Development of hyaluronan-based dissolving microneedle arrays for dermal vaccination

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Chapter 1

General introduction, aim and outline of this thesis

GENERAL INTRODUCTION

Vaccination

Vaccination is one of the most effective healthcare interventions lowering mortality and morbidity and as a result increasing life expectancy [1]. The introduction of vaccines led to the eradication of smallpox, while eradication of polio is on its way and other important infectious diseases such as measles are under control in vaccinated populations [2]. Although many successes can be related to effective vaccines and vaccination programs, each year almost 3 million children still die of diseases caused by infections that could be prevented by vaccination [2]. A large proportion of deaths occurs in low-income countries [1]. Vaccination costs related to the production, transport, need for cold-chain and for trained personnel capable to administer the vaccine all contribute to total system costs, making vaccination too expensive for developing countries [3, 4]. A reduction in the vaccination coverage is also related to avoidance of injections because of fear for pain, needle-phobia and stress experienced during vaccination [5]. To overcome these problems, research on a minimally invasive strategy of vaccine administration has been addressed focusing on nasal [6], oral [7], pulmonary [8] and intradermal route of vaccination [9]. Intradermal vaccination has been considered particularly attractive because the skin is easy to access and a very immune competent organ containing antigen presenting cells (APCs) as Langerhans cells (LCs) and dermal dendritic cells (dDCs) [10, 11], making the skin an attractive vaccination site. However, the skin presents a barrier located in the upper layer, the *stratum corneum*, that protects the body against the entrance of foreign substances [12]. An effective method to enable vaccine administration via the skin is the use of microneedles (MNs), conceived for the first time by ALZA corporation and described in a patent in 1976 [13].

Microneedles

MNs have a size of up to 1 mm in length. They are designed to pierce the physical barrier, the *stratum corneum*, and to avoid the generation of pain because the MN tips do not reach pain receptors in the skin [14-16]. A further potential advantage of MNs consists in reducing the vaccination costs by i) avoiding the need of trained personnel to be applied [14, 17] and ii) resulting in a dose-sparing potential as the skin is a potent immune competent organ [18-21].

Generally, MNs are classified in six groups [22-25]:

i) Solid MNs designed for pretreatment of the skin. After the formation of microholes in the skin, the MNs are removed and a vaccine loaded patch is applied on the pierced skin to allow the diffusion of the vaccine along the conduits into the skin. Although a very straightforward method, the diffusion of the vaccine from the skin surface to and through the microholes made by the MNs is slow and inefficient due to the small diameter of the microholes and the limited number of microneedles in the patch. Thus, over a reasonable time

period, only a small fraction of the applied vaccine is delivered into the skin. Furthermore, the microholes may close soon after the removal of the MNs limiting the diffusion time [26]. To avoid this inconvenience, new MN systems have been developed including hollow, coated, porous, dissolving and hydrogel-forming MNs.

ii) Hollow MNs contain a bore through which the vaccine formulation can be injected into the skin. One of the advantage of using hollow MNs is that the skin depth and injection volume and injection rate can be precisely controlled [15]. However, one big challenge is to avoid leakage due to the short needle length inserted in the skin. Furthermore, dry formulation need a reconstitution step previous injection [17]. Additionally, the use of harmful chemicals, as hydrofluoric acid and concentrated sulfuric acid, may be included in the production process of hollow MNs [27, 28].

iii) Coated MNs have the vaccine coated on their surface and upon insertion the vaccine is released into the skin. Although only a very low amount of vaccine can be coated, due to the limited surface area of MNs, this may be sufficient to evoke a protective immune response [29]. Coatings are usually applied by: i) dip coating method [30, 31], ii) gas jet drying approach producing a thin coating and thus reducing vaccine wastage [32] or iii) layer-by-layer coating approach in which the intended amount of vaccine can be coated by adjusting the number of layers [33-35].

iv) Porous MNs with the vaccine deposited in a porous MN matrix release the vaccine by diffusion of the vaccine in solution from the pores into the skin after piercing. However, porous structures are generally more fragile than solid structures [36] and may easily break remaining in the skin. This may be a problem when the matrix is made of no-biodegradable material as silicon [37, 38]. Therefore, biodegradable materials are preferred such as microporous calcium phosphate coating stainless steel solid MNs [39].

v) Hydrogel-forming MNs that after insertion into the skin take up interstitial fluids from the tissue that triggers the release of the vaccine into the skin. These MNs are prepared from crosslinked polymers that rapidly take up skin interstitial fluid upon skin insertion to form continuous hydrogel conduits from the array to the dermal microcirculation. With this approach, the delivery of vaccine is controlled by the crosslink density of the hydrogel system [40].

vi) Dissolving MNs are made of hydrophilic material, such as polymers or sugars, mixed with the active pharmaceutical ingredient. After insertion into the skin, the MNs can completely dissolve thereby releasing the vaccine.

This thesis focuses on dissolving microneedles (dMNs). They have the advantage to completely dissolve in the skin avoiding sharp needle waste left behind after use [17] and thus avoiding potential infections due to the needle re-use especially in developing countries. Moreover, for vaccine in solid state, as for coated and dissolving MNs, it may be

possible to circumvent the need for a cold-chain to keep the antigen stable during storage and shipping [30].

Microneedle application

Microneedles can be applied onto the skin either manually or by the use of an applicator. The manual application, however, especially for short MNs (< 550 μm), may results in both a low penetration efficiency in the skin and a low reproducibility of skin piercing [41, 42] and therefore an applicator is required for this type of microneedles for a controlled application [43] in terms of penetration force or velocity.

AIM AND OUTLINE OF THIS THESIS

The aim of this thesis was to develop dissolving MNs for dermal vaccine delivery. The study objectives include:

1. An overview of dMNs for a proper understanding of the dMN subject present in the following research chapters;

2. The use of a digitally-controlled applicator as a tool to investigate mechanical variables and their effect on insertion efficiency of a wide variety of MNs in the skin and consequent delivery of the antigen in the skin;

3. The optimization of dMN fabrication and investigation of antigen loading capacity of dMNs;

4. The evaluation of the effects of the molecular weight of hyaluronan (dMNs matrix material) on the immune response for a proper choice in the fabrication of dMNs;

5. The determination of the effects of different antigen(-adjuvant) administration modalities by dMNs, such as single dose or repeated fractional doses administration, on the immune response.

Chapter 2 provides a review of the current status of dMN development. This includes a screening of the materials used for the fabrication of dMNs, a description of the dMN manufacturing methods and an analysis of the parameters that must be evaluated to assess dMN quality. Furthermore, this chapter describes the immunogenicity of antigens administered by dMNs both in preclinical and in clinical studies.

The research described in *Chapter 3* focuses on the investigation of penetration efficiency and antigen release in the skin after MN application by the use of a digitally-controlled

applicator for a reproducible piercing of the skin. To this end, different application settings were set digitally in the applicator to determine the optimal insertion parameters for a variety of MN arrays from different manufacturers.

In *Chapter 4*, optimisation of the manufacturing of dMNs is described. A novel dMN fabrication method was developed and the effects of ovalbumin loading on dMNs physicochemical properties, such as dMN appearance, antigen stability after dMN fabrication, penetration efficiency of dMN in the skin, dissolution time of dMN in the skin, quantification of the amount of antigen delivered in the skin after dMN dissolution and mechanical integrity of dMNs after storage, were investigated. Finally, immunogenicity of OVA delivered by dMNs was determined in mice and compared with the immunogenicity of OVA administered via the conventional subcutaneous route.

The selected base material for the dMNs studied in this thesis is high molecular weight hyaluronan (HA). In the literature examples of immune modulating properties of low-molecular weight HA have been reported. The effect of HA molecular weights (HA-MWs) on the immune response against OVA are described in *Chapter 5*. After assessment of potential immune-modulatory effects following immunization with fluid formulations containing HA with different molecular weight, the suitability of HA-MWs to fabricate mechanically robust and functional dMNs was assessed.

Even though dermal immunization is in general very effective, adjuvants may be needed to optimise immune responses either quantitatively or qualitatively. Therefore, in *Chapter 6* an adjuvant screening for dermal vaccination was performed by vaccine microinjections using hollow MNs. Diphtheria toxoid was used as antigen. After selection of two promising adjuvants, dMNs loading diphtheria toxoid (DT), in the presence or absence of adjuvants, were fabricated and characterized *in vitro* and *in vivo*. In addition to a single full dose immunization, the effect of administering several fractional doses in several days was tested.

Chapter 7 provides a summary of the main findings of this thesis and the future prospects of dMNs-based immunization are discussed.

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