Targeting the Skin for Microneedle Delivery of Influenza Vaccine

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Abstract

Influenza infection represents a major socioeconomic burden worldwide. Skin represents a new target that has gained much attention in recent years for delivery of influenza vaccine as an alternative to the conventional intramuscular route of immunization. In this review we describe different microneedle vaccination approaches used in vivo, including metal and dissolving microneedle patches that have demonstrated promising results. Additionally we analyze the immunological basis for microneedle skin immunization and targeting of the skin's dense population of antigen presenting cells, their role, characterization, and function. Additionally we analyze the importance of inflammatory signaling in the skin after microneedle delivery.

Keywords

Microneedles • Skin immunization • Influenza • Dendritic cells • Langerhans cells

13.1 Influenza Virus and Influenza Vaccination

13.1.1 Influenza Virus and Disease

Influenza virus represents one of the most common respiratory viral pathogens and is a major cause of morbidity and mortality worldwide [1, 2]. The virus is responsible for annual epidemics of influenza with seasonal outbreaks in the USA from October through April. The CDC estimates that more than 200,000 hospitalizations in the USA are attributed to influenza infection, annually [3, 4].

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P.D. Katsikis et al. (eds.), *Crossroads Between Innate and Adaptive Immunity IV*,
Advances in Experimental Medicine and Biology 785, DOI 10.1007/978-1-4614-6217-0_13,

In several cases, the magnitude of lung inflammation and respiratory distress can lead to serious complications and even death. It is estimated that more than 40,000 deaths in the USA alone are related to influenza infection or complications following the infection [5–7], while the number of deaths associated with influenza infection account up to 1.5 million worldwide [8–10]. The World Health Organization (WHO) estimates that each year 10–20% of the world's population is being infected by influenza virus [11]. Seasonal influenza infection can affect all age groups and genders [12]. The severity of influenza infection or complications associated with it are greater in certain high risk groups [13–15]. According to the CDC these groups include children younger than 5 years of age and particularly affected the ones younger than 2 years old [16–18], elderly individuals 65 years old and above [19–23], pregnant women [24, 25], and people with certain underlying medical conditions such as asthma [26, 27], chronic lung disease [28, 29], heart disease, diabetes [22, 30–32], immunocompromised individuals [33–35], and some others [36]. Additionally, people who live in nursing homes and long-term care facilities [30, 37, 38] as well as health care workers [39–42] are at high risk from influenza infection.

Influenza virus is a single-stranded negative sense RNA virus. There are three different serotypes of influenza viruses that can cause disease in humans, A, B, and C, distinguished by their antigenic differences in their nucleocapsid (NP) and matrix (M) protein. Influenza types A and B have eight separate segments encoding at least ten different proteins, they can spread easily among human population and are responsible for seasonal epidemics every year [43–58]. Influenza type C is very rare, it has seven separate different segments encoding nine proteins, and although it may cause mild respiratory disease it is not responsible for epidemics [59]. Influenza type A viruses have common internal antigens but can be divided into several different subtypes based on the antigenic properties of the two major proteins in their surface, the hemagglutinin (H) and neuraminidase (N) proteins. These two proteins also represent the two major surface antigens of influenza viruses. So far 17 different hemagglutinin and 10 neuraminidase proteins have been identified circulating in nature [60–63]. The two influenza A subtypes that cause seasonal influenza infections in humans are the H1N1 and H3N2 influenza viruses [63, 64]. Influenza B viruses have a limited host range (humans and seals) and are not divided into subtypes like influenza A subtypes but are classified based on their strain differences [65].

Influenza viruses exhibit a great ability to introduce minor or major changes in their two major surface proteins, the hemagglutinin and neuraminidase. Minor changes in the influenza virus genome are more common and are induced by the constant selective pressure caused by the host immune responses. These minor changes (antigenic drift) are characterized by point mutations in the HA and NA genes. Due to these changes, the host's preexisting immunity may only partially recognize the HA and NA proteins of a new strain resulting in decreased protection and subsequently higher infection rate [66–68]. Influenza A viruses circulate among humans as well as different animals, including ducks, chickens, pigs, horses, etc. This constant circulation of influenza viruses among different species results occasionally in genome recombination inside a reservoir host between different strains and in the appearance of an antigenically new influenza virus (antigenic shift) [69–72] that the human immune system has never encountered before and hence has no or little protection against it. Due to lack of preexisting immunity, the new virus spreads quickly causing pandemics and affecting millions worldwide. The five major pandemics of the twentieth century and the first pandemic of the twenty-first century, the swine origin A/California/07/09 strain, resulted from such antigenic shifts. Influenza represents a significant socioeconomic burden, leading to increased health care cost, high levels of work absenteeism, disruption in work, and productivity loss [73].

In the USA, annual influenza epidemics result in an average of 3.1 million hospitalization days and 31.4 million outpatient visits, while the total direct and indirect economic burden of annual influenza epidemics amounts to 87.1 billion

dollars [74]. The WHO places the number of infected individuals at high risk from influenza infection as more than 1.2 billion worldwide including 385 million elderly, 140 million infants, 700 million adults and children with underlying health conditions including pregnant women, and approximately 24 million health care workers [11].

13.1.2 Influenza Vaccination

Vaccination represents the best method of prevention and protection from influenza infection and its related complications, improving herd immunity, and reducing morbidity and mortality rates worldwide [2, 75–79]. Currently there are two different types of commercially available influenza vaccines on the market: (a) the trivalent inactivated influenza vaccine (TIV) administered intramuscularly with syringes, approved for use in infants older than 6 months and (b) the live attenuated influenza vaccine (LIV) given as nasal spray, approved for use only in healthy individuals from 2 to 49 years of age who are not pregnant [80]. The trivalent inactivated vaccine is the most widely utilized worldwide. There are three different types of inactivated influenza vaccine: whole virus vaccine, virus vaccine split after detergent treatment, and subunit vaccine consisting of purified HA and NA proteins. In the USA the current influenza vaccines are the split and subunit ones; both contain 15 μg of H1 and H3 hemagglutinins of the circulating seasonal influenza A subtypes and of type B influenza virus. These vaccine formulations were studied in the 1970s and proven to be safe, with reduced reactogenicity when compared to the whole inactivated influenza vaccine used until then [81–86]. Despite the excellent safety profile provided by the split and subunit influenza vaccines, the immune response following vaccination has been proven to be short-lived and not fully protective, especially in high risk groups such as the elderly, children, and immunocompromised individuals [14, 33, 87–90]. Thus studies have shown that the antibody titers to influenza wane within 7–8 months post-vaccination and that children unprimed to influenza require two vaccine doses to elicit protective immune responses. According to FDA guidelines an influenza vaccine is considered protective when the vaccinee develops anti-influenza hemagglutination inhibition titers above 40 [91]. In addition, the efficacy of these vaccines depends on how well matched the influenza strains in the vaccine are with the ones in circulation. According to the CDC, in randomized controlled trials conducted among healthy adults less than 65 years of age, the efficacy of inactivated influenza vaccines has been estimated to be between 50 and 70% during seasons in which the vaccine components were well matched to the circulating influenza viruses. Under conditions of suboptimal match, the efficacy of the inactivated vaccines fluctuates between 48% among high risk groups and 60% among healthy adults. In cases where the influenza vaccine and the circulating influenza viruses are poorly matched, the effectiveness of these vaccines is further reduced [92, 93]. All these facts strongly suggest the need for better vaccines or vaccine delivery approaches to improve protection, duration and breadth of immunity, as well as vaccine acceptance for worldwide coverage.

13.2 Skin as an Immunological Organ

13.2.1 Skin Structure, Functions, and Resident Cell Populations

A new vaccine delivery target that has gained more attention in recent years is the skin [94]. The skin is one of the most complex structures and the largest immunological organ of the human body [95]. Its main function is protective, serving as a physical barrier from numerous pathogens but also from injuries and UV radiation. It is also part of the body's homeostatic mechanism and an important sensory organ. It is composed of two primary layers, the epidermis and dermis [96]. The epidermis represents the most outer layer of the skin. It is 50–100 μm thick and it is divided into several sublayers; (1) *stratum corneum* which is the outer layer of epidermis, (2) *stratum germinativum*, (3) *stratum lucidum* that appears

in certain parts of the body, (4) *stratum granulosum* which contains squamous cells and filaggrin and prevents loss of nutrients, (5) *stratum spinosum* that further enhances structural support and prevents skin abrasion, and (6) *stratum basale* that contains epithelial cells which undergo rapid mitosis to replenish dead cells from upper layers. These layers are mostly consisting of keratinocytes, melanocytes, and Langerhans cells (LCs) [96]. Langerhans cells are present in all layers of the epidermis and are in close proximity to the *stratum corneum* [97]. These are immature APCs produced from bone marrow precursors that reach and populate the skin through the peripheral circulation [98]. The dermis lies beneath the epidermis and contains hair follicles, sweat and endocrine glands, lymphatic vessels, blood vessels, and several nerve endings. It is largely populated by dermal dendritic cells (DDCs) that are distinct from the epidermal Langerhans cells populations based on their surface markers. LCs express differential levels of CD11b, $CD205^{int/high}$, and more specifically CD207 (Langerin) while DCs express $CD11b^{high}$, and $CD205^{low/int}$ and CD207 negative [99, 100]. Additionally, these two populations are characterized by differences in chemokine receptor expression especially during the maturation and migration of LCs from tissues to draining lymph nodes [101–104]. The presence of two types of antigen presenting cells, LCs and DDCs, classify the skin as an immunological organ [105]. Additionally, the expression of Toll-like receptors [106, 107] (TLRs) on LCs, DDCs, and keratinocytes make it an ideal target for vaccine delivery [105]. These two types of APCs, in combination with other immunologically active cells residing in the skin including LC-like DCs, monocytes, and macrophages [108], recognize and take up the antigen upon delivery in the skin, and migrate while undergoing maturation to the proximal lymph nodes where they prime naïve T and B cells thus initiating and shaping the adaptive immune responses [97]. Both LCs and DDCs are involved in the process of T cell activation [97]. Studies have demonstrated that in the absence of a stimulus, epidermal LCs and dermal DCs express low levels of major histocompatibility molecules MHC class I and II and co-stimulatory or adhesion molecules [109]. For LCs it is possible that passive transfer and diffusion is involved in the process of antigen uptake or a more active mechanism has been proposed where LCs reach out and extend their arms in order to capture the antigen [110, 111]. Dermal DCs have also been shown to be actively involved in the antigen presenting process as well and to be immunologically highly active [105, 112]. Two subpopulations have been identified: dermal $langerin^{+}$ dendritic cells and dermal $langerin^{neg}$ dendritic cells [113]. Dermal DCs occur in higher numbers than epidermal LCs, they express high amounts of MHC class II molecules on their surface, and they are as potent in antigen presentation in naive T cells playing an important role in the regulation of skin immune response [105, 111, 113].

13.2.2 The Role of Inflammation During Skin Vaccination

The inflammatory environment and inflammatory response induced upon antigen entry into the skin seems to be very important and play a crucial role in the immune response. Several studies have demonstrated that LCs and DDCs can produce large amounts of IL-12, TNF-α, and type I interferons (IFNs) as well as attract and activate other innate lymphocytes such as NK cells, NKT cells, and γδ T cells that secrete large amounts of IFN-γ. A recent study by Martin et al. [114] demonstrated the importance of local responses induced after skin vaccine delivery. In this study, Martin et al. observed the upregulation of several important chemokines and cytokines after microneedle delivery and particularly interleukin 1β (IL-1β), macrophage inflammatory protein 1 alpha (MIP-1α), macrophage inflammatory protein 2 (MIP-2), tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1). These cytokines have been shown to contribute to the regulation and migration of LCs and DDCs in the draining lymph nodes. Furthermore other cytokines important to the proliferation, activation, and recruitment of neutrophils and monocytes such as

granulocyte colony-stimulation factor (G-CSF), interferon gamma induced protein 10 (IP-10), and cytokine-induced neutrophil chemoattractant (CXCL-1) were also increased after skin vaccination for influenza. These data demonstrate the numerous complex mechanisms activated upon delivery of the antigen into the skin that may be important for the improved immunological responses of the vaccine recipient. All these immunological advantages and mechanisms seem to favor skin delivery of influenza antigen compared to the conventional intramuscular immunization. Current inactivated influenza vaccines are administered intramuscularly in the deltoid muscle area. Several studies have demonstrated that myocytes contain low numbers of APCs and lack MHC class II expressing cells leading to poor antigen-dependent T cell activation and reduced humoral and cellular immune responses [115, 116]. All these limitations can potentially be overcome by skin immunization because of the many professional APCs populating the epidermis and the dermis, and thus achieving an improved quantitative and qualitative immune response when compared to intramuscular immunization.

13.3 Microneedle Vaccination

One of the most promising novel vaccine delivery platforms that takes advantage of the skin's immunological potential is microneedle technology [94, 117–120]. This technology relies on rapid delivery of the antigen into the skin epidermis and/or the dermis layers with high precision, and without causing any discomfort or irritation. The materials of choice used for fabrication are metals or polymers, both FDA approved and already applied in several other medical devices [116, 121–123]. Metal microneedle arrays coated with whole inactivated influenza virus (WIV) or monovalent subunit vaccine and polymer (PVP) microneedles encapsulating WIV have been successfully tested in vivo and have generated promising results for vaccine delivery methods of influenza antigen through the skin [116, 121–123].

13.3.1 Solid Metal Microneedle Arrays

Metal microneedle arrays are fabricated from stainless steel sheets by laser cutting. These are arrays of hundreds of microneedles projecting a few hundred microns from the base of the patch. To deburr and clean the microneedle edges and to make the tips sharp, microneedles are electropolished in an appropriate solution. Each needle is approximately up to 700 μm long. The microneedles are coated using a dip-coating process with different formulated coating solutions that ensure stability of the vaccine. The coating is performed using an appropriate apparatus and monitored by a video camera attached to a microscope. These metal microneedle arrays coated with the antigen, when applied onto the skin, pierce microscopic holes in the skin's epidermis with a thickness of 10–20 μm for antigen delivery [122, 124–126]. Several studies have demonstrated that by piercing the skin, transdermal permeability increases by as much as four orders of magnitude.

We have previously demonstrated that delivery of whole inactivated influenza vaccine using metal microneedles coated with the antigen can improve the duration of protective immune responses and lead to serological memory [116, 122]. In our latest studies using metal microneedle arrays we demonstrated successful delivery of influenza subunit vaccine in the mouse model in vivo [121], and we observed improved immune responses when compared to the conventional intramuscular administration of the vaccine. Microneedle immunized animals demonstrated enhanced humoral immune responses compared to intramuscularly immunized mice as shown by anti-influenza IgG titers, hemagglutination inhibition titers, and neutralizing antibody titers 9 months after a single dose of vaccine delivery [121] suggesting long-lived immune responses. Their functional antibody titers (HAI and NT) were maintained at levels that are indicative of protection (>40) even at 9 months post-immunization. These findings correlated well with the numbers of bone marrow influenza specific IgG secreting cells which were significantly higher in the microneedle immunized group. Furthermore in the same group the IgG1 and IgG2a isotype

profile showed a more balanced response when compared to the isotype profile induced after intramuscular vaccination, which predominantly induced IgG1 responses. The IgG2a isotype profile is indicative of cellular Th1 immune responses. A more balanced IgG1/IgG2a ratio observed after microneedle immunization could indicate the induction of cellular immune responses after vaccination [121]. Overall these data strongly suggest that delivery of subunit influenza vaccine through the skin can lead to improved humoral immune responses.

It is well established that split and subunit influenza vaccines are poor inducers of cellular immune responses [127]. Investigation of IFN-γ cells in the spleen of microneedle immunized animals revealed higher frequency of these cells indicating improved cellular immune responses [121]. Activation of both the humoral and cellular immune system can potentially provide improved protection when compared to the intramuscular route of vaccination. Indeed, studies have demonstrated a much more rapid clearance of the virus from the lungs of mice infected with 10×LD50 of homologous mouse adapted influenza virus after skin vaccine delivery as well as improved longevity of the immune response and improved protection [116, 121].

13.3.2 Dissolving Microneedle Patches

In contrast to coated metal microneedle arrays where the antigen is being coated on the surface of the needles, the polymer microneedles encapsulate the antigen [123, 128–130]. During delivery into the skin, the whole microneedle array (shaft and tip) dissolves delivering the vaccine cargo into the skin rapidly, eliminating biohazard sharps. This type of needle requires optimal geometry in order to achieve structural rigidity and stability during insertion into the skin [131]. Sullivan et al. designed and fabricated dissolving microneedle patches [123, 131]. The polymers used for microneedle manufacturing were FDA approved and used in several other medical applications. A slurry of vinylpyrollidone was mixed with lyophilized WIV rehydrated to the desired concentration and the mixture was polymerized at room temperature. This process was found to preserve vaccine antigenicity and prolonged shelf life while the microneedles were mechanically strong to ensure skin insertion, rapid dissolution of the needle into the skin, and successful vaccine delivery. Sullivan et al. showed successful dissolution of the microneedles up to 90% within the first 5 min of application into guinea pig skin [123]. This approach has several advantages, delivery of the vaccine to an easily accessible target such as the skin, elimination of biohazard sharps improving public safety and potential for self-administration [128], rendering influenza vaccination more attractive to the population thus ensuring better coverage, stability [124, 132], and rapid distribution of the vaccine. We demonstrated that dissolving microneedle patches induced robust protection in the mouse model after a single immunization with a low antigen dose, at least as good as the one observed after the systemic immunization. Skin delivery of influenza vaccine was followed by higher number of IFN-γ secreting cells in the spleen of microneedle immunized mice when compared to conventional intramuscular vaccine delivery and faster lung virus clearance after infection [123].

Overall, these studies demonstrate that a new platform technology for rapid and easy administration of influenza vaccine through the skin using metal microneedle arrays coated with the antigen or dissolving microneedle patches encapsulating influenza vaccine can be used for successful delivery of the antigen and improved immune responses and protection in vivo [121, 123].

13.3.3 Other Types of Skin Delivery Systems

There are other several types of devices in development from different groups that can be used for delivery of different antigens. One of these designs involves hollow microneedles [120, 131, 133–136]. In this case after delivery and insertion of the microneedles into the target organ the drug/antigen is delivered by a continuous flow into the skin after which the hollow microneedle

patch is being removed. Recent studies have demonstrated that a skin penetration depth of 750 μm to 1.5 mm is ideal for intradermal delivery of drugs and antigens including insulin, anthrax vaccine, and even influenza vaccine [120, 121, 131, 134, 137–143]. Another microneedle design that has been successfully tested in vivo is the Nanopatch [144, 145]. This is an array of densely packed projections that are dry-coated with influenza vaccine formulation and applied to the skin for 2 min. In this case delivery of influenza antigen through the skin induced improved immune responses when compared to the conventional intramuscular route of delivery with the additional advantage of a dose sparing effect [145]. Currently there is one FDA approved influenza vaccine in the market for intradermal delivery, Fluzone manufactured by Sanofi Pasteur. Intradermal delivery relies on the same principles of targeting similar populations of antigen presenting cells in the skin that microneedle delivery is based on. Early results from clinical trials demonstrate that intradermal delivery of the Fluzone vaccine through the skin induced similar seroconvertion rates as that induced after intramuscular delivery but with a dose sparing effect; from 15 μg HA per strain (45 μg HA total) used for the conventional intramuscular injection the dose was reduced to 9 μg HA (27 μg HA total) [146]. These results confirm the hypothesis that targeting skin APCs improves immune responses and support the promise of skin vaccination for various drugs and vaccines.

13.4 Conclusions

The complex structure of the skin and the quantity and quality of immunologically active cells that it contains [96, 105] establishes this organ as an ideal target for vaccine delivery. After several years of investigation, significant advances have been made indicating the importance of innate cell populations residing in the skin and the mechanisms behind antigen uptake. Numerous microneedle devices are in development exploiting the unique features of skin. The selection of the best design relies mostly on the type of drug to be delivered and on the type of antigen presenting cells that must be targeted. In the case of influenza vaccination very simple designs have been successfully tested in vivo and show promising results that are in the process to be advanced in clinical trials. Several important advantages make this method ideal for large-scale immunization programs. The simplicity of the method makes it ideal for self-administration. Since the skin is an easily accessed organ, and the method can eliminate biohazardous sharps, it can be completed without the need for highly trained personnel. Additionally preliminary data in humans demonstrate dose sparing further reducing the cost of this vaccination route [146]. Taking under consideration the immunological advantages achieved after microneedle delivery, the data suggest that this method could be an alternative to the conventional intramuscular route of immunization. The logistical advantages such as the ease and the simplicity of administration, the high safety profile, the better acceptance by the public [119, 120, 131, 147], and the immunological advantages [121] make this approach an important future direction in influenza vaccination.

Acknowledgments We thank for the collaboration Dr. Mark R. Prausnitz, Dr. Vladimyr G. Zarnitsyn, Dr. Harvinder Singh Gill and Dr. Sean P. Sullivan, School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA.

Research was supported by 1U01 AI074579-01/NIH and 1U01 EB012495/NIBIB grants.

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