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The success of microneedle-mediated vaccine delivery into skin

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Abstract

Microneedles (MNs) are designed to specifically target the outermost, skin barrier layer, the *stratum corneum*, creating transient pathways for minimally invasive transcutaneous delivery. It is reported that MNs can facilitate delivery without stimulating the pain receptors or damaging blood vessels that lie beneath, thus being perceived as painless and associated with reduced bleeding. This immunocompetence of the skin, coupled with its ease of access, makes this organ an attractive vaccination site. The purpose of this review was to collate primary scientific literature pertaining to MN-mediated *in vivo* vaccination programmes. A total of 62 original research articles are presented, compiling vaccination strategies in 6 different models (mouse, rat, guinea pig, rabbit, pig, macaque and human). Vaccines tested span a wide range of viral, bacterial and protozoan pathogens and includes 7 of the 13 vaccine-preventable diseases, as defined by the WHO. This review highlights the paucity of available clinical trial data. MN-delivered vaccines have demonstrated safety and immunogenicity in pre-clinical models and boast desirable attributes such as painless administration, thermostability, dose-sparing capacity and the potential for self-administration. These advantages should contribute to enhanced global vaccine access.

1. Introduction

1.1 Vaccine delivery

Vaccines are conventionally administered using a hypodermic needle [1]. This form of administration provides a rapid and direct method of vaccine delivery. Despite familiarity, widespread use and proven efficacy, the hypodermic needle is associated with accidental needle stick injury, spread of blood-borne infections [2-4], as well as phobias, pain and significant anxiety [5-8]. In addition, these needles are not easily self-administered, unless the individual has received specialised training on injection technique and needle disposal [9]. Oral vaccination is an attractive alternative [10] and a limited number of oral vaccines have been approved for human use [11-15]. However, this mode of

immunisation can be less effective, as vaccine antigens undergo digestion in the gastrointestinal tract prior to induction of an adequate immune response [10] and research on their use has been limited almost exclusively to protection against mucosally transmitted pathogens, with some notable recent exceptions [15]. The transdermal route, based on diffusion, has also been investigated. However this route limits delivery only to lipophilic, low molecular weight potent products [16] and would prevent a vaccine from crossing the skin due to the presence of the relatively impermeable, outer *stratum corneum* (SC) layer. Intradermal vaccination is not a novel concept. In 1910, French physician Charles Mantoux published his clinical research on the intradermal injection of tuberculin as a diagnostic test for tuberculosis disease [17]. This diagnostic technique formed the basis for intradermal vaccination, a technique still in use for vaccines such as rabies [18] and BCG [19]. However, intradermal delivery is technically challenging, requiring significant operator training [20] and has been associated with adverse events such as pain, inflammatory changes [21] and the development of abscesses [22].Taking into account the limitations of parenteral, oral and traditional transdermal and intradermal vaccination, the concept of the microneedle (MN) emerged as a solution to these issues. MNs can be 1µm in diameter and range from 50µm to 1000µm in length, while mini-needles range from 1000µm to 1500µm [23]. They are designed to specifically target the outermost, rate limiting, skin barrier layer, the SC, creating transient pathways for minimally invasive transcutaneous delivery [24]. There are four different types of MNs: solid, coated, hollow and dissolving. It is reported that MNs can facilitate delivery through SC interruption without stimulating the pain receptors and blood vessels that lie beneath, thus being perceived as painless and associated with a reduction in bleeding [1, 25, 26]. Other advantages of microneedle-mediated delivery include avoidance of first pass metabolism; potential for highly targeted administration to individual cells [26, 27]; improved patient compliance [28]; dose sparing [29, 30]; thermostability of certain platforms [31-33] and potential for self-administration.

1.2 The skin: an immune organ and vaccine target

Skin is the largest immune organ in the human body [34], composed of two primary layers, the epidermis and dermis [35]. These layers provide a protective interface between internal organs and the external environment, encountering a host of toxins, pathogenic organisms and physical stresses [36]. The skin functions as more than just a physical barrier. It is capable of mounting a potent immune response due to the residence of specialised antigen presenting cells. Langerhans cells are abundant in the epidermis, comprising 2% to 4% of epithelial cells [36], while more classical dendritic cells are found in the dermis [36-40]. Other immune-competent accessory cells residing in the skin include keratinocytes, epidermal cells which play a role in initiating cell-mediated immune responses through the release of cytokines and the expression of cellular adhesion molecules to

facilitate movement and coordination with other immune cells; T lymphocytes; melanocytes, epidermal pigment cells which produce a number of cytokines that mediate inflammation and mast cells, leukocytes which modulate host innate immune response through the release of granular and secreted mediators and recruit multiple inflammatory cells through the production of chemotactic factors [36, 41-43]. The resident professional APC are adept at antigen capture, and upon appropriate activation through intracellular interaction, migrate to proximal lymph nodes to activate B and T lymphocytes and mediate initiation of an adaptive immune response [37, 44]. This immunocompetence, coupled with its ease of access, makes the skin an attractive vaccination target.

2. Literature review

The purpose of this review is to collate literature detailing the success of MN-mediated *in vivo* vaccination programmes. Keywords including 'microneedle', 'solid microneedle', 'coated microneedle', 'hollow microneedle' 'dissolvable microneedle', 'dissolving microneedle', were combined with 'vaccine', 'vaccination' and 'immunisation'. Using Google as a search engine, these keywords were combined in various permutations and combinations to search PubMed. This yielded a total of 748 results. Following removal of duplications, 180 results remained. The title and abstract of each result were examined and included or excluded in the final review based on the criteria outlined in Table 1. A total of 62 results were included in the final review [29, 45-105] .

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	**Exclusion Criteria**
Original research articles	Review articles
MN-mediated vaccine delivery	MN-mediated non-vaccine delivery
Published in English language	MN fabrication studies
in vivo MN administration	MN stability studies
Article available in full	

2.1 Solid MNs

The simplest forms of MNs are solid devices. Solid MNs create transient micropores in the SC, thereby increasing permeability of the barrier layer. Vaccine applied onto the treated surface diffuses into the skin (from a loaded patch or semi-solid formulation) through the pores created by MN pre-treatment. The applied vaccine can exert a local effect in the skin and a systemic effect following uptake [1]. Solid MNs have been used to deliver vaccines for diphtheria [45, 46, 48], influenza [46], hepatitis B [47, 49, 50] and malaria [51, 52] in mice. Microenhancer array devices were developed to cause mild abrasion of the SC. These devices scrape the skin with blunt-tipped

microneedles and have been used to increase the delivery of an anthrax vaccine in mice and rabbits [53], a Japanese encephalitis vaccine in cynomolgus monkeys [56] and a rabies vaccine in humans [57, 58]. While these devices were shown to be effective, intradermal injection of the vaccine in each of these studies was significantly more effective, potentially due to inefficient delivery into the skin from the formulation. In spite of immunogenicity, the popularity of solid MNs has reduced in recent years, potentially due to the requirement for a multi-step administration process, the lack of consistency and the increased number of advantages of other MN systems.

2.2 Coated microneedles

Advancement on solid MNs was the development of coated devices. Solid MNs are pre-coated with a vaccine in a formulation suitable for coating and dissolution [1], thus resulting in a one-step delivery process. The vaccine coated MNs are inserted into the skin, where dissolution of the vaccine occurs. Vaccine delivery via coated MNs is limited by the dimensions of the MN shaft and tip [106, 107]. Successful vaccine coated MNs include influenza [54, 55, 59-77], human papillomavirus [78, 79], chikungunya virus [80], West Nile virus [80], rotavirus [81], herpes simplex virus [82] and hepatitis C [83] in mice, influenza virus [84] and bacillus Calmette-Guérin in guinea pigs [85], hepatitis B virus in pigs [86], and measles [87] and polio [88] viruses in rats. This literature search did not reveal any clinical trials pertaining to vaccine delivery via coated microneedles.

2.3 Hollow microneedles

Hollow MNs provide a pre-defined conduit for vaccine delivery into the skin or other tissue. Currently there are two hollow MN designs: a single MN or mini-needle, which mimics the conventional hypodermic needle [108] or an array of multiple MNs [109]. The latter permits simultaneous application of a vaccine formulation over a wider area of skin, potentially resulting in higher bioavailability and increasing the likelihood of lymphatic uptake of presented antigens [110]. Vaccine may be delivered by passive diffusion through the MN. Conversely, a syringe may be attached to the MN, permitting active vaccine delivery. There are several commercially available hollow MN systems; Soluvia® is licensed for use [57, 91] and MicronJet® is being clinically tested [29]. Soluvia® is a pre-fillable microinjection system with a single 1500µm hollow silicon MN, while MicronJet® is composed of four 600µm hollow silicon MNs arranged on a plastic adaptor for attachment to a standard syringe barrel [23]. Hollow MNs have been successfully developed to immunise human subjects with polio [89, 90] or influenza [29, 91, 92] vaccines, to immunise mice against plague [93] and to administer polio vaccine to rats [94].

2.4 Dissolving microneedles

The final, most advanced and complex MN is the dissolving MN. Dissolving MNs are polymeric and encapsulate vaccine within their matrix [1, 111, 112]. Insertion of the MNs into the skin catalyses the degradation of the polymeric compound, subsequently releasing the vaccine [112]. Unlike the alternate MN platforms already discussed, dissolving MNs are fully biocompatible and do not generate biohazardous waste, a distinct advantage [113, 114]. Other advantages include robustness and scalability [115, 116]. However, unlike hollow MN, a limitation is placed on the amount of vaccine that can be incorporated into the system [117] and vaccinees may be obliged to wait for extended periods of time to ensure complete MN degradation [114]. Dissolving MNs have been developed to incorporate vaccines for influenza virus [95-100], hepatitis B [101, 102], tetanus [97], diphtheria [97], malaria [97] and HIV [103] in mice and measles [104] and polio [105] in rhesus macaques, with a long term aim to create a thermostable, self-administration platform. Although an attractive platform, dissolvable microneedle (DMN) systems for vaccine delivery have required more time to reach clinical trials compared to hollow or solid microneedles. Hollow and solid MN devices have a traditional medical device classification. In contrast, DMN patches will likely be seen, from a regulatory perspective, as a combination product of a medicinal product (the vaccine) and a device (potential backing layers and/or applicators). However, as a new dosage format, the product specifications, critical quality attributes of each product and regulatory pathway of DMN systems has not yet been defined. Furthermore, to ensure the quality of vaccine-loaded DMN patches that will be clinically used, they must be produced in the appropriate environment that complies with, good manufacturing practice (GMP). These processes, guidelines and regulatory strategies are only recently being defined [118].

3. Discussion

The purpose of this review was to collate primary scientific literature pertaining to MN-mediated *in vivo* vaccination programmes, according to the inclusion and exclusion criteria outlined in Table 1. A total of 62 original research articles are presented, compiling vaccination strategies in 6 different models (mouse [45-52, 54, 55, 59-83, 93, 95-103], rat [87, 88, 94], guinea pig [84, 85], rabbit [53], pig [86], cynomolgus [56] or rhesus macaque [104, 105]) and in human subjects [29, 57, 58, 89-92]. The review highlights MN compatibility with live, inactivated, subunit and DNA vaccines. Vaccines tested span a wide range of viral, bacterial and protozoan pathogens; including influenza [29, 46, 54, 55, 59-77, 84, 91, 92, 95-100], hepatitis B [47, 49, 50, 86, 101, 102], Japanese encephalitis [56], rabies [57, 58], human papillomavirus [78, 79], chikungunya virus [80], West Nile virus [80], rotavirus [81], herpes simplex [82], hepatitis C [83], measles [87, 104], polio [88-90, 94, 105] and HIV [103], bacterial illnesses including diphtheria [45, 46, 48, 97], anthrax [53], tuberculosis [85], plague [93]

and tetanus [97] and protozoan illnesses including malaria [51, 52, 97], as summarised in Table 2. This list includes 7 of the 13 vaccine-preventable diseases, as defined by the WHO [119]. This review highlights the paucity of clinical trial data, with only 11.29% of the 62 trials presented conducted in human subjects.

Table 2: Disease targets for microneedle-mediated vaccine delivery

Model	Virus	Bacteria	Protozoa
Mouse	Hepatitis B [47, 49, 50, 101, 102] Influenza [46, 54, 55, 59-77, 95-100] Human papillomavirus [78, 79] West Nile virus [80] Chikungunya virus [80] Rotavirus [81] Herpes simplex [82] Hepatitis C [83] HIV [103]	Diphtheria [45, 46, 48, 97] Anthrax[53] Plague [93] Tetanus [97]	Malaria [51, 52, 97]
Rat	Measles [87] Polio [88, 94]		
Guinea Pig	Influenza [84]	Tuberculosis [85]	
Rabbit		Anthrax [120]	
Pig	Hepatitis B [86]		
Macaque	Japanese encephalitis [56] Measles [104] Polio [105]		
Human	Influenza [29, 91, 92] Rabies [57, 58] Polio [89, 90]		

3.1 Influenza: a popular vaccine target

The influenza virus vaccine is as a popular vaccine target, being the pathogen of interest in 32 of the 62 research articles presented. Influenza is a highly contagious respiratory illness, with influenza A and B viruses causing annual seasonal epidemics and sporadic pandemics of disease, leading to hospitalisations and occasionally death [121-125]. In the US, it is estimated that influenza resulted in greater than half a million hospitalisations, 18,491-95,390 intensive care admissions and 4,915-27,174 deaths per year between 2010 and 2013 [126]. Investment in the development of an influenza vaccine offers significant commercial and technical gain. Unlike other vaccines, which offer life-long immunity with a single dose, influenza immunity requires annual re-vaccination as a result of antigenic variation of the virus [127]. The target end-user of a microneedle patch-based influenza

vaccine is the adult population and not the paediatric, thus reducing the barrier to clinical use. Vaccination is effective in preventing infection [128]. Furthermore, unlike many other vaccines, serological correlates of protection exist and the CHMP criteria are accepted to measure immunogenicity. However, coverage rates in target populations are far below the WHO-recommended 75% [129-131]. In addition, there are unmet needs associated with current influenza vaccines [132]. This motivates the development of alternate delivery systems such as MNs that may offer enhanced vaccine uptake and acceptance [133]. If a MN-based vaccine exhibited enhanced stability and lower vaccine doses could be used [29, 30], then this could be attractive to vaccine manufacturers. From a user perspective, the prospect of a painless, potentially self-administered vaccine may lead to improved vaccination coverage [28]. However all of these features still remain to be rigorously tested and developed in a clinical context.

3.2 Improving vaccine coverage in developing countries

Even though vaccination programmes are frequently cited as one of the most low-cost, high-impact public health measures [134], 1.5 million children die every year as a result of vaccine preventable illnesses, including some of those presented in this review. Vaccines are temperature sensitive biological products, requiring refrigeration. In many developing world countries, a cold-chain infrastructure is almost prohibitively expensive thus preventing adequate vaccine distribution [135, 136]. The thermostability of MN vaccines eliminates cold-chain requirements, thus reducing logistic costs and potentially improving distribution [31-33]. This thermostability would permit stock-piling in regular drug distribution networks, combatting the frequently encountered issue of supply shortage. In addition to being thermolabile, conventional vaccines often require administration by trained personnel. In LMIC countries, there are shortages of medical personnel at all levels of training [137]: Africa has 2.3 healthcare workers per 1000 population, compared to 24.8 per 1000 in the Americas [138]. Therefore the previously discussed potential for self-administration with MN vaccines could further improve vaccine coverage in these countries, in tandem with other public health efforts. However, most paediatric vaccines in the Expanded Programme of Immunization are adjuvanted. Pharmaceutical, immunological, safety and efficacy issues of incorporating licensed adjuvants into solid dosage formats of microneedles must be addressed before this technology will be licensed and deployed for these vaccines. Significant research and development effort is being focussed in these areas to resolve these concerns.

3.3 Translation into clinical use

This review presents a variety of MN vaccines in the pre-clinical development stage, demonstrating safety and immunogenicity in animal models but also highlights the scarcity of clinical trial data. There is a progression from evaluation in small animal models such as mice, to higher animal models

such as rhesus macaques, prior to transition to clinical development and evaluation in human subjects [139]. While preclinical research answers basic questions, it is not a surrogate for clinical research. It is hoped that the MN vaccines presented in this review, especially those that have undergone assessment in non-human primates, will progress through the developmental stages, ultimately leading to vaccine licensing and introduction into clinical use. An issue that needs to be assessed is the habituality of hypodermic needle-mediated vaccination. Despite the aforementioned disadvantages, traditional immunisation has repeatedly demonstrated efficacy and safety. Familiarity breeds acceptance. Therefore a paradigm shift is required to drive the transition of MN-vaccines into clinical use. Increased end-user acceptability of MN-based vaccines will be required for widespread adoption. Positive attributes such as pain-free, bloodless administration must be rigorously tested and defined and acceptance of this technology by the end-user must be assessed, understood and the technology adapted to incorporate end-users' needs. MN fabrication considerations include scalability and dose loading capacity must also be addressed so that the vaccine manufacturer can assimilate the technology into their fill-finish systems. The majority of MN research has been conducted at laboratory scale in small quantities and the development of alternate fabrication approaches has begun to demonstrate scalability [140, 141]. There is an inherent dose loading capacity associated with some MN technologies, whereby there is a limit to the amount of vaccine that can be coated on or incorporated in the MN [1]. The inclusion of adjuvants may reduce the vaccine dose required to elicit an appropriate immune response [86, 142], although their inclusion will also necessitate appropriate validation and production in GMP environments. Finally, there is a need for the development of universal acceptance criteria and Good Manufacturing Practice specifications, permitting MN characterisation and subsequent commercialisation [118].

This review presented the research pertaining to *in vivo* MN vaccines. Vaccines have been delivered via solid, coated, hollow and dissolving MNs. The dissolving MN offers a significant advantage over other MN platforms: the elimination of sharp, biohazardous waste after vaccination. MNs have the potential to improve vaccine access in developing countries. These vaccines have demonstrated safety and immunogenicity in pre-clinical models. The paucity of clinical data presented in this review highlights the need to incentivise vaccine research in human subjects. The technology possesses desirable attributes for the end-user including painless administration and potential for self-application, which may increase compliance and subsequent vaccine coverage, as well as benefits for the manufacturer including thermostability and dose-sparing capacity. All of these advantages demonstrate the high potential for microneedle technologies to have a positive impact on global immunisation programmes in the future.

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