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Technology update: dissolvable microneedle patches for vaccine delivery

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Abstract: Despite vaccination representing one of the greatest advances of modern preventative medicine, there remain significant challenges in vaccine distribution, delivery and compliance. Dissolvable microarray patches or dissolving microneedles (DMN) have been proposed as an innovative vaccine delivery platform that could potentially revolutionize vaccine delivery and circumvent many of the challenges faced with current vaccine strategies. DMN, due to their ease of use, lack of elicitation of pain response, self-disabling nature and ease of transport and distribution, offer an attractive delivery option for vaccines. Additionally, as DMN inherently targets the uppermost skin layers, they facilitate improved vaccine efficacy, due to direct targeting of skin antigen-presenting cells. A plethora of publications have demonstrated the efficacy of DMN vaccination for a range of vaccines, with influenza receiving particular attention. However, before the viable adoption of DMN for vaccination purposes in a clinical setting, a number of fundamental questions must be addressed. Accordingly, this review begins by introducing some of the key barriers faced by current vaccination approaches and how DMN can overcome these challenges. We introduce some of the recent advances in the field of DMN technology, highlighting the potential impact DMN could have, particularly in countries of the developing world. We conclude by reflecting on some of the key questions that remain unanswered and which warrant further investigation before DMNs can be utilized in clinical settings.

Keywords: microneedle patches, dissolvable, vaccine, cold chain, hazardous sharps waste, skin

Introduction
Vaccination is one of the greatest medical advances of modern preventative medicine. It is the most effective means of controlling the incidence of infectious disease, as evidenced from the elimination of smallpox and estimated avoidance of 2.5 million deaths per year from diphtheria, tetanus, whooping cough and measles.1 Despite this, current vaccination approaches face a number of challenges which impact upon vaccination compliance. Most vaccines are administered via intramuscular (IM) or subcutaneous (SC) injection, causing pain and discomfort and, in many cases, leading to poor compliance as a result of needle phobia.2,3 Hypodermic needles result in the creation of biohazardous sharps waste, requiring safe disposal to ensure needles are not reused, either intentionally or accidentally. This is notably problematic in the developing world, where the use of unsafe or inappropriate injection practices leads to the transmission of infectious diseases. Specifically, it is estimated that up to 33,800 HIV infections, 1.7 million hepatitis B infections and 315,000 hepatitis C infections arise every year as a result of unsafe
injection practices. Additional challenges arise due to ineffective supply chains and vaccine wastage due to multi-dose vials or failures in cold chain systems.

Oral vaccination is an alternative to that of parenteral, and has been approved for human use for a number of vaccines. Nonetheless, oral delivery of vaccines presents significant challenges, including decreased immunogenicity as a result of antigen digestion and degradation in the gastrointestinal tract, prior to the induction of appropriate immune responses. Thus, oral vaccination has been confined to a relatively small number of licensed vaccines including rotavirus, typhoid, cholera and some poliovirus vaccines. Transdermal vaccine delivery has also been investigated. The skin, however, by virtue of its protective function, serves as a barrier for delivering drugs or vaccines through the topical route. The skin’s stratum corneum acts as a barrier for topically applied drugs or vaccines, allowing only certain molecules such as those of low molecular weight (usually <350 Da) or lipophilic drugs to pass across it. Intradermal (ID) injection as an alternative delivery system into the viable skin layers is technically challenging, necessitating specialist training of health care providers. Considering the above-mentioned challenges with parenteral, oral and traditional ID delivery routes, recent research efforts have focused on addressing the urgent and unmet requirement for simplified, alternative methods for vaccine delivery.

Microneedle (MN) patches have been proposed as an innovative vaccine delivery platforms and a viable means of circumventing the challenges associated with conventional vaccine delivery. MNs are minimally invasive devices that consist of an array of microscopic needles attached to a base support or backing (Figure 1A and B) and are categorized into five main types, namely, hollow, solid, coated, swellable and dissolving MNs (DMN). DMNs show particular promise for vaccination purposes due

![Image of Microneedle (MN) patches](https://example.com/image)

**Figure 1** (A) Scanning electron microscope images of DMN, 500 µm in height and with 300 µm width at base. (B) A DMN, prior to application to the skin. (C, D) Representative optical coherence tomography (OCT) images showing DMN insertion in skin (C) and DMN dissolution in skin at time 0, 15 mins and 60 mins (D). Reproduced from Rodgers AM, McCrudden MT, Vincente-Perez EM, et al. Design and characterisation of a dissolving microneedle patch for intradermal vaccination with heat-inactivated bacteria: a proof of concept study. *Int J Pharm*. 2018;549(1–2):87–95. Creative commons license and disclaimer available from: [http://creativecommons.org/licenses/by/4.0/legalcode](http://creativecommons.org/licenses/by/4.0/legalcode).

**Abbreviation:** DMN, dissolving microneedles.
to their self-disabling nature, prohibiting re-use. DMN are fabricated from fast dissolving materials such as polymers or sugars and the vaccine is incorporated within the matrix.\textsuperscript{15,16} These MNs have a “pin cushion” appearance and are applied to the skin in a manner similar to that of a plaster, bandage or conventional transdermal patch. Upon insertion of the needles in the skin, they come into contact with skin interstitial fluid and dissolve, simultaneously facilitating delivery of vaccines to the skin’s epidermis and dermis (Figure 1C and D).\textsuperscript{17,18} Therefore, DMN are the most promising MN for clinical use as they can overcome any safety issues caused by broken MNs from solid arrays which may remain in the skin post application.\textsuperscript{19} The needles of the array are typically 50–900 µm in length and as such, are long enough to penetrate the dermis, but are, in most cases, short and narrow enough to avoid stimulation of dermal nerves or puncture dermal blood vessels. Accordingly, DMNs facilitate a painless means of drug or vaccine delivery that is well accepted by patients.\textsuperscript{20}

This review summarizes the barriers faced by current vaccination approaches. We introduce the field of DMN technology, providing an overview of the advantages this technology offers for ID vaccine delivery, in comparison to that of traditional SC, IM or ID injections. Specifically, the advantages of DMN from an immunological perspective are drawn upon. With this as a starting point, we subsequently provide an update on the recent advances which have been made using DMN for vaccination purposes, highlighting the potential and novelty of this technology. Extensive research efforts have been devoted to the development of innovative fabrication methods of DMN for vaccination purposes, with the aim of improving vaccine loading into needles and subsequent delivery.\textsuperscript{21–23} It is beyond the scope of this review to cover such aspects; however, we direct the reader to a number of recently published reviews which provide an insight into some of the challenges faced in the fabrication of DMN.\textsuperscript{24,25} Herein, we finally conclude by reflecting on some of the key questions which remain to be addressed for adoption of DMNs in a clinical setting.

**Circumventing vaccine delivery challenges: is the solution in the patch?**

Despite the global impact vaccination has played in public health, there remain significant challenges with current vaccination approaches. As previously highlighted, the hypodermic needle and syringe are most commonly utilized for vaccine administration into the muscle tissue, despite the fact that the muscle is not a highly immunogenic organ.\textsuperscript{26} For this purpose, health care providers must acquire training for correct vaccine reconstitution and subsequent administration. This is particularly problematic in countries of the developing world where there are significant barriers to effective vaccination (as reviewed in\textsuperscript{27}).

In short, this is primarily due to logistical and economic challenges in relation to geographical accessibility and insufficient numbers of appropriately trained health care workers. The estimated cost of cold chain storage is $200–$300 million annually and failures in this system often result in vaccine shortages.\textsuperscript{10} As many vaccines require booster injections for induction of appropriate immune responses, this can also be difficult to implement in the developing world due to limited access to health care and difficulty in implementing vaccination programs. Accordingly, safer, cost-effective, innovative vaccine formulations are warranted, in order to improve coverage of current vaccines and help control the incidence of infectious disease.

**Vaccine delivery to the skin via DMNs**

The skin is an attractive site for vaccination. The physiology and anatomy of the skin has been well characterized and reviewed elsewhere.\textsuperscript{10,27} This organ serves as the first line of defense against pathogens, containing an abundant population of immune cells, including epidermal Langerhans cells (LC) and dermal dendritic cells (dDC), which can provide excellent targets for vaccine delivery.\textsuperscript{28} Since the work of Glenn and collaborators in the early 2000s, several clinical studies have shown the potential of transcutaneous (TC) administration route for vaccination.\textsuperscript{29–32} A Phase I trial by Combadière et al\textsuperscript{33} was the first study to demonstrate the superiority of this route, in comparison to that of IM. Specifically, it was shown that delivery of inactivated influenza vaccine via the TC route resulted in more efficient induction of influenza-specific CD\textsuperscript{8} T cell responses, in comparison to that delivered via the IM route. Due to the substantial numbers of APCs present in the skin, vaccine delivery by this route may result in dose-sparing effects, thus permitting the induction of enhanced immune responses using lower doses of vaccine.\textsuperscript{34} As illustrated in Figure 2, DMNs inherently target these immune cells, providing a unique opportunity for enhanced vaccine immunogenicity and dose-sparing effects,\textsuperscript{35,36} concomitant to additional

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**References**

1. Rodgers et al. Medical Devices: Evidence and Research 2019:12.
logistical advantages which will be discussed in further detail in the following subsections.

Opportunity for vaccine administration by untrained personnel

At present, the administration of vaccines necessitates delivery by a health care provider. This presents a challenge in situations whereby there is limited access to health care providers and facility-based care, particularly within the developing world countries. DMNs overcome the requirement for trained personnel for correct vaccine delivery because they are simply inserted by hand or utilizing an applicator device. This may be especially beneficial during, for example, mass vaccination campaigns, whereby vaccine administration by patients themselves, or lesser-trained health care providers would expand access to lifesaving vaccines. Concurrently, if vaccines were to be self-administrated by patients themselves, or by lesser-trained personnel, this could have significant cost savings.

Considering this, authors have developed different studies to explore the opinions of health care professionals and the public on the use of MN. In a publication by Birchall and co-workers, all focus groups were in favor of MN, in comparison to that of hypodermic injections, although concerns were raised regarding the need for feedback mechanisms to reassure the user of successful MN insertion and delivery. More recently, Marshall et al published a literature review of perception and acceptability of the MN technology for vaccination, particularly in the pediatric population. In general, the review highlights the positive perceptions of the technology both in the general public and in health care professionals, listing a variety of advantages commonly associated with this approach. Nevertheless, concerns about unfamiliarity with the technology and inability to ensure accurate vaccine delivery were also highlighted and require further efforts by researchers and companies.

In contrast to taking medicine orally or via an injection whereby delivery is complete within minutes, DMN must be worn until the needles have dissolved within the skin. This is dependent upon the DMN formulation and can range from minutes to hours. Thus, an innovative means of monitoring DMN delivery may be beneficial to the end users, providing assurance of correct usage and assisting in the future translation of the technology to clinical use. In light of this, the use of a low-cost pressure-indicating sensor film to provide feedback upon MN application has been evaluated. To elaborate, human volunteers self-applied MNs, with and without a pressure-sensing film. Following this, optical coherence tomography was used to visualize MN insertion in skin and to monitor the insertion depth into the skin layers. The pressure-sensing film facilitated colorimetric analysis to provide feedback on MN insertion. Assessment of participants’ opinions indicated that 75% preferred the MNs with the pressure-sensing film. It
must be noted that these experiments were conducted with hydrogel-forming MNs, rather than DMN, although the principles of insertion remain the same between both modalities. Another study by Norman et al. assessed the usability and acceptability of stainless steel MN for vaccination against influenza utilizing a snap device as a force feedback to users. To elaborate, 91 subjects were recruited and received either placebo MN given by the investigator or by self-administration or an IM injection of saline. Of the MN group, MNs were given three times and 70 of the participants inserted MN with thumb pressure alone while the remainder used the snap-based device that closed shut at a certain force. Thereafter, skin staining and acceptability was measured with an adaptive-choice analysis. Results indicated that the best usability was evident with the snap device, with users inserting a median value of 93–96% of MN over three repetitions.

**Overcoming pain and needle phobia**

Needle-phobia can, in many cases, be an impediment to patient adherence to vaccination programs. Needle gauge and the mechanics of needle insertion, including force and mechanical workload of the hypodermic needle, have all been found to correlate with the frequency of pain. As DMNs are fabricated to be short and narrow enough to avoid stimulation of dermal nerves, there is no pain associated with vaccine administration via this route. Therefore, patient adherence to vaccination programs via DMN is likely to increase and as highlighted previously, DMN are, in general, well accepted by patients.

**Potential for reduced cost vaccination**

It is anticipated that DMN will result in reduced vaccination costs due to the prospect of self-vaccination or vaccination by untrained personal, the elimination of hazardous sharps waste and simplification of the supply chain. It will be important to develop an understanding of DMN manufacturing requirements and subsequent costs, which are likely to be vaccine-specific, to fully compare DMN vaccination to other conventional approaches. It is also worth to consider that if applicator devices are used to facilitate DMN insertion, this will undoubtedly increase DMN vaccination costs.

**Elimination of hazardous sharps waste**

As DMN are fabricated from water-soluble, biocompatible materials that dissolve in the skin post-insertion, they overcome the generation of biohazardous sharps wastes and any material that remains on the skin may be discarded in non-sharps waste. Thus, this circumvents the risk of injury and disease transmission from used or contaminated needles.

**Improved thermostability, simplified supply chain and subsequent increased vaccine coverage**

DMN are smaller in size than traditional hypodermic needles and syringes, thus offering simplified supply chains, storage and distribution. Most vaccines necessitate storage at specific temperatures from the point of manufacture, through transportation, storage and administration. This results in significant economic challenges, particularly in developing world countries where the infrastructure requirements for cold chain storage are usually difficult to meet. Moreover, failures in the cold chain and vaccine exposure to temperatures outside of recommended ranges can result in decreased vaccine potency and subsequent lack of protection against the vaccine-preventable illness. DMN are fabricated such that the vaccine is contained within the DMN in its dried form, in many cases in combination with suitable excipients to improve thermostability. Accordingly, because of their solid-state formulation, DMN can be stored at ambient temperatures, overcoming the requirement for cold chain storage completely or partially. In the case of the latter whereby only partial thermostability is achieved, the DMN may be refrigerated during storage but may not require cold chain storage during distribution to remote locations or during mass vaccination campaigns.

A number of reported studies have demonstrated the thermostability of vaccines in DMN. As an example of this, Mistilis et al. developed a thermostable DMN for influenza vaccination. It was demonstrated that a number of DMN formulations were stable during storage at room temperature for up to 6 months. Following this, a more recent study reported by the same research team assessed the long-term stability of DMN containing influenza vaccine during storage outside the cold chain and when exposed to potential stresses found during manufacturing and storage. In short, it was demonstrated that influenza vaccine in DMN lost no significant activity during exposure to 60°C for 4 months, multiple freeze-thaw cycles or electron beam irradiation. The thermostability of adenovirus-based vaccines and measles vaccines has also been demonstrated. Moreover, Kolluru and co-workers recently developed a thermostable DMN for administration of inactivated polio vaccine with improved thermostability, in comparison to that of the conventional liquid inactivated polio vaccine (IPV). A number of excipients were screened for their ability to improve stability and combinations of maltodextrin and D-sorbitol in histidine buffer were...
found to be effective in preserving activity of IPV. The resultant DMN maintained the stability of IPV in storage at up to 40°C, with more than 40% of activity maintained post-storage for 2 months, and more than 20% after 1 year. The time required for a vaccine to maintain its thermostability in storage conditions depends both on the vaccine itself and on the location where it will be distributed and administered. For example, in Bihar, vaccines stable for 1 month were used in advance of expiry, whilst in Mozambique, vaccines required a longer time interval (2 months) to be used before expiry.88

DMN vaccination: trends, progress and recent applications

The first successful vaccination with DMN was reported by the Prausnitz group in 2010.55 In this study, DMN were fabricated from liquid vinyl pyrrolidone monomer, with needles of 650 µm in height, and containing 3 µg of lyophilized inactivated influenza virus vaccine. The DMN were inserted into mouse skin by hand pressure and dissolved within minutes. It was demonstrated that the DMN could induce protective immune responses, greater than those observed following IM vaccination with the same dose. Specifically, it was shown that DMN induced enhanced antibody and cellular responses, resulting in lung viral clearance post lethal influenza challenge. Following this initial study, Kendall and co-workers developed the Nanopatch™ and demonstrated successful delivery of Quil-A adjuvanted ovalbumin and influenza vaccine.56 Concurrent with the reports by Prausnitz et al,55 Kendall and co-workers showed that DMN were more efficient at inducing antibody titers against ovalbumin in mice than the conventional IM injection. In the case of the influenza vaccine, authors report strong antibody responses generated in mice immunized with DMN, using a much lower dose than that of the IM injection control. Following these initial publications which evidenced the promising potential of the technology, a plethora of studies have reported the successful delivery of various vaccine antigens, with significant progress being made in the field of DMN vaccination. A summary of published preclinical studies utilizing DMN for vaccination is presented in Table 1. As shown, this has included a variety of viral, bacterial and model antigens, with overall promising results.15,19,52–55,57–78

Viral vaccines

A wide range of publications have reported on the use of DMN for vaccination against polio, measles, influenza, HIV, hepatitis B and enterovirus 71 (EV71), the causative agent of hand-foot-and-mouth disease (HFMD), as highlighted above.

In 2015, Edens and colleagues developed DMN from gelatin/sucrose to deliver IPV to rhesus macaques (Macaca mulatta). DMN contained 100 needles, each 650 µm in height and rhesus macaques were vaccinated with DMN or via IM injection. DMN were well tolerated by the monkeys and neutralizing antibody titers were equivalent among both immunization strategies for IPV types 1 and 2. Following this work, the same group published results on the development of polymeric DMN for measles vaccination in rhesus macaques.54 In this case, DMN were fabricated from sucrose and carboxymethyl cellulose (CMC) and contained the standard dose of measles vaccine (1000 TCID50). The rhesus macaques were vaccinated with DMN or SC injection and results showed that both groups generated comparable levels of neutralizing antibody responses to measles. DMN were found to retain their potency post-storage at elevated temperatures suggesting improved thermostability in comparison to that of standard lyophilized vaccine. Subsequent studies by the same group further explored the stability of other vaccines in DMN and this included subunit and inactivated vaccines,50,80 with influenza receiving particular attention.51,52

Influenza has been indeed the focus of the majority of preclinical studies concerning the delivery of viral vaccines with DMN, mostly using mice as the animal model. In 2016, Vrdoljak and co-workers proposed the use of polyvinyl alcohol (PVA) and trehalose in DMN with different array geometries and needle heights (12×12, 280 µm long and 5×5, 550 µm long) for single-dose influenza immunization in mice.15 Using this approach, significant dose-sparing was observed in comparison with conventional IM injection, with broadly neutralizing antibody responses that were even able to tackle heterosubtypic viral strains and non-stalk regions of hemagglutinin. Similarly, another study reported protective neutralizing antibody responses against two influenza virus strains (PR8 and Vac-3) in mice immunized with DMN.59 In this case, one of the strains required a boost immunization to generate protective responses, evidencing the need for specific strategies to be developed for each particular vaccine. DMN have also been used as a boost strategy following priming with the inactivated virus58 or as a supplementary vaccination approach in combination with IM vaccine injection,61 with strong, longer-lasting, protective antibody responses being achieved in comparison with

Table 1

| Vaccine type | Antigen | Dosage | IM control | DMN | Results |
|--------------|---------|--------|------------|-----|---------|
| Polio        | Poliovirus | 4 µg   | 4 µg       | 4 µg | Enhanced responses |
| Measles      | Measles vaccine | 10 µg | 10 µg | 10 µg | Equivalent responses |
| HIV          | HIV vaccine | 1 µg   | 1 µg       | 1 µg | Equivalent responses |
| Hepatitis B  | Hepatitis B vaccine | 2 µg | 2 µg | 2 µg | Equivalent responses |
| EV71         | Enterovirus 71 vaccine | 10 µg | 10 µg | 10 µg | Equivalent responses |

Note: Results are compared against standard IM injection control.
Table 1 Preclinical vaccine studies with DMN

| Vaccine          | DMN characteristics | Animal model | Vaccination scheme | Main findings                                                                 | Ref  |
|------------------|---------------------|--------------|--------------------|------------------------------------------------------------------------------|------|
| Amyloid β peptide | Sodium hyaluronate  | Mouse        | Weekly (1st month), then biweekly for 12 weeks | Little improvement in cognitive behaviour and in Th2-dominant immune responses; induced anti-Aβ1–41 responses | 75   |
| Enterovirus 71 (EV71) | Sodium hyaluronate | Mouse        | Prime +2 boosts | DMN with 10-fold lower dose than IM induced comparable antibody and antibody-secreting cell levels and protection from lethal EV71 challenge | 76   |
| Hepatitis B      | Hydroxyethyl starch, chondroitin sulfate | Pig          | Single-dose Prime + boost | Single-dose DMN immunization (adjuvanted with QS-21 in liposomes) induced antibody responses that were inferior to 2 doses of IM commercial vaccine; results were equivalent between 2 doses of adjuvanted DMN, IM prime + DMN boost and 2 doses of IM commercial vaccine | 77   |
| HIV              | Gantrez® AN-139, Polysorbate 80 | Mouse        | Prime +3 boosts (via DMN, intranasal or intravaginal) | DMN prime with intranasal boosts led to IgG levels and lymphocyte proliferation equivalent to subcutaneous regimen; also induced high antigen-specific vaginal IgA levels | 78   |
|                  | Na-CMC, sucrose     | Mouse        | Single dose        | DMN preserved immunogenicity of antigen encoded in live recombinant human adenovirus vector; induced CD8+ T cell expansion and cytokine responses equivalent to conventional injection routes | 53   |
| Influenza        | PVP                 | Mouse        | Single dose        | Immunization with DMN elicited strong humoral and cellular responses even at low antigen dose, granting protection against lethal challenge; results with DMN were similar to or even stronger than IM injection | 55   |
|                  | Na-CMC, trehalose   | Mouse        | Prime + boost      | Hemagglutination inhibition titres obtained with DMN were significantly higher than with IM immunization (after boosting); immunization with trivalent vaccine induced strain-specific antibody responses, comparable to IM | 57   |
| Vaccine     | DMN characteristics | Animal model | Vaccination scheme | Main findings                                                                                                                                                                                                 | Ref  |
|-------------|---------------------|--------------|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Influenza   | Trehalose, PVA      | Mouse        | Single dose       | DMN immunization enabled significant dose-sparing in comparison with IM injection; no significant differences in terms of hemagglutination inhibition except for a delay on its induction in the DMN group; antibody responses elicited by DMN were broadly neutralizing, including against heterosubtypic virus strains and non-stalk regions of hemagglutinin | 15   |
|             | Hydroxyethyl starch, chondroitin sulfate | Mouse        | Single dose (PR8 strain) Prime + boost (Vac-3 strain) | Vaccination with DMN induced higher neutralizing antibody responses and more efficient protection from challenge in comparison with SC injection, irrespective of the influenza strain used (PR8 or Vac-3); DMN immunization with the whole vaccine was better than with the split virion, particularly at low doses | 59   |
|             | Na-CMC, vaccine stabilizer (s), PVA, sucrose | Mouse        | Single dose       | Trivalent vaccine loaded in DMN and stored for over a year at 25 °C induced equivalent or higher antibody levels than fresh liquid vaccine delivered ID; vaccine in DMN did not lose activity when stored at 60 °C for 4 months, or when exposed to multiple freeze-thaw cycles or to electron beam radiation | 52   |
|             | Na-CMC, [arginine + heptaglucosane] or sucrose, PVA | Mouse        | Prime (inactivated virus) + boost (DMN or IM) | DMN-boosted group showed higher antibody levels against both influenza strains tested in comparison with IM-boosted; DMN-boost allowed longer-lasting protection, stronger cellular response, higher lung virus inhibition and clearance and cross-protection against a different influenza strain, in comparison with IM-boost | 58   |
|             | PVA, sucrose        | Mouse        | Prime + boost     | Co-administration of peptide-only nanoparticles in DMN patch and IM inactivated influenza virus led to improved immunogenicity, with more efficient lung virus clearance and enhanced cellular recall responses after lethal challenge, in comparison with IM-only immunization | 61   |
|             | PVA, CMC, sucrose   | Mouse        | Single dose       | Focused on the effect of statin therapy in the immune response against an influenza vaccine in mice with different ages; antibody titres declined with age and more significantly in IM-immunized groups; statin therapy led to reduction in the immune response of IM-immunized mice, while DMN-immunized animals showed much higher total IgG and hemagglutination inhibition levels | 60   |
| Vaccine                     | DMN characteristics                  | Animal model | Vaccination scheme | Main findings                                                                                                                                                                                                 | Ref |
|----------------------------|--------------------------------------|--------------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Influenza                  | PVA, Sucrose, Trehalose, BSA         | Mouse        | Single dose        | Focused on evaluating GM-CSF as an adjuvant for DMN influenza vaccines; showed inclusion in DMN does not alter GM-CSF biological activity and induces cross-reactive responses, not seen in IM or ID; this approach also showed increased long-term antibody responses | 62  |
| Measles                    | Sucrose, threonine, CMC, PVA         | Rhesus       | Single dose        | DMN induced immune responses similar to SC injection, with all animals seroconverted and showing neutralizing antibody levels correlated to protection; no adverse reactions were observed in DMN group and the vaccine retained activity when stored at high temperature | 54  |
| Neisseria gonorrhoeae      | Trehalose, maltose, PVA, HPMC        | Mouse        | Prime +2 boosts    | Whole-cell gonococci encapsulated in cross-linked albumin microparticles and delivered via DMN patch induced higher antibody levels than SC injection of a vaccine suspension; T cell proliferation similar in all groups and higher than negative controls | 63  |
| OVA (model antigen)        | Chitosan, PVA, PVP                   | Rat          | Single dose        | Chitosan DMN acted as an implanted depot, releasing antigen for up to 28 days; DMN immunization with low antigen dose induced higher antibody levels than IM with high dose, with or without a chitosan solution, allowing 2.5-fold dose sparing | 64  |
|                            | Chitosan, sodium hyaluronate, PVA, PVP| Rat          | Single dose        | Aimed at emulating a prime and boost scheme by releasing OVA quickly from hyaluronate tips and then slowly from chitosan needle shafts, for up to 4 weeks; the combination of hyaluronate and chitosan showed higher antibody levels than chitosan-only DMN and SC injection of antigen with the polymers in solution; responses were also higher and longer-lasting than repeated or double-dose SC injection of OVA | 65  |
|                            | CMC, sodium hyaluronate              | Rat          | Prime +2 boosts    | DMN immunization led to higher antibody levels than SC injection at the same antigen dose, irrespective of the time of application (10 mins or 4 hrs) and of the polymer used in the fabrication of the patch (CMC or sodium hyaluronate) | 19  |
|                            | Polymacrylic acid                    | Mouse        | Prime + boost      | Aimed at developing a lymph node-targeting vaccine, using amphiphilic antigen and CpG adjuvant administered via DMN; strategy led to higher lymph node accumulation, higher levels of CD4⁺ T cells and IgG in comparison with ID injection | 66  |
Table 1 (Continued).

| Vaccine | DMN characteristics | Animal model | Vaccination scheme | Main findings | Ref |
|---------|---------------------|--------------|-------------------|---------------|-----|
| Poliovirus | [Sucrose + threonine] or maltodextrin, gelatin | Rhesus macaque | Prime + boost | Trivalent vaccine in DMN elicited strong immune responses against IPV types 1 and 2, similar to IM injection; responses against IPV type 3 were lower than those obtained with IM injection | 67 |
| Porcine circovirus type 2 (DNA vaccine) | PVA, bPEI, PVP | Mouse | Prime + boost | Mice immunized with DMN showed much higher antibody titres than those receiving the vaccine via IM injection; in-situ formation of polyplex with DNA vaccine and bPEI within the needle shaft increased transfection efficiency and protected the vaccine from degradation | 68 |
| Pseudomonas aeruginosa | Gantrez® S-97 | Mouse | Single dose | Better bacterial infection control after challenge was achieved with mice immunized with DMN loaded with heat-inactivated bacteria | 10 |
| Rabies | Sucrose, PVA | Dog | Prime + boost | First in-dogs clinical trial for veterinary applications; DMN vaccination elicited similar antibody levels to IM injection (boost required in both groups); no evidence for 10-fold dose sparing | 70 |
| Staphylococcus aureus | Chondroitin sulfate, trehalose | Mouse | Prime +2 boosts | DMN immunization significantly extended antigen retention time in vivo, inducing high and protecting specific antibody levels; animals receiving DMN vaccine were protected from lethal challenge; results were overall better than with IM administration | 71 |
| Tetanus | PVA, CMC, sucrose | Mouse | Single dose | Immunization of pregnant animals with DMN led to offspring with detectable antigen-specific antibody levels for up to 12 weeks of age, with complete protection against lethal challenge up to 6 weeks of age, in contrary to IM immunization | 79 |
| Tuberculosis | Sodium hyaluronate | Mouse | Single dose | Developed DMN with a “cave” to accommodate powder vaccine and then covered with hyaluronate; DMN immunization completely avoided the local adverse reactions associated with ID injection and induced cytokine production and antibody levels to similar levels as ID injection | 72 |
| Sodium hyaluronate | Mouse | Prime + boost | Antibody levels elicited by DMN were equivalent or higher than those obtained with IM injection, depending on the antigen dose; similar results observed for cellular responses, bacteriostatic ability and survival against challenge | 73 |
| Tetanus + Diphtheria, Malaria, Influenza | Sodium hyaluronate | Mouse, rat | Prime + variable number of boosts (antigen-specific) | Immunization with DMN induced effective immune responses, similar to conventional injection routes (IM, ID, SC); no mucosal responses induced; response was Th2-skewed for TT+DT but not for influenza | 74 |

Abbreviations: IM, intramuscular; ID, intradermal; SC, subcutaneous; Na-CMC, sodium carboxymethyl cellulose; CMC, carboxymethyl cellulose; PVP, polyvinylpyrrolidone; PVA, polyvinyl alcohol BSA, bovine serum albumin; HPMC, hydroxypropyl methylcellulose; bPEI, branched polyethyleneimine; GM-CSF, granulocyte-macrophage colony-stimulating factor; IPV, inactivated poliovirus; TLR, Toll-like receptor; TT, tetanus toxoid; DT, diphtheria toxoid.
animals receiving IM-only immunization. Finally, some authors have also extended the application of this strategy, as is the case of Vassilieva and co-workers, who studied the effect of statin therapy in the immune response against DMN-based influenza vaccines. In this study, the authors observed a positive effect of DMN vaccination in overcoming the declining of antibody titers observed in animals treated with statins, in comparison with IM immunization. The same group has also explored the inclusion of adjuvants, such as the granulocyte-macrophage colony-stimulating factor (GM-CSF) in influenza-delivering DMN, with promising results.

To move toward the evaluation of the suitability of DMN use in human subjects, this approach has also been investigated in higher animals. For example, Arya and co-workers assessed the safety and immunogenicity of DMN vaccination utilizing a rabies DNA vaccine in dogs, with the aim of preventing the complex and expensive post-exposure vaccination scheme in humans. The vaccine was stable during formulation and storage, for at least 3 weeks at 4°C. DMN were applied to the ears of dogs by hand and while mild erythema was observed, complete resolution occurred within 7 days post-vaccination and no systemic adverse reactions occurred. Importantly, DMN were found to be as immunogenic as the IM vaccine injection, as seen by serum antibody titers. In another example, DMN were used for immunization of pigs against hepatitis B virus, with QS-21-loaded liposomes included in the formulation as an adjuvant. The antibody responses generated in this study were equivalent between groups receiving two doses of DMN vaccine, IM prime and DMN boost or two IM doses of the commercial vaccine, reinforcing the potential of this approach even in other animal models besides the common rodents.

Following the abovementioned studies, the clinical evaluation of these DMN vaccination approaches was published by a couple of research groups. In 2015, Hirobe et al reported a study with 40 healthy male volunteers, receiving trivalent seasonal influenza hemagglutinin antigens either with DMN or through SC injection. In this case, DMN were applied using a handheld applicator and the safety and efficacy of the immunization was evaluated only in subjects showing at least 50% of needle dissolution after application. Nevertheless, DMN immunization led to equivalent or superior immune responses against the antigens in comparison with the conventional SC injection, inducing activation of T cells and antigen-specific IFN-γ-producing cells.

On the other hand, the Prausnitz group has more recently published the results of a randomized, partly blinded, placebo-controlled, Phase 1 study on the safety, immunogenicity and acceptability of DMN vaccination against influenza. DMN delivery (20 mins) of influenza vaccine was compared to that of IM administration of the same vaccine. Both participants and health care workers administered the vaccine by DMN (Figure 3A and D). The mean geometric antibody titers, as determined by hemagglutination inhibition antibody assay, were similar at day 28 between the DMN and the IM treatment groups for the three virus strains employed (H1N1; H3N2 and B strain). Importantly, similar titers were observed for the DMN self-vaccination group, highlighting the simplicity of this vaccination approach. Post DMN vaccination, local reactions were evident in the skin (Figure 3E and F); however, these quickly resolved and participants were in favor of the DMN vaccination over IM vaccinations. Importantly, participants found DMN to be less painful than that of IM injection (Figure 3F).

Bacterial vaccines
While most published studies with DMN have focused on viral infections, a small number of studies have also investigated DMN vaccination against bacteria. As an example of this, Esser and co-workers tested the hypothesis that DMN could potentially overcome the immunotolerance induced during pregnancy and enhance protective immunity to tetanus in mothers and their newborns. DMN were prepared using a two-step fabrication process from a formulation of PVA, sucrose and CMC. The resultant DMN were 650 µm in height and 250 µm in diameter at the base and were used for the delivery of unadjuvanted tetanus toxoid to the skin of pregnant mice. The study demonstrated that the DMN were superior to IM injection, with mice born to DMN vaccinated mothers showing detectable tetanus-specific IgG antibodies for up to 12 weeks of age and complete protection to tetanus challenge up to 6 weeks of age. On the contrary, mice that were vaccinated via the IM route failed to survive challenge.

In an innovative strategy, Chen and co-workers approached the well-known local adverse reactions elicited by the commercial tuberculosis vaccine (BCG) by developing DMN with an internal “cave” where the powder BCG vaccine could be accommodated and released in the ID space. With this strategy, the authors were able to completely avoid the local reactions in mice, while inducing immune responses similar to those achieved with ID injection. Other studies looked at...
DMN-based immunization against *Neisseria gonorrhoeae* and *Staphylococcus aureus*, with promising results. In the first case, DMN-immunization of mice with whole-cell gonococci encapsulated in albumin microparticles led to higher antibody levels than an SC injection of the vaccine suspension. Similarly, Liu and co-workers reported extended antigen retention in mice, higher antibody levels and stronger protection against challenge using DMN for vaccination against *S. aureus*, in comparison with IM vaccination.

More recently, our research group has reported the successful ID vaccination of mice using DMN fabricated from Gantrez® S-97 and containing heat-inactivated bacteria. Specifically, it was demonstrated that the incorporation of heat-inactivated *Pseudomonas aeruginosa* into DMN (500 µm in height) and subsequent vaccination of mice, resulted in these mice having a greater capability to control bacterial infection following challenge. This proof-of-concept work demonstrated the potential of DMN for ID vaccination against a bacterium for which there is...
currently no licensed vaccine. Moreover, this is a cost-effective approach that could easily be implemented in developing countries, allowing a rapid and simplified approach to anti-bacterial immunization.

Model and novel vaccines
In addition to DMN delivery of vaccines currently in use, researchers have also investigated the use of DMN for the delivery of alternative and model vaccine agents. In the field of model antigens, ovalbumin (OVA) is definitely the most common choice for vaccine delivery studies. In the last few years, several authors have reported on the development of DMN-based vaccination strategies with OVA as a model antigen, mainly in rodent models (mice and rats). In this scope, An and co-workers have published the development of polyacrylic acid DMN in a 10×10 array, with 500 µm-long needles and amphiphilic OVA, aiming at a lymph-node targeting vaccine. This approach led to high accumulation of the antigen in the lymph nodes and stronger humoral and cellular responses than those achieved with ID injection. Similarly, other authors have described the use of chitosan-based DMN for the immunization of rats with OVA, in two subsequent studies. In the first one, chitosan DMN acted as an implanted depot, with the needle tips left in the animals’ skin to release OVA for up to 28 days. This led to a 2.5-fold dose-sparing effect observed with DMN immunization in a single-dose approach, in comparison with IM injection. Subsequently, the same group reported the modification of DMN to achieve a “prime-boost” effect, with antigen loaded into faster-dissolving sodium hyaluronate needle tips and slower-dissolving chitosan needle shafts. With this strategy, the immune responses elicited were stronger and longer-lasting than those obtained with chitosan-only DMN or repeated or double-dose SC OVA injection, thus reinforcing the potential of this novel DMN formulation.

Yan and colleagues recently evaluated the ability of DMN fabricated from sodium hyaluronate (HA) to vaccinate mice against tuberculosis (TB). These HA DMN had 500 µm in height and a base diameter of 250 µm, and were formulated to contain a DNA vaccine encoding the secreted protein Ag85B of Mycobacterium tuberculosis (Figure 4A–C). The developed DMN were successfully inserted in murine skin (Figure 4D), as confirmed also by histological analysis of the application site tissue (Figure 4E). DMN vaccination was compared to that of IM injection. While no significant difference was observed between DMN and IM groups with low dose (4.2 µg), DMN induced better antibody responses than those of IM injection at a higher dose (12.6 µg). Similar results were obtained in terms of cellular immune response, by measuring cytokines in splenocytes. Mice vaccinated either by DMN or IM injection were subsequently challenged by tail vein injection with 5×10^5 colony forming units (CFU) of the H37Rv strain of M. tuberculosis, 4 weeks post-vaccination. Analysis of CFU post-challenge demonstrated that the bacterial load in the lungs and spleens of the DMN group was significantly lower than that of the control groups (Figure 4F and G). Analysis of survival post-vaccination showed that the DMN group had prolonged survival, compared to that of the DMN without DNA (MNAWD), while the IM group did not elicit a significant change in survival rate. Collectively, these data demonstrate that DMN vaccination may provide more effective protection against this pathogen compared to that induced by IM vaccination.

DMN have also been investigated for vaccination against Alzheimer’s disease. Matsuo et al used DMN for delivery of amyloid-beta peptide, composed of amino acid residues 1-41 (Aβ1-41), to a mouse model of Alzheimer’s disease. Amyloid-beta had previously shown therapeutic efficacy in mouse models but human clinical trials had to be stopped due to serious adverse reactions, specifically incidences of meningoencephalitis caused by Th1 cell activation and infiltration into the brain. Various studies have suggested that TC vaccination would likely trigger Th2-dominant immune responses and thus the research team in question hypothesized that DMN delivery of amyloid-beta could be effective at treating Alzheimer’s disease, potentially circumventing the previously encountered adverse reactions. Accordingly, the researchers fabricated DMN from hyaluronate as the base material (MicroHyala). The resultant DMN were cone-shaped and had needle lengths of 300 µm or 800 µm. While this approach induced little improvement in cognitive function and Th2-dominant immune responses, DMN vaccination did induce anti-Aβ1-41 immune responses but further modifications of the delivery platform were required.

DMN vaccination to other tissues
The studies described herein highlight the swath of work that has been conducted to date using DMN for skin vaccination purposes. Inspired by such successes with DMN for ID vaccination, DMN have more recently been used for delivery at other tissues, including the oral and vaginal mucosa. As an example of this, Wang and co-workers combined different types of multifunctional
liposomes loaded with ammonium bicarbonate to fabricate DMN as a vaccine adjuvant delivery system for the vaginal mucosa. To elaborate, mannosylated lipid A-liposomes (MLLs) and stealth lipid A-liposomes (SLLs) were loaded with a model antigen (OV A) and formulated with ammonium bicarbonate forming proSLL/MLL MN (proSMMA), that upon rehydration dissolved rapidly. Vaccination of mice with proSMMAS applied to the vaginal mucosa resulted in robust mucosal and systemic antigen-specific humoral and cellular immunity. The surface antigen gD of herpes simplex virus (HSV2) was also loaded in proSMMAS, and vaccination of mice resulted in successful protection against HSV2 virus challenge, a virus that infects over 20 million people annually. The authors concluded that the developed proSMMAS showed promising potential for the dual delivery of antigen and adjuvant to the vaginal mucosa, and could potentially be loaded with various antigens for the development of vaccines, particularly against sexually transmitted infectious agents.

The oral mucosa has also been investigated as a site for MN vaccination; however, such studies have focused on the use of coated MN rather than DMN. Moving forward, development of DMN for vaccination at other tissues will necessitate novel DMN designs to account for different tissue anatomy, physiology and biomechanics. Lessons learned from previous studies conducted with DMN for ID vaccination will undoubtedly prove valuable in this regard.

**Safety considerations for adoption of DMN in a clinical setting**

DMNs differ from conventional transdermal patches in that the needles of the array breach the SC barrier,
Consequently, crossing the membranes...

For the reported... C. albicans, P. aeruginosa, S. aureus...

While polymers used in membranes, we demonstrated that the total...

Given the antimicrobial properties of the...

In 90–89...

be minimal. We have previously demonstrated that MNs...

Accordingly, while it is unlikely that DMN will cause infection, it will be of pivotal importance to ensure the components within DMN do not alter or modulate the immune response to the antigen.

Information gained from research to date implies that appropriate use of DMNs is unlikely to result in skin infections. The skin, by virtue of its protective function against the external environment, is subjected to a multitude of microscopic insults as a result of injuries such as scratches or trauma, from which the skin repairs itself, without infection occurring. According to some published studies, the ability of microorganisms to traverse the holes created in the skin as a result of DMN insertion appears to be minimal. We have previously demonstrated that MNs allow lower microbial penetration than that of the traditional hypodermic needle in vitro. Specifically, by employing Silescol™ membranes, we demonstrated that the total numbers of C. albicans, P. aeruginosa and S. epidermidis crossing the membranes were significantly lower with solid silicon MN, in comparison with the use of a 21G hypodermic needle. In another study, using polymeric DMN, we have also showed that repeated application of these patches to mouse skin in vivo did not lead to any significant alteration in biomarkers of infection, immunity and inflammation. Given the antimicrobial properties of the skin, it is thus likely that the appropriate use of DMN would cause neither local nor systemic infection in immune-competent individuals. Concurrent with results in this study, another study by Wei-Ze et al reported that rats treated with solid silicon MNs did not become infected post incubation with S. aureus. However, it should be highlighted that the needle length in this study was only 70–80 µm, leading to smaller pores formed in the skin and potentially less probability of microbial penetration.

Additional safety concerns include the biocompatibility of the materials selected for DMN formulation and fabrication. DMN may be fabricated from a range of polymeric materials (as reviewed in). Of paramount importance will be an assurance that no local or systemic reactions occur in the skin as a result of the polymers used in the fabrication of DMN. Biocompatibility and safety studies are warranted to investigate this. To date, there is little knowledge on the long-term effects of repeatedly penetrating the skin with DMN. As previously described, DMN result in polymer deposition in the skin, due to dissolution of the vaccine-containing needles, the effects of which are currently unknown. The polymers used for DMN formulation should, therefore, be able to be eliminated from the body by polymer degradation if biodegradable or by excretion via glomerular filtration if non-degradable. For the latter, polymer molecular weight will be particularly important, as excretion will only be possible if the polymer size is below the glomerular filtration size threshold, as shown for polyvinylpyrrolidone. While polymers used in DMN fabrication have widespread use in other common pharmaceutical and cosmetic applications, they have never been used ID in the clinic. Accordingly, the long-term effects of polymer deposition in the skin are not fully understood. The deposition of polymers which are not excreted could result in polymer accumulation in tissue, potentially causing local erythema or granuloma formation or accumulation in clearance organs in the body. While it is anticipated that repeated long-term applications will be unnecessary for vaccine delivery, the impact of polymer deposition will still need to be fully elucidated. Moreover, while prime-boost regimes may be necessitated, it is unlikely that the DMNs would be inserted into the exact same insertion site on the skin’s surface.

**Future perspectives**

DMN have undoubted potential, as evidenced by the significant body of work published in the field, on the microfabrication, vaccine delivery and subsequent immunogenicity of vaccines delivered via this route. For
adoption of DMN in clinical practice, a number of questions remain to be addressed. The scale-up of DMN production for industrialization and mass production will necessitate considerable thought. A wide range of different manufacturing methodologies for DMN fabrication in a small-scale laboratory setting have been reported; however, there remain significant barriers for adoption of these approaches on an industrial scale.\textsuperscript{97,98} Often, DMN fabrication requires multiple fabrication steps for localization of vaccine antigen and adjuvant in certain parts of the DMN array, for improved delivery efficacy and immunogenicity.\textsuperscript{99,100} Adoption of such methodologies to larger scale could pose significant challenges and it would be necessary for industry to make significant investments in both equipment and processing capabilities. Manufacturers will need guidance in terms of good manufacturing practices, pharmacopeial standards and standardized tests for DMNs production and characterization.\textsuperscript{101} Of note, LTS Lohmann Therapie-Systeme AG, the world’s largest manufacturer of transdermal patches, holds now a manufacturing license for MN. Considering that DMN contain vaccine within the matrix of the array, each DMN may possess different characteristics and thus it will be required that each DMN be tested to ensure it is fit for purpose. The current lack of regulatory guidance in this area presents a problem for DMN product development. If DMN are to be implemented to clinical use, regulatory guidelines pertaining to patient use are warranted. The PATH Centre of Excellence for Microarray Technology aims to address regulatory issues and quality control tests in order to progress this technology. Such factors requiring consideration are packaging, disposal, ease of use, confirmation of insertion and subsequent delivery, in addition to the previously mentioned safety concerns.\textsuperscript{95}

To date, there are contradictory opinions as to whether DMN will need to be used in combination with an applicator device. Several researchers and companies have reported the development of applicators for MN patches and the topic has been reviewed elsewhere.\textsuperscript{102–105} In the case of DMN, numerous studies have clearly demonstrated that DMN can be easily and reliably inserted into the skin by minimally trained personnel and patients, but further in-depth studies are required to ascertain if variability exists between users. This will undoubtedly have implications for vaccine delivery and the resultant immune response. Indeed, the introduction of an applicator device would ensure consistent application forces between end users but this would have also significant additional cost implications, above the previously reported acceptable estimates of $1 USD + API.\textsuperscript{30} The use of alternative feedback mechanisms, for example, a pressure-indicating film, would provide a less expensive alternative option.\textsuperscript{42} It will be of pivotal importance to assess the opinions of the end-users of DMN in this regard and to include their insights in the co-design of future MN devices.

An investigation into the potential implications of polymer deposition in the skin will also require consideration. Factors such as polymer-induced irritation, changes in skin barrier function and in-depth studies of microbial penetration must be conducted. Additionally, the biodistribution and subsequent polymer accumulation in the skin will need to be fully understood before DMN are routinely used in clinical settings. Similarly, studies on the antigen bioavailability following transdermal administration evaluating the actual antigen dose delivered to the skin and reaching the blood circulation could be interesting to broaden the knowledge on the mechanism of action and efficacy of this immunization approach.

A scheme of the mechanism of vaccine delivery using DMN as well as a list of the main advantages and challenges associated with this immunization approach are summarized in Figure 5.

**Conclusion**

Vaccination is considered to be one of the most significant health interventions for the control of infectious disease. The skin, being the largest organ of the human body, has garnered substantial interest as a site to facilitate ID vaccination via DMN. The recent Phase I clinical trial using DMN for influenza vaccine delivery published by the Prausnitz group, in combination with the breadth of ongoing work being conducted throughout the globe, exemplifies the tremendous potential DMN could have if brought to clinical use. Moving forward, there remain a number of questions that will need to be addressed if DMN are to be adopted for clinical use. To date, the commercial translation of DMN for ID vaccination has been limited by the relatively small number of clinical studies conducted in humans, in combination with implementation of large-scale, cost-effective manufacturing processes. Despite the aforementioned hurdles which must be addressed in advance of the large-scale clinical exploitation of DMN, the valuable impact these delivery devices could have, particularly in the developing world, can already be predicted.
Abbreviations
APC, antigen-presenting cell; dDC, dermal dendritic cell; CMC, carboxymethylcellulose; DMN, dissolving microneedle array; EV71, Enterovirus 71; GM-CSF, granulocyte-macrophage colony-stimulating factor; HA, sodium hyaluronate; HFMD, hand-foot-and-mouth disease; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus; ID, intradermal; IPV, inactivated polio vaccine; IM, intramuscular; LC, langerhans cells; MLLs, mannosylated lipid A-liposomes; MN, microneedle array; OCT, optical coherence tomography; PV A, polyvinyl alcohol; SC, subcutaneous; SLLs, stealth lipid A-liposomes; TC, transcutaneous.

Disclosure
The authors report no conflicts of interest in this work.

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Figure 5 Schematic representation of the process of vaccine delivery using dissolving microneedle (DMN) arrays and summary of the main advantages and challenges associated with this potential vaccination strategy.

Advantages
› Abundance of immune cells in the skin
› Opportunity for administration by untrained personnel
› Overcoming pain and needle phobia
› Potential for reduced cost
› Improved thermostability and simplified supply chain

Challenges
› Assurance of sterility and no influence on immune response against the antigen
› Biocompatibility of materials used in DMN fabrication
› Scale-up of manufacturing for mass production
› Assurance of insertion (need for an applicator?) and effective dose delivery (for patients and healthcare professionals)
› Lack of information on antigen bioavailability following DMN administration

Vaccine-loaded DMN
DMN dissolution
Vaccine release
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