Intradermal delivery of nanoparticulate vaccines using coated and hollow microneedles

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Chapter 1

General introduction, aim and outline of this thesis

General introduction

Vaccination is the most effective tool to fight against infectious diseases [1]. With vaccination smallpox was eradicated in 1979, which had killed millions of people in 20th century and before [2]. The incidence of other devastating diseases such as polio, tuberculosis and tetanus has substantially declined owing to the routine vaccination programs [3]. However, there is still a need for new and better vaccines against infectious diseases [4]. Nowadays, in addition vaccination gains increasing attention for therapeutic use against established diseases, such as cancer and chronic auto-immune disorders [5].

Traditional vaccines are derived from attenuated organisms or inactivated pathogens. These vaccines can induce potent immune responses, but safety issues including the administration of potentially harmful components and reversion to virulent forms have restricted their application. Nowadays, subunit antigens containing only antigenic parts of a pathogen are being extensively investigated because of their better safety. However, they are generally less immunogenic than traditional vaccines. In order to improve their immunogenicity, immune modulators and nanoparticle delivery systems can be used [6-9].

Microneedle technology for vaccine delivery

Most vaccines are administered by intramuscular or subcutaneous injection, but these methods can cause pain and stress due to the fear of injection [10]. Furthermore, there is a risk of infection due to the reuse of needles especially in developing countries [11]. Lastly, the delivery of vaccines to antigen presenting cells may be inefficient as these delivery sites are not rich of antigen presenting cells.

As an alternative, intradermal delivery has gained attention because of its potential for needle-free administration. Additionally, the skin is easy to access and contains a large number of antigen presenting cells, such as Langerhans cells in epidermis and dendritic cells in dermis, making the skin an attractive site for vaccination [12]. However, the stratum corneum, the uppermost layer of the skin, forms the main barrier and prevents most foreign substances from entering our body. To effectively overcome the skin barrier, microneedles have been developed [13]. Microneedles are micro-sized needle structures which can be used to penetrate the skin in a non-invasive and pain-free way, as they only pierce the upper layers of skin without touching the deeper nerves and blood vessels [14].

Microneedles were first used for pretreatment of the skin [15]. After the conduits were made and the microneedles were removed, the vaccine-loaded patch or formulation was applied onto the microneedle-penetrated skin. However, the diffusion of vaccines through the microneedle-made conduits is slow due to the small diameter of the conduits and small surface area of the pretreated skin. As a result, only a small fraction of the antigen is delivered into the skin. Furthermore, the conduits may close soon after the removal of the microneedles, limiting the time available for diffusion [16]. To increase the delivery efficiency of vaccines and avoid the separate application of vaccines after the removal of the microneedles, several types of new microneedle systems have been developed in the past twenty years, including coated, hollow and dissolvable microneedles [13, 14, 17, 18].

In case of coated microneedles, antigens are coated onto the surface of microneedles. After the microneedles are inserted into the skin, the antigen should be quickly released. One disadvantage of using coated microneedles is that the coating amount of antigen is limited due to the small surface area of microneedles. At the same time, there could be a waste of antigen during the coating process, as the coating efficiency is normally much lower than 100%.

Several methods have been reported for the coating of antigen on microneedles. Gill et al. described a dip coating method and found that the surface tension and viscosity of the coating solution had a major influence on the uniformity and thickness of the coating, respectively [19, 20]. Chen et al. proposed a gas jet drying approach to reduce vaccine wastage and produce a thinner coating of antigen [21]. In our group, we developed pH-sensitive microneedles by modifying their silicon surface with pyridine groups. The microneedles can adsorb negatively charged antigens at slightly acidic conditions and release them at neutral pH [22]. Finally, in some studies a layer-by-layer coating approach was used, in which the amount of coated antigen can be tailored by adjusting the number of coating layers [23, 24].

Dissolvable microneedles are made of water soluble polymers or low-molecular-weight sugars and the antigen is loaded inside the solid microneedle matrix [25]. When the microneedles are inserted into the skin, they start to dissolve, thereby releasing the antigen [17, 25]. The advantage of using dissolvable microneedles is that there is no sharp waste after the application of the microneedles. The mostly used method for fabrication of dissolvable microneedles is micromolding [25]. The micromolds are first filled with an aqueous polymer/sugar solution, and through evaporation of the solvent the polymer/sugar will be solidified to form the microneedles [26]. Recently, efforts have been made to load vaccines only in the tips of dissolvable microneedles, aiming to maximize the delivery/release of the loaded antigen during the application of microneedles [27, 28].

Hollow microneedles contain conduits through which the liquid formulation of vaccines can be injected into the skin. The advantage of using hollow microneedles, compared to coated and dissolvable microneedles, is that the injection volume, rate and depth can be precisely controlled [14, 29]. Furthermore, there is no need to optimize the loading process of vaccines in the microneedles and in case of liquid vaccine formulations the formulation buffer does not have to be developed. A disadvantage is that dry vaccine formulations need to be reconstituted in water or buffer before injection [13]. Hollow microneedles can be made from metal, polymer or silicon [13]. Davis et al. reported a laser micromachining method in which nickel was coated onto a polymer mold made by laser drilling. The prepared hollow microneedles were released by etching the polymer mold [30]. In our group a method was developed to prepare hollow microneedles by etching fused silica capillaries with hydrofluoric acid [31].

Microneedles can be penetrated into skin manually or by using an applicator. As the elastic skin will stretch and deform when being pressed, the manual application may result in a low and variable penetration efficiency of microneedles [32]. By using an applicator, the microneedles can be applied with a fast, controlled rate with the penetration force precisely controlled. However, the disadvantage is that a sophisticated and expensive applicator needs to be developed.

Nanoparticles for vaccine delivery

Nanoparticle delivery systems have been extensively studied for vaccination, as they are able to enhance the immunogenicity of antigens by protecting them from degradation, increasing their uptake by antigen presenting cells and co-delivering them with adjuvants [8]. Furthermore, the immune responses can be potentially modified by tuning the properties of nanoparticles such as size, surface charge, loading and release of antigens [33]. Different types of nanoparticles have been studied for vaccination, such as polymeric nanoparticles, liposomes, inorganic nanoparticles and emulsions [7, 8, 33-38].

Polymeric nanoparticles are prepared from synthetic or natural polymers such as poly(lactic-co-glycolic) (PLG), poly(lactic-co-glycolic acid) PLGA, chitosan and gelatin [39]. The most often studied polymeric nanoparticles are PLGA nanoparticles and the antigens are normally encapsulated inside the PLGA matrix. The antigens will be released from the nanoparticles during the PLGA degradation process. Liposomes are made of lipids and the antigens can be adsorbed on the surface of liposomes, loaded in the core or incorporated in between the lipid bilayers. Polymeric nanoparticles and liposomes have been extensively investigated for vaccine delivery because of their excellent biocompatibility and biodegradability. Inorganic nanoparticles have been studied for the delivery of vaccines because of their rigid structure and excellent thermo stability. Recently, mesoporous silica nanoparticles (MSNs) have gained increasing attention for vaccine delivery because of their excellent biocompatibility and multiple options for surface functionalization. Additionally, the large pores and surface area of MSNs allow for efficient loading of relatively large amounts of antigens [40, 41].

Studies have shown that the physicochemical characteristics of nanoparticles such as size, surface charge and release property of antigens have an important influence on the immunogenicity of the encapsulated antigens. Nanoparticles with a size below 200 nm have been shown to be more efficiently taken up by dendritic cells [33, 42]. Nanoparticulate vaccines with a positive zeta potential have been reported to enhance the activation of antigen presenting cells and the subsequent immune responses [43]. Besides, the sustained release of antigen together with the depot effect of nanoparticles on cell surface could allow longer interaction of antigen with the antigen presenting cells [6, 44].

An increasing number of studies focuses on the use of nanoparticles for co-delivery of antigen and Toll-like receptor (TLR) ligands [46-54]. These ligands can function as pathogen-associated molecular patterns and selectively bind to the TLRs of antigen-presenting cells, thereby enhancing the immune responses. Among different types of TLR ligands, poly(I:C) and CpG, which are ligands for TLR3 and TLR9 respectively, have been extensively investigated. Both of these adjuvants are capable of enhancing Th1/$CD8^+$ T cell responses [9]. Recent reported studies have shown that antigen and poly(I:C)/CpG co-encapsulated nanoparticles elicited superior Th1/$CD8^+$ T cell responses compared to mixture of antigen and adjuvant solution [46, 48-54]. The co-delivery of antigen and immune modulator to the same antigen presenting cells is likely responsible for this [47, 52].

Combination of microneedles and nanoparticles for intradermal delivery of vaccines

The intradermal delivery of nanoparticulate vaccines was first studied by using traditional hypodermic needles [46, 48, 50, 53-56]. Some studies have shown that the intradermal injection of these vaccines induce stronger IgG2a [54] or $CD8^+$ T cell responses than subcutaneous injection [56]. Other studies further have shown that the antigen and adjuvant co-encapsulated nanoparticles induce higher IgG2a titers [46, 53] and $CD8^+$ T cell responses [50] compared to antigen and adjuvant solution after the intradermal delivery by hypodermic needles.

Nowadays, researchers are trying to combine the utilization of microneedles and nanoparticles for intradermal delivery of vaccines. Initially, microneedles were used for pretreatment of skin and the nanoparticles were applied onto the pretreated skin after removal of the microneedles. Microneedle-assisted administration of nanoparticle vaccines has been shown to induce stronger immune responses than antigen solution [57, 58]. However, the reported results were conflicting. Another study showed that antigen-loaded liposomes did not enhance immune responses compared to antigen solution in microneedle pretreated mice, most likely

because the transport of the nanoparticles in the conduits made by microneedles was limited [59].

To improve the delivery efficiency of nanoparticulate vaccines, coated, hollow and dissolvable microneedles were used [24, 49, 60, 61]. For coated microneedles, the processes of the encapsulation of antigen into nanoparticles and the coating process of nanoparticles onto microneedles need to be optimized, in order to reach a sufficient antigen dose coated on the microneedles. Demuth et al. described the coating of antigen-loaded lipid nanocapsules onto PLGA based microneedles by using a layer-by-layer coating approach [24]. The coated multilayers were rapidly released into the skin after the application of microneedles into the skin, resulting in a balanced response of multiple IgG isotypes, whereas the immunization with soluble antigen only induced a weak IgG1-biased immune response. In case of dissolvable microneedles, it is important to study whether the loading of nanoparticles in the microneedles does not affect the properties of dissolvable microneedles, such as the mechanical strength or dissolution rate of microneedles in the skin. It has been reported that antigen-loaded PLGA nanoparticles were successfully encapsulated into methylvinylether and maleic anhydride (PMVE/MA) based dissolvable microneedles [60, 61]. The prepared microneedles were able to penetrate the skin and dissolve quickly within 15 min. The dissolvable microneedles loaded with antigen encapsulated PLGA nanoparticles induced higher Th1/$CD8^+$ responses than antigen solution [60, 61]. In case of hollow microneedles, a suspension of nanoparticulate vaccine can be directly injected into the skin. Siddhapura et al. reported the use of hollow microneedles for intradermal injection of tetanus toxoid -loaded chitosan nanoparticles into mice. This was found to induce a higher IgG2a response and stronger expression of Th1 cytokines than a commercial vaccine delivered intradermally [62].

From the above it can be concluded that microneedle-mediated intradermal delivery of nanoparticulate vaccines is a promising approach for effective intradermal vaccination. However, there are no systematic studies reported yet focusing on 1) optimization of microneedle-based systems for the delivery of nanoparticulate vaccines, 2) the effect of physicochemical characteristics of nanoparticles on the immunogenicity of encapsulated antigens after microneedle-mediated intradermal vaccination.

As described above, coated microneedle arrays with pyridine modified pH-sensitive surface and hollow microneedles prepared from fused silica capillaries have been developed in our lab and used successfully for intradermal delivery of various protein antigens [14]. The results of the immunization studies have shown that the microneedle mediated immunization groups induced comparable immune responses as compared to subcutaneous or intramuscular group [22-23, 31, 63-65]. Therefore it is attractive to combine these microneedle approach with nanoparticles to improve ithe immunogenicity of the antigens.

Aim and outline of this thesis

The aim of this thesis is to determine whether a combination of microneedles and nanoparticles can be used to elicit potent immune responses after intradermal administration and whether the immune response can be tailored by the choice of the nanoparticles. For these purposes, two types of microneedles, namely coated and hollow microneedles, and several types of nanoparticles, covering a broad range of physicochemical parameters, were used.

In Chapter 2, the development of a new type of MSNs for the encapsulation of ovalbumin (OVA) is described. Additionally, the coating of OVA-loaded MSNs onto pH-sensitive microneedle arrays was investigated. In Chapter 3, another type of nanoparticles, namely PLGA nanoparticles, are used for the encapsulation of OVA and an adjuvant (poly(I:C)). T

cell responses induced by these nanoparticles after hollow microneedle-mediated intradermal immunization in mice were studied. In Chapter 4, hollow microneedles are used to study the effect of nano-encapsulation of OVA and poly(I:C) on humoral and cellular immune responses. In this study, four types of nanoparticles, namely MSNs, PLGA nanoparticles, liposomes and gelatin nanoparticles, were compared. In Chapter 5, hollow microneedles are used to examine the effect of encapsulation manner of diphtheria toxoid (DT) and poly(I:C) in liposomes on the antibody responses in mice. In Chapter 6, the antibody responses of DT-loaded MSNs after coated and hollow microneedle-mediated intradermal vaccination are compared. In Chapter 7, the main findings of the studies described in this thesis are summarized and discussed. The future prospects of using microneedles for intradermal delivery of nanoparticulate vaccines are briefly discussed.

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