The Applications of Dissolving Microneedles in Vaccine Delivery and Cancer Therapy Over the Last Decade: A Review[1]

Mais Salim Saadallah*, Zahraa Hussein Ali, Amal F.Al-Dulaimi

Department of Pharmaceutics, College of Pharmacy, University of Mosul, Al-Hadbaa Street, 41002, Mosul, Iraq.

**drmais@uomosul.edu.iq*

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ABSTRACT

Microneedles (MNs) are characterized by micron size projections having a length from hundreds of microns up to 1000 µm with the ability to avoid the pain fibers in the skin and increase skin permeation. According to their shape, microneedles can be divided into four types; hollow MNs, coated MNs, dissolving MNs, and solid MNs, with different approaches to drug delivery into the skin. Recently, dissolving MNs has been of great interest among the other types of microneedles due to their unique advantages including easy fabrication and high loading capacity of drugs. In the last decade, dissolving MNs covered a wide area of applications, especially in cancer therapy, and vaccination delivery as well as various miscellaneous applications. This review highlights the types of microneedles, their modes of drug release, and the applications of dissolving MNs over the last decade regarding vaccine delivery and cancer therapy.

Keywords: *Transdermal drug delivery, dissolving microneedles, transcutaneous immunization, skin penetration, superficial antitumor therapy.*

INTRODUCTION

The skin is considered as the largest organ in the human body; consisting of three layers with protective function mainly against injuries. These layers are the Epidermis which is mainly composed of keratinocytes, dermis, and hypodermis [1]. The stratum corneum or the outermost layer of the skin is the main barrier against foreign materials entering into the skin as well as excessive water loss from the skin [2]. Regarding recent drug delivery technologies; the skin offers a painless way to deliver drugs both for topical and systemic drug administration [3]. An alternative and non-invasive way for drug administration via the skin is known as the transdermal drug delivery system (TDDS). TDDS offers several advantages over the oral route such as by-passing first-pass effect with controlled and sustained release of drugs. However, the stratum corneum barrier of the skin remains a big challenge for formulation scientists and needs to be overcome [4]. Various physical and chemical methods have been developed to modulate the stratum corneum obstacle. Chemical methods can enhance skin permeation of the drugs passively, for example; nanocarriers [4]. Physical methods involve active enhancement of skin penetration by the application of external stimuli such as sonophoresis, iontophoresis, and microneedles. Among the physical methods, microneedles represent a convenient, safe, and painless way to enhance the transdermal permeation of

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drugs [5]. Microneedles (MNs) are characterized by micron size projections having a length from hundreds of microns up to 1000µm with the ability to avoid the pain fibers in the skin and increase skin permeation effectively [6,7]. According to their shape, microneedles can be divided into four types; hollow MNs, coated MNs, dissolving MNs, and solid MNs, with different approaches to drug delivery into the skin (figure 1) [8]. Microneedles cover a wide range of applications including vaccination, cosmetic, and therapeutics delivery [9]. Recently, dissolving MNs have been of great interest among the other types of microneedles due to their unique advantages including easy fabrication and high loading capacity of drugs [10].

This review highlights the types of microneedles, their modes of drug release, and the applications of dissolving MNs over the last decade regarding vaccine delivery and cancer therapy.

TYPES OF MICRONEEDLES

Hollow microneedles

With a structure resembling that of conventional hypodermic needles, this type uses a" poke and flow" approach for drug delivery [11]. It represents a-holes at the tips of the needles by which a drug solution or dispersion can accommodate and the flow rate of drugs needs to be controlled [12]. This type of microneedles is difficult to design due to its fragility; sometimes a metal coat is added around the tip to make it stronger [12]. Hollow MNs have a diversity of applications and due to their unique structure, they have been used in the blood extraction process. Li *et al.* have developed an optimized hollow microneedle for blood extraction. They developed a microneedle that is long and sharp enough to reach blood vessels and at the same time avoid nerve contact, respectively. The produced microneedles were strong enough to pierce into the skin and they were used successfully in the extraction of mouse blood [13]. Another study reported by Suzuki *et al.* included the fabrication of hollow microneedles from chitosan and it has been concluded that the design of hollow microneedles using the dip-coating technique was successful. Besides, the utilization of the produced chitosan-hollow MNs was successful in the aspiration of blood from a frog, and the aspirated amount was enough to perform a blood glucose test [14].

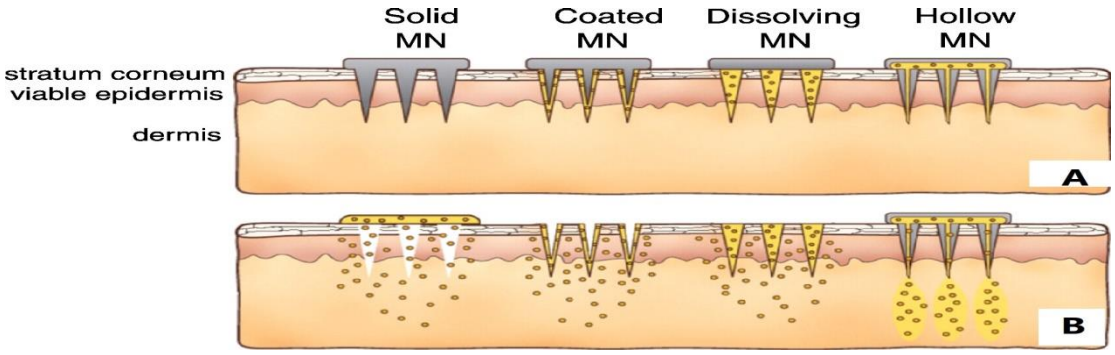

Figure 1. Types of microneedles. A: Before application. B: After application. Adopted from [12].

Coated microneedles

This type uses a "coat and poke" approach of delivery; resembling solid MNs in structure with a layer coating the needle tip containing the drug solution or dispersion [15]. Coated MNs have been used in vaccine delivery simply by the application of vaccine solution around a solid MN tip followed by the subsequent release of the vaccine into the dermal layer of the skin and taken by the antigen-presenting cells; producing an immune response. Generally, the magnitude of the coated drugs depends on the specific thickness of the coating layer as well as the size of the MNs which is usually very small [16]. Choi and co-workers fabricated poly-lactic acid (PLA) MNs array coated with second-generation smallpox vaccine around the MNs tip by dip-coating technique. It has been shown that the produced smallpox-coated MNs vaccine has induced both cellular and humoral-mediated immune responses in the mice, suggesting

the usefulness of the MNs approach in vaccination; representing a good alternative to intramuscular and subcutaneous injections [17]. Another study reported by Kapoor *et al*. included the use of coated MNs in macromolecules delivery; particularly peptide A. approximately 250 µg have been successfully coated around the MNs tip and the delivery from these MNs has produced an absolute bioavailability comparable to that of subcutaneous delivery. Besides, the stability of peptide A within the MNs patch was improved, showing little degradation when stored at room temperature in comparison with the subcutaneous formulation [18].

Solid microneedles

Solid MNs use a "poke and patch" approach of delivery, were firstly reported in 1971, and are mainly made of metals. Other materials such as silicon and polymers can be used to fabricate this type as well [19]. Solid MNs are composed of a bunch of tips that generally don't contain any medication or excipients; it is used to pretreat the skin before formulation application by creating micron-size pores in the skin. After their removal, the permeation of the intended formulation can be enhanced dramatically [20]. Ananda and co-workers have developed and for the first time the anti-malarial drug primaquine as a transdermal patch and explore the increment of *ex-vivo* permeability utilizing the approach of solid MNs, dermaroller®. *In-vitro* release and *ex-vivo* permeation studies revealed that 31.31 ± 5.25% and 22.55 ± 4.35% of primaquine were released from transdermal patches. The permeation profile showed improved *ex-vivo* permeation of primaquine when combined with dermaroller®, up to 45.89 ± 5% after 24 hours [21]. Although MNs approach has been used greatly to enhance the permeation of vaccines [22] and macromolecules for example insulin [23], there is limited research on their ability to enhance ions permeation via skin. I. Abaindu and K. Ita have investigated the effect of solid microneedles in enhancing potassium chloride (KCL) permeation via pig skin. Permeability study results showed that the cumulative amount of KCL permeated via pig-ear skin was higher after microneedles application compared to the control (untreated with MNs), after 12 hours of the experiment. Besides, the transdermal flux of KCL permeated via skin-treated with MNs was 10 times greater when compared to untreated skin [24].

Dissolving microneedles

These MNs use the "poke and release" approach of delivery. The dissolving tips are made of biodegradable polymers; which makes this type poses superiority over other types by not creating waste hazards. Besides, they are considered a one-step administration system by eliminating the need for MNs removal after application [25]. Dissolving MNs characterized by being easily fabricated, mainly based on micro molding. Alkhiro A. and Ghareeb M. formulated Lornoxicam as a dissolvable MNs patch by a micro-molding technique using polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) polymers. *Ex-vivo* permeability study results showed that the cumulative amount of the drug permeated from the MNs patch was 67% compared to only 24% from the control patch (containing the same amount of drug, polymers composition of the MNs patch) and the total increment in the steady-state flux that obtained by the MNs patch was approximately 2.7 folds when compared to that obtained by the control patch [26]. It is worthy to mention that polymer type effect on release pattern; hydrophilic polymers are not preferred in the case of long-acting drug delivery; instead hydrophobic polymers are used. Poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) have been used in dissolving MNs manufacture in case of long-acting drug delivery in a sustained manner for example levonorgestrel, methotrexate, and etonogestrel [27–29].

The applications of dissolving microneedles

Recently, the development of transdermal delivery has derived great attention since it can provide a convenient and painless way for drug administration; avoiding many of the adverse events associated with oral and parenteral therapy.

Over the last decade, due to their unique advantages dissolving MNs have been widely used in vaccine delivery [30,31], and cancer therapy [32,33].

1. Dissolving microneedles for vaccine delivery

Vaccination is the exclusive mean for prophylaxis against various infectious diseases and it is the only method that can protect people from inevitable death [34]. The traditional vaccination technique involves the use of injections, however, these methods are painful and require trained healthcare providers for their administration with increased risk of needle-linked diseases and medical problems [34–36]. The successful vaccination process requires the availability of a cold chain to ensure the proper storage of the antigen solutions, proper elimination of biohazardous disposed products, the demand to improve the vaccine-related immune response, maximize cost-effectiveness and minimize the dependence on trained medical personnel [36,37]. All of these barriers should be covered in a full resource setting.

One of the revolutionary approaches to overcome the obstacles associated with needle-based vaccination is through the application of transcutaneous immunization (TCI), particularly with the use of dissolving microneedles [36]. In this method, the antigen solution is directly introduced to the epidermal layer and dermis [with the penetration through the stratum corneum) [35,36]. These skin layers are rich in dendritic cells and Langerhans which can present the administered antigens near the immunological cells. Therefore, greater immunogenicity could be created with TCI as compared to the traditional intramuscular vaccination along with a dose-sparing advantage [35,37]. In addition, dissolving MNs (DMNs) vaccination is pain-free, easy to administer (with the feasibility of self-administration), reproducible, free from sharp disposed of products and the antigens could be formulated as thermostable which enables easier vaccine storage and transportation [36,38].

In recent years, there is a strong focus on the development of DMNs loaded with thermostable antigen formulations [7]. Different types of antigens were studied for dermal vaccination using DMNs to produce sufficient immunization against many pathogens [36]. These antigens could be classified into nucleic acid-based, protein-based, live attenuated, whole inactivated, and viral vector-based antigens [36]. A graphical representation of the the types of antigens are demonstrated in figure (2).

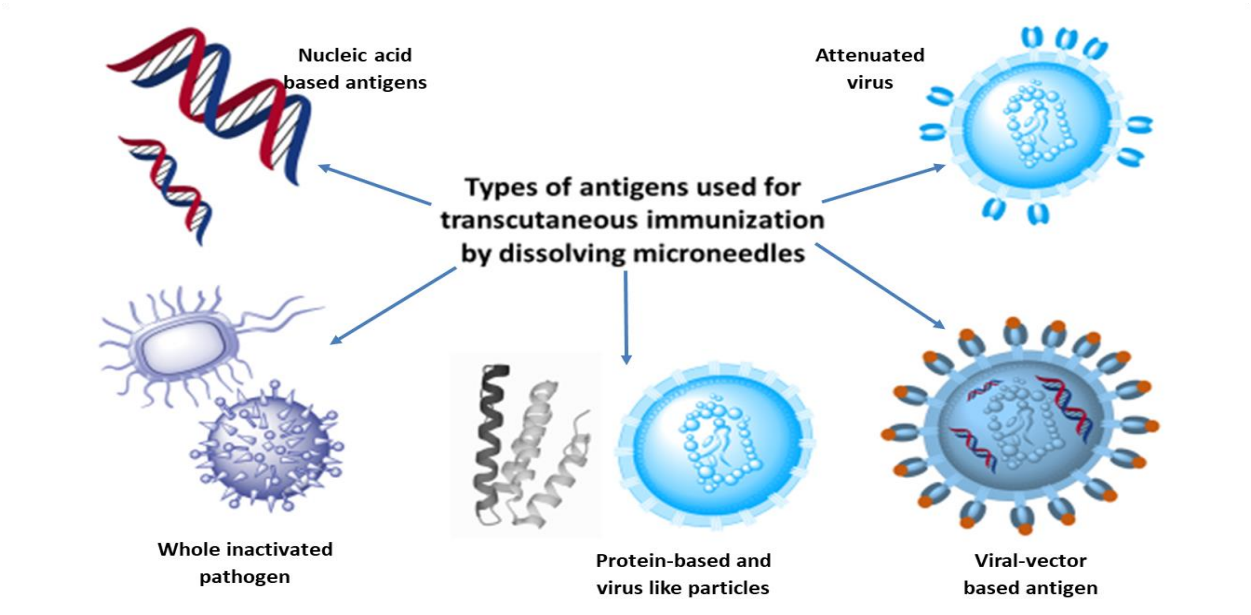

Figure 2. Types of antigens studied for immunization using dissolving microneedle.

In addition to antigens, many DMNs studies investigated the effect of the inclusion of adjuvants within the formulations to augment the produced immunological response [37,39–42]. Adjuvants are

substances that can potentiate the potency of the immune reaction against a specific antigen, prolonging its duration and making the response earlier [43,44]. All of these effects could be achieved with few toxic effects and without the ability to initiate an immune reaction by themselves [43,44]. Based on their mechanism of action, two categories of adjuvants could be recognized including the immune stimulants and the vehicles [43,44]. Saponins, cytokines, TLR ligands, and exotoxins are some of the examples of immune stimulants which can augment the response to specific antigen directly [44]. On the other hand, the vehicles (such as emulsions, liposomes, mineral salts, polymer microspheres, and others) are adjuvants that act by optimizing how the antigen is presented to the immune cells [44].

NUCLEIC ACID-BASED ANTIGENS

DNA-based antigens are easy to produce and their application in TCI would generate strong immune responses with relatively lower antigen doses [45,46]. They can induce both humoral and cellular immune responses [45].

Qinying *et al*. developed promising DMNs arrays for immunization against tuberculosis. They utilized a DNA-based antigen encoding for the Ag85B protein which is secreted by *Mycobacterium tuberculosis.* The microneedles were produced by micro molding technique using sodium hyaluronate as the casting material. The immunization study was conducted on mice using two doses of the antigen (either 4.2 or 12.6 µg). The immune response obtained was comparable to the IM immunization at a low immunizing dose while a high immunizing dose produced a superior immune response [47].

Jaya *et al*. generated an innovative DMNs patch with DNA based vaccine for immunization against rabies in dogs. The DMNs arrays were fabricated through a two-step micro molding technique using PVA and sucrose as the backing materials. The immunization study was executed on beagle dogs through the manual insertion of a DMNs patch into ear skin. The animals were divided into three groups: the IM group (50 µg of antigen) and two TCI groups using two different doses (5 and 50 µg). TCI with the high dose resulted in an immune response comparable to the IM immunization with the same dose. However, TCI with the low dose led to a much lower response [48].

Table 1. The available studies on TCI using DMN arrays containing protein-based antigens that are published over the last decade.

Targeted illness/ pathogen	Antigen(s) (dose)	Adjuvant(s) (dose)	DMNs type	Administration regimen	Animal model, application site	Ref.
Canine influenza virus	A/canine/VC378/2012 antigen (5 µg).	--------	Novel (insertion responsive) DMNs prepared using hyaluronic acid at the tips.	3 immunization doses at 2-weeks interval were given manually with the thumb pressing for 10 seconds and then detached from the skin.	Guinea pigs, Dorsal skin.	[51]
Diphtheria	Diphtheria toxoid (0.36 µg).	Nanoparticulate aluminum salts: - Gibbsite (0.36 µg). - Poly (I:C) (0.36 µg).	Hyaluronic acid was used as a base.	Immunization was performed as a single full dose or as fractional doses given on three consecutive days. Three immunization process was repeated on days 0, 22, and 43.	BALB/c Mice, Left flank skin.	[40]
Hand, foot, and mouth virus	Virus-like particles of enterovirus 71 (1 µg).	------	Micro mold casting technique with the use of hyaluronic acid as the backing material.	3 doses (at week 0, 2, and 4) applied by a spring-application device (home-made) using 10 N force for 2 mins.	BALB/c mice, Dorsal skin.	[52]
Hepatitis B	Hepatitis B virus surface antigen	------	The dual release platform was fabricated using tips	A single dose was applied using a clip that exert a force of about	Mice, Dorsal skin.	[53]

	(0.15 µg in the CMC coating for bullous release and another 0.15 µg within the tips for slow release).		made of poly lactic acid (for the slow release). These tips were coated with CMC (for immediate bullous release).	20 N and held the patch in place for 30 mins.		
	Hepatitis B antigen (1.2 µg in mice), (24 or 48 µg in macaques).	-------	Trehalose and CMC-based DMNs fabricated by sequential casting technique.	- Mice: 2 doses at 3 weeks interval, each dose composed of 2 patches applied manually with pressure for 10 mins - Macaques: single-dose composed of either 3 patches or 6 patches applied by thumb pressing for 30 secs and left for 20 mins.	- BALB/c Mice, Back skin. - Rhesus macaques, Back skin.	[54]
	Hepatitis B surface antigen (20 µg).	Saponin QS-21 (15 µg).	Hydroxyethyl starch and chondroitin sulphate based DMN.	2 doses 4 weeks apart were applied by handheld application system using 5 N force to hold the patch in place for 10 mins.	Duroc swine, Flank skin.	[55]
	Recombinant hepatitis B surface antigen (0.5 µg).	-------	The antigen was encapsulated within lipid A cationic liposomes by an emulsification-lyophilization method. Then this formulation was filled into micro holes of the mold by inverse molding technique under decreasing pressure using PVP and sucrose as a backing material.	Single-dose applied manually.	Kunming mice, Oral mucosa.	[56]
Human immune deficiency (HIV)	Recombinant CN54gp140 (10 µg).	TLR4 (20 µg).	DMNs arrays were prepared by micro molding technique (laser engineered) using Gntrez (methyl vinyl ether/maleic anhydride copolymer) as backing material	4 immunization doses at 2-weeks intervals were applied by thumb pressing and held in place overnight.	BALB/c mice, Ear skin	[37]
Influenza	H1N1 hemagglutinin subunit (1 or 3 µg).	Recombinant murine granulocyte-macrophage colony-stimulating factor GM-CSF (100 ng).	PVA-based DMN fabricated by sequential casting technique.	Single-dose immunization applied by direct pressure with thumb for 1 min and held in position for 20 mins.	BALB/c Mice, Caudal area of dorsum.	[57]
	Nucleoprotein peptide/4M2e peptide of influenza A (7.5 µg).	-------	Peptide layered nanoparticles (composed of nucleoprotein peptide at the core with M2e peptide at the coating) were loaded onto a DMNs platform made of PVA and sucrose.	A single-dose patch was given as supplemental immunization 4 weeks after IM immunization.	BALB/c Mice, Not reported.	[58]
	Aichi, PR8 and M2e antigens (1.6, 1.6, and 3.2 µg, respectively).	---------	2 steps molding technique was used for fabrication of DMNs based on PVA and sucrose platform.	A single dose of the tricomponent DMNs was applied manually by pressing for 1 min and left on the skin for 30 mins.	BALB/c Mice, Dorsal skin.	[59]

	Subunit A/Brisbane/59/07 of H1N1 (2.7 µg).	------	PVA and CMC were used as the DMNs platform.	Single-dose.	BALB/c Mice, Not reported.	[60]
	Subunit A/Brisbane/59/07 of H1N1 (2.5 µg).	-------	2 steps micro molding technique.	A single dose was applied manually by pressing for 1 min and left in place for 10 mins.	BALB/c mice (pregnant and non-pregnant), Caudal area of the dorsal skin.	[61]
	4M2e-tFlic fusion protein [16 µg).	-------	2 steps molding technique using PVA and sucrose as backing materials.	A single dose of DMNs was given as a booster 4 weeks after the IM immunization. The patch was applied manually, pressed for 1 min, and held in place for 20 mins.	BALB/c mice, Dorsal skin.	[62]
	-B/Brisbane/60/08 antigen (2.8 µg). - A/Brisbane/10/10 antigen (3.8 µg). - Trivalent vaccine (3.8 µg).	------	DMNs patches were prepared using PVA and sucrose as the backing materials.	A Single-dose of the freshly prepared or the stored patches was applied by thumb pressing for 1 min and held in place for 20 min with the aid of adhesive at the patch backing.	BALB/c mice, Back skin.	[63]
	- A/Brisbane/59/07 (2.5 or 10.8 µg). - A/Victoria/210/09 (2.5 or 10.8 µg). - B/Brisbane/60/08 (2.5 or 10.8 µg).	------	2 steps casting method with the use of acetonitrile solution of PLGA as an insoluble backing layer.	The spring-based applicator was used for the delivery of the DMNs patches. 2 doses were given at 21 days intervals.	Hartley guinea pigs, Dorsal skin flap.	[64]
	- A/Brisbane/59/07 antigen (3 µg). - A/Victoria/210/09 antigen (3 µg). - B/Brisbane/60/08 antigen (3 µg).	-------	2 steps molding technique using sucrose and fish gelatin as the backing materials.	A Single-dose was applied manually by pressing for 1 min and held in place for 10 mins.	BALB/c mice, Dorsal skin.	[65]
	H1N1 PR8 HA (0.4 µg).	------	PVP base DMNs prepared by UV irradiation-induced polymerization in the presence of azobisisobutyronitrile as an initiator of free radicals.	A Single-dose. The skin was treated with a non-ablative fractional laser (NAFL) before the DMNs application.	Swiss Webster mice, Dorsal skin.	[66]
Leishmania	Recombinant LiHyp1 (10 µg).	TLR9 agonist CpG (cytosine-phosphate-guanine oligodeoxyneoclutide) (18 µg).	The DMNs platform contains either free antigen or encapsulated within cationic liposomes	Each patch contains 5 µg of LiHyp1, 2 patches were used for each vaccination. Three doses (14 days apart) were given. Manual application with the	BALB/c mice, Each patch is applied over the	[67]

			(with or without adjuvant).	thumb pressing for 10 secs and then the patches were left overnight.	dorsal area of each ear.	
Malaria (*Plasmodium falciparum*)	Pfs47 recombinant surface protein.	TLR9 agonist CpG.	2 steps molding technique using sucrose and fish gelatin as the backing materials.	No immunization study was performed.	--------	[68]
	SE36 protein (0.1 or 2 µg).	------	Sodium hyaluronate based DMNs arrays in two lengths (300 and 800 µm) were prepared by a micro casting technique.	4 immunization doses every 14 days were applied manually by pressing and left in place for 6 hrs.	BALB/c mice, Back skin.	[69]
Staphylococci	Recombinant staphylococcal enterotoxin B (13 or 26 µg).	---------	The Micromolding technique was used. The platform is composed of chondroitin sulphate and trehalose.	3 immunization doses were given at 3 weeks intervals and applied to the skin with the aid of an applicator.	BALB/c mice, Dorsal skin area.	[70]
SARS-2-Co-V	S1 subunit Receptor-Binding-Domain (S-RBD) (25 µg)	------	Low molecular weight hyaluronic acid base using micromolding technique.	Three doses are given on days 0, 3, and 7. Given by thumb pressing for 10 sec.	BALB/c mice, Back skin.	[71]
Scrub typhus	Recombinant scrub typhus antigen (5 µg).	------	CMC-based DMNs was prepared by 2 methods: droplet-born air blowing technique and centrifugal lithography technique.	3 doses at 2-weeks intervals applied by microlancer implantation device.	C57BL/6 mice, Not reported.	[72]
Tetanus	Tetanus toxoid (5 flocculating units (Lf)).	-----	2 steps micromolding technique using PVA na sucrose as the backing material.	A single dose was applied with about 40 N force and held in place for 20 mins.	BALB/c mice (pregnant and non-pregnant), Caudal area of the dorsal skin.	[73]
Zika	ZIKV-rEfl antigen (20 µg).	------	CMC base DMNs.	2 doses at 2-weeks interval.	C57BL/6 mice, Not reported.	[74]
Diphtheria/ Tetanus divalent vaccination	- Diphtheria toxoid (20 µg). - Tetanus toxoid (10 µg).	-----	Sodium hyaluronate based DMNs prepared by micromolding technique.	DMNs patches either freshly prepared or stored for 1 year at different temperatures (4, 25, and 40 °C) were given by a handheld application system and left in place for 6 hrs. the vaccination was repeated 5 times within 2-weeks interval.	Wister ST rats, Back skin.	[75]
	- Diphtheria toxoid (1 or 3 µg). - tetanus toxoid (1 or 3 µg).	-----	Sodium hyaluronate based DMNs arrays in two lengths [300 and 800 µm) were prepared by a micro casting technique.	5 immunization doses every 14 days were applied manually with pressing and left in place for 6 hrs.	Hairless rats, Back skin.	[69]

Ebola virus infections result in serious illness with relatively high mortality rates [49]. Hung-Wei *et al.* fabricated an immunization system against the Ebola virus. The system is composed of PLGA-PLL/γPGA (poly lactic-co-glycolic acid-poly-L-lysine/poly-γ-glutamic acid) based nanoparticles on which the DNA based antigen of the Ebola virus was linked then these nanoparticles were loaded on DMNs patch by two steps casting approach using PVA and PVP as the backing materials. The immunization study was

executed in mice with four immunization doses of about 18 µg given every four weeks and the results revealed that there was no significant response between the TCI and IM immunization [49].

Yuqin *et al.* studied the application of DMNs patch for vaccination against hepatitis B virus through the use of DNA-based antigen along with CpG ODN (as an adjuvant) both of which were encapsulated within a cationic liposomal system. At the end of the study, it was found that TCI using these delivery systems would result in a better Th2/Th1 balanced immune response [46].

PROTEIN-BASED ANTIGENS (SUCH AS VIRUS-LIKE PARTICLES (VLP))

Protein-based antigens are superior to nucleic acid-based antigens and whole inactivated pathogens in terms of vaccine safety and the development cost [50]. However, these antigens have limited antigenicity when used alone therefore adjuvants are required to give the desired long-lasting immune response [44,50].

Protein-based antigens include various substances such as detoxified toxins/toxoids, recombinant/isolated proteins, glycoproteins, virus-like-particles (VLP), and others [50]. A representation of the available studies on protein-based vaccines targeting various diseases using DMN arrays over the last decade is summarized in table 1.

In addition, Sachico *et al.* conducted a clinical study on human volunteers to evaluate the TCI using a DMNs patch containing trivalent influenza antigens (15 µg of each). The arrays were developed by micromolding technique using sodium hyaluronate as the backing material. The immunization study was conducted on healthy male volunteers who were divided (randomly) into two groups: the TCI group and the SC immunization group. Each individual received two immunization doses of about 15 µg of each antigen which were given at 3-weeks intervals by either method. The DMN patches were applied to the upper area of the left arm and held in place for 6 hrs with the aid of a hand-held applicator. At the end of the study, they found that the TCI efficacy for vaccination was comparable to the SC immunization as measured by anti-HA antibody titers [34].

OVALBUMIN AS A PROTEIN-BASED ANTIGEN MODEL

Ovalbumin (OVA) is one of the most available of the four allergens that exist in the white protein of hen egg [76]. OVA has many immunological characteristics to be used as an antigen model such as T-cell and B-cell mediated immune responses [76]. Therefore, OVA is one of the most studied proteins as an antigen model for dermal vaccination studies [76].

Jihui *et al.* developed a new technique for the production of DMNs using an automated dispensing system that delivers nano-sized droplets to decrease antigen loss. The efficiency of loading was tested using OVA as an antigen model. The results demonstrated a shorter production time with a massive reduction in antigen wastage [77].

Sachiko *et al.* [78] studied the mechanism, properties, and dynamics of the immune response produced following DMNs application in mice. OVA was used as the model antigen and the DMNs arrays were fabricated by micromolding technique using sodium hyaluronate as a backing material. In another study, Sachiko *et al.* [42] evaluated the effect of CpG-oligonucleotide as an adjuvant on the immune response caused by the application of OVA-loaded DMNs. The DMNs arrays were fabricated by the two-step casting method using sodium chondroitin sulphate as the casting material. The vaccination study was conducted on mice through the application of the OVA-loaded DMNs on the back skin for 5 mins and immediately followed by the application of the CpG-oligonucleotide loaded hydrogel for 24 hrs on the same area on which the patch was applied. The results illustrated an augmented immune response in groups that received adjuvanted vaccination protocol as indicated by the higher Th1 and Th2 titers along with the greater immune cell populations as indicated through draining lymph nodes (dLNs) analysis.

Mara *et al.* investigated the effect of the molecular weight of hyaluronan as a biodegradable polymer on

the characteristics and the antigenicity of the produced DMNs using OVA as a model antigen. The DMNs were produced by micromolding methods with hyaluronan ranging in molecular weight from 4.8 kDa up to 1.8 mDa as the casting material. The vaccination study was conducted on mice. The results indicated that the molecular weights of hyaluronan did not have a major influence on the immune response as manifested through the antibody titers but they have an effect on the characteristics of the fabricated DMNs, particularly, the time required for their complete dissolution after application. The higher the molecular weight the longer the time required for dissolution but very low molecular weight hyaluronan was not suitable for DMNs manufacture (since it resulted in very loose microneedles that could not pierce the skin during application) [79].

Stephen *et al*. were the first in developing undercut DMNs intended for dermal vaccination using OVA as antigen and poly (I: C) as an adjuvant. The microneedles molds were fabricated by micro-additive manufacture technique and the resulting molds were utilized for the fabrication of undercut DMN loaded with antigen (with or without adjuvant) through the application of the spin casting method. The resulting DMNs were 750 µm in height, pyramid in shape, with a sharp apex (30° angle), undercut stem area, and filleted base. This design was specifically appealing in terms of the mechanical strength which was required for manufacture and during skin application [80].

To investigate the efficacy of DMNs in the delivery of particulate vaccines, Juha *et al.* developed hyaluranon-based DMNs containing PLGA nanoparticles loaded with OVA (as an antigen) and poly(I: C) (as an adjuvant). The immunogenicity of OVA from these delivery systems was compared to the immunogenicity of the same antigen using the same dose (1 µg) delivered in hollow microneedles and the study was conducted on mice. They found that the immune response triggered by the DMNs-loaded nanoparticles was inferior to the response obtained from hollow microneedles loaded with nanoparticles [81].

Ning *et al.* utilized two types of multifunctional liposomes (the mannosylated lipid A-liposomes (MLLs) and the stealth lipid A-liposomes (SLLs)) for the encapsulation of both OVA (as a model antigen) ammonium bicarbonate. These liposomes were loaded on DMNs arrays which were fabricated by centrifugation casting technique using a mixture of sucrose, PVP, and CMC as the casting ingredients. The produced arrays were tested for their immunization capability through the manual application of them on the vaginal membrane of mice. The results demonstrated the ability of these adjuvant-aided vaccination systems to produce both cellular and humoral immunological reactions [82].

To achieve sustained-release cutaneous vaccination through DMNs, Peter *et al.* developed a special type of DMNs composed of a silk tip held on polyacrylic acid (PAA) foundation. The tip is loaded with OVA antigen. After skin insertion, the PAA base rapidly dissolves leaving the persisting depot made of silk hydrogel. These depots would release the antigen very slowly over about 7-14 days such release resulted in ten times higher immune response when compared to the traditional injectable immunization [83].

In a different study, Mei-Chin *et al.* developed chitosan-based DMNs with the purpose of sustained-release cutaneous vaccination using OVA as an antigen model. The DMNs are composed of chitosan tips and poly(L-lactide-co-D, L-lactide) (PLA) based supporting arrays. When inserted into the skin, the PLA base dissolves rapidly releasing the chitosan tips that are loaded with the antigen which would act as a depot that slowly releases the antigen for up to two weeks. Such delivery resulted in a prolonged and much higher immune response as compared to the traditional needle-based immunization [84].

Finally, Lei Guo *et al.* studied the utilization of cationic liposomes for the encapsulation of antigen and adjuvant to improve the TCI through the application of DMNs arrays. In this study, they use OVA as an antigen model and CpG ODN as an adjuvant and the immunization was conducted on rats. The results showed an enhanced immune response using the cationic liposomes [39].

LIVE ATTENUATED PATHOGEN-BASED ANTIGENS

Jessica *et al.* utilized a combination of attenuated measles and rubella antigens to a vaccine delivered through DMNs arrays for simultaneous immunization against both measles and rubella infections. The microneedles arrays were developed by two steps casting method with the application of a mixture of PVA, sucrose, and deionized water as the backing constituents. Infant macaques were used as the animal model for the vaccination study. The animals were divided into four groups. The first two groups were challenged with measles immunoglobulin (by IP administration) 48 hours before their immunization by either SC injection or patch application. The remaining two groups received either SC or dermal vaccination without previous challenge. The DMNs patches were applied to the inner thigh region with pressure for 30 seconds and held in place for 15 minutes. The non-challenged groups developed sufficient antibody titers against the two pathogens and the results were superior to those following SC immunization. However, pre-challenged groups failed to generate a protective immune response in both immunization groups [85].

Fan *et al.* studied the applicability of using DMN arrays for the delivery of powdered (freeze-dried) live attenuated antigen of *Mycobacterium tuberculosis* (Known as Bacille Calmette–Guerin (BCG)) with the aim of TCI. The MNs arrays were fabricated by a micro-molding approach (using sodium hyaluronate as the casting material) in a certain manner that enabled the generation of a small cave at the bottom of each needle in which the powdered antigen was packed. Following dermal application, the MNs shaft dissolves rapidly leaving the powder exposed to the epidermal tissues which would absorb the interstitial fluids, dissolve and move through the epidermal tissues provoking the immunological response. Mice were used for the immunization studies. The DMN patches were applied manually (with thumb pressure) on the dorsal skin area and removed after 15 minutes. Comparable immunological responses (as measured by cellular or humoral immune responses) were obtained following the regular SC immunization and the novel DMN loaded with the BCG powdered antigen [86].

For vaccination against measles using micron scaled needles, Chris *et al.* developed DMN arrays containing attenuated measles vaccine. The arrays were produced by a two-step micro-molding approach using sucrose and PVA mixture and the immunization study was performed on rhesus macaque by the application of a DMN-based patch on the upper back skin with manual pressing for 10 minutes then the patch was removed. The immunological results were equivalent between the groups that received the TCI and those who took SC vaccination [87].

WHOLE INACTIVATED PATHOGEN-BASED ANTIGENS

To determine the feasibility of the co-vaccination against poliovirus and rotavirus through the application of TCI with microneedles, Sung-Sil *et al.* fabricated DMN arrays using either CMC or MC as the backing materials loaded with the whole inactivated antigen of poliovirus and rotavirus either alone or in combination (at full or half dose levels). Rats were used as animal models for evaluating the vaccination efficiency of the produced patches. The vaccination was performed through the co-administration of the two antigens either separated in individual patches or combined in a single patch using the two-dose strengths. Each group received three immunization doses given at 21-days intervals. It was found that there was no significant difference in the immunization efficiency when the two antigens are given simultaneously by either way and there was no negative interaction between these two antigens when given together within the same patch. Therefore, this delivery system was a very promising co-vaccination tool [88].

To study the suitability of using a combination of trehalose and pullulan as a backing material in the production of stable DMN for influenza vaccination, Tian *et al.* developed microneedle arrays loaded with the whole inactivated influenza virus (as an antigen). The arrays were formulated by using five different (trehalose/pullulan) weight ratios (00:100, 20:80, 30:70, 40:60 and 50:50). The antigen was loaded in

two doses (1 and 12.5 µg) in the form of suspension. Mice were utilized for the vaccination study using two types of the prepared DMN (the freshly prepared and those that were stored for 1 month). The patches were applied on the flank skin through the use of a special applicator that operates digitally. The results demonstrated good antibody titers with no difference from the titers obtained after IM immunization using similar doses suggesting that this combination was suitable for DMN fabrication [89].

To incorporate lyophilized antigen into DMN patches, Yoo Chun *et al.* fabricated microneedle arrays for influenza vaccination using an organic suspension of the freeze-dried WIV as an antigen. Chloroform was utilized as the organic solvent in which the antigen was suspended. The arrays were developed by a double casting approach with the use of a solution of PVP in chloroform as the backing material. It was found that the vaccine microneedles maintained their antigenicity during development and after storage for up to 3 months. A potent immune response was triggered during the vaccination study that was conducted on mice using the produced arrays in which the patches were applied manually on the caudal dorsal skin area and held in place for 15 minutes. This research ensured the possibility for freeze-dried stabilized vaccines to be administered by DMN arrays [90].

Vaccination against poliovirus is challenging with a demand for the production of DMN arrays into which the inactivated poliovirus was included. Agnese *et al.* formulated DMN containing trivalent Sabin inactivated poliovirus as an antigen. A combination of trehalose and PVA was utilized as a platform for the DMN arrays which were produced using a specific technique through the application of a syringe pump attached to a micron-sized capillary tube (to deliver the formulation into the mold). Rats were utilized for immunization studies in which about 1.5 of the single human dose of the trivalent antigen was delivered by manual application of the patches to either flank or ear skin area. The TCI resulted in a potent immune response as manifested by antibodies against two serotypes of poliovirus (type 1 and 3) in titers comparable to those obtained following IM immunization using higher antigen doses [91]. In an earlier study, Chris *et al.* developed DMN patches loaded with inactivated poliovirus in an attempt to replace the oral vaccination protocol against poliovirus infections. The arrays were fabricated through the application of a micro-molding approach using a blend of gelatin and sucrose as the backing material. The *in-vivo* immunization studies were conducted on rhesus macaque with the manual application of patches on the upper back area for 15 minutes and then removed. The collected results indicated good immunological responses which were comparable to the results obtained from IM vaccination concerning poliovirus serotypes 1 and 2 while a much weaker immune response was observed against serotype 3 (as compared to the IM immunization) [92].

On the other hand, Rekhav *et al*. succeeded in formulating novel nanotechnology-based DMN arrays loaded with a whole inactivated antigen of *Neisseria gonorrhoeae*. Biodegradable nanoparticles with albumin matrix were used to encapsulate the spray-dried antigen which was then loaded into a platform composed of trehalose, maltose, PVA, and HPMC and molded as microneedle arrays. To investigate the immunogenicity of the produced microneedles, three doses (1 starting dose and 2 booster doses each containing 100 µg of the antigen) were given at one-week intervals by manual application with moderate pressure for 20 minutes. At the end of the study, they found a significant increase in the titers of gonococci-specific antibodies in groups that received the antigen-loaded microneedles as compared to the negative group suggesting an interesting vaccine delivery system [93].

On the other hand, Aoife *et al.* studied the possibility of utilizing DMN arrays for the delivery of another whole inactivated bacterium with the purpose of vaccination. A certain strain of *Pseudomonas aeruginosa* (PA01) was inactivated by heating and then lyophilized to be used as an antigen. Methyl-vinyl-ether copolymer with maleic acid was used as the backing material in the formulation of DMN arrays and in which the antigen was suspended. Mice were used for the in-vivo immunization studies in which each animal received two DMN patches applied on either dorsal ear area and a week after the vaccination the animals were challenged with fresh bacterium suspension. One day following the challenge, the

lungs of the treated animals were removed for analysis and the results demonstrated significantly lower bacterial loads in the lungs of the vaccinated animals as compared to the non-vaccinated ones [94].

Streptococcus suis is another bacterial species that were investigated as an antigen in the design of DMN. Kai-Jen *et al.* developed DMN arrays loaded with the whole inactivated *Streptococcus suis* to determine its capability of improving the efficiency of streptococcal serotype 2 immunization using a mouse as an animal model. Two steps molding method was applied for the production of the DMN with PVA as the backing material. At the end of the vaccination and lethal challenge studies that were carried out on mice, it was found that a single dose immunization was sufficient to elicit a potent immune response with full protection against the exposure to streptococci lasting for up to seven months [95].

To determine the difference between using split virion or whole inactivated virus as an antigen for TCI via DMN arrays, Akihiro *et al.* fabricated DMN and loaded them with either one of these two types of antigens derived from two types of influenza viruses (namely the H1N1 and the H1N5). The arrays were produced by two steps casting method using an aqueous solution of chondroitin (in which polyethylene terephthalate was dissolved) as the backing solution. After performing an immunization followed by lethal challenge investigations that were conducted on mice, it was found that the TCI with DMN arrays containing the whole inactivated virus as an antigen could induce a potent immune response with adequate protection against lethal challenge at a much lower antigen dose compared to TCI via DMN loaded with split virion antigen [96].

Anto *et al.* developed a novel approach for the fabrication of DMN arrays to overcome some of the obstacles that occur with the conventional production methods. In this study, they described a drop-dispensing technique that was microfluidic that could be applied in the production of DMN with the unique possibility of including microneedles containing different constituents within the same patch (which is called heterogeneous arrays). Whole inactivated trivalent influenza antigen was used as an antigen model and the immunization studies were conducted on mice through the application of the produced DMN patches on the ear skin area which were left in place for 18 hours. In the end, they revealed that lower doses of the antigen were required to induce a significantly stronger immune response as compared to the administration of the same antigen by IM injection [97].

To evaluate the three months stability of vaccine loaded into DMN patches, Leonard *et al*. prepared DMN patches loaded with whole inactivated influenza vaccine and performed *in-vitro* and *in-vivo* stability assessments of the vaccine. PVA and sucrose were utilized as the casting material in the formulation of the DMN arrays into which the soluble antigen was included. The results indicated that there was at least a 40% loss of antigenicity occurred during the microneedle production but after that, the vaccine maintained its antigenicity for up to 3 months of storage at various temperatures thus confirming the capability of the DMN to ensure the stability of antigen at higher temperatures without the need of cold chain [98]. In a different study to estimate the stability of whole inactivated influenza vaccine incorporated into DMN arrays, Mathew *et al.* investigated the effect of different formulation excipients (stabilizers and surfactants) on the stability of the manufactured DMN vaccines. Each surfactant was added at a concentration greater than its critical micelles concentration. At the end of the study, they concluded that certain excipients (some amino acids and polysaccharides) could increase the stability of the vaccine formulation, particularly those that contain calcium heptagluconate or arginine. Finally, they concluded that the proper formulation of the vaccine-loaded DMN had a significant impact on the stability of the delivery system [99].

At last, we should mention that there are two phase-1 clinical trials regarding the use of DMN patched for vaccination using a whole inactivated virus as an antigen. The first one was a randomized study conducted by Hiroaki *et al.* which was a partly blinded (active-controlled) phase-1 clinical trial to investigate the use of DMN for vaccination against Japanese encephalitis. Young healthy adults (who were neither previously infected nor vaccinated against the

Japanese encephalitis virus) were enrolled in the study. They were randomly divided into three groups: the SC injection group (2.5 µg), the high-dose DMN (0.63 µg), and the low-dose DMN (0.25 µg). each individual received two doses at a 21-days interval. The antibody titers were determined 42 days after the first vaccination and they were found to be dose-dependent. The vaccination with the DMN was dose sparing which suggested a possible reduction in the amount of antigen required for efficient vaccination. Therefore, this study proved that Japanese encephalitis vaccination using DMN arrays was safe and efficacious with the possibility for antigen dose reduction [100].

The second phase-1 clinical trial for the use of whole inactivated vaccine encapsulated within DMN arrays was for vaccination against the influenza virus and it was performed by Nadine *et al.* the study was a partly-blinded (placebo-controlled), randomized clinical trial. Young, immunocompetent, non-pregnant individuals were enrolled in the study. The participants were assigned randomly into one of the four groups: the vaccine DMN, placebo DMN, IM vaccine (all of them vaccinated by trained health care providers), and the last group received vaccine DMN by self-administration. Each participant received a single-dose vaccination. The anti-HA antibodies were measured on day 28 of the vaccination. There was no serious adverse effect linked to the vaccine administration. The antibody titers at day 28 were higher in all vaccination groups as compared to the placebo group. Also, there was no significant difference in the antibody titers between groups who received vaccination by health –care providers (the DMN vaccination and the IM vaccination) and those who took the DMN patch by themselves. These results indicated that the influenza vaccination using DMN patches is generally safe, well-tolerated, and efficacious [101].

VIRAL VECTORED-BASED ANTIGENS.

Modern technologies enabled the generation of viral vectors which act as vehicles that are capable of delivering the desired antigen [102]. Compared to the live-attenuated-based vaccines, viral vectored antigens offer the advantage of being thermostable so their storage would not demand the availability of a consistent cold chain [103]. Also, they can elicit a long-term immune response [102,103]. However, the production of viral vectors is associated with many challenges such as the difficulty of scaling up the production technique and the possibility of generating a virulent phenotype as a result of the interaction between the viral vector and the circulating virus [102].

Eun *et al.* fabricated DMN arrays for immunization against coronavirus infection using one of the recombinant proteins that resemble the structure of the virus (MERS-S1f, MERS-S1fRS09, MERS-S1ffliC, SARS-CoV-2-S1 or SARS-CoV-2-S1fRS09). These proteins were engineered by codon optimization followed by fusion with the foldon trimerization subunit. Then, the microneedle arrays (loaded with the antigen) were produced by a spin-drying approach which was conducted through two steps with the use of CMC-based hydrogel as the backing material. The immunization study was performed on BALB/c mice and resulted in a potent immunological response [104].

Geza *et al.* developed a three-dimensional platform for TCI loaded with adenoviral vectored antigen encoding both ovalbumin (as a model antigen) and poly(I: C) (as an adjuvant) to elicit a stronger immunological reaction. The microneedle arrays were generated by a spin-casting technique using CMC and trehalose as the casting materials. Immunization studies were conducted on mice and revealed that the combined effect of OVA with the adjuvant which was encoded through adenovector resulted in improved humoral and cellular immunological responses [105].

To investigate the possibility of using adenovirus vector engineered antigens for TCI against HIV, Marija *et al.* produced DMN arrays loaded with adenovectors encoding the HIV-1 subunit. The arrays were produced by the multilayered centrifugation casting technique. The tips were composed of sodium CMC in which antigen was incorporated. The second layer contained sodium CMC along with lactose and the basal layer was a pre-made layer composed of sodium CMC which was attached to the bottom of the needles. BALB/c mice were used for immunization studies and the DMN patches were applied manually to the dorsal skin area. It was found that these vaccine

delivery systems provoked a long-lived immune response [106].

In another study, Eun *et al.* succeeded in fabricating an adenoviral vectored vaccine for TCI against zika viral infections through the use of CMC-based DMN arrays. These adenovector antigens were capable of encoding the extracellular part of the virus envelop gene. Two immunization doses (given at 14-days intervals) were administered to mice by manual application. The results demonstrated a good humoral immune response. In addition, the lethal challenge study was performed on pups of immunized mice and revealed complete protection against zika virus infection without any undesirable effects [74].

Pablo *et al.* utilized an adenovirus vector to engineer HIV-1 gag for TCI using DMN arrays against HIV infection with the aim of the generation of long-lived memory of $CD8^+$ T-cells. The DMN arrays were fabricated by centrifugation casting approach using sodium CMC (as the casting material) and sucrose (as a stabilizer). The immunization studies were performed by the application of the DMN patch on the dorsal area of C57BL/6 mice. The lymph node analysis detected $CD8^+$ T-cells for about two years following the TCI with the DMN arrays [107].

The aim of producing long-lived $CD8^+$ T-cells for immunization against HIV infection was also investigated by Veronique *et al*. who manufactured DMN arrays loaded with adenoviral vectors encoding for HIV-1 gag through the utilization of sodium CMC and sucrose as the casting material. The analysis of the draining lymph nodes revealed that these delivery systems are capable of generating long-lived cellular immunity [103].

DISSOLVING MICRONEEDLES FOR CANCER THERAPY

In the last decade, the ascending increment in cancer occurrence necessities the development of treatment regimens with better drug delivery systems to tumor cells [108].

The properties of dissolving microneedles make it a good candidate for delivering some anticancer therapy to the tumor site. The easy non-invasive biodegradable topical application could exclude many of the systemic side effects by avoiding the leaking of the drug to the normal tissues surrounding cancerous cells. Besides, dissolving MNs could use to deliver different types of antitumor therapy including chemotherapeutics agents (doxorubicin), phototherapeutics agents (5-aminolevulinic acid), and immune-trigger, gen-trigger cancer vaccinations like ovalbumin and pDNA, respectively (figure 2) [109].

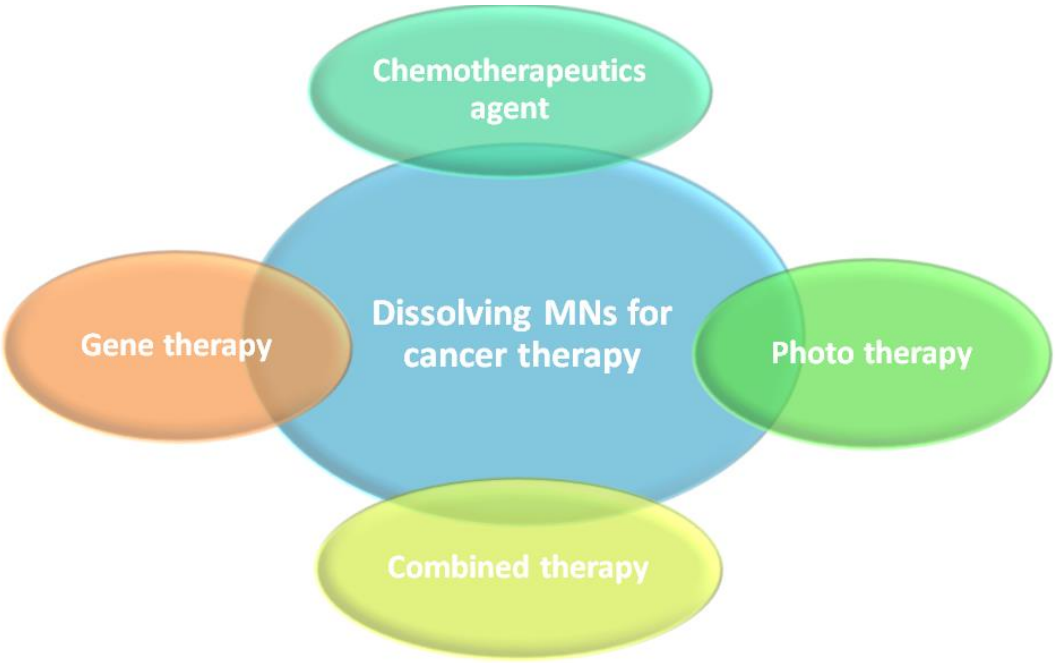

Figure 2. The different types of antitumor therapy that could be included in dissolving MNs.

DISSOLVING MNS CONTAINING CHEMOTHERAPEUTIC AGENTS

Doxorubicin is one of the first-line chemotherapeutic agents used to treat different types of cancer [110]. It has been formulated by various drug delivery systems like hydrogels, microneedles, and liposomes to increase its efficacy and limited its side effects [111].

Nguyen *et al.* formulated doxorubicin with biodegradable polymer polyvinyl alcohol (PVA) as DMN. The viscosity of PVA has been controlled to change the location and speed of drug release from the MNs along with the drug deposition profile. Ethanol was used to facilitate the doxorubicin dissolution in PVA mold. The in-situ dissolution profile of doxorubicin MNs showed rapid dissolution of the needle tips while the bases dissolved gradually and the dissolution continued for 24 h depending on skin moisture. The *in-vitro* release of doxorubicin from PVA microneedles relies on the location of the drug within the DMN. The human cadaver skin was used in histology studies, it showed that doxorubicin delivery across the skin from PVA- dissolving MNs was significantly higher in comparison with the passive diffusion. Further *in-vivo* studies on animals were required to approve the chemotherapeutic activity of doxorubicin from such a delivery system [112].

Bhatnagar *et al.* prepared doxorubicin and docetaxel (which is analog to paclitaxel) as DMN using a super-disintegrant polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA). They conducted different studies to measure the mechanical strength, characterization, skin insertion, release, and *ex-vivo* skin permeation of these DMN in a mouse model. The results showed an inverse relationship between the mechanical strength and the dissolution rate of the doxorubicin/docetaxel DMN. The comparison between the transdermal applied doxorubicin/docetaxel DMN and inter-tumor injection in the tumor beard mice indicated a higher decrease in the volume and the weight of tumor in the mice treated with the DMN in addition to the enhancement in survival rate [33].

Fabrication of hyaluronic acid-based DMN containing doxorubicin by using transfersome was performed by Yang *et al*. They proposed that after the insertion of the DMN efficiently into rat skin, the release of doxorubicin drug was mediated through self-dissolution and a significant accumulation of the drug was seen in the lymph nodes of the tumor-bearing rat.

The good doxorubicin blood bioavailability along with the lymph node accumulation provides promising future uses of the DMN in destroying the metastasis of cancerous cells [113].

DISSOLVING MNS CONTAINING PHOTOTHERAPEUTIC AGENTS

The use of phototherapies in tumor management has increased in the last decade due to their advantages over conventional treatments. They can invade cancerous cells only which in turn enhances the therapeutics' effectiveness and declines the side effects by avoiding the destruction of normal cells around the tumor.

Photodynamic therapy is a type of phototherapy that consist of light-energy combined with a reactive-oxygen generating compound called photosensitizer [114].

5-Aminolevulinic acid is a compound converted in the mitochondria after multiple reactions to an endogenous photosensitizer called protoporphyrin IX. The topical application of 5-aminolevulinic acid showed poor skin penetration [115].

5-Aminolevulinic acid was prepared as DMN with a biocompatible polymer, hyaluronic acid, which provides a good acidic environment free from oxygen due to its containing many carboxylic groups. These prepared MNs acted as efficient delivery of the loaded 5-aminolevulinic acid into the tumor cells with good stability at ambient conditions due to the properties of hyaluronic acid [116]. Champeau *et al.* fabricated the hyaluronic acid-based DMN that contains 5-aminolevulinic acid through the usage of the aluminum master structure. They developed two different heights of these MNs, the 400 μm height contained 20 mg of 5-aminolevulinic acid while the 750 μm height contained 100 mg. Stability, mechanical strength along with skin penetration were tested for this type of 5-aminolevulinic acid DMN and the results showed a good skin penetration with a dissolving rate of about 45 min; further *in-vivo* test is required to study the pharmacological activity of such MNs on cancerous bearing rats [117]. So, Champeau *et al.* conducted another research aimed to evaluate the dissolution and penetration efficacy of the prepared MNs mentioned previously on a skin lesion introduced in the 40 rats model. The results of Histology studies and pharmacokinetics tests showed a keratosis lesion in the pre-epitheliomatous area. The dissolution rate on rats' skin was equal to 60 min [118].

In another way to enhance the transdermal delivery of 5-aminolevulinic acid, Requena *et al.* formulated it in three different concentrations as DMN with an aqueous mixture consisting of two polymers which are: methyl vinyl ether plus maleic anhydride in 20% w/w. This study was conducted to compare the DMN with cream of the same concentration of 5-aminolevulinic acid. The results indicated the better transdermal delivery of 5-aminolevulinic acid through mice skin compared with the cream in addition to its possible lower cost as its required lesser drug concentration. However, further experimental studies about the effectiveness of such microneedles in tumor killing are required [119].

Another form of phototherapy depends on the conversion of the energy produced from light absorption in cancerous cells into heat and this lead to the burning of the tumor cells [120]. This type of treatment is called photothermal therapy and it consisted of a radiation source with photosensitizer agents like aggregation-induced emission luminogens (AIEgen). The problem in this therapy is the localization of the effect inside the cancerous area only without affecting the surrounding normal cells. One of the good solutions to such a problem is the formulation of AIEgen as DMN with pH-responsive polymers. These DMN had been evaluated for the treatment of superficial tumor cells such as melanoma, they showed an obvious erosion of cancerous cells after a single dose application combined with laser irradiation [121].

DISSOLVING MNs CONTAINING GENE ANTICANCER THERAPY

The use of gene therapy in cancer treatment has increased lately [122]. Dissolving MNs containing nanoparticles of prototype DNA were fabricated by combining RALA (cationic cell-penetrating peptide) with PVP and tested on mice infected with cervical cancer. The result showed a significant reduction in tumor size in mice treated with such a system [123]. A

similar system was developed by induction of an immune response against different cancerous cells [124]. Cole *et al.* produced nanoparticles of RALA/pDNA and formulate those nanoparticles as DMN. In the beginning, they tasted the activity of the formulated MNs on cervical cancer. They found the increase in specific IgG titer was around 1.7 fold in MNs containing nanoparticles compared with intramuscular ones which indicated the better activation of the immune system when RALA/pDNA nanoparticles were delivered by MNs system [125].

In another study, the RALA/pDNA nanoparticles-MNS system showed a successful activation of T-cell mediated immune response as confirmed by an *in-vitro* study by using model cells (HEK-293 and DC 2.4 cells) that could be used for the prevention and treatment of prostate cancer without the need for additional adjuvants [126].

Melanoma, the cancer of melanocytes could be treated by gene therapy containing small interfering RNA (siRNA). siRNA causes activation in the STAT3 gene (signal transducer and activator of transcription 3) which in turn effected a cascade of gene expression in melanoma cells. Hyper activation of the STAT3 gene will cause downregulation and inhibition in cell proliferation. The problem that hinders the subcutaneous delivery of siRNA is related to its hydrophilicity and high molecular weight. One of the effective solutions to such problems is to formulate it as a DMN using hyaluronic acid, dextran, and PVP. Small interfering RNA including DMN showed a promising anti-melanoma activity due to the tumor necrosis observed in the histological study on the infected animals [32].

Antigen-containing DMN were used to boost the immune response in cancer treatment. Activation of dendritic cells through the application of ovalbumin-containing DMN was performed by Lee *et al*. These MNs were prepared by a method called (droplet-born air blowing) and fabricated by using a hyaluronic acid solution with ovalbumin. The transdermal delivery of these MNs provoked an immune response in the tumor cell that was mediated through ovalbumin antigen activation of cytotoxic T and Th1 cells [127].

DISSOLVING MNs CONTAINING COMBINED THERAPY

A combination of two types of anticancer therapies to increase the efficacy of tumor treatment had been used widely. The development of light-activated dissolving MNs was reported by Chen *et al.*. The DMN were fabricated by embedding the doxorubicin drug in plasmonic nanomaterials of lanthanum hexaboride (LaB6) with a mixture of PVA and PVP. Applying a near-infrared (NIR) laser to this DMN inserted in cancer-bearing mouse cause activation of LaB6 which will convert the laser radiation into heat and this leads to the release of doxorubicin from the DMN. These MNs can deliver to the tumor not only anticancer drugs but also heat which leads to tumor necrosis and is supposed to reveal a complete eradication of the tumor after a single application within 1 week [128].

Dong *et al.* prepared DMN containing gold Nano-cages [AuNCs) and doxorubicin drug. These MNs were fabricated using hyaluronic acid as a polymer. The AuNCs is a photothermal agent able to convert NIR laser radiation applied to the MNs into thermal energy. The killing of tumor cells in superficial skin cancer will occur synergistically by the combination of NIR laser with the chemotherapeutic effects of doxorubicin [129].

Pei *et al.* fabricated a composite MNs patch. They contained a mixture of doxorubicin hydrochloride and photosensitizer (indocyanine green). The indocyanine green was formulated as mesoporous-silica nanoparticles to improve its stability. After that, it was loaded along with doxorubicin as DMN using PVP as solvent. Application of this MNs patch to the site of the tumor exhibited a good penetration to the skin. The *in-vitro* results were obtained by applying the MNs patches on MG-63 cells (human osteosarcoma cells), which indicated the death of human osteosarcoma model cells due to the synergistic effects of combining the doxorubicin along with photosensitizer as a composite MNs patch [130].

The synergistic effects of combining chemotherapeutic agent (doxorubicin) with indocyanine green lead to prepare them as separable DMN by using a rapid dissolving supportive base of PVP and PVA mixture. Insertion of these separable

MNs on the site of melanoma cancer in the mice model indicated the rapid liberation and good penetration of the chemotherapeutic drug (doxorubicin) due to the rapid dissolution of the supportive base. The application of NIR irradiation to the embedded MNs arrowheads leads to indocyanine green photo thermal conversion and inhibition of tumor proliferation [131].

A possible effective strategy for local treatment of melanoma was developed by Peng *et al.* through the formulation of DMN that contain a combination of the chemotherapeutic agent (Paclitaxel) and indocyanine green. This chemo-phototherapy was fabricated as functionalized nanoparticle consisting of a biphasic mixture of $\boldsymbol{\alpha}$-tocopheryl succinate being introduce in poly lactic-co-glycolic acid to increase the porosity and facilitate the release of the drug. The *in-vivo* results showed a superior antitumor activity of loaded MNs. Such a combination of nanoparticles in DMN gives a promising new technique for the treatment of superficial cancers [132].

Another Paclitaxel-based topical treatment for melanoma tumors was prepared by Qin *et al.* Paclitaxel was formulated as nanoparticles with a photothermal agent (IR-780) to increase the drug stability by providing a lipid-based platform. These solid lipid nanoparticles were inserted into DMN. The release of this system was spatiotemporally and pulsatile controlled. The results indicated the rapid release of the combined antitumor agents after the NIR irradiation on tumor-bearing cells (B16 cells) and tumor site localization in comparison with intra-tumoral and IV injections. This drug localization indicated the benefit of higher bioavailability with limited systemic side effects. In addition to that, the tumor-killing efficacy was high, and almost complete tumor eradication was obtained within one month [133].

Fu *et al.* fabricated MNs contained with photosensitizer (IR820). The preparation of cisplatin (lipid-soluble chemotherapeutic drug) and IR820 as DMN were facilitated by using a copolymer of polyvinylpyrrolidone and vinyl acetate. The insertion of these MNs in breast cancer cells indicated the rapid release of both drugs within 5 min. Upon laser radiation, the IR820 agent generated active oxygen species that ceased cell proliferation and induce apoptosis of cancerous cells along with cisplatin [134].

To enhance the penetration of immune cells and drug therapy to the melanoma cancer site, He *et al*. designed DMN that contained modified hyaluronidase nanoparticles with polyinosinic/polycytidylic acids which act as immunity adjuvant [135].

CONCLUSION

Transdermal drug delivery represented a convenient and painless way of drug administration; avoiding many of the adverse events associated with oral and parenteral therapy. For most low molecular weight drugs, various penetration enhancing strategies are successfully employed to increase their permeation into the skin. Among the physical methods, microneedles represent a smart approach to enhancing transdermal delivery and overcoming the stratum corneum barrier. Dissolving MNs showed amazing results in cancer therapy and vaccination delivery during the last decade; holding much promise in transdermal delivery of therapeutics.

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