An Alternative Diagnostic Method Using Microneedles For Sampling The Immune System *In Situ*

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Friday, May 19th, 2017

ABSTRACT

Current protocols for immune system monitoring involve the collection of cells from blood or cerebrospinal fluid. However, since major populations of immune cells reside within tissues, these invasively-obtained body fluid samples are, at best, indirect indicators of the status of the immune system. Direct tissue sampling through biopsies is difficult to incorporate into long-term, repetitive, longitudinal immune monitoring. Whereas delayed-type hypersensitivity tests (e.g., Mantoux tuberculin test) query the presence of antigen-specific cells in the skin, but do not provide information about the phenotype and functional characteristics of responding immune cells.

Here we present a technology that addresses several of these challenges simultaneously, with the synergistic goals of providing enhanced diagnostic methods for sampling and analyzing the function of the immune system, and providing a greater insight into the status of the immune system than state-of-the-art assays. We designed hydrogel-coated, immune-monitoring, sampling microneedles that are capable of sampling non-recirculating immune cell populations present in the skin and permitting the quantification of biomarkers present in collected dermal interstitial fluid, thus enabling the parallel monitoring of both cellular and humoral immune responses.

We focused, first, on optimizing the materials for fabricating sampling microneedles with the requisite properties of mechanical integrity and robustness, reproducible fabrication, effective skin penetration, ability to include bioactive cell-signaling molecules in the MN sampling platform and a compartment within the platform for sample collection and retention. Next, we utilized two animal models: an immunization model in which mice were vaccinated with model antigen ovalbumin, and an infection model in which mice were infected, via tail-skin scarification, with vaccinia-virus expressing SIVgag. We established that including adjuvants and antigen as cargo in lipid nanocapsules embedded in the hydrogel coating of the microneedles elicit the recruitment and sampling of not only antigen-specific cells, but also non-recirculating tissue resident memory cells. In both models, we demonstrated that even at long times post antigen exposure, sampling microneedles consistently recruited for higher proportions of antigen-specific cells than those present in blood. Finally, we also showed that the dermal interstitial fluid collected via sampling microneedles, could be reliably quantified for biomarkers such as antigen-specific IgG.

The technology of sampling microneedles allows *ex vivo* analysis of cells retrieved directly from the local tissue environment and enables the investigation of antigen-specific cells for diagnostic purposes as well as answering spatio-temporal questions related to immunology in local tissue environments. This simple, painless and minimally-invasive sampling approach should facilitate longitudinal monitoring of antigen-specific immune cell populations in the skin relevant for a variety of infectious and autoimmune diseases, and aid the process of vaccine design.

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