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Controlled release of a model vaccine by nanoporous ceramic microneedle arrays

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ABSTRACT

Current vaccination technology can advance from the use of novel ceramic nanoporous microneedle arrays (npMNA), where the material serves as a storage reservoir for vaccines. Moreover, npMNA will enhance vaccine efficacy by more precisely reaching skin dendritic cells, the kickstarters of T and B cell immunity. In the present study we assessed the efficacy of vaccination using npMNAs by *in vivo* application of $OVA_{257-264}$ peptides mixed with agonistic anti-CD40 antibodies as adjuvant. The induction of OVA-specific CD8$^+$ T cells *via* npMNA was comparable with the frequency induced *via* intradermal injection using needle-syringe. However, only when expanding the vaccination area by using two npMNAs the frequencies of induced IFN-γ-specific effector CD8$^+$ T cells were comparable with those induced *via* needle-syringe injection. Analysis of vaccine release from npMNA in a human *ex vivo* skin explant model revealed that $OVA_{257-264}$ peptides were indeed delivered intradermal, and release also increased by prolonging the npMNA application time on the human skin. Together, our studies demonstrate the potential of npMNA for vaccine delivery in human skin and *in vivo* induction of CD8$^+$ effector T cell responses.

1. Introduction

CD8$^+$ T cell responses are important in the immune defense against tumors and viral infections. Vaccination using tumor- or virus-derived antigens is a powerful way to induce immunity against these anomalies, inhibiting the disease progression due to induction of specific cytolytic effector T cells (CTLs) (Appay et al., 2008). Dendritic cells (DCs) are key cells in this process as these provide essential signals to naive CD8$^+$ T cells that induce their activation and subsequent differentiation into CTLs. Upon activation DCs present antigen-derived peptides in MHC molecules, express costimulatory molecules and secrete pro-inflammatory cytokines, all necessary for induction of effective CTL responses (Janeway and Medzhitov, 2002; Banchereau et al., 2000). To accomplish DC activation, vaccines contain adjuvants. Besides the classical adjuvants (*e.g.*, aluminum-based or oil-in-water emulsions), also synthetic Toll-like receptor ligands and agonistic antibodies that bind DC-activating molecules, such as CD40, are currently being explored as adjuvant (Mbow et al., 2010; Nicholls et al., 2010; Vonderheide and Glennie, 2013). The agonistic anti-CD40 antibody triggers the maturation of DCs which subsequently leads to the priming of antigen-specific CD8$^+$ T cells. Using combinations of melanoma antigen-encoded peptides and agonistic anti-CD40 antibody successful induction of tumor-specific T cell responses have been achieved in preclinical models (Fransen et al., 2011) and are currently tested in patients with both hematologic cancers and solid tumors (Vonderheide et al., 2001; Bensinger et al., 2012; Byrd et al., 2012).

With a dense network of DCs and draining lymphatics, the skin provides an ideal portal for vaccine delivery. Multiple DC subsets are present in skin, including DCs in the dermis and Langerhans cells (LCs) in the epidermis (Haniffa et al., 2012; Klechevsky et al., 2008; Segura et al., 2012). DCs in the skin are easily accessible for vaccination purposes. Indeed, by using an *ex vivo* human skin model, intradermal applied vaccines were shown to be taken up by skin DCs and to enhance CTL immune responses (Fehres et al.,

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2013). Moreover, intradermal vaccination has shown significant advantages with respect to dose-sparing and immunogenicity in comparison to other routes (Kenney et al., 2004; Quan et al., 2010). While vaccines containing classical adjuvants are administered intramuscularly or subcutaneously, as intradermal administration of these vaccines likely causes local irritation, induration, skin inflammation and granuloma formation (Wahl and Hermodsson, 1987; Vogelbruch et al., 2000), the use of novel adjuvants, like agonistic anti-CD40, facilitates the use of the intradermal route as they potently trigger the activation of skin DCs. Furthermore, while vaccines containing the classical adjuvants predominantly induce neutralizing antibodies and are successfully used to prevent microbial infections, the novel types of adjuvants additionally trigger potent $CD4^+$ helper T cell- and $CD8^+$ cytotoxic T cell-mediated immunity, which makes them suitable for therapeutic treatment of chronic infections and cancer. Moreover, the elicitation of effector $CD4^+$ helper T cells not only optimizes the quality and durability of antibody responses, they also aid the generation and function of effector $CD8^+$ T cells to lyse intracellular pathogens and tumors.

However, intradermal vaccination using standard needle and syringe is technically challenging and inaccurate administration of vaccines can even result in adverse side effects. A promising technical alternative to standard needle and syringe injection is the use of microneedle arrays (MNAs) to deliver vaccines into the skin (Prausnitz et al., 2009; Prow et al., 2010; Kim and Prausnitz, 2011; van der Maaden et al., 2012; Verhoeven et al., 2012; Kim and Sharpless, 2012). Intradermal vaccination with MNAs has shown significant advantages compared to vaccination using standard needle and syringe. Due to their highly defined but ultra-small geometry, MNAs can go into the skin at very low insertion forces and controlled insertion depth, facilitating effective and pain-free application of vaccines as pain receptors are not reached. Moreover, the number of injection sites is increased using MNAs compared to a single needle injection site, which enables simultaneous delivery of the vaccine to more DCs (Fig. 1). Different approaches of MNA technologies have all been confirmed feasible. For example, initial comparisons demonstrated the use of MNAs as very efficient for insulin delivery in type 1 diabetes patients as well as influenza vaccination or delivery of anti-TNFα antibodies in animal studies (Gupta et al.,2009; Nordquist et al., 2007; Harvey et al., 2011; Levin et al., 2014; Norman et al., 2014).

Amongst other approaches by using MNAs for delivery, solid MNAs have been used as a pre-treatment device to create temporary pores through the stratum corneum *via* which the applied vaccine can enter the skin (van der Maaden et al., 2012). However, this strategy requires multiple steps. In case of vaccination, where only a relatively low dose is required, another approach for delivery is to apply a dry coating that contains the antigen directly at the top of the surface of a solid MNA (Gill and Prausnitz, 2007; Chen et al., 2009; Kim et al., 2010; McGrath et al., 2011). On the other hand, not all vaccines may be administrated in a dry formulation and ease-of-use should be facilitated by a reservoir-integrated skin interface that allows microneedle-guided transport of the vaccine while inserted in the skin. For such liquid formulations, this approach can be realized, for example, with so-called hollow out-of-plane MNAs, which are defined by a back plate seamlessly integrated with microneedles vertically protruding from the surface of the back plate (Gardeniers et al., 2003). Various hollow MNA designs and their fabrication technologies have been developed. The fabrication of such hollow MNAs can be performed by using combinations of several advanced microelectronics processing techniques or more classical routes of precision engineering and assembly (Gardeniers et al., 2003; van der Maaden et al., 2014; Indermun et al., 2014).

Instead of miniaturizing the concept of a traditional needle by hollow microneedles, a more elegant method will be the manufacture of seamlessly integrated MNAs with reservoir function directly from a nanoporous material. Such a material encloses a vaccine compound in its interior structure and yet allows the controlled release thereof upon application of the microneedles into the skin due to molecular gradient exchange such as differences in concentration and osmotic pressure. An MNA with these design features can act as a nanofluidic portal in the skin. To realize nanoporous (np)MNAs, Bystrova et al. developed an out-of-plane MNA mold fabrication process and replication technique with well-defined ceramic slurry (Bystrova and Luttge, 2011). The most distinguishing feature of this novel npMNA is its generic cargo reservoir, formed by an intrinsic nanoporous structure, in combination with chemical stability and mechanical robustness of the material. Subsequently, the nanopores can contain a dry or liquid compound formulation. Using MyLife Technologies ceramic npMNA technology, we previously demonstrated the delivery of antibodies to human skin DCs (Verhoeven et al., 2012). However, it is not known how the use of these novel ceramic npMNAs for vaccine application impacts the induction of T cell-mediated immunity. We therefore expanded our initial studies (Verhoeven et al., 2012) and tested these novel npMNAs *in vivo* by applying $OVA_{257\text{-}264}$ peptide mixed with agonistic anti-CD40 antibodies to study the induction of OVA-specific effector $CD8^+$

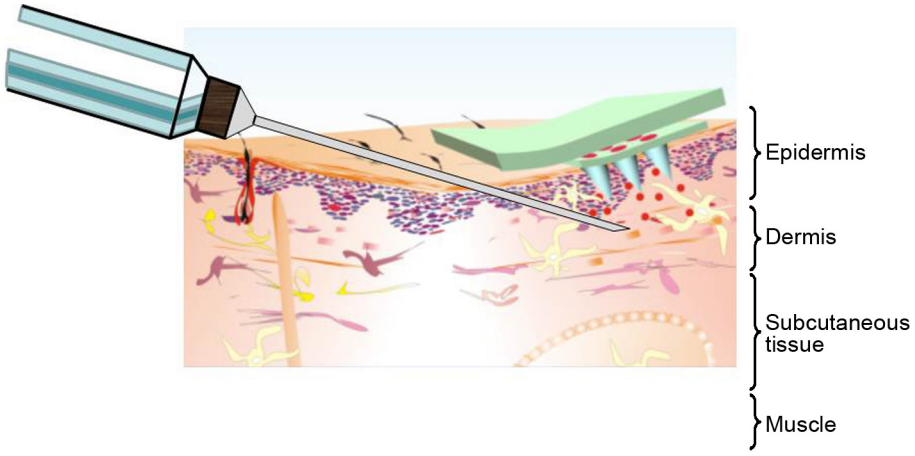

Fig. 1. Schematic representation of intradermal vaccination with MNA versus standard needle and syringe injection. The antigen presenting cells LCs and DCs are present in epidermis and dermis layers of the skin, respectively. These cells are ideal for inducing antigen-specific immune responses *via* antigen vaccination strategies. Because of multiple injection sites and ease-of-use, MNA are a promising alternative to needle and syringe.