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Enabling skin vaccination using new delivery technologies

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Abstract

The skin is known to be a highly immunogenic site for vaccination, but few vaccines in clinical use target skin largely because conventional intradermal injection is difficult and unreliable to perform. Now, a number of new or newly adapted delivery technologies have been shown to administer vaccine to the skin either by non-invasive or minimally invasive methods. Non-invasive methods include high-velocity powder and liquid jet injection, as well as diffusion-based patches in combination with skin abrasion, thermal ablation, ultrasound, electroporation, and chemical enhancers. Minimally invasive methods are generally based on small needles, including solid microneedle patches, hollow microneedle injections and tattoo guns. The introduction of these advanced delivery technologies can make the skin a site for simple, reliable vaccination that increases vaccine immunogenicity and offers logistical advantages to improve the speed and coverage of vaccination.

Introduction

Morbidity and mortality due to infectious diseases have been dramatically reduced by improved vaccination, which is the most cost-effective public health measure to prevent spread of disease (Lambert et al., 2005, Levine and Sztein, 2004). Most vaccines are given by intramuscular injection, even though the muscle is not a highly immunogenic organ (Hutin et al., 2003, Hohlfeld and Engel, 1994). The skin, in contrast, is a much more attractive site for vaccination from an immunologic perspective, because of its many resident dendritic cells and efficient drainage to lymph nodes (Debenedictis et al., 2001, Kupper and Fuhlbrigge, 2004). However, skin vaccination has made relatively little impact on medical practice because intradermal injection requires specialized training and, even with training, does not reliably target the skin (Flynn et al., 1994, Mitragotri, 2005).

Skin vaccination was used heavily during the smallpox eradication campaign, by employing the bifurcated needle with two sharp vaccine-holding prongs that are repeatedly inserted into the skin to deposit a dose of live vaccine in the skin (Baxby, 2002). Although the bifurcated needle is easy to administer, the small and variable dose it delivers limits its continued use for vaccination. Bacillus Calmette-Guérin (BCG) vaccine against tuberculosis is currently administered intradermally using the Mantoux method, in which a conventional hypodermic needle is inserted at a shallow angle into the skin (Andersen and Doherty, 2005, Flynn et al., 1994). This method requires specially trained personnel and typically achieves inconsistent delivery, which has motivated some to recommend abandoning intradermal injection in favor of a simple-to-use percutaneous puncture device (Hawkridge et al., 2008). Since 1991,

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the World Health Organization (WHO) has recommended intradermal injection using the Mantoux method as a cost-saving measure in developing countries for vaccination against rabies, because fractional doses of vaccine are effective when injected in the skin (WHO, 2010).

Many more vaccines would be candidates for vaccination via the skin if simple, reliable methods of intradermal delivery were available. Because skin is often the first organ of the body to face microbial or viral invasion, skin protects the body from infection using not only its physical barrier of the stratum corneum layer, but also its strong immunological function enabled by resident antigen-presenting cells (Kupper and Fuhlbrigge, 2004). Langerhans cells in epidermis and dermal dendritic cells in the dermis are the main immunological skin cells with the essential role to capture foreign antigens and present them in draining lymph nodes. Additionally, skin keratinocytes and other cells in epidermis and dermis produce cytokines and chemokines, which can stimulate and control immune responses. Antigen trafficking studies have shown that vaccination through the skin leads to more efficient antigen migration into lymph nodes than conventional intramuscular delivery (Steinman and Banchereau, 2007, Valladeau and Saeland, 2005, Sugita et al., 2007).

Vaccine dose-sparing by delivery via the skin is well established in clinical practice for rabies vaccine and has been demonstrated in many clinical trials for a number of other vaccines as well, which is a practical outcome of skin's enhanced immunogenicity (PATH, 2009). More recently, intradermal influenza vaccine has been introduced in Europe and other parts of the world due to its improved protective immunity seen in the elderly compared to conventional intramuscular vaccination (Arnou et al., 2009). In this case, intradermal injection of a reformulated vaccine is achieved by a novel microneedle syringe that enables medical personnel to reliably inject into the skin with minimal additional training (Laurent et al., 2007). As discussed below, a number of novel non-invasive and minimally invasive technologies have been developed for skin vaccination. These technologies are poised to now make the skin a viable route for vaccination.

Non-invasive skin vaccination

Hypodermic needles are not only difficult to use for intradermal injection, but their intentional re-use and unintentional needle sticks cause more than one half million deaths annually due to transmission of HIV and hepatitis B and C from dirty needles (WHO, 2004). Thus, an ideal skin vaccination method would not only be reliable, but would also eliminate the dangers and pain associated with hypodermic needles. Non-invasive skin vaccination methods seek to achieve this ideal by eliminating the needle and replacing it with methods to increase skin permeability that do not involve the generation of sharps waste (Mitragotri, 2005).

Liquid jet injection is the best known needle-free vaccination method, which involves directing a pressurized liquid to make a pathway into the skin, and thereby deposit vaccine intradermally. This method was in widespread use in the mid-20th century for intramuscular and subcutaneous vaccination, and has been adapted for intradermal injections too. Intradermal jet injection is gaining renewed interest and is the subject of clinical trials for inactivated poliovirus vaccination (Mohammed et al., 2010).

Epidermal powder immunization (EPI) is conceptually similar to liquid jet injection using high pressure flow, but accelerates dried-powder particles of vaccine, rather than liquid, into the skin at supersonic speed. Immunization via this route has been shown to derive similar immune responses compared to intramuscular immunization in human study (Dean and Chen, 2004). As another variation on this theme, particle-mediated epidermal delivery (PMED) administers DNA vaccine coated on gold microparticles that are shot into the skin.

Clinical studies of PMED immunization showed promising results, but less immunogenic responses compared to standard vaccine delivery methods (Jones et al., 2009).

These first methods described above actively deposit vaccine into the skin. Other approaches seek to remove the skin's main barrier, stratum corneum, as a first step, and then allow vaccine entry into the skin by subsequent diffusion from a patch or other topical formulation. The stratum corneum can be removed by abrasive methods, such as tape-stripping or sanding with emery paper. Transcutaneous immunization in this way induced robust humoral and mucosal immune response and migration of activated antigen presenting cell from skin to draining lymph nodes (Guebre-Xabier et al., 2003). Clinical trials using this approach seek to develop a new vaccine against traveler's diarrhea and influenza (Frech et al., 2005, Frerichs et al., 2008). Skin abrasion using a razor and a toothbrush also showed promising human clinical results (Van Kampen et al., 2005). Microdermabrasion is a cosmetic approach to remove stratum corneum; a recent study demonstrated vaccination using this approach in animals (Gill et al., 2009).

Other approaches developed for transdermal delivery of drugs have also been adapted for skin vaccination. For example, thermal ablation generates microscopic holes in stratum corneum by vaporizing it at high temperature produced by highly focused thermal energy (Park et al., 2008). Resistive heating from electrical energy or radiofrequency have been developed and shown to enable protective immune responses to vaccine antigens in animal studies (Bramson et al., 2003, Fagnoni et al., 2008).

Ultrasound has also been shown to increase skin permeability and thereby used for vaccine delivery into the skin. The effects of ultrasound not only enhanced the vaccine delivery but also stimulated activation of antigen-presenting cells in the epidermis in animals (Dahlan et al., 2009).

Electroporation has been used for transdrmal drug delivery by increasing skin permeability and one study delivered peptide-based vaccine using an electroporation as a permeability enhancing tool (Zhao et al., 2006), but mostly electroporation has been used primarily to increase permeability of skin cells to enhance intracellular delivery of DNA vaccine for increased cell transfection for effective antigen protein production (Drabick et al., 2001). Electroporation has been used to derive effective immune responses in DNA vaccine studies in animal and, more recently in human clinical trials for DNA vaccination against prostate cancer (http://clinicaltrials.gov/ct2/show/NCT00859729).

The use of chemical enhancers is the best known method to disrupt stratum corneum lipid structure and thereby increase skin permeability, mostly only for small molecules. However, recent study has shown the potential use of mixtures of chemical enhancers as a vaccination tool that, when properly optimized through high-throughput screening, can not only increase skin permeability to the vaccine antigen, but also play a novel role as an adjuvant (Karande et al., 2009).

Overall, non-invasive vaccination methods mostly fall into two categories. The high-velocity, needle-free injection systems are generally able to quickly and efficiently drive vaccines into the skin, while avoiding the dangers of hypodermic needles. These methods do, however, often require bulky devices, can cause pain similar to hypodermic needles, and sometimes do not achieve reliable injections (Baxter and Mitragotri, 2006, Hogan et al., 2010). The other non-invasive methods increase skin permeability to varying extents and then require a slow and often inefficient process of vaccine antigen diffusion into the skin that typically leaves a large fraction of the vaccine behind on the skin surface (Prausnitz and Langer, 2008).

Minimally invasive skin vaccination

To overcome the limitations of non-invasive skin vaccination methods, while still avoiding the dangers and unpleasantness of hypodermic needles, minimally invasive methods to administer vaccine to the skin have been developed, primarilyusing very small hollow and solid needles. Because this approach directly and actively deposits vaccine in the skin, it can deliver vaccine doses faster and more reliably than non-invasive vaccinations.

Most work on minimally invasive skin vaccination has involved the use of microneedles, which are long enough to cross the stratum corneum barrier, but short enough to avoid pain and to reliably remain within the skin for targeted delivery (Gill et al., 2008). There are four different types of microneedles that have been studied for vaccine delivery: hollow, solid, coated, and dissolving microneedles (Prausnitz et al., 2009).

Hollow microneedles look like miniature hypodermic needles that can be inserted at an angle perpendicular to the skin, which permits healthcare professionals to give intradermal injections without special training. Hollow microneedles have been used as single needles or as multi-needle arrays for vaccination against influenza, anthrax and other diseases in animals and human subjects (Arnou et al., 2009, Van Damme et al., 2009, Mikszta et al., 2005, Dean et al., 2005). A single hollow microneedle device has recently been introduced into clinical practice for intradermal influenza vaccination because skin vaccination was shown to provide superior immunogenicity in the elderly, who have the highest rates of morbidity and mortality from seasonal influenza (Holland et al., 2008). Additional human trials with hollow microneedles also showed significant dose-sparing compared to intramuscular immunization (Leroux-Roels et al., 2008, Van Damme et al., 2009).

Solid microneedles have been used to pierce holes in the skin for subsequent delivery of vaccine from a topical formulation. This approach is similar to many of the non-invaisve methods that rely on slow and typically inefficient delivery by diffusion into the skin. Microneedles have been used in this way for transcutaneous vaccination using diphtheria toxoid, but did not generate strong immune responses for influenza vaccine (Ding et al., 2009). Scraping the skin with solid microneedles by a method similar to the non-invasive abrasive techniques has also been used for DNA vaccine delivery, which derived better humoral and cellular immune responses against hepatitis B than intramuscular or intradermal vaccination by injection (Mikszta et al., 2002).

Recently, microneedles have been coated with vaccine for rapid dissolution in the skin within minutes. Metal microneedles have been coated with a number of different types of influenza vaccines, including inactivated virus (Kim et al., 2010a, Fernando et al., 2010) and virus-like particle (Quan et al., 2010b) as well as other antigens, such as hepatitis C (Gill et al., 2010) and ovalbumin (Matriano et al., 2002). Vaccination in mice using inactivated influenza virus vaccine induced similar virus-specific IgG antibody response, hemagglutination inhibition titer, and neutralizing activity as a primary response to vaccination and generated robust protective immunity to influenza after challenge compared to conventional intramuscular immunization (Kim et al., 2009, Kim et al., 2010c). Notably, influenza virus was cleared from the lungs of microneedle-immunized mice after challenge much more efficiently than intramuscularly immunized mice. This was explained by enhanced humoral and cellular recall immune responses among microneedle-immunized mice. Microneedle vaccination also induced significantly higher levels of antibodies and MHC II-associated $CD4^+$ T helper cells post-challenge (Kim et al., 2009, Kim et al., 2010c).

Mice vaccinated by coated microneedles stored at room temperature for one month generated similar antibody responses to those of mice vaccinated by freshly coated microneedles, which suggests the possiblility of a thermostable vaccine that does not require

refrigeration (Kim et al., 2010b). In addition, microneedle vaccination generated dose-sparing effects using an influenza virus-like particle vaccine and a model ovalbumin vaccine (Matriano et al., 2002, Quan et al., 2010a)

Dissolving microneedles have been developed to offer the simplicity and effectiveness of coated metal microneedles, but eliminate the generation of sharp microneedle waste because dissolving microneedles made of water-soluble polymers and sugars completely dissolve away in the skin. In a recent vaccination study, dissolving-microneedle immunization induced similar humoral and cellular immune responses compared to intramuscular immunization and showed better lung virus clearance and enhanced cellular recall responses after challenge, which is similar to the results seen with coated metal microneedle immunization (Sullivan et al., 2010).

Finally, tattooing is well-established method of depositing materials in the skin for cosmetic purposes, which has now been adapted for DNA vaccination in the skin. High-frequency oscillating tattoo needles can pierce the skin to deliver vaccine in the dermis. DNA tattooing induced better humoral and cellular immune response than intramuscular immunization in animal studies (Bins et al., 2005).

Minimally invasive methods of skin vaccination, notably through the use of various microneedle designs, offer advantages over non-invasive approaches by actively delivering vaccines into the skin in a rapid, reliable and efficient manner. They also offer advantages over hypodermic needles, by reducing or eliminating pain, biohazardous sharps waste and the need for specially trained medical personnel. Minimally invasive methods are, nonetheless, invasive and therefore have greater safety concerns and sterility requirements than non-invasive methods do.

Logistical advantages of skin vaccination methods

In addition to the immunological advantages of vaccination in the skin, skin vaccination can also offer important logistical advantages compared to conventional hypodermic injection. Most of the skin vaccination methods described in the preceding sections avoid the pain and apprehension felt by patients when receiving hypodermic injections, eliminate or reduce the risk of needle-stick injury and re-use of needle and/or syringe, and may be administered by minimally trained personnel or possibly by patients themselves, thereby enabling increased vaccination coverage. This becomes especially important during rapid mass vaccination against a possible pandemic or during immunization campaigns, because hypodermic injection is relatively slow and assembly of populations at centralized sites for vaccination risks cross-contamination and spread of pathogens.

Many of the skin vaccination methods, including EPI, many transdermal patch designs and solid microneedles, use vaccine in a dry state. This enables vaccine stabilization in the dry state, possibly without the need for refrigeration, and avoids the need for vaccine reconstitution by medical personnel, which it time consuming, can lead to vaccine wastage, and is a source of medical errors. Reformulation of the vaccine for dry-state presentation, however, will incur additional costs and technical challenges (Hickling et al., 2010).

Skin vaccination methods also often reduce the package size of the dosage form compared to a hypodermic needle, syringe and vaccine vial, which facilitates storage, transportation and disposal. This reduces cost and infrastructure needs, and, for vaccines requiring refrigeration, this reduces the space required in the cold chain.

Finally, skin vaccination has the potential to reduce the cost of vaccination due to these logistical advantages, as well as possible dose sparing and increased vaccine

immunogenicity that reduces overall healthcare costs by better preventing disease. These savings need to be balanced against the possibly increased cost of the vaccination method. In most cases there will be initial costs to introduce the new technology, but at steady state mass production many of the skin vaccination methods need not cost more than manufacturing of conventional injectable vaccines. This is because the dominant cost is often the cost of the antigen and its sterile manufacturing, whereas the novel dosage form costs no more than a needle, syringe and vial. However, in other cases, especially when a reusable device is involved, there may be added costs.

Conclusion

Overall, skin vaccination has the potential to improve both the immunology and logistics of vaccination. Although skin-targeted immunization has been used for a long time, its application beyond a few vaccines has been hindered due to the lack of simple and reliable skin vaccination technology. In recent years, a number of technologies developed largely for drug delivery has been applied to skin vaccination and shown to administer vaccines easily and reliably into the skin. Non-invasive technologies offer the safety of a needle-free method in either a rapid, high-velocity injection format or a slow, diffusion-based patch. Minimally invasive methods, mostly in the form of various microneedle designs, offer simple, rapid and efficient vaccination that have advantages over non-invasive methods, but with the trade-off of introducing safety concerns associated with their invasive nature. With these vaccine delivery tools, we can now administer vaccines to the skin for possible application in the clinic, carry out studies to better understand skin immunology, and design skin-based vaccines that harness and optimize skin immunology for improved immunization. Skin vaccination can now transition from a topic of immunological interest with limited clinical utility to a viable method of vaccination to increase vaccine immunogenicity and broaden vaccination coverage.

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