disease, and we successfully captured NS1 from these mice using noninvasive sampling/extraction on the Nanopatch.

**RESULTS AND DISCUSSION**

**Vaccination:** Using a trivalent split virus influenza vaccine (Fluvax) – directly compared the immunogenicity and protection generated by Nanopatch delivery compared to standard intramuscular injection. **Figure 2** summarizes the results, showing that over 100 times less antigen was needed to be delivered to the skin to achieve an equivalent response when compared with intramuscular injection by needle and syringe. This represents a marked improvement – an order of magnitude greater than reported by others – without reliance on an added adjuvant and with only a single vaccination. In my presentation, I will extend by reporting on improved immunogenicity generated by Nanopatch application with other antigens (e.g. HPV) and thermostabilization data (12 months storage at room temperature, with no loss in vaccine activity).

**Diagnostic extraction:**

**Figure 3** shows titrated serum level of NS1 detected by the Nanopatch selective biomarker extraction from the skin of live mice. Detection was achieved in the mouse injected with 300 μg of NS1, which at the time of sampling had the circulating serum level 8 μg/mL. Sampling of NS1 at this concentration is consistent with levels detected in clinical serum/plasma samples. I will discuss this data, and extend into presenting successful extraction of antibodies from skin and their collective importance for practical devices for the diagnosis of infectious disease within the field.

**CONCLUSION**

A microprojection array-based patch method has been developed (Nanopatch), in which the dynamic application leads to penetration into the skin’s rich immune cell environment. This approach has been successfully applied to achieve targeted vaccine delivery (with significantly improved immunogenicity) and, separately, selective extraction of biomarkers for the diagnosis of disease. The next step is translating these approaches to practical devices for human use.