# Microneedle-Based Vaccine Delivery: Review of an Emerging Technology

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## Abstract

Vaccination has produced a great improvement to the global health by decreasing/eradicating many infectious diseases responsible for significant morbidity and mortality. Thanks to vaccines, many infections affecting childhood have been greatly decreased or even eradicated (smallpox, measles, and polio). That is why great efforts are made to achieve mass vaccination against COVID-19. However, developed vaccines face many challenges with regard to their safety and stability. Moreover, needle phobia could prevent a significant proportion of the population from receiving vaccines. In this context, microneedles (MNs) could potentially present a solution to address these challenges. MNs represent single dose administration systems that do not need reconstitution or cold-chain storage. Being self-administered, pain-free, and capable of producing superior immunogenicity makes them a more attractive alternative. This review explores microneedles' types, safety, and efficacy in vaccine delivery. Preclinical and clinical studies for microneedle-based vaccines are discussed and patent examples are included.

KEY WORDS Microneedle · Transdermal · Vaccine · Needle phobia

## INTRODUCTION

Infectious diseases caused by different bacteria, viruses, parasites, and fungi are considered the leading cause of death worldwide. The most contributing agents are human immunodeficiency virus (HIV) and acute respiratory infections. For example, lower respiratory tract infections remained the world's most deadly communicable disease, ranked as the 4th leading cause of death. In 2019, it claimed 2.6 million lives (1). Moreover, viral infections have a very significant impact on global health as viral infections affect millions of people globally (2). According to HealthyPeople.gov, viral hepatitis, influenza, and tuberculosis remain among the leading causes of illness and death in the USA and account for substantial expenditures (3).

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Recently, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected millions of people worldwide, causing the coronavirus disease 2019 (COVID-19) pandemic. High infectibility is attributed to the lack of symptoms during the incubation period, among other factors (4). Since its outbreak in 2019, there have been 384.86 million recorded cases and 5.7 million deaths around the globe as of early 2022. In the USA, the number of infected individuals reached a staggering 75.68 million cases with a death toll of 894,320 as of early 2022, making it the most affected country affected by the outbreak (5).

Despite the decrease in CO13D-13 transmission by non-pharmaceutical interventions (social distancing), a great effort is still needed to combat the current pandemic (6). In this context, vaccination is considered one of the safest and most cost-effective means of preventing illness and related disability and death from certain infectious diseases (7). Many vaccines were developed in the last two centuries and had a fundamental role in controlling infectious diseases such as diphtheria, smallpox, and polio. Some other vaccines to eliminate pathogens (acute respiratory infections, malaria, HIV) are yet to be developed (8). In the meantime, vaccination represents the best available option to prevent COVID-19-associated morbidity and mortality. Additionally, vaccination may combat future virus variants (9). Vaccination is crucial for the general public to develop "herd immunity" which requires vaccinating about 70% of the total population (9).

A vaccine is a biological product that contains a weakened or killed microorganism or its toxins or surface antigens (10). Recently, nucleic acid-based vaccines, either DNA-based (as plasmids) or RNA-based (as messenger RNA (mRNA)) vaccines, have been developed for safe and efficacious biologics to stimulate cell-mediated immunity (11).

Vaccines stimulate the host's natural defenses to recognize and combat foreign agents. Immunization confers resistance to vaccinated individuals reducing their risk of getting the disease or spreading it. Additionally, the more vaccinated individuals, the less likely the infection will spread, protecting individuals who cannot get vaccinated due to health, age, or other factors (12).

The primary requirements for an ideal vaccine are safety, effectiveness, and optimal induction of "sterilizing" immunity. Moreover, an ideal vaccine should also possess secondary requirements such as low cost, thermal stability, long-lived immunity, and ease of administration (13).

Most vaccines are administered via injection as vials for single, multi-dose administration or prepackaged syringes for hypodermic injection. But some are administered orally such as adenovirus, rotavirus, and typhoid. However, using the oral route for vaccination comes with challenges related to absorption, degradation, and first-pass metabolism, among others (14). Vaccine delivery via other routes of administration has been investigated without producing tangible results as these of needle-based vaccines (15). Therefore, parenteral administration is still the most common route for vaccine administration.

Parenteral administration of vaccines includes intramuscular (IM), subcutaneous (SC), and intradermal (ID) inoculation, delivered via hypodermic needles or needle-free injections. Intravenous administration is not considered for immunization due to inadequate immune response and risks related to severe allergic reactions (16).

ID delivery carries benefits associated with the abundance of immune cells in the skin compared to muscle or subcutaneous tissue. Skin is densely populated with several antigen-presenting cells (APCs), such as dendritic cells (DCs) in the dermis, and Langerhans cells in the epidermis. These cells capture the foreign antigens and transport them to the draining lymph node to present antigens to T cells. This leads to antigen-specific T-cell and B-cell activation (17, 18).

Despite its efficacy in eliciting an immune response, ID administration can lead to localized adverse reactions, which tend to be more severe than SC and IM administration. The latter has the least local reactions among the three. Therefore, IM administration is recommended to avoid local tissue irritation, induration, and inflammation associated with SC and ID vaccine administration (16). Moreover, IM injections are widely used for vaccine delivery as the muscles are richer in blood vessels than the skin (19). However, muscular tissues contain relatively fewer resident APCs than the skin (17, 18).

Although injection represents a cost-effective, rapid solution for vaccine, and drug delivery, it suffers many limitations. For example, as injectable dosage forms, vaccines require medically trained administration personnel. Additionally, needles generate sharp waste that poses a risk to staff and patients and could lead to the spread of bloodborne diseases. According to the World Health Organization (WHO), about 33,800 HIV infections, 1.7 million hepatitis B infections, and 315,000 hepatitis C infections annually were caused by unsafe injection practices. Additional needle-limiting challenges include needle phobia and pain (20, 21).

Needle phobia is described as the anxiety associated with needles or injection use. However, needle phobia can lead to a series of physiological processes that end with fainting. This emotional fear increases heart and respiratory rates, activating other physiological alterations, including sweat, headache, and gastrointestinal discomfort. Fainting and blurred vision then follow. Severe physiological changes can occur in high levels of needle phobia causing hemodynamic instability or even death as reported in documented cases (22). Needle phobia results in avoiding and hampering treatment, diagnosis, and vaccination efforts towards numerous illnesses (23).

The prevalence of needle phobia varies among different groups. For example, teenagers' prevalence is 20–50%, while the range is between 20 and 30% in young adults. Additionally, 16% of adult patients, 27% of hospital staff, and 18% of workers in long-term facilities avoided influenza vaccination due to fears related to needles. The latter results also extended to other types of vaccines. Results from a representative sample of US adults highlighted that 19% and 20% of the study population did not receive pneumococcal and tetanus vaccines, respectively (24).

The stability of vaccines available as liquid injectable dosage forms is another major concern. A vaccine must be kept refrigerated from the time of manufacture till administration (this refers to the "cold chain" process). The estimated cost of cold chain storage is $200–$300 million. Deviations in this process, such as temperatures above or below 2 to 8 °C, will render the vaccine ineffective (25). This has elevated the risk of vaccine shortage due to cold chain failure, especially in low-income countries where electricity for refrigeration is limited (20, 21). Furthermore, the WHO reported that 50% of all globally manufactured vaccines are wasted and attributed most of this waste to cold-chain failure, particularly in developing countries (25).

## RATIONALE FOR THE USE OF MICRONEEDLES IN VACCINES

For these reasons, attention was given to other delivery methods for vaccine administration. Utilizing skin as a route for vaccination presents microneedles (MNs) as an alternative delivery system. MN is considered a minimally invasive transdermal drug delivery technique without pain during administration. This is because MNs are long enough to pierce the skin without reaching nerve endings responsible for pain (26, 27).

MNs can deliver vaccines by physically penetrating the stratum corneum (28), allowing pain-free drug delivery, therefore avoiding poor compliance associated with hypodermic needles. Moreover, there will be a reduced need for trained healthcare staff (21) while eliminating the risk of accidental needle injuries and the need for safe disposal. MNs work by delivering antigens to the epidermis and dermis using APCs in the skin (27, 29). Vaccines delivered intradermally by MNs successfully elicited immune responses comparable to those produced by SC or IM injections across several trials (18). Moreover, a high dose of the vaccine can be delivered to the skin microenvironment using MNs. This, in turn, can result in a dose-sparing effect, which would decrease the vaccine dose required for adequate immunization and thereby decrease the cost and toxicity (17).

From a stability point of view, vaccine delivery by MNs has several advantages over conventional methods. MN vaccines can be formulated and stored in a solidified form which is generally more stable at elevated temperatures than liquefied forms of the vaccine. The dry state of the vaccine, together with the stabilizing effect of excipients, can enhance vaccine thermostability (18). This eliminates reconstitution and reduces costs associated with the cold chain (30). Therefore, MNs present a preferable option to conventional injections (31).

## Microneedles Types

MNs generally fall in four different types depending on their delivery strategy; solid, coated, hollow, dissolving, and hydrogel-forming.

Solid microneedles (SMNs) are drug-free micron-sized arrays with tapered tips capable of penetrating the stratum corneum, creating micro-channels/holes for subsequent drug application (32). It can be fabricated from metals or silicon and is considered a pretreatment step that creates temporary micro-channels in the skin. Upon removing SMNs, the drug is applied onto the skin in the form of a solution, cream, or patch where absorption takes place by passive diffusion (33). However, SMNs suffer certain limitations, including lack of uniform drug delivery (34), need for a two-step process, a hazardous residue requiring special disposal, and rapid micro-pores healing (32).

Coated microneedles (CMNs) were proposed to overcome the complexity of the two-step process in SMNs. Herein, MNs tips are coated by the drug that is absorbed rapidly after MN application (33, 35). CMNs are made from metals and silicon coated with the drug (35). Since the antigen dose needed to trigger an immune response ranges from nanograms to micrograms, CMNs can be used to deliver vaccines (36). For instance, an influenza vaccine was developed using MNs coated with influenza virus-like particles inserted into the skin for ID immunization (37). Also, Choi et al. reported successful smallpox vaccination using CMNs (38). However, CMNs are suitable only for potent therapeutics with doses > 1 mg. Additionally, coating formulation must maintain stability and adhesion during storage and application. Moreover, coating can decrease the tip's sharpness, decreasing skin penetration efficiency (36).

In case of hollow MNs (HMNs), therapeutic agents are embedded in the hollow space located in MN tips, where MNs act as a drug reservoir. It is made of metal, glass, ceramics, or silicon (39). HMNs permit higher drug dose and dose precision than SMNs and CMNs (33). The drug in the channels/hollow space is directly released into the epidermis upon insertion (39). HMNs have been tested for insulin and vaccines delivery. In a trial, four nanoparticulate systems loaded with model antigen (ovalbumin) were delivered intradermally by HMNs to elicit an adequate immune

response in mice (39). However, HMNs suffer an increased risk of needle clogging by tissue upon insertion, hindering drug flow. Needle breakage, difficulty in delivering dry formulation, and expensive manufacture also limit its use (40, 41).

The aforementioned MN types pose a risk of possible needle breakage in the skin and the presence of sharp needle waste. This led to the development of dissolving MNs, which are made of water-soluble biocompatible/biodegradable polymers or sugars. By adjusting polymer type and ratio in the formulation, drug release can be sustained for months. Upon insertion, polymers dissolve/degrade in the interstitial fluid releasing encapsulated drug (42, 43). Furthermore, these self-dissolving MNs leave no sharp waste and thus eliminate the possibility of secondary infection by repeated MNs use. However, dissolving MNs tips must maintain sufficient strength to penetrate the stratum corneum. Otherwise, incomplete skin penetration would occur, leading to dose waste (37). Dissolving MNs can be used to deliver various types of therapeutics and vaccines. Lee et al. reported transdermal insulin delivery using gelatin/carboxymethylcellulose (CMC) MNs with efficient hypoglycemic effect (44). Rodgers et al. presented ID vaccination against *Pseudomonas aeruginosa* via MNs. Heat-inactivated bacteria was incorporated into dissolving polymethylvinylether/maleic acid MNs (27). It permitted prolonged vaccine release leading to enhanced outcomes (45).

Finally, the fourth type of MNs is hydrogel-forming MNs, which absorb skin interstitial fluids resulting in hydrogel formation. The drug is released in a manner determined by the hydrogel's crosslinking degree, giving rise to slow release over several days (39). It is made of mixtures of polymers such as polymethylvinylether/maleic acid, CMC, and amylopectin (41).

## Factors Affecting Microneedles' Efficiency

Enhanced efficiency of MNs requires an optimal design (a low insertion force and high fracture force) (46). This optimal design depends on different factors including MN geometry, tip diameter and sharpness, application velocity and force, length, and density and MNs interspace as seen in the next section.

### MN Geometry

Geometry is an important factor to be considered while developing MNs, because it influences mechanical force, penetration efficiency, and patient compliance. There is a direct relation between the number of polygon vertices and MN mechanical strength (47). MNs show a pyramidal or conical shape with a 1:1–1:3 width-to-height ratio (38).

Studies indicate that sharp edges of triangular- and square-based MNs are superior in their insertion depth to hexagonal based microneedle (47, 48).

### Tip Diameter and Sharpness

Tip diameter affects MN insertion depth, while its sharpness affects and controls.

the penetration force. However, sharpness can reduce the structural strength of the microneedles, leading to its breakage (49). Römgens et al. concluded that sharp microneedles (< 15 μm) are very necessary for efficient and controllable delivery of drugs especially vaccines (50).

### Application Velocity and Force

Application velocity and force are key factors for successful penetration of MNs. Appropriate force is needed to pierce SC tissue and form micropores to successfully deliver therapeutic agents (51). Two different studies reported that insertion forces of 15–20 mN per microneedle were sufficient for appropriate MN insertion (52, 53). However, controlled application or applicator may be required to eliminate variations resulting from manual delivery of therapies (49).

### Length

MN length is significant as MNs have to be long enough to avoid drug loss on the skin. MNs of appropriate length will deliver the drug to the dermal layer, providing suitable drug delivery. Increased length leads to increased volume, which allows higher drug loading. However, unduly long MN will cause pain due to nerve contact. Minimum pain is produced when MN length is less than 750 μm (54–56). MNs length should be tailored for therapeutics. In case of drugs of higher perfusion capacity, shorter MNs may be used. However, if the goal of therapy is the rapid delivery, it is better to use longer MNs to reach the dermis, where blood vessels are located (49).

### Density and MNs Interspace

Increasing the number of needles in a densely packed array increases the number of microchannels through which the drug diffuses. This, in turn, permits higher drug loading. However, the penetration is inhibited by the "bed of nails" effect. In this effect, the force required for insertion will be divided among needles, meaning a higher pressure is needed for insertion. Such effect can be overcome by using different MN lengths in one array, thereby reducing the required insertion force (55). Olatunji et al. reported that as the interspacing increased, the resistance to penetration decreased and so the normal stress at MN tip decreased (57).

## Microneedles' Safety

Despite the fact that MNs do penetrate the skin, they are labeled "safe" due to their small size. Microneedles' safety can be assessed by the resultant pain, degree of skin irritation, stability during storage, and *in vivo* degradation (58).

## Microneedle and Pain

Pain receptors are embedded deeply in the dermis that microneedles cannot reach. Therefore, MNs cause less/no pain compared to hypodermic needles. Pain degree depends on MNs number, length, and shape (59). Pain can be assessed by a validated subjective measure known as the Visual Analog Scale for Pain (VAS) (60). A study was performed on 180 volunteers who received IM and MNs influenza vaccine. The pricking pain with MNs was significantly lower, as reflected by a lower VAS score than IM (61). However, the pain did not significantly differ between MN and IM groups during administration. Moreover, in many studies, solid and dissolving MNs did not reveal any pain or discomfort at the application site (62–64).

## Irritation Assessment

At the application site, erythema and/or edema can be provoked either due to the nature of the material used or by MN remains (mechanical failure or fracture). Thus, irritation level is measured to ensure the safety of MNs (65).

Irritation degree can be evaluated by the Draize method (assessment technique performed by applying test substance on the eye/skin of the animal followed by observing certain irritation signs) (66). Al-Kasasbeh et al. evaluated the degree of irritation of hydrogel-forming MN patch on 11 volunteers (34). Clinical visual scores were graded from "no" to "severe erythema." Most volunteers exhibited a certain degree of skin erythema immediately after the patch removal, but it was short-lived (34). Contrarily, dissolving MN patches containing polyvinylpyrrolidone and sucrose were investigated on 15 volunteers. Volunteers reported no swelling, pain, or erythema at the application site. This can be due to the biocompatibility of MN materials (67).

## *In Vivo* Degradation

Full MN insertion within the skin is needed to overcome skin deformation and ensure effective delivery of the intended dose. Created microchannels should close quickly to avoid any dose permeation and control skin infection. Microchannels can be measured visually by applying a liquid bandage to create an inverse copy of the holes or by a transepidermal water loss (TEWL) test (68). The time of skin resealing is important and depends on skin condition and needle geometry. Skin resealing is evaluated by TEWL or staining (69).

Park et al. evaluated polymeric MNs *in vivo* degradation by a fluorescent dye. Rhodamine 6G was incorporated within MNs followed by a histological examination at different time intervals, which showed biphasic release of dyes (70). Chen et al. tested the behavioral delivery of dissolving MNs using *in vivo* fluorescence images (71). All polymers showed the same fluorescence intensity at the sites of application, which decreased over time due to the gradual diffusion of encapsulated drugs (71).

## Storage Stability

Vaccine stability is crucial to maintain its efficacy. MN use in vaccination is advantageous as they make vaccines less reliant on cold chain storage (72). This improves vaccine distribution, which is highly valued in developing countries with a refrigeration infrastructure shortage. Furthermore, stability can be enhanced by adding various polymers and sugars such as polyvinylpyrrolidone, trehalose, sucrose, and heptagluconate (73).

Microneedles of peptide-coated parathyroid hormone achieved 2-year shelf-life stability through sucrose addition as a stabilizer, outgassing formaldehyde from device components and controlling oxygen and moisture (74).

Mistilis et al. maintained the stability and activity of biodegradable polymeric MNs coated with influenza vaccine by adding arginine/heptagluconate mixture (24 months at 25 °C and 4 months at 60 °C) (75).

## Efficacy of Microneedle-Based Vaccines in Preclinical and Clinical Studies

There have been many studies evaluating the efficacy of MN-based vaccines. Herein, we will explore the effectiveness of these vaccines in both preclinical and clinical settings against various infectious agents. Supplementary Table 1 includes patents of MN-based vaccine delivery (76–85).

### Adenovirus and Vaccinia Ankara Virus

Human adenovirus (HAdV) is a DNA virus that causes various conditions varying from mild respiratory conditions to acute respiratory infections and more. This virus is responsible for 2–5% of all respiratory infections around the globe (86).

In a study evaluating the delivery of live vaccines in mice, solid MN arrays were loaded with vectors of live recombinant adenovirus (AdV) and vaccinia virus Ankara. The study revealed that the CD8 + T cell response and the antibody response to AdV in MN array group were similar to that of ID group that was delivered using a needle and syringe (87).

## COVID-19

The coronavirus-2019 has wreaked havoc across the globe since it became a pandemic. The use of MN-based COVID-19 vaccine has been explored by Kim et al. (88). The researchers had experience producing Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) dissolvable MN array that achieved long-lasting effect in mice. Kim et al. then utilized the same technology to produce an effective vaccine against COVID-19. This technology involved subcloning the SARS-CoV-2S1 subunit gene using a pmaxCloning vector and subsequent purification of recombinant proteins. Significant antigen-specific immunoglobulin G (IgG) antibodies response was elicited as early as 2 weeks post-vaccination, highlighting the potential use of this delivery technique in vaccination. Another study utilized a separable MN patch to deliver polymer encapsulated spike (or nucleocapsid) protein encoding DNA vaccines and immune adjuvant for efficient immunization. Deoxycholic acid conjugated with polyethylenimines as an amphiphilic polymer was designed to encapsulate the hydrophobic R848 (immunostimulant) in the core of nanoparticles. Then a plasmid DNA for expressing S- or N-protein (pCOV-S or pCOV-N) was absorbed on the nanoparticles through electrostatic interaction. The endo/lysosome escaping capability of nanoparticles was investigated by labeling pCOV-S and pCOV-N with fluorescence dye and confirmed by flowcytometry. The expression of S- and N-protein in both RAW264.7 and DC2.4 was confirmed with both enzyme-linked immunosorbent assay and Western blot. Ex vivo imaging of lymph node collected form sacrificed mice confirmed DNA vaccine migration to the lymph nodes. *In vivo* cellular immune responses showed that IFN-γ + CD4/8 + and IL-2 + CD4/8 + T cells or virus-specific IgG were significantly increased after vaccination (89).

## Ebola

Ebola virus (EBV) has a high fatality ratio reaching as high as 54%. Since there is no approved vaccine yet, Yang et al. (90) sought to investigate the use of nanoparticle-prepared EBV DNA vaccine to determine its safety and immunogenicity. The developed vaccine was compared to the naked DNA vaccine delivered via dissolvable MN patch or IM injection. The study revealed that antigen-specific antibodies did not differ significantly between both vaccines and routes of administration, except that MN-delivered naked DNA vaccine had lower IgG titers. However, the nanoparticle-prepared MN-loaded vaccine's neutralizing antibodies activity against EBV was highest.

## HIV

HIV is a virus that impacts the host's immune system, causing acquired immunodeficiency syndrome (AIDs). Since its discovery, the virus has claimed the lives of about 32 million individuals and caused the suffering of over 74 million people worldwide (91). One of the major goals in developing a vaccine against HIV is to have a properly folded and stabilized envelope trimers that mimic the original viral envelope glycoproteins (92).

Boopathy et al. (93) examined the use of implantable silk MN array patch loaded with HIV envelope timer antigen in mice. This MN patch was designed in a way that ensures the release of antigens over 2 weeks since sustained release of the antigen results in significantly enhanced humoral immunity and improved germinal center (GC) B cell responses. The study demonstrated that compared to traditional bolus injection, vaccine delivery using MN array patches yielded a 16-fold rise in the bone marrow plasma cells, a 1300-fold increase in serum levels of the MD39-specific (immunogen) IgG that was sustained for months, and increased GC responses. Currently, there are no approved HIV vaccines, highlighting the potential of utilizing MN technology in creating a vaccine that elicits a suitable immune response to confer protection against the virus. Despite the lack of vaccines, testing of mRNA-based HIV vaccine on animals has demonstrated promising results (94).

## Influenza

### Preclinical Studies

Ever since identified, the influenza virus has substantially contributed to mortality worldwide. Influenza-associated lower respiratory tract infections caused the death of 99,000–200,000 people in 2017 (51).

The use of MNs to deliver vaccines has been explored by Alarcon et al. (95), where they evaluated the use of ID stainless steel MNs in delivering 3 different types of influenza vaccines to rats, including a full inactivated influenza virus, a trivalent split-virion human vaccine (H1N1, H3N2, and B strains), and a plasmid DNA that encodes hemagglutinin (HA) portion of the influenza virus. In the groups that received the full inactivated virus, the elicited immune response was either comparable to or greater than IM injections groups. In the trivalent split-virion vaccine groups, results revealed that rats injected with a low dose of the vaccine using MN technique had a non-significantly different response against the H1N1 strain compared to groups receiving a high dose of the vaccine via both IM and MN injections. Regarding the H3N2 and B strains, results demonstrated that both the MN and IM injections elicited similar responses. Overall, the study found that compared to IM injection, dose-sparing benefit in MN injection was 100-fold in the whole inactivated virus, tenfold in H1N1 strain groups, and fivefold in the DNA vaccine groups (95).

In another study that evaluated the use of dissolving MN patches to deliver influenza vaccine, Sullivan et al. (96) discovered that upon testing on mice, results revealed that IgG1 titers in the MN group were more pronounced than these in the IM group on day 14. Additionally, MN vaccination

provided a 1000-fold more effective lung virus clearance than the IM vaccine, which correlates to reduced morbidity and mortality.

The previous results were in line with the results obtained by Mistilis et al. (75), who assessed the potency of influenza-loaded dissolvable MN patch vaccine that has been stored for one year at 25 °C in comparison with ID fresh liquid vaccine in mice. The study results revealed a higher immunogenic response in the H3N2 strain in the MN group than the ID injection, with no difference between both techniques with regard to the H1N1 strain.

## Clinical Studies

A clinical study has explored the use of MNs on human subjects. In a randomized study conducted by Vandamme et al. (61), 180 subjects were divided into three groups; the first received a full standard influenza vaccine dose using IM injection, while the second and third groups received intermediate and low ID vaccine doses using silicon crystal MNs, respectively. Results of the study demonstrated a non-significant difference in response to both IM and ID administration routes.

In another study by Hirobe et al. (97), forty healthy men received a trivalent influenza vaccine using either dissolving MN patches or a traditional SC flu shot. The study concluded that results obtained using MN patches were not different from these obtained using conventional SC injection. Another randomized trial performed by Rouphael et al. (98) yielded similar results.

## Measles

Measles is one of the preventable causes of morbidity and mortality in children. Recently, the WHO has reported a surge in cases, driven by the rise in numbers in countries in Asia, Europe, and South America (99).

Edens et al. (100) loaded and stabilized the measles virus on a stainless-steel MN patch and administered it to cotton rats. The study revealed that neutralizing antibody levels similar to these resulting from standard human dose were generated. Further investigation revealed that on the 10th day, antibody titers were significantly higher in MN patch groups than SC vaccine group, suggesting a more rapid antibody response.

In another study, a standard measles vaccine was loaded on a dissolvable polymeric MN patch and was administered to rhesus macaques because of the strong correlation between the immune response of the macaques to that of humans. This study revealed that both techniques yielded antibody titers that were significantly higher than the 12 mIU/ml level deemed as the titer level needed to confer protection in humans (101).

## Polio

Poliomyelitis is a viral disease with grave consequences, such as paralysis or death. The efforts in controlling the disease have been successful. The incidence of polio has decreased by over 99.9% from 1988 to 2020. However, progress could be lost if this momentum is not maintained (102).

Inactivated polio vaccine (IPV), containing antigen types I, II, and III, loaded on an MN patch was investigated by Edens et al. (103) to determine the efficacy. In the study, inactivated polio vaccine was administered to rhesus macaque using IM injection or dissolving MN patches. The study revealed that the antibody response of antigen types I and II was strong in both MN and IM groups. Concerning IPV type III, the immunogenic response in MN patch group was lower than that of IM injection group. The authors concluded that MN patches provided a potent immunogenic response against IPV types I and II with the need to find suitable techniques to improve immunogenicity towards IPV type III.

## Rabies

Vaccines against the human rabies virus exist and can prevent the disease. However, despite our best efforts, the disease still causes the death of around 59,000 people worldwide every year. Once symptomatic, the disease causes fatality in 99.9% of cases (104).

The use of dissolving MN patches to deliver rabies vaccine has been explored. Arya et al. (105) loaded MN patches with rabies DNA vaccine for administration in dogs. Results demonstrated that on day 56, the mean antibody titer was higher in MN group than IM group for the same dose of the vaccine. Overall, the study found that 100% of dogs were seropositive in MN and IM groups.

## Tuberculosis

Tuberculosis (TB) is a disease caused by bacterial infection, and it represents the leading cause of death attributed to a single infectious agent. In 2018, the disease was responsible for 1.5 million deaths, showing the significant burden of the disease (106).

Bacille Calmette-Guérin (BCG) is the only available vaccine against TB to this date. This vaccine is delivered via ID route and carries several limitations. To address the limitations, Hiraishi et al. (107) designed a BCG-coated stainless-steel MN patch vaccine to improve ID delivery and tested it on guinea pigs. Results revealed that at week 12 of the trial, MN-induced antibody response was significantly higher in MN group compared to that of the hypodermic vaccine. Results obtained in the previous trial were consolidated by

Chen et al. (108), who tested MN-delivered BCG vaccine in mice. The investigators concluded that using MNs to deliver vaccines represents an innovative technique with numerous benefits.

## VACCINE CHARACTERISTICS AND LIMITATIONS Of MNs IN VACCINE DELIVERY

From literature, MNs have been used to deliver different types of vaccines, including whole inactivated virus, subunit, DNA plasmid, recombinant protein, and antigen (89, 95, 109). However, ideal vaccine for MN delivery should be thermostable and maintain its antigenicity during manufacturing and sterilization processes (73).

Moreover, each type of MNs selected for delivery has its pros and cons. For example, hollow MNs can be suitable for liquid vaccines. But, this type of MNs requires injection device and trained personnel. Moreover, stability of liquid formulation must be taken into consideration. Coated MNs are easy to administer and can deliver a stable solid vaccine formulation. However, an applicator and specific formulation steps may be needed for successful delivery. Finally, dissolving MNs share the same features with the advantage of dissolving into the skin without leaving sharp wastes (109). Furthermore, the accuracy of dose in MN is generally less than with hypodermic needles. MNs should be carefully administered in a vertical position to avoid loss of dose and maintain uniform skin penetration. Variation in the depth to which dose is delivered may result from variation in the stratum corneum thickness among individuals. Skin condition may further affect the dose delivery/bioavailability (110). In addition, solid MNs may leave metal traces within the skin which may cause erythema and swelling (73, 110).

## Challenges Associated with MN-Based Vaccines Scale-Up

The scale-up of microneedles is considered a critical issue in clinical translation of MN-based vaccines. This is indicated by low number of clinical studies and clinical data available. Large scale production of MNs requires consideration of different aspects. First, although MN-based vaccines do not necessitate cold-chain storage, vaccine thermostability in MNs must be investigated (111). Second, the sterilization of MN-based vaccine can be challenging as the filtration method adopted for vaccine sterilization is not suitable for MNs. This demands aseptic condition for manufacturing to maintain vaccine sterility and antigenicity which is expensive when utilized commercially (73, 111). Furthermore,

special packaging, need of desiccants, or protectant from moisture may be needed to enhance storage stability (45). Finally, the regulatory aspects of MN-based vaccines represent other challenges as regulatory authorities consider such product a combination of biological product and a mechanical device. Therefore, MN-based vaccine should fulfill the requirements of each constituent part and the product as a whole (73).

## A Glimpse of Marketed Microneedle Products

Despite the limitations and challenges in the scale up productions, the commercial use of microneedles in delivering vaccines has been present for over a decade. To our knowledge, Sanofi Pasteur was the first company to file for marketing authorization of a microneedle-based vaccine. The European Medicine Agency (EMA) granted Sanofi Pasteur's Intanza® a marketing authorization within the European Union in February 2009 (112). The vaccine was intended to vaccinate adults aged 18–59 years against seasonal influenza. It is important to note that Intanza® is no longer marketed in the EU, as the marketing authorization was withdrawn based on Sanofi Pasteur's request for commercial reasons (113). In the USA, the first approval of a microneedle-based vaccine was granted by the Food and Drug Administration (FDA) to Sanofi Pasteur's Fluzone® on May 2011, a vaccine that is identical to Intanza® (112).

## CONCLUSION

In light of recent events, injectable vaccines have provided hope to battle the ongoing COVID-19 pandemic. In addition to comparable immunogenic effect, microneedle-based vaccines offer many advantages over traditional vaccines delivered via hypodermic needles. Apart from being less painful on administration, MNs offer safe (eliminates hazardous waste and the need of trained personnel for administration), cost-effective (reduces the dose needed to trigger an immune response), and a more stable (less cold chain supply dependence) alternative to traditional vaccination techniques. Most preclinical and clinical studies suggest that MNs will be a suitable alternative to the currently available vaccination means. MN-based vaccine product of an affordable price taking into consideration formulation, validation, and regulation is crucial for successful mass production. The use of microneedles to deliver therapeutic agents could usher in a new era of compliance and improve patient outcomes.

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## Declarations

**Conflict of Interest** The authors declare no competing interests.

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