HHS Public Access

Author manuscript

Expert Rev Vaccines. Author manuscript; available in PMC 2021 January 19.

Published in final edited form as:

Expert Rev Vaccines. 2020 February ; 19(2): 175–194. doi:10.1080/14760584.2020.1732215.

The potential role of using vaccine patches to induce immunity: Platform and pathways to innovation and commercialization

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Abstract

Introduction: In the last two decades, the evidence related to using vaccine patches with multiple short projections (≤ 1 mm) to deliver vaccines through the skin increased significantly and demonstrated their potential as an innovative delivery platform.

Areas covered: We review the vaccine patch literature published in English as of March 1, 2019, as well as available information from key stakeholders related to vaccine patches as a platform. We identify key research topics related to basic and translational science on skin physical properties and immunobiology, patch development, and vaccine manufacturing.

Expert opinion: Currently, vaccine patch developers continue to address some basic science and other platform issues in the context of developing a potential vaccine patch presentation for an existing or new vaccine. Additional clinical data and manufacturing experience could shift the balance toward incentivizing existing vaccine manufactures to further explore the use vaccine patches to deliver their products. Incentives for innovation of vaccine patches differ for developed and developing countries, which will necessitate different strategies (e.g., public-private partnerships, push or pull mechanisms) to support the basic and applied research needed to ensure a strong evidence base and to overcome translational barriers for vaccine patches as a delivery platform.

Keywords

vaccine; human skin; microneedle; microarray patch

1. Introduction

The history of vaccine development includes exploration of vaccine delivery to humans through all possible routes of entry into the body using a wide range of strategies [1]. The

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Declaration of interest

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earliest vaccination technique involved applying virus particles directly to disrupted skin (i.e., variolation with smallpox). Currently, although a limited number of licensed oral vaccines (e.g., oral poliovirus vaccine, oral rotavirus vaccine) and aerosol vaccines (e.g., FluMist™) exist [2], the use of syringe and needle that carries the vaccine through the skin barrier represents the dominant current vaccine delivery strategy. Delivery of vaccines by syringe and needle is generally well-accepted by vaccine recipients, even though they may experience some fear (i.e., needle phobia), pain associated with receiving injections, and/or in rare instances of injuries, such as shoulder injuries related to syringe and needle vaccine administration [3] or adverse events from lyophilized vaccine reconstitution errors. Health systems also broadly accept syringe and needle delivery of vaccines and benefit from the interchangeability and stability of a supply chain supported by multiple suppliers. However, syringe and needle vaccine delivery require the use of trained, skilled healthcare workers to administer the vaccines, and even with sufficient training these workers face risks of needle-related occupational injuries. In addition, the disposal of used syringes and needles leads to system costs and risks.

The evaluation of delivering vaccines into different skin strata and underlying tissues dates back at least to the 1930s [4]. Vaccine administration can occur via injection into a muscle (i.e., intramuscular [IM]), the dermis (i.e., intradermal [ID]), the hypodermis (i.e., subcutaneous [SC]), or onto the epidermis (i.e., topical or transcutaneous) [1]. Given the size of the molecules in vaccines and the need to deliver them past immunological skin defenses in the epidermis, most vaccine injection occurs either into the IM or SC layer, with the recommended needle size varying by vaccine recipient age and body mass, injection site, and target. Some ID vaccine delivery into the relatively shallow dermis use needles and require training for proper vaccine administration (e.g., BCG, and historically, smallpox, which used a bifurcated needle that left a signature scar). A comprehensive review of the literature shows mixed success with the use of jet injector devices for ID delivery, with multiple studies suggesting effective use, but some reporting issues with cross-contamination [1]. More recent disposable-syringe jet injectors with sophisticated applicators avoid some of risks of cross-contamination and offer a needle-free immunization opportunity [5–8].

Transcutaneous immunization (TCI) involves the application of vaccine antigen (sometimes in the presence of an adjuvant) directly to the skin [9]. Some TCI methods involve direct application of liquid or dry vaccine to intact skin, and sometimes using a hydrated patch which disrupts the stratum corneum. Other TCI methods apply a vaccine antigen prior to or following disruption of the stratum corneum by scraping, electroporation, nonablative fractional laser (NAFL), or another mechanism that allows the antigens in a vaccine applied topically to the skin to pass into the epidermis. Some TCI methods include covering the skin coated with vaccine with a patch that occludes the area for some time. Despite extensive investment in the development of TCI dating back to 2000 [10], the technique showed inferiority in human clinical trials, including a phase 3 trial for an Enterotoxigenic *Escherichia coli* traveler's diarrhea vaccine [11], a phase 1/2 trial for measles vaccine [12], and a graded phase 1 trial for influenza vaccine [13]. The history of successes and failures of alternative vaccine delivery systems generally offer some important lessons for the development of vaccine patches, which fall beyond the scope of this review.

In the last two decades, new materials and technologies explored the use of micron-sized (≤1 mm) projections arranged as an array or matrix that can deliver vaccine past the stratum corneum, but not typically beyond the dermis. As the evidence base for this new vaccine patch technology continues to expand, we appreciate the need to review the literature to understand the current status of and challenges to development of vaccine patches as a delivery platform. For this review, we define a vaccine patch as a product that applies a vaccine antigen (with or without adjuvants) using a single array or matrix of projections less than 1 mm in height applied directly to the skin. Thus, our definition of a vaccine patch requires skin penetration by a submillimeter projection array (i.e., it goes beyond a singled, bored ID hollow needle). The focus of our systematic review differs from other reviews of related topics that focused specifically on TCI [9], ID vaccine delivery [14], microneedles [15–17], dissolvable microneedles [18], clinical trials for non-invasive vaccine delivery [2], low- and middle-income country markets [19,20], or other topics. We note that parallel development of patches to deliver other therapeutic agents also impacts this technology (e.g., by potentially establishing design, fabrication, manufacturing, and/or regulatory precedents), but for purposes of this review we focus on the platform issues related to human vaccines. We evaluated the literature to date and identified unresolved platform research issues that would benefit from coordinated research funding and activities, including basic research related to immunology and skin characteristics, and applied research related to production, licensing, and acceptability.

2. Conceptual Framework

The pathway to develop a successful vaccine patch, like other therapeutic agents, involves multiple stakeholders and includes many stages to get from the concept to a licensed product. Figure 1 depicts key stages for vaccine patch development in the US regulatory context [21] for an existing licensed vaccine product, and it shows the anticipated intensity of efforts for three key workstreams by: (1) immunobiologists and other basic scientists, (2) patch developers, and (3) vaccine manufacturers required to converge to develop a single vaccine patch product. Figure 1 highlights the unique challenge in vaccine patch commercialization in that a technology handoff is currently required between patch developers and vaccine manufacturers mid-cycle of the commercialization path. This differs from the development of incremental improvements of existing vaccines (e.g., dose sparing using adjuvants, reformulation, etc.) or improvements in patch fabrication technologies in which the development cycle is largely driven by one or the other industry. Furthermore, recognizing vaccine patches as a generalizable pharmaceutical platform leads to appreciation that some of the early research and development in the process of developing one vaccine patch (i.e., for a specific antigen) will likely influence the entire platform. For vaccine patch development, the following sections provide a systematic review of the literature to date and then discusses key platform concepts across the three streams of work in Figure 1 that need to converge to see the realization of vaccine patches in the market.

3. Systematic Literature Review

We performed a systematic literature review by searching Web of Science and PubMed for papers published in English prior to March 1, 2019 that included a combination of the terms:

"vaccine" and "patch" or "microneedle." The review process included screening the titles and abstracts of the search results to create a database of all studies that explored the development of vaccine patches for one or more specific vaccines. The review process included the extraction of information about the patch developer, the vaccine(s), and characteristics of the vaccine patch. We reviewed the full text for any papers that lacked enough information in the abstract. The search included the identification of prior reviews, and the review process included any relevant studies not captured in our search that we identified in the references of these reviews or in the references of the papers identified by our search. We included papers that reported the results of pre-clinical or clinical testing of vaccine patches for identified pathogens. We excluded studies that described experiments for non-specific genetic materials (DNA, RNA, plasmids), proteins (ovalbumin [OVA]), or other materials (non-specific laboratory bacterium or virus strains or components). Although these studies may provide generic information relevant to vaccine patches, we wanted to characterize the state of the literature specific to vaccines that target identified human pathogens. We excluded all studies not relevant to the review, and we report the numbers of excluded studies according to broad exclusion categories. We recognize that synergistic innovation in the use of patch technology for general pharmaceutical product delivery (non-vaccine products) may help to stimulate vaccine patch development and adoption over time [15], but we do not include consideration of this in our review. For the included studies, we extracted the specific vaccine used and its status as licensed or experimental, the group that conducted the study, the nature of the vaccine patch (i.e., height, number, and arrangement of projections, and whether the projections were coated with the vaccine, dissolving (and containing the vaccine), or hollow (such that the vaccine flowed through them), the species used for the tests, and the method of patch application and time applied to the skin (i.e., wear times).

Figure 2 provides a summary of the systematic literature review search process that led to the extraction of information from 116 studies [22–137]. Our review overlapped some with prior reviews (e.g. compared to the 2016 review by Marshall et al. [17], our 116 studies included 46 of the same studies ([22–26,29–37,42–49,51,53,54,56,62–65,83,85,86,99,100,103,108,109,113,115,116,121,123,126,127]) but we excluded TCI studies that it included (e.g., [138]).

Table 1 summarizes some key characteristics of included studies. Studies involving coated (N=69) and dissolving (N=39) projections dominate. The literature continues to increase with time, with half of the studies (N=64) published in the last 5 years. Multiple patch developers contributed to the literature, but 2 groups contributed the majority of the studies (i.e., Georgia Institute of Technology/Emory University/Micron (N=57) and Queensland University/Vaxxas/Nanopatch (N=14)). We identified 31 human vaccines with studies by at least one vaccine patch developer, more than 4 papers for some vaccines, and the same relatively small number of patch developer groups reporting on the use of essentially the same (albeit evolving) technology for more than one vaccine. With nearly half of the studies (N=68) focused on influenza, the use of a vaccine patch presentation for the delivery of influenza vaccine appears likely to lead the vaccine platform as the first product to progress through late-stage development and potential licensure as a product. Most studies reported pre-clinical results of tests using experimental animals. The potential opportunity to save on

antigen costs using vaccine patches to offset other costs of their development represents a key consideration for some studies. As shown in Table 1, 44 of the 116 studies (38%) demonstrated or suggested the potential for dose sparing with the use of vaccine patches compared to syringe and needle, while 6 studies looked for but did not find evidence of dose sparing.

Table 2 provides some of the details extracted from the 116 included studies organized by patch developer groups with the most-to-least studies. As reflected in the number of studies identified, the review shows 2 predominant strategies emerging: dissolving microneedles of approximately 0.5–0.7 mm in height, and coated microneedles of approximately 0.1–0.3 mm height. The review also shows a wide range of administration approaches, applicators (if used), and times of application. Some patch designs without an applicator include a built-in force feedback indicator to give audible or visible cues for the vaccine administrator. The design of applicators (if used) reflects different factors considered by patch developers to date related to the expected immunological performance of the projections and/or other factors (e.g., requirements for consistent delivery, requirements of the products to prevent choking for small objects). The use of different species in part reflects the appropriateness of different animal models for various vaccines; ideally the optimal animal model for each antigen is used that allows for a measurable immune response as a proxy indicator for the induction of immunity in humans using vaccine patches. As noted by others, the relatively small number of human studies for vaccine patches limits the opportunities to advance vaccine patch technology and get it into clinical practice [17,139].

Our review of the vaccine patch literature summarized the published information regarding the characteristics of human skin and strategies to induce immunity in the human skin needed to support the development of vaccine patches. The next section provides a brief review of the key concepts of the microscopic anatomy of normal human skin, which provides context relevant to the depth of vaccine patch projection penetration and the state of the evidence of skin immunobiology, and then discusses key platform concepts related to inducing immunity through the skin.

4. Key platform concepts and issues related to skin characteristics and inducing immunity

As the largest and most accessible of all body organs, extensive studies of skin date back centuries. Physical properties of skin vary as a function of body site, gender, race/ethnicity, environmental exposure (e.g., the sun) and age, and selective studies are required to address the pathobiology of skin in various systemic conditions that specifically affect the skin such as systemic sclerosis, sun damage, and inflammatory dermatoses. Changes in physical properties of skin as a function of other environmental parameters such as hydration and temperature, physical conditioning (e.g., shaving or alcohol sterilization), or injury remain less well-studied. Although discussion of the vast literature on the cellular, molecular, and tissue biology of the human skin falls beyond the scope of this review, we provide a high-level discussion of microanatomy and antigen presentation in the human skin to introduce key cellular constituents of the skin immune system and their anatomy relevant to the

development of vaccine patches. We go into sufficient detail in this section to address key gaps in basic sciences that have emerged as a consequence of recent advances in vaccine patch development. A recent review highlights an urgent need for technical consistency across models and platforms used by vaccine patch developers [140]. Meaningful comparisons will help to promote successful development, testing, and commercialization of vaccine patches in human skin.

Skin Variability as a Function of Age and Body Site

The available evidence provides limited information about the nature of physiological and structural changes in skin as a function of age. Some studies show measurable differences in human skin morphology as a function of age [141,142], but an *in vivo* study reported no significant functional or physiological differences in the skin barrier function [143]. Uncertainty remains about the extent to which observed morphological changes result in equivalent changes in physical properties and/or physiological functions of the skin in various sites. On average, most body sites studies show an epidermal thickness of approximately 60 microns, except for the forehead with an epidermal thickness between 75 and 80 microns.

Several studies that focused on the dermal thickness as a function of age, gender, body site and/or body mass index (BMI) showed some general differences across multiple categories, including subcutaneous fat as a function of gender and body site [144–147]. A study of the epidermal thickness in multiple sites in the body using Reflectance Confocal Microscopy showed variation between sites in the thickness of stratum corneum, granular layer, and papillary length, but they also noted the presence of a “striking variation at any one body site for a single individual” [148], representing 50–74% of total morphological variation measured at any given body site. Despite these studies, insufficient evidence exists for the pediatric and elderly (i.e., ≥65 years) age groups to characterize skin structure and physiology as a function of age across the full CDC-recommended vaccination schedule to make definitive conclusions about individual vaccines, and the existing studies do not provide sufficient cellular, functional, or physiological data.

Microscopic Anatomy of Normal Human Skin

The microscopic anatomy of normal human skin is commonly described in the form of several flat structural layers that in combination form an “integument” at the interface of the body and the outside environment. Starting from the outside, these include the keratin layer (also known as stratum corneum), epidermis, papillary dermis, reticular dermis, and subcutis (also known as hypodermis). At the physical scale and biological scope of vaccine patch development, the epidermis, papillary dermis, and reticular dermis represent the three most relevant subsystems of the human skin.

Figure 3 shows a histological preparation of excised human skin with micron-level resolution that reveal specific cells and compartments by general morphology and selective immunostaining. The staining technique in Panel A shows keratinocytes as the majority of cells visible, but intraepithelial lymphocytes and other cell types (e.g., Langerhans cells [LCs]) are not specifically identifiable. The dense collagen bundles seen as broad

eosinophilic clumps at the bottom of the image characterize the reticular dermis, and the more delicate region of connective tissue between the reticular dermis and the epidermis is the papillary dermis, which shows multiple vascular structures, including capillaries (arrows) and lymphatics (arrowheads). The image does not provide sufficient information or detail to identify specific other cell types or structures (e.g., small nerves). Panel B shows immunostaining of an adjacent section, in which brown pigment shows on the surface of cells that express CD1a. Stained cells include intraepithelial LCs (arrowheads). CD1a-positive cells in the dermis (arrows) are also antigen presenting cells, but CD1a does not have sufficient specificity in the dermis to distinguish circulating LCs from other dermal antigen presenting cells (APCs). Morphological studies combined with selective immunohistochemistry and related techniques have the ability to characterize skin constituents with increasing sophistication. Although detailed catalogues of normal human skin as a function of age, gender and other variable are not generally available, various compositive pictures can be developed by combining data across multiple studies and techniques, some of which are described in more detail below.

Immunobiology of the Normal Human Skin

As suggested in Figure 3, normal human skin harbors a variety of APCs, the majority of which belong to the general category of conventional dendritic cells (DCs). Skin DCs show extensive immunophenotypic diversity relevant to both health and disease, but their subclassification into epidermal DCs, also known as LCs, and dermal DCs (DDC) serves as a useful high-level subdivision [149]. LCs, reviewed in detail by others [150,151], are resident epidermal APCs responsible for T-cell priming to antigens encountered in the epidermis. The ability of LCs to migrate to regional lymph nodes where classical T-cell priming takes place facilitates their significant immunological function. Although LCs and DDCs share many morphological and functional similarities, LCs also share many similarities with macrophages, and may behave as resident tissue macrophages with the ability for local self-renewal, as opposed to conventional DCs that derive from hematopoietic stem cells [150]. Immunophenotypically, human LCs express high levels of CD1a, which is an MHC-related membrane protein involved in the presentation of lipid antigens [151]. An endosomal protein called CD207/Langerin also expresses at high levels in humans and is a constituent of Birbeck granules, which may play a role in the internalization of viruses [152].

Kashem et al. [151] offer a catalogue of human LCs immunophenotypes, although detailed pathobiology remains an active area of investigation. Dermal APCs include a highly heterogenous population of cells [149–151,153] with at least 4 different subgroups recognized in humans [151]. First and the most prevalent of the DDCs in human skin, CD1c + conventional DCs are classically involved in Th2 cell differentiation and immunity against parasites and helminths. CD1c+ DDCs migrate to regional lymph nodes and generally co-localize with resident lymph node DCs in proximity to the sinus endothelium of paracortex. Second, CD141+ DDCs are a minor population of conventional DCs thought to induce Th1 and cytotoxic T lymphocytes in response to a variety of antigens, including fungi, intracellular pathogens, and tumor antigens. CD207/Langerin is expressed in mouse homologs of CD141+ DDCs, creating a complex picture involving CD1c+ DDCs that may

also express Langerin, and migrating LCs also express Langerin. CD141+ DDCs turn over at a high rate and migrate rapidly into the deep T cell zone of regional lymph nodes. Third, plasmacytoid DCs (pDCs) circulate throughout the body and can be found in human skin under inflammatory conditions. Morphologically, pDCs resemble plasma cells and lack dendritic extensions typical of LCs and other DDCs. Finally, monocyte-derived macrophages represent the last known category of dermal APCs in human skin. Dermal macrophages are well adapted for phagocytosis but are inferior to DDCs in antigen presentation to T cells. Despite the wealth of evidence, as summarized in Table 3, uncertainty (or possibility biological variability) remains about some of the markers of human immunophenotypes for some APCs in some parts of the skin relevant to vaccine patches. In addition, testing of vaccine patches in animals must consider the different sets of immunophenotypes relevant to those species and locations (e.g., comparison of mice to humans [9], and choose appropriate animal models for human vaccine-preventable diseases [81,113,154]).

In addition to normal APCs in the human skin, the role of the disruption of the physical barrier in triggering of an immune response represents a key consideration in vaccine patch immunobiology. As immune sentinels, keratinocytes can sense physical injury (e.g., abrasion, puncture wounds) induced by micron-sized projections and produce pro-inflammatory cytokines that in turn activate DDCs [153,155]. Similarly, dermal fibroblasts and other dermal components can activate various components of the immune system in response to puncture or other forms of physical trauma. Review of the existing literature demonstrates that the application of vaccine patches can induce an inflammatory response, as evidenced by skin erythema and other macroscopic inflammatory changes [68,156]. However, the extent and pattern of local immune system activation secondary to vaccine patch application and its role in inducing immunity remains a topic in need of further study. Of note, one of the 2 leading vaccine patch development groups (i.e., Queensland/Vaxxas/Nanopatch) uses very dense arrays of coated and relatively short projections intended to kill epidermal cells while depositing the vaccine, because the immunogenic signals that are released from impacted cells may help with dose sparing [96,157].

Non-Invasive Skin Imaging

As shown above, *ex vivo* microscopy in the form of conventional histology, or in various other forms such as immunofluorescence or electron microscopy, represents the gold standard for cellular and subcellular study of the human skin. However, *ex vivo* microscopy is an invasive procedure requiring tissue removal, often in the form of a skin punch or excisional biopsy. Although skin biopsy represents a minor medical procedure, healthy human volunteers, especially in the pediatric age group, do not commonly undergo biopsy for purely investigational purposes. Post-mortem studies at the time of an autopsy offer a more practical option for baseline human studies, but post-mortem changes add complications, which depend on the typically not-controlled post-mortem time interval and co-morbid conditions that often served as the primary reason for medical autopsies. Forensic autopsies for accidental death can potentially overcome some of these limitations, but baseline studies in forensic autopsies are rarely performed. Because of these limitations, many recent dermal vaccine and vaccine patch studies rely on non-invasive imaging by high

resolution ultrasound or *in vivo* optical technologies. Many studies used high-frequency ultrasound scanners of 10–50 MHz to image skin because they offer a reasonable tradeoff between axial resolution and penetration depth [144,145,158]. However, ultrasound devices generally prove inferior to optical methods in measuring submillimeter structures that may be necessary for detailed characterization of cellular and/or microvascular morphology in the submillimeter penetration depth, which is the relevant scale for vaccine patch applications. Ultrahigh frequency ultrasounds (70–100 MHz) can counter this limitation, but they are rarely used in skin measurements. In addition, ultrahigh frequency ultrasound methods typically lose some of the benefits of general ultrasound techniques, such as deeper penetration depth. In one comparative study involving ultrahigh frequency ultrasound and optical coherence tomography (OCT), the optical method showed better axial resolution than 100 MHz ultrasound (5.5 μm and 16 μm, respectively), while both had limited penetration depth in the range of approximately 1 mm [158]. More generally, OCT gave better lateral resolution, while ultrasound provided better contrast of the lesion to surrounding tissue [158].

The inability to include multiplex investigations such as immunophenotyping for cellular or subcellular studies represents a limitation of *in vivo* imaging technologies. However, *in vivo* imaging methods provide a significant opportunity for live cell and/or dynamic studies, which is difficult in animal models and virtually impossible in humans with invasive skin biopsies. Bachy et al. present a highly informative animal study of the dynamics of immune system activation by live adenovirus microneedle arrays that sheds light on the immunobiology of the system and provides a nice animal model for *in vivo* imaging of microneedle dissolution dynamics and skin repair [159]. In a separate study, Liu et al. used OCT to successfully characterize dynamics of the controlled release of dissolving silk microneedles in mice [160]. Unfortunately, the various methodologies developed in animal studies do not immediately apply in human subjects without limitation or further development. Bal et al. used an *in vivo* confocal microscopy technique to study the dynamics of a fluorescent dye tracer through conduits produced by microneedles of similar length but with various shapes in a small group of adult volunteers [161]. They observed that microneedle geometry, but not the manner of application affected the shape and depth of the conduits and the penetration of the fluorescent dye. Using a comparable imaging strategy of multiphoton microscopy, Wei et al. characterized the diffusion of rhodamine-conjugated dextrans applied to excised human skin through microneedle arrays [162]. As such, they successfully characterized the rate of dissipation of dextran macromolecules as a function of molecular weight at the imaged skin layers. Although more limited in nature than the animal imaging studies, human subject studies that combine *in vivo* imaging with dynamic biochemical or cellular studies are increasingly needed to better characterize the interaction between various vaccine patches and the human subjects.

Multiple research groups used other imaging modalities such as scanning electron microscopy (SEM) and fluorescence microscopy in a variety of circumstances [31,92,159,161–166]. These invasive techniques offer super-high resolution, generally for static and morphologically well-defined objects such as the projections of the vaccine patch, but they appear to provide limited value in dynamic, large scale or *in vivo* studies.

Figure 4 provides a high-level view of some of the physical issues and imaging constraints described here. Panels A-D show shows the representative performance of four imaging technologies in the context of typical vaccine patch projections, which are depicted in Panel E. Panel A shows a hematoxylin and eosin stained section of paraffin-embedded human skin showing the relative proportions of keratin, epidermis, papillary dermis, reticular dermis and subcutaneous adipose tissue (hypodermis), respectively. (For ease of reference, these layers are highlighted in the right-hand side of Panel A using pseudocolors of red, pink, blue, no color, and yellow, respectively.) The scale bar represents the 1 mm scale across all panels, corresponding to the cut off used for needle size in this review. Panel B (adapted from [145] and redrawn to scale) represents the high frequency ultrasound (40 MHz) methodology commonly used to assess skin thickness in various body sites. Although the epidermis, dermis, and subcutaneous layers are generally discernable, the image shows insufficient resolution to assess structural details within each layer, particularly at the scale relevant to vaccine patches. Panel C (adapted from supplementary data in [167] and redrawn to scale) shows increased morphological detail in the dermis and superficial subcutaneous tissue by ultrasound imaging at ultrahigh frequencies (70 MHz shown). The increased resolution at higher frequencies generally comes at the expense of shallower penetration depth for comparable probes, as seen by the relative loss of signal below the dermis compared to Panel B. At frequencies approaching 100 MHz (not shown), ultrasound and optical imaging technologies reach comparable resolution at the superficial layers of the skin [158]. Panel D (adapted from [168] and redrawn to scale) shows simultaneous dual-band line-field confocal OCT. Unlike typical ultrasound images, this high-resolution OCT image provides significant cellular detail at the level of epidermis and superficial papillary dermis (inset), but the penetration depth is not sufficient for full thickness imaging of the entire dermis. Panel E facilitates comparison of the images in Panels A-D by redrawing to scale a single dissolving microneedle (adapted from [64] on the left) and a single projection of a coated nanopatch (adapted from [92] on the right). The black rectangle drawn at the base of the needle corresponds to the position of the corresponding patch inner surface.

5. Key platform concepts and issues for patch developers

Over the course of the last 15 years, vaccine patch developers explored and addressed numerous concepts related to the fabrication and design of the vaccine patches, which supported the 116 published studies identified in section 3 of our review (with multiple published reviews of different fabrication and design approaches, including [15,169,170]). Based on our review of the literature and discussions with key stakeholders, this section discusses key platform issues that we identified for patch developers.

To become viable commercial products, innovative vaccine patches will need to prove safe and effective, and meet non-inferiority criteria when compared to any existing vaccines. Several recent reviews and studies [18,20,139,171] identified key issues that influence design choices made by patch developers. These reviews highlight opportunities to identify desirable product attributes and to develop target product profiles as a means for potential users to provide guidance about their preferences for different product attributes. The ideal site placement and duration of wear for vaccine patches will influence their initial design and guide their subsequent redesign ahead of clinical deployment. Research that identifies

the optimal delivery sites for each product with respect to the induction of immunity and acceptability by vaccine recipients could reduce the need for testing each product by guiding development and potentially offering some standardization across the platform. In the absence of platform-related guidance, vaccine patch developers and other researchers continue to demonstrate safety and acceptability of their designs [68,94,156,172–175], with published literature reviews of acceptability studies now available [20,176]. In addition to recommendations related to ideal vaccine patch site placement, guidance related to vaccine patch delivery that would help across the platform includes recommendations related to wear time duration, characterization of recipient tolerance of vaccine patch application force by age and body site, skin site preparation needs (e.g., hair removal, cleaning, etc., which must not neutralize live vaccines or react with any components and should be consistent with the design of any clinical trials performed), post-application site care (if needed), and requirements related to the ability to confirm successful delivery (i.e., at the time of injection and/or long-term). The innovation of potential long-term recording of vaccine delivery [177] could add costs and affect acceptability (e.g., positively by helping health systems and individuals track their immunity status and/or negatively by leaving permanent markers that individuals may not want). Additional guidance, including development of international consensus guidelines and standards as appropriate, necessary, and/or useful [20,140], would also help individual vaccine patch developers prioritize their designs to achieve required or desirable attributes, and allow for trade-offs on product attributes that matter less. For example, vaccine patches could be designed to offer increased efficacy, increased thermostability, eliminate the need for reconstitution, make vaccine delivery easier, require less training, reduce or eliminate sharps, decrease medical waste, offer single-use and single-dose administration, reduce vaccine wastage, reduce or eliminate the pain and risks of injections, save costs, etc., if any such requirements were known *a priori* and included in the initial design and fabrication. The ideal target product profile may vary for developed and developing countries and/or for different vaccines [20,139], and any such differences could also be recognized and incorporated early in the development stage. The World Health Organization (WHO) recently published an example of a target product profile for measles-rubella (MR) combination vaccine [178]. Current research on patch delivery for other pharmaceutical products (i.e., non-vaccine) may provide information relevant to the vaccine patch platform, particularly for products that target healthy children and adults.

Vaccine patches will require high-quality, cost-effective, and reliable processing under good manufacturing practice (GMP) conditions. Vaccines are highly-regulated given their use in healthy children, which will imply significant investments of regulatory compliance costs. In addition, significant uncertainty remains about the need for sterile vs. low bioburden production, and the processes regulatory authorities will find acceptable [20,139,140,179]. Low bioburden production means not requiring sterility for vaccine patches (due to their administration of vaccine to non-sterile skin) but would limit any organisms in the final product to very low levels. Low bioburden production would save significantly on production costs, because sterile production processes require isolators and other costly equipment. Production and design choices will determine the cost of vaccine patches, and thus the cost premium relative to existing syringe and needle or other presentations for existing vaccines.

Developing low-cost, scalable, and reliable designs for manufacturing of vaccine patches currently represents a translational hurdle [20]. Developing mass production processes typically occurs in the context of proprietary activities by companies who will need to make significant financial investments that they will need to be convinced that they can recover [139]. Lessons learned and technology transfer from the commercial development and large-scale production of non-vaccine patch products may help to support some vaccine patch development. For effective and timely clinical translation, vaccine patch developers will need to partner with vaccine manufacturers or decide to become vaccine manufacturers themselves, for example by purchasing a vaccine manufacturer to ultimately deliver a licensed product. We emphasize that the process for patch developers to become a vaccine manufacturer *de novo* would take many years under current regulatory pathways.

6. Key platform concepts and issues for vaccine manufacturers

The research laboratory processes used by vaccine patch developers to create the patches they use for clinical trials will influence the starting point for vaccine manufacturers as they evaluate the potential for mass production. Mass production of vaccine patches will also require overcoming significant design challenges related to operating at scale, including the need to perform inline inspection and quality control (QC) testing, establish and maintain environmental controls (temperature and humidity), and manage the logistics of chemistry, manufacturing, and control (CMC) processes. A recent review highlighted the absence of standardized techniques and equipment used by patch developers to demonstrate mechanical properties of vaccine patches, and suggested an “urgent need” for standards that would support consistent comparisons to promote innovation and successful commercialization of vaccine patches [140].

For all vaccine manufacturers, commercializing a new product will likely require the payment of significant costs for any late-stage (e.g., phase 2 or 3) clinical trials to demonstrate efficacy, non-inferiority, and durability of immunity required to support licensing the new vaccine patch product, and they will need to perceive a reasonable expectation of recovering these costs. In contrast to new vaccines, the development of vaccine patches for existing vaccines may only require bridging studies, but it could also lead to changes in required formulation or concentrations (e.g., excipients, stabilizers), which may ultimately require extensive and expensive regulatory changes for the vaccine itself [20]. Few financial incentives for innovation seem apparent for current low-cost, well-established vaccines, in some cases licensed many years ago, suggesting potential high costs and risks to reopening safety profiles or changing production processes to meet current regulatory requirements [20]. In addition, for vaccines already produced at large scale, vaccine manufacturers will not see the need for dose sparing, which would only serve to make some of their existing vaccine production capacity redundant. However, if one manufacturer dominates a particular market, then incentives may exist for alternative new products offered by other manufacturers that allow them to compete for market share.

The stability of the supply chain will represent a critical consideration for vaccine manufactures. Manufacturers will most likely prefer to co-locate the patch and vaccine production, but they will need to evaluate the cost-effectiveness of performing some

production elsewhere. As with all vaccine manufacturing processes, evaluation of the supply chain will include consideration of raw material sourcing (e.g., particularly those derived from animals like bovine and porcine components), which may raise issues related to the acceptability of the ultimate vaccine patch product. For example, the use of a porcine-based component in a rubella-containing vaccine led to acceptability issues in Indonesia that significantly affected coverage [180], whereas vaccines containing bovine components may not be acceptable in other countries (e.g., India).

In addition to any specific issues related to the vaccine patch presentation, vaccine manufacturers will need to address the issues that typically come with the production and licensing of vaccine products. These include managing the nature of the batch processing, QC, packaging, labeling, and storage of vaccines, and the time delays related to regulatory processing, licensing, compliance, and WHO pre-qualification (for those seeking to sell to markets that require it), all of which will require resource investments. All the details related to labeling and packaging requirements (e.g., required language or codes, temperature deviation monitors similar in purpose to vaccine vial monitors, desiccants, etc.) represent areas that will need development. Vaccine manufacturers will also face decisions related to pursuing single or multiple antigens in the vaccine patch formulation and on making trade-offs associated with product attributes based on their perceptions and expectations about the future market. For large multi-national companies that work with multiple national regulatory authorities, seeking and obtaining approval for process changes can represent a time-consuming and expensive undertaking, which may also impact the willingness of vaccine manufacturers to adopt unfamiliar technologies. In addition, the innovative nature of vaccine patches implies the need for a new regulatory pathway, and currently no consensus exists across functioning national regulatory authorities (NRAs) on such a pathway, which will mean extensive discussions with multiple NRAs and imply associated costs and time delays.

The nature of the vaccine market, which includes relatively few manufacturers and a relatively small number of large buyers, influences incentives and leads to segmentation. Specifically, segmentation translates into lower-priced multi-dose vaccines for developing countries, and relatively higher-priced vaccines targeted at developed countries (e.g., single dose, combination vaccines), often produced by only one or two manufacturers. In developed countries, the existing market incentives are relatively favorable, with some incentives for innovation coming from the opportunity of gaining significant market share for superior products (i.e., more effective, safer, easier-to-deliver, and/or more cost-effective options than any currently available). As discussed above, influenza appears likely to be the first potential vaccine patch product to complete phase 3 clinical trials. In addition, with potential demand for the entire population for annual administration of seasonal influenza vaccine that changes in formulation annually, a flu vaccine patch could prove to be a potentially viable vaccine patch product with significant consumer appeal. The development and licensure of FluMist™ as a nasal delivery method suggests a willingness by vaccine manufacturers in developed countries to pursue alternatives to the traditional needle injections despite the estimated $1 billion cost (i.e., $340 million to license FluMist™ and an estimated total costs of 2–3 times higher by the time patients started receiving it [181]). However, innovation in vaccine delivery for some products targeted for developed countries

may require some support from push incentives, which subsidize development costs, and/or pull incentives, which reward manufactures for offering the desired product, or a public-private partnership (e.g., for pandemic vaccines), particularly in the context of highly uncertain demand and expected relatively low margins for vaccine products compared to other therapeutic agents.

Based on the current status of vaccine patch development and discussions with key stakeholders, the business case for vaccine patches does not currently support the required investments by vaccine manufacturers that supply the developing country markets to expect licensed vaccine patches to come to the market soon [20]. For developing countries, prior experience with new vaccine products will influence manufacturer willingness to engage in development activities. For example, the rotavirus vaccine market currently includes multiple products now pre-qualified by WHO with different attributes, and it does not show a willingness of vaccine purchases to pay a price premium for a thermostable vaccine (i.e., to prefer thermostable ROTASIIL®[182] to less thermostable but cheaper alternatives [183]) to date. Public-private partnerships can bring investments that share the costs and risks of the vaccine development process, particularly for products with thin profit margins or relatively smaller markets. For example, public-private partnerships were required to support the development of vaccine products needed in developing countries, including a meningitis vaccine for central Africa (i.e., for MenAfriVac™ [184])), oral cholera vaccine [185], and other vaccines [186,187] However, such partnerships do not always lead to commercial success. For example, the partnership that sought to develop an aerosol formulation for MR vaccine invested significant resources in the conduct of several clinical trials, including a trial that ultimately showed the aerosol formulation did not meet the non-inferiority criteria compared to syringe and needle presentation [188].

The licensure and successful marketing of the first vaccine patch product will establish the platform and set precedents that create opportunities and/or challenges for future vaccine patch products. Consumer demand for multiple vaccine patches could follow the establishment of a licensed vaccine patch product that receives wide public acceptance and establishes the platform. In this regard, health system and consumer experience with the first vaccine patch product will likely influence acceptance and demand for other vaccine patch products, as may any experience with other (i.e., non-vaccine) patch-delivered pharmaceutical products. Notably, if the first vaccine patch product leads to greater acceptance of and demand for vaccination and thus higher coverage, then this could increase population immunity and demonstrate the potential for significant benefits. In addition, the first product to market will establish the first regulatory path for the platform, packaging requirements (e.g., protection of projection integrity and the vaccine antigen, the need for desiccants, waste disposal), acceptability of the product within the health system (e.g., costs, ease of use, administration times and skills needed, storage, potential sharps, residual active ingredients after delivery, confirmation of delivery signals), acceptability by individual vaccine recipients, and other firsts. In addition, widespread use of the first vaccine patch will present the first opportunity to observe potential new adverse events (e.g., skin reactions, unintended use or ingestion of a vaccine patch, packaging, etc.), as well as any potential injury due to application/misapplication. With seasonal influenza as the apparent front runner for vaccine patch development, we note the potential for issues unrelated to the

vaccine patch itself to impact the product. For example, following the recommendation and adoption of FluMist™ by the US, choices related to the strains used in the formulation led to the temporary suspension of its recommended use in the US market [189–191], which had nothing to do with the delivery mechanism.

7. Conclusion

Over the past two decades, significant advances in engineering supported the development of vaccine patch technologies with the potential to deliver vaccines through the skin while taking advantage of other favorable properties (e.g., increased thermostability, no reconstitution or field preparation, etc.) [15,18,192,193]. At the same time, we now have a detailed understanding of antigen presentation in the human skin [149–151], and development of non-invasive imaging modalities have enabled *in vivo* studies of the human skin with submicron resolution [145,158,160,168]. A coordinated convergence of the three disciplines discussed here could help to develop the baseline basic science necessary to characterize and model the immunobiology of various vaccine patch technologies and accelerate progress in vaccine patch platform development. For instance, further research could help develop broad reference standards for physical characteristics of skin across multiple categories of age, gender, site, BMI, as well as other potential variables such as nutritional status and ethnicity, or physical conditions such as humidity and temperature. Specific vaccine patch delivery methodologies could be refined to specific human skin characteristics relevant to the given technique. For instance, BMI and gender may be relevant to longer projections that potentially penetrate through the dermis at a given site, while age and body site may be more relevant to shorter projections that either stay within the epidermis or superficially penetrate the papillary dermis. Several studies also demonstrate that the design of the projections can impact pain [194–196].

This review highlights tremendous progress to date in the development of vaccine patches, and provides a glimpse into the many future opportunities to deploy vaccine patches in broad use. Innovative vaccine delivery technologies offer many promises for increasing immunization coverage. Given the apparent lack of incentives that exist for vaccine manufacturers to pursue the development of vaccine patches for developing country markets, public-private partnerships will likely be needed if any key stakeholder wants to realize (or accelerate) the licensure of vaccine patches for widespread use, particularly for existing, low-margin vaccines. Although developed countries continue to explore the use of vaccine patches to support disease eradication efforts that require reaching difficult-to-access populations in developing countries, the willingness-to-pay a premium for vaccine patches remains uncertain, even in an eradication context. The opportunity for new vaccines, and for expensive antigens that would benefit from dose-sparing, might improve the value proposition for vaccine patches and make investments in their development attractive to vaccine manufacturers in some cases. Overall, the incentives that vaccine manufacturers perceive and realize will depend on the value that consumers and health systems ultimately place on the set of attributes that each product brings to the system.

8. Expert Opinion

Despite substantial advances in microneedle technology and basic science, progress towards clinical deployment of microneedle skin patches for vaccination remains relatively slow, which reflects current financial incentives. The lack of a perceived sufficient return on investment for vaccine manufacturers to make incremental improvements in vaccine delivery technology broadly, and for low-cost, legacy vaccines specifically, represents a critical barrier or translational valley of death [197,198]. The lack of competitive forces that would create financial incentives for patch developers to become first movers and disrupt the market by also taking on the vaccine manufacturing aspects of the products (i.e., becoming vaccine manufacturers themselves) similarly means vaccine patch development will likely depend on external financing or financial incentives.

Similar to other situations discussed above, the development and adoption of vaccine patch products for developing country markets will likely require the support from public-private partnerships and/or other incentives, such as targeted national or international investments, to overcome the early economic barriers. In this context, financial partners may influence the overall timing of availability of vaccine patches to the market. This could accelerate progress (e.g., by supporting the establishment of large-scale GMP manufacturing processes and facilities in parallel with phase 2 clinical testing [20]) and/or slow it down (e.g., by requiring multiple patch developers to all reach each specific stage before proceeding such that the trials generate comparable results by using the same settings and criteria for advancement to the next stage). Thus, while some patch developers may complete initial development with respect to clinical formulation, processing, and assessing prototype stability, they may experience delays in the clinical testing timeline at multiple points if they need to wait for others to finish the prior phase. In addition, all patch developers will need to wait for as any financial partners evaluate results and makes stage-gate decisions about continuing to the next step. The establishment of a public-private partnership to develop the general platform would further allow the public health and donor partners to negotiate some controls on the prices of the final products, particularly if the financial support that they provide shares the costs and risks of product development.

For developed country markets, sufficient private financing (e.g., venture capital) may already exist and the competitive nature of the market may alleviate the need for a public-private partnership. Specifically, vaccine patches may provide an opportunity to differentiate a product from other competitors, which could offer a significant market advantage. Nevertheless, national research funding mechanisms (e.g., a National Institutes of Health study section, and/or a targeted funding program, and/or support from Biomedical Advanced Research and Development Authority in the US) could significantly stimulate basic science and translational research for vaccine patches and accelerate their use. The creation of broad funding mechanisms for the patch platform (including non-vaccine applications) could help to resolve some shared questions, while specific funding to support vaccine patches would likely better support the basic science immunobiological questions relevant to specific vaccines.

Although we discuss a number of current issues, these represent surmountable hurdles that can be overcome with sufficient resources, and they should not be viewed as barriers to innovation or clinical deployment. Vaccine patches could support efforts to significantly increase immunization coverage [20]. Future reviews will likely show a continued acceleration of vaccine patch research and development activity and insights related to many of the topics discussed in this review. Innovation in the use of patch technology for general pharmaceutical product delivery (non-vaccine products) may further patches as a delivery platform broadly for pharmaceutical products, which may help to accelerate vaccine patch development [15]. Health systems and consumers could easily adapt to the platform and could prefer immunization delivered by vaccine patches, although perceptions about efficacy and actual efficacy will impact uptake [175]. We anticipate that within the next 10 years, the commercial viability of a vaccine patch for influenza vaccine will become clear, and that efforts to develop vaccine patches as a delivery platform will mature. Progress will depend on the investments made to support multiple streams of work by numerous stakeholders.

Acknowledgments

The initial draft of this article was prepared when Kamran Badizadegan was serving as the Chair of Pathology at Nationwide Children's Hospital and professor of pathology at The Ohio State University Wexner College of Medicine. We thank Shanda Boyle, Gitte Giersing, David Hoey, Suresh Jadhav, Ravi Menon, Michael Royals, and multiple anonymous individuals for helpful comments and discussions.

Funding

This paper was supported by Cooperative Agreement Number 5NU2RGH001913-03-00 funded by the Centers for Disease Control and Prevention (first and last authors). The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Appendix

Histological preparations shown in Figures 3 and 4 represent normal portions of skin in pediatric patients who underwent surgical excision for a pigmented skin lesion located at least 2 mm away from the normal skin represented in the images. All tissues were fixed in neutral-buffered formalin for at least 24 hours before being processed routinely for paraffin embedding and sectioning at 4–5 microns followed by hematoxylin and eosin staining. For immunohistochemistry, paraffin sections were deparaffinized with xylene and hydrated through a graded series of alcohol. After antigen retrieval in 0.1M citrate buffer (pH 6.0) in a microwave oven for 10 min, inhibition of endogenous peroxidase activity was performed by immersion in 3% hydrogen peroxidase in methanol. The sections were then incubated with the primary antibodies, followed by thorough washing in phosphate-buffered solution (PBS), incubation with the biotinylated secondary antibody, followed by the avidin-biotinylated horseradish peroxidase complex, and finally developed using 3,3'-Diaminobenzidine as chromogen. The nuclear counterstaining was accomplished using Mayer's hematoxylin. All reagents, antibodies and instruments used to process and stain the tissue were from Leica Microsystems Inc. (Buffalo Grove, IL). Photographs were taken on a BX43 microscope (Olympus America, Center Valley, PA) coupled to an Infinity2–5 camera (Lumenera, Ottawa, Ontario). Images were captured in Photoshop (Adobe Systems, San Jose,

California), optimized for brightness and color to generally maximize the width of the image histogram. Final images were cropped to assemble the composite images shown here.

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9.

Article highlights

- Systematic review of the literature reveals substantial progress in the development of vaccine patches
- Efforts to translate research and development into commercial products will depend on success convergence of multiple streams of work
- Different incentives exist for commercialization of vaccine patches in developed and developing countries
- Financial incentives for the platform will impact vaccine patch design
- Standardizing models and techniques would help to promote successful commercialization of vaccine patches in human skin

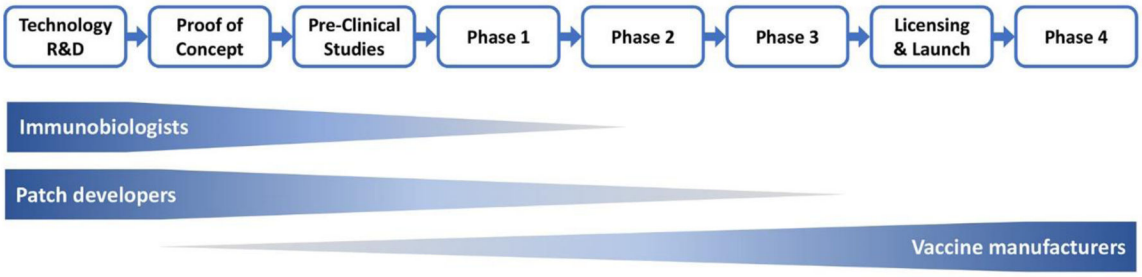

Figure 1:

Vaccine patch development pathway and intensity of efforts (indicated by triangles) required from stakeholders that need to resolve key issues and converge to commercialize a clinical vaccine patch, including successful handoff from patch developers to vaccine manufacturers.

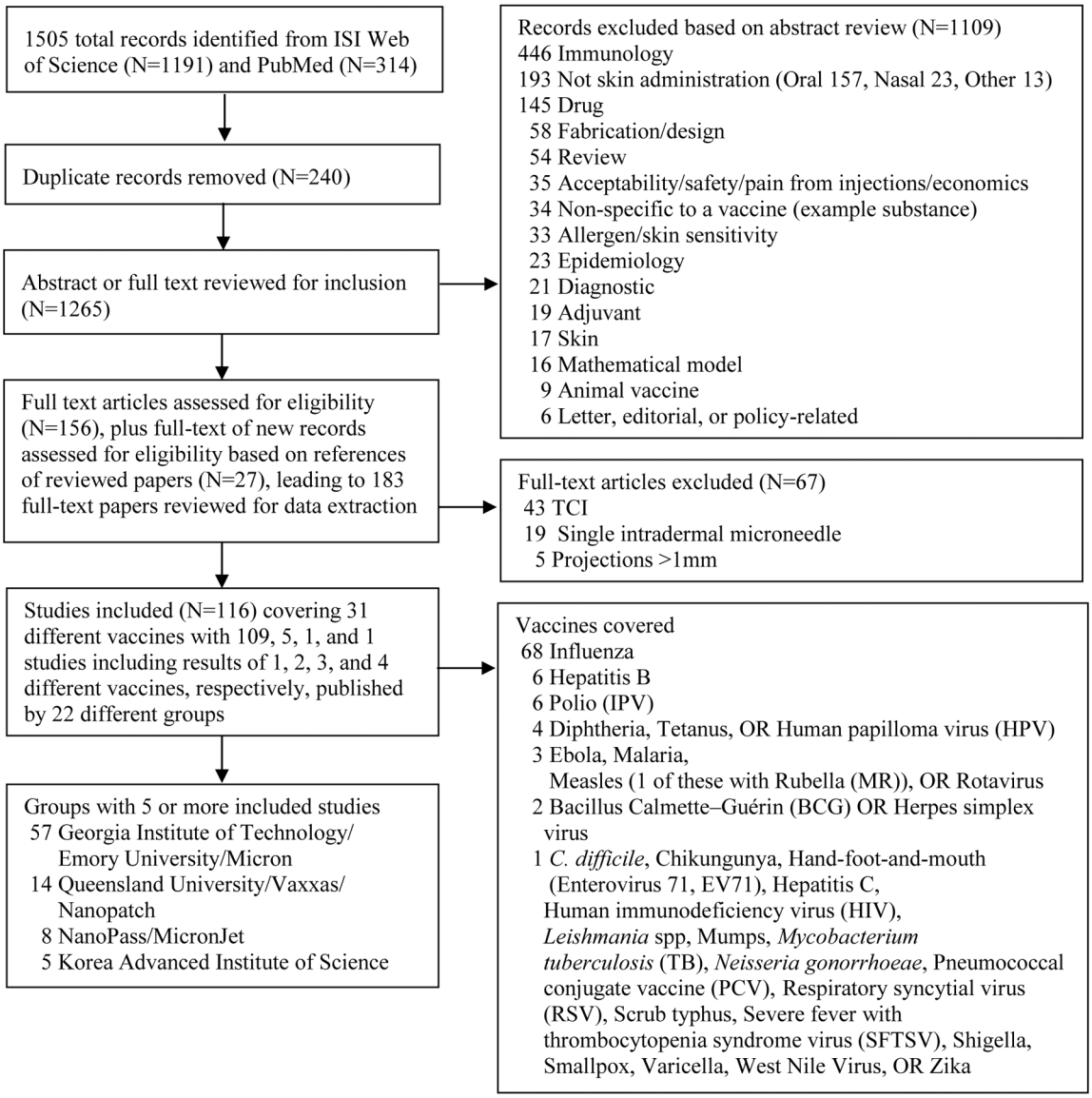

Figure 2:

Process used to identify 116 relevant peer-reviewed vaccine patch papers published in English that met the inclusion criteria.

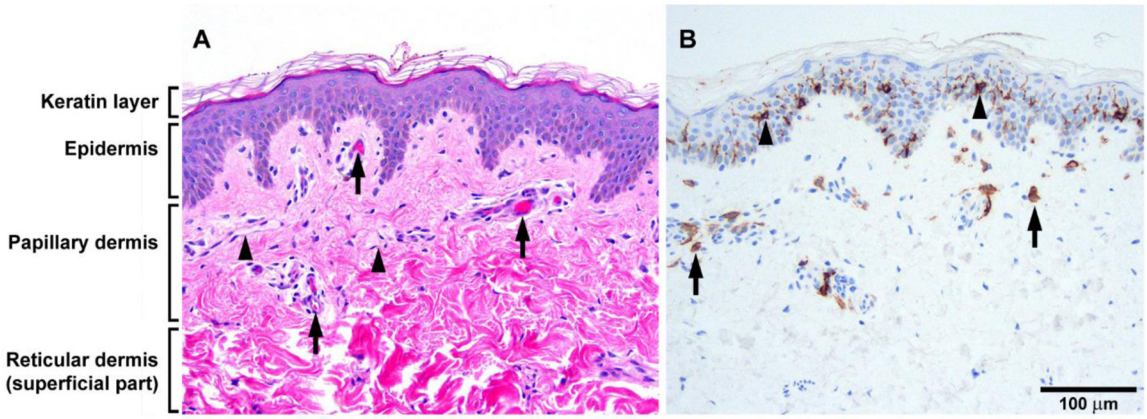

Figure 3:

Histological preparation of the excised human skin for which the solid horizontal bar represents 100 microns in both panels. Panel A shows a hematoxylin and eosin stained section of paraffin-embedded human skin (original magnification 200x), with capillaries noted by arrows and lymphatics by arrowheads. Panel B shows immunostaining of an adjacent section using antibodies to CD1a with intraepithelial Langerhans Cells noted by arrowheads and CD1a-positive cells in the dermis noted by arrows.

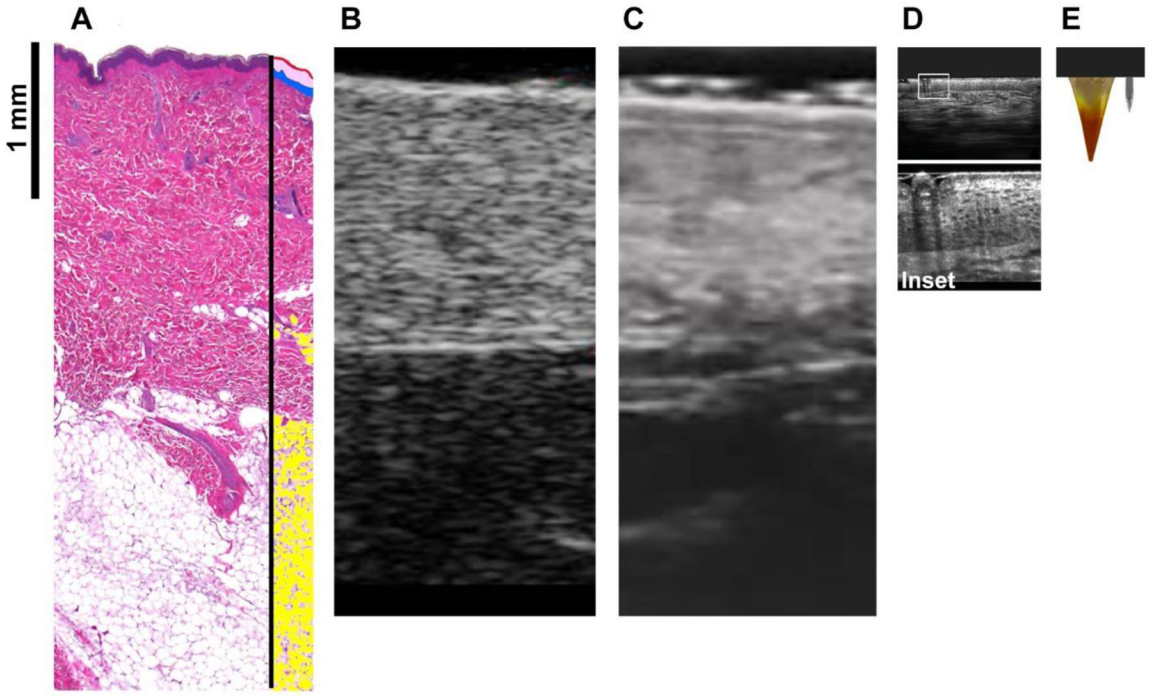

Figure 4.

Scale drawing (bar represents 1 mm) with Panel A showing a hematoxylin and eosin stained section of paraffin-embedded human skin showing the relative proportions of keratin, epidermis, papillary dermis, reticular dermis, and subcutaneous adipose tissue, respectively, highlighted in the right-hand side by pseudocolors of red, pink, blue, no color, and yellow, respectively. Panel B shows an image from high frequency ultrasound (40 MHz) (adapted with permission from [145]), Panel C shows detail in the dermis and superficial subcutaneous tissue by ultrasound imaging at ultrahigh frequencies (70 MHz shown) (adapted with permission under Creative Common license http://creativecommons.org/licenses/by/4.0/ from supplementary data in [167]). Panel D shows simultaneous dual-band line-field confocal optical coherence tomography (OCT) (adapted with permission from [168] © The Optical Society) that provides very high resolution (note the significant cellular detail in the inset below at the level of the epidermis and superficial papillary dermis not visible in Panels B and C), which comes at the expense of relatively short penetration depth. Panel E shows a single dissolving projection (adapted with permission from [64]) on the left, and a single coated nanopatch projection (adapted with permission under Creative Common license http://creativecommons.org/licenses/by/4.0/ from [92]). Finally, the black rectangle drawn at the base of the needle corresponds to the position of the corresponding vaccine patch inner surface.

Table 1:

Summary characteristics of 116 included vaccine patch studies

Patch projection type *
Coated (N=69) [22–61,81,83–94,103–107,109–112,119,121,125,128,132,133] [62,108] *
Hollow (N=10) [108] *[95–102,134]
Dissolving (N=39) [63–80,82,113–118,120,122–124,126,127,129–131,135–137] [62] *
Publication Year
2005–2009 (N=8) [22–26,95,108,125]
2010–2014(N=44) [27–53,63,81–88,96–98,113,114,116,119,121]
2015–2019 (N=64) [54–62,64–80,89–94,99–107,109–112,115,117,118,120,122–124,126–137]
Patch Developer
Georgia Institute of Technology/Emory University/Micron (N=57) (2009–2019) [22–80]
Queensland University/Vaxxas/Nanopatch (N=14) (2010–2018) [81–94]
NanoPass MicronJet (N=8) (2009–2016) [95–102]
Korea Advanced Institute of Science and Technology (N=5) (2015–2017) [103–107]
Leiden University (N=4) (2009–2019) [108–111]
Osaka University/MicroHyala (N=4) (2012–2017) [112–115]
Queen's University (N=3) (2012–2018) [116–118]
Cork/ImmuPatch (N=2) (2012–2016) [119,120]
Novartis (N=2) (2013–2015) [121,122]
Wellman Laboratory (N=2) (2015–2017) [123,124]
Anhui Medical University (N=1) (2015) [126]
Chinese Academy of Sciences (N=1) (2016) [127]
China State Institute of Pharmaceutical Industry (N=1) (2016) [129]
GSK Fujifilm (N=1) (2017) [130]
Hamamatsu University School of Medicine, ASTI (N=1) (2018) [134]
Hokkaido University/Fujifilm (N=1) (2017) [131]
Mercer (N=1) (2018) [135]
Tufts University/Massachusetts Institute of Technology/Vaxxes (N=1) (2017) [132]
USAMRID Easy Vax™ (N=1) (2007) [125]
University of Pittsburgh (N=1) (2016) [128]
University of Navarra/Cardiff University (N=1) (2017) [133]
Yonsei (N=1) (2018) [136]
Zhejiang University of Technology (N=1) (2018) [137]
Vaccine **
Influenza (N=68)
H1N1 (N=29) [24–26,28–33,39,41,43–46,49–52,55,57,63,66,69,70,91,94,105,106]
H1N1,H3N2,B (N=18) [62,68,73,81,82,87,92,95,96,98,101,104,112,113,115,120–122]
H1N1,H3N2,B,B (N=2) [112,132]
H1N1 Pandemic (N=5) [40,97,103,111,123]
H3N2 (N=4) [23,38,78,108]

H5N1 (N=3) [34,35,42]
H1N1,H5N1 (N=2) [27,131]
H1N1,H3N2 (N=1) [53]
H1N1 Pandemic,H3N2,A other (N=1) [54]
H1N1,H3N2,H5N1,H7N9 (N=1) [79]
H1N1,H3N2,H1N1 Pandemic (N=1) [72]
H1N1,H3N2,H1N1 Pandemic,A other (N=1) [61]
H1N1-like (A other) (N=1) [77]
Hepatitis B surface antigen (HepBsAg) (N=6) [22,75,80,126,127,130]
Polio (N=6) [65,90,93,99,100,109]
Diphtheria toxoid (N=4) [108,110,113,114]
Tetanus toxoid (N=4) [67,110,113,114]
Human Papilloma virus (HPV) (N=4) [56,85,117,118]
Ebola (N=3) [58,59,71]
Malaria (N=3) [88,113,119]
Rota (N=3) [48,76,102]
Bacillus Calmette–Guerin (BCG) (N=2) [37,124]
Herpes simplex virus (N=2) [83,84]
Measles (N=2) [47,64]
C. difficile (N=1) [132]
Chikungunya (N=1) [86]
Gonorrhoeae (N=1) [135]
Hand-foot-and-mouth disease (Enterovirus 71, EV 71) (N=1) [129]
Hepatitis C (N=1) [36]
Human immunodeficiency virus (HIV) (N=1) [116]
Leishmania spp (N=1) [133]
Measles rubella (MR) (N=1) [74]
Mumps (N=1) [134]
Pneumococcal conjugate vaccine (PCV) (N=1) [89]
Respiratory syncytial virus (RSV) (N=1) [60]
Scrub typhus (N=1) [136]
Severe fever with thrombocytopenia syndrome virus (SFTSV) (N=1) [107]
Shigella (N=1) [132]
Smallpox (N=1) [125]
Tuberculosis (non-BCG) (N=1) [137]
Varicella (N=1) [134]
West Nile Virus (N=1) [86]
Zika (N=1) [128]
Species
Mice (N=87) [23–36,38–46,48–63,66,67,69–73,76–89,91,92,103–108,110,111,116–120,124–129,131–133,135–137]
Human (N=10) [68,94–101,115]
Rats (N=7) [47,90,93,109,112,114,134]
Rhesus macaques (N=3) [64,65,74]

Pigs (N=3) [22,102,130]
Guinea pigs (N=3) [37,121,122]
Mice, rats *** (N=1) [113]
Mice, pigs *** (N=1) [123]
Mice, rhesus macaques *** (N=1) [75]
Dose sparing (N=50)
Demonstrated (N=22) [23,32–35,37,43,44,69,76,81,83,90,91,93,96,98,101,103,108,129,134]
Suggestive (N=22) [25,27,36,38,49,53–55,62,70,75,77,84,85,95,97,111,115,120–122,132]
Looked for but not found (N=6) [48,64,65,99,100,133]

* More than one patch projection type (coated and dissolving [62], coated and hollow [108])

** Studies with multiple vaccines (diphtheria and tetanus toxoids, influenza, and malaria [113], *C. difficile*, influenza, and Shigella [132], diphtheria and tetanus toxoids [110,114], diphtheria toxoid and influenza [108], mumps and varicella [134], Chikungunya and West Nile Virus [86], and 2 types of influenza [112])

*** Studies with more than one species

Table 2:

Details extracted from 116 included studies organized by group with the most to least studies

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
Georgia Institute of Technology/Emory University/Micron				
2009 [22]	Pigs	HepBsAg	C: Square (5×10), 0.6 mm, SA=0.5 cm^2	30 min
2009 [23]	Mice	Influenza (H3N2)	C: Line (5×1), 0.7 mm, SA=NR	2 min
2009 [24]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2009 [25]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2009 [26]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	5 min
2010 [27]	Mice	Influenza (H1N1,H5N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [28]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [29]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [30]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [31]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [32]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [33]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [34]	Mice	Influenza (H5N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [35]	Mice	Influenza (H5N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2010 [36]	Mice	HepC	C: Line (5×1), 0.7 mm, SA=NR	10 min
2011 [37]	Guinea pigs	BCG *	C: Line (5×1), 0.7 mm, SA=NR (x10 patches)	10 min
2011 [38]	Mice	Influenza (H3N2)	C: Line (5×1), 0.75 mm, SA=NR	5 min
2011 [39]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2011 [40]	Mice	Influenza (H1N1 Pandemic)	C: Line (5×1), 0.7 mm, SA=NR	5 min
2012 [41]	Mice	Influenza (H1N1)	C: Line (5×1), 0.75 mm, SA=NR	10 min
2012 [42]	Mice	Influenza (H5N1)	C: Line (5×1), 0.7 mm, SA=NR	20 min
2012 [43]	Mice	Influenza (H1N1)	C: Line (5×1), 0.75 mm, SA=NR	20 min
2012 [44]	Mice	Influenza (H1N1) ** (Novartis)	C: Line (5×1), 0.75 mm, SA=NR	5 min
2012 [45]	Mice	Influenza (H1N1) ** (Novartis)	C: Line (5×1), 0.7 mm, SA=NR	5 min

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
2012 [46]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	5 min
2013 [47]	Cotton rats	Measles	C: Line (5×1), 0.75 mm, SA=NR	10 min
2013 [48]	Mice	Rotavirus	C: Line (5×1), 0.75 mm, SA=NR (x5 patches)	10 min
2013 [49]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2013 [50]	Mice	Influenza (H1N1)	C: Line (5×1), 0.75 mm, SA=NR	NR
2013 [51]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	20 min
2014 [52]	Mice	Influenza (H1N1) ** (Novartis)	C: Line (5×1), 0.7 mm, SA=NR	5 min
2014 [53]	Mice	Influenza (H1N1,H3N2)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2015 [54]	Mice	Influenza (H1N1 Pandemic H3N2,A other)	C: Line (5×1), 0.7 mm, SA=NR	NR
2015 [55]	Mice	Influenza (H1N1) ** (Novartis)	C: Line (5×1), 0.7 mm, SA=NR	NR
2015 [56]	Mice	HPV	C: Line (5×1), 0.7–0.75 mm, SA=NR	2 min
2015 [57]	Mice	Influenza (H1N1)	C: Line (5×1), 0.75 mm, SA=NR (x5 patches)	NR
2018 [58]	Mice	Ebola	C: Line (5×1), 0.7 mm, SA=NR (2 doses, 4-week interval)	2 min
2018 [59]	Mice	Ebola	C: Line (5×1), 0.7 mm, SA=NR (2 doses, 4-week interval)	2 min
2018 [60]	Mice	RSV	C: Line (5×1), 0.7 mm, SA=NR (x2 patches)	10 min
2019 [61]	Mice	Influenza (H1N1,H3N2, H1N1 Pandemic, A other)	C: Line (5×1), 0.65 mm, SA=NR	10 min
2015 [62]	Mice	Influenza (H1N1,H3N2,B)	C: Line (5×1), 0.7 mm, SA=NR D: Square (10×10), 0.65 mm, SA=1 cm^2	C; 5 min D; 10 min
2010 [63]	Mice	Influenza (H1N1)	D: Square (10×10), 0.65 mm, SA=0.5 cm^2	15 min
2015 [64]	Rhesus macaques	Measles	D: Square (10×10), 0.6 mm, SA=0.29 cm^2	10 min
2015 [65]	Rhesus macaques	IPV ** (GSK)	D: Square (10×10), 0.6 mm SA=1.29 cm^2 (x2 patches for IPV serotype 1, 1 patch each for serotypes 2 and 3)	15 min
2016 [66]	Mice	Influenza (H1N1)	D: Square (10×10), 0.6 mm, SA=NR	NR
2016 [67]	Mice	Tetanus ** (Serum Institute of India)	D: Square (10×10), 0.65 mm, SA=NR (x0.5, half of patch used)	2 min held in, left 20 min
2017 [68]	Human (Phase 1)	Influenza (H1N1,H3N2,B)	D: Square (10×10), 0.65 mm, SA=NR	20 min, force feedback indicator
2017 [69]	Mice	Influenza (H1N1)	D: Square (10×10), 0.65 mm, SA=0.56 cm^2 (x0.5, half of patch used)	1 min held in, 10 min
2017 [70]	Mice	Influenza (H1N1)	D: Square (10×10), 0.7 mm, SA=NR	10 min, force feedback indicator
2017 [71]	Mice	Ebola	D: Square (10×10), 0.7 mm, SA=0.56 cm^2 (4 doses, 4-week intervals)	5 min

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
2017 [72]	Mice	Influenza (H1N1,H3N2, H1N1 Pandemic)	D: Square (10×10), 0.65 mm, SA=NR (booster dose only)	1 min held in, 21 min
2017 [73]	Mice	Influenza (H1N1,H3N2,B)	D: Square (NR), NR, SA=NR	1 min held in, 21 min
2018 [74]	Rhesus macaques	MR	D: Square (10×10), 0.7 mm, SA=1 cm^2	30 sec held in, 15 min
2018 [75]	Rhesus macaques and Mice	HepBsAg	C: Line (5×1), 0.75 mm, SA=NR (x2 patches) D: Square (10×10), 0.65 mm, SA=1 cm^2	C: 10 min D: 20 min, held 30 sec, explored different levels
2018 [76]	Mice	Rotavirus	D: Square (10×10), 0.85 mm, SA=1 cm^2	30 sec held in, 15 min
2018 [77]	Mice	Influenza (A other (H1N1-like))	D: Square (NR), NR, SA=NR	1 min held in, 20 min
2018 [78]	Mice	Influenza (H3N2)	D: Square (10×10), 0.65 mm, SA=NR	20 min
2018 [79]	Mice	Influenza (H1N1,H3N2, H5N1,H7N9)	D: Square (10×10), 0.65 mm, SA=NR	1 min held in, 30 min
2019 [80]	Mice	HepBsAg	D: Square (10×10), 0.6 mm (x2 patches)	NR
Queensland University/Vaxxas/Nanopatch				
2010 [81]	Mice	Influenza (H1N1,H3N2, B)** (Seqirus Pty Ltd)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches)	2 min, spring applicator (1.96 m/s)
2010 [82]	Mice	Influenza (H1N1,H3N2, B)** (CSL Limited)	D: Square (58×58), 0.116 mm, SA=0.16 cm^2 (x2 patches, except x1 for low dose group)	5 min, spring applicator (1.96 m/s)
2010 [83]	Mice	Herpes simplex virus	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches) (3 doses, 3-week intervals)	5 min, spring applicator (2 m/s)
2010 [84]	Mice	Herpes simplex virus	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches) (3 doses, 3-week intervals)	5 min, spring applicator (2 m/s)
2010 [85]	Mice	HPV** (Merck)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches)	5 min, spring applicator (2 m/s)
2010 [86]	Mice	West Nile, Chikungunya	C: Square (58×58), 0.065, 0.11 mm, SA=0.16 cm^2	5 min, spring applicator (2 m/s)
2012 [87]	Mice	Influenza (H1N1,H3N2, B)** (CSL Limited)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches)	2 min, spring applicator (2 m/s)
2013 [88]	Mice	Malaria	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches) (2 doses, 2- or 8-week interval)	5 min, spring applicator (1.96 m/s)
2015 [89]	Mice	PCV** (Pfizer)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (2 doses, 2-week interval)	2 min, spring applicator (3.1 m/s)
2016 [90]	Rats	IPV** (Bilthoven Biologicals)	C: Square (58×58), 0.23 mm, SA=0.16 cm^2 (x2 patches) (3 doses, 3-week intervals)	2 min, spring applicator (3.1 m/s)
2016 [91]	Mice	Influenza (H1N1)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches)	2 min, spring applicator (NR m/s)
2016 [92]	Mice	Influenza (H1N1,H3N2, B)** (bioCSL)	C: Square (58×58), 0.11 mm, SA=0.16 cm^2 (x2 patches)	2 min, spring applicator (2.3 m/s)

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
2017 [93]	Rats	IPV** (Bilthoven Biologicals)	C: Square (58×58), 0.23 mm, SA=0.16 cm^2 (x1 patch for each serotype) (3 doses, 3-week intervals)	2 min, spring applicator (3.1 m/s)
2018 [94]	Human (Phase 1)	Influenza (H1N1) ** (Seqirus Pty Ltd)	C: Square (100×100), 0.25 mm, SA=1 cm^2 (x2 patches)	2 min, spring applicator (20 m/s)
NanoPass MicronJet				
2009 [95]	Human (Phase 1)	Influenza (H1N1,H3N2, B) ** (GSK)	H: Line (4×1), 0.45 mm, SA=NR	NR
2012 [96]	Human (RCT)	Influenza (H1N1,H3N2, B) ** (Sanofi Pasteur)	H: Line (3×1), 0.6 mm, SA=NR	NR
2012 [97]	Human (RCT)	Influenza (H1N1 Pandemic) ** (Sanofi Pasteur)	H: Line (4×1), 0.45 mm, SA=NR	NR
2014 [98]	Human (Phase 2)	Influenza (H1N1, H3N2,B) ** (Crucell)	H: Line (4×1), 0.45 mm, SA=NR	NR
2015 [99]	Human (RCT)	IPV ** (Sanofi Pasteur)	H: Line (3×1), 0.6 mm, SA=NR	NR
2015 [100]	Human (RCT)	IPV ** (Netherlands Vaccine Institute)	H: Line (3×1), 0.6 mm, SA=NR (2 doses, 8-week interval)	NR
2016 [101]	Human (Phase 1)	Influenza (H1N1,H3N2,B)	H: Line (3×1), 0.6 mm, SA=NR	NR
2016 [102]	Pigs	Rotavirus	H: Line (3×1), 0.6 mm, SA=NR (3 doses, days 0, 10, 21)	NR
Korea Advanced Institute of Science and Technology				
2015 [103]	Mice	Influenza (H1N1 Pandemic)	C: Line (5×1), 0.7 mm, SA=NR	10 min
2016 [104]	Mice	Influenza (H1N1,H3N2,B)	C: Line (5×1), 0.7 mm, SA=NR (2 doses, 4-week interval)	10 min
2017 [105]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR (3 doses, 4-week intervals)	15 min
2017 [106]	Mice	Influenza (H1N1)	C: Line (5×1), 0.7 mm, SA=NR	10–15 min
2017 [107]	Mice	SFTSV	C: Line (5×1), 0.7 mm, SA=NR (2 doses, 4-week interval)	15 min
Leiden University				
2009 [108]	Mice	Diphtheria, Influenza (H3N2)	C: Square (4×4), 0.3 mm, SA=0.5 cm^2 H: Square (4×4, 9×9), 0.245 mm, SA=0.5 cm^2	1 s, electronic applicator (3 m/s)
2015 [109]	Rats	IPV	C: Square (24×24), 0.2 mm, SA=0.25 cm^2 (x3 patches) (2 doses, 3-week interval)	1 min, 3lectronic applicator (3 m/s)
2017 [110]	Mice	Diphtheria, Tetanus	C: Circular (105 total projections), 0.475 mm, SA=0.75 cm^2 (x3 patches) (2 doses, 3-week interval)	30 min, handheld applicator (4N)
2019 [111]	Mice	Influenza (H1N1 Pan)	C: Circular (105 total projections), 0.475 mm, SA=0.75 cm^2 (x3 patches) (2 doses, 3-week interval)	30 min, handheld applicator (4N)
Osaka University/MicroHyala				

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
2017 [112]	Rats	Influenza (H1N1,H3N2,B or H1N1,H3N2,B,B) ** (Biken)	C: Circular (481 total projections), 0.27 or 0.3 mm, SA=0.785 cm^2 (2 doses, 3- or 4-week interval)	30 or 60 min, spring applicator
2012 [113]	Rats and Mice	Diphtheria, Tetanus, Influenza (H1N1,H3N2,B), Malaria	D: Square (40×40), 0.2, 0.3, or 0.8 mm, SA=0.8 cm^2 (number, height, dosing, and interval depends on vaccine)	6 hr, handheld applicator (12.8N/20 projections)
2013 [114]	Rats	Diphtheria, Tetanus	D: Square (200 total projections), 0.2, 0.3, or 0.8 mm, SA=0.8 cm^2 (5 doses, 2-week intervals)	1 or 6 hr, spring applicator
2015 [115]	Human (Pre-clinical)	Influenza (H1N1,H3N2,B)	D: Square (40×40), 0.2, 0.3, or 0.8 mm, SA=0.8 cm^2 (2 doses, 3-week intervals)	6 hr, handheld applicator (12.8N/20 projections)
Queen's University				
2012 [116]	Mice	HIV	D: Square (19×19), 0.6 mm, SA=1 cm^2 (4 doses, days 0, 14, 28, and 42)	Overnight
2017 [117]	Mice	HPV	D: Square (19×19), 0.6 mm, SA=1 cm^2 (x2 patches) (3 doses, 2-week interval)	5 mins held in, 24 hours
2018 [118]	Mice	HPV	D: Square (19×19), 0.6 mm, SA=1 cm^2 (3 doses, 2-week interval)	5 mins held in, 24 hours
Cork University				
2012 [119]	Mice	Malaria	C: Square (5×5), 0.2 mm, SA=NR	4 hr
2016 [120]	Mice	Influenza (H1N1,H3N2,B)	D: Square (5×5 or 12×12), 0.5 or 0.28 mm, SA=1 cm^2	10–20N, 18 hr
Novartis				
2013 [121]	Guinea pigs	Influenza (H1N1,H3N2, B) ** (Novartis)	C: Hexagonal (320 total projections), 0.5 mm, SA=1.29 cm^2 (2 doses, 3-week interval)	15 min, spring applicator (8 m/s)
2015 [122]	Guinea pigs	Influenza (H1N1,H3N2,B) ** (Novartis)	D: Circular (low dose 2520 projections, SA=0.9 cm^2 or high dose 5600 projections, SA=2 cm^2), 0.2 mm (2 doses, 3-week interval)	5 min, spring applicator (NR m/s)
Wellman Laboratories				
2015 [123]	Pigs and Mice	Influenza (H1N1 Pandemic)	D: Rectangular (6×9), 0.6 mm, SA=1 cm^2 (x2 patches for pigs, x1 for mice)	15 min (some with nonablative fractional laser (NAFL))
2017 [124]	Mice	BCG *	D: Rectangular (6×9), 0.6 mm, SA=1 cm^2 (x2 patches)	15 min
All others				
2007 [125]	Mice	Smallpox	C: Rectangular (8×10), 0.45 mm, SA=NR (x4 patches)	1 sec, skin electroporation device (6 pulses of 100 Volts, 100 S pulse duration and 125mS pulse interval)
2015 [126]	Mice	HepBsAg	D: Square (6×6), 0.66 mm, SA=0.36 cm^2	NR
2016 [127]	Mice	HepBsAg	D: Square (6×6), 0.65 mm, SA=0.36 cm^2 (2 doses, 3-week interval)	3 min, 2N
2016 [128]	Mice	Zika	C: Square (10×10), 0.75 mm, SA=NR (2 doses, 2-week interval)	NR

Year	Species	Vaccine	Type: Projection shape (array density), height, SA (dosing details for studies using more than 1 patch)	Administration time and applicator (if used)
2016 [129]	Mice	EV 71	D: Square (15×15), 0.55 mm, SA=NR (3 doses, 2-week interval)	spring-driven applicator, ~10N/ patch
2017 [130]	Pigs	HepBsAg ** (GSK)	D: Square (10×10), 0.6 mm, SA=1 cm^2 (2 doses, 4-week interval)	10 min, handheld applicator (0.5J), 5N
2017 [131]	Mice	Influenza (H1N1,H5N1)	D: Square (3×3), 0.43 mm, SA=1 cm^2 (H1N1 1 dose, H5N1 2 doses, 4-week interval)	5 min
2017 [132]	Mice	Influenza (H1N1,H3N2,B,B)	C: Square (20×20), 0.7 mm, SA=2.25 cm^2 (2 doses, 2-week interval)	24 hr
2017 [133]	Mice	Leishmania spp	C: Rectangular (3×10), 0.4 mm (0.15 mm penetrating), SA=NR (3 doses, 3-week intervals)	10 min
2018 [134]	Rats	Mumps ** (Takeda Pharmaceutical), Varicella (Biken)	H: Line (6×1), 0.41 mm, SA=NR (2 doses, 1-week interval)	NR
2018 [135]	Mice	Gonorrhoeae	D: Square (10×10), 0.6 mm, SA=1 cm^2 (3 doses, weeks 0, 4, 6)	20 min
2018 [136]	Mice	Typhus	D: Square (5×5), 0.44 mm, SA=1 cm^2 (3 doses, 2-week intervals)	Microlancer implantation system
2018 [137]	Mice	Tuberculosis (non-BCG)	D: Square (8×8), 0.25 mm, SA=1 cm^2 (x1(low), x3(high, (2 doses, 4-week interval)	NR

Abbreviations: BCG, Bacille Calmette–Guerin; C, Coated; D, Dissolving; EV 71, Enterovirus 71; H: Hollow, HepBsAg, Hepatitis B surface antigen; HIV, Human immunodeficiency virus; HPV, Human Papilloma virus; HepC, Hepatitis C; IPV, inactivated poliovirus vaccine; MR, measles rubella; N, newtons; NAFL, nonablative fractional laser; NR, not reported; PCV, pneumococcal conjugate vaccine; RSV, respiratory syncytial virus; SA, surface area; SFTSV, severe fever with thrombocytopenia syndrome virus

Notes:

* Pre-clinical reference standard

** Study used a licensed vaccine (vaccine manufacturer)

Table 3:

Summary of evidence about the location, Antigen Presenting Cell type, and typical immunophenotype characteristics

	Langerhans Cell	CD1c+ Conventional Dermal Dendritic Cell	CD141+ Conventional Dermal Dendritic Cell	Plasmacytoid Dendritic Cell	Macrophage
Primary Skin Location	**Epidermis**	**Dermis**	**Dermis**	**Dermis**	**Dermis**
Mouse Homolog	LC	cDC2	cDC1	pDC	Macrophage
CD1a	++	+	−/+	−/+	−
CD1c	+	+	−	−/+	+
CD11b	+/−	+	−	−	+/−
CD11c	+/−	+	+/−	−	+
CD14	−	−	−	+	−
CD141	−	−/+	++	−/+	−
CD207/Langerin	++	+/−	−/+	−	−
Other Unique Characteristic	Birbeck Granule				CD163+ CD68+

*Grayed out boxes indicate markers that positively identify each category. Variability in expression and/or lack of consensus about human immunophenotype is indicated using +/− (likely positive) and −/+ (likely negative).