IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

MICRONEEDLE ARRAY: A VIABLE ROUTE FOR TRANSDERMAL MACROMOLECULE DELIVERY

ANAMIKA PALAK[1*], AYUSH GANDHI[2], SUREKHA[3]
Dasmesh College of Pharmacy, Faridkot, Punjab, India-151203

Abstract

As far as bodily organs go, the skin is king. It is a major route for topical and transdermal drug delivery. Transcutaneous delivery has numerous benefits over the oral and parenteral route of administration. However, the stratum corneum remains the main barrier in the effective delivery of wide collection of therapeutic molecules in the blood, specifically macromolecules. As the ideal characteristics to be delivered through the skin,the drugs should be sufficiently lipophilic and non ionic and should have lower molecular weight i.e. <500 da. Numerous studies have shown that, macromolecules require external sources of energy for their penetration through the skin. Although, these techniques are employed with additional assembly and require more investment in terms of money and time. Microneedles have recently been investigated for the delivery of macromolecules through a minimal invasive, painless procedure that penetrates the stratum corneum and creates micropores in the skin toenablethe successful passage of molecules through the skin into the systemic circulation.This method may prove cost-effective and more compliant as far as drug administrations by hypodermic needles are concerned.To portray the exceptional utility of these methods in transcutaneous delivery of hydrophilic or very large molecules, the present review aims to bring forward the current development in microneedle-based drug delivery and describe the essential features of these systems that are enabling them to transform the present and the future of transdermal drug delivery approaches. Future investigations on microneedle arrays may contribute to the advancement of the therapeutic efficiency of other molecules that are yet hard to deliver through the transcutaneous route following passive diffusion.

Keywords: Transdermal, Micro-needle, Macromolecules, Advanced Macromolecule delivery, Vaccine, Protein and peptide

1. Introduction

One tenth of a human body is covered by skin, the biggest organ and the first line of defense against environmental hazards. (1,2) Because it is easily accessible, can be managed, and does not come into touch with the harsh environment of the gastrointestinal tract, the skin is an intriguing administration region for medicine distribution(3).The skin is divided into three regions, Epidermis includes layering system that consists of five layers,

all composed of keratinocytes. The outermost layer is the dead skin layer resemble wall composed of brick and mortar. It acts as a barrier for the penetration of the skin. Dermis layer is thicker and contains ~70% collagen by weight, consisting of some connective cells, a bundle of immunological cells, blood, various secretary glands, hair follicles, and nerve impulse endings. Subcutaneous(SC) underneath the dermis and inside the layer contains the adipose tissue to a larger extent(1).

Transdermal delivery of drugs is being used for a long time. It transports various therapeutic active agents via the skin pores to the local or to capillaries present beneath the skin, that is, aa painless, reliable, targeted, and cost-effective therapeutic regimen for patients. It is successful in numerous applications like hormonal replacement, pain management,and smoking cessation(4).However, there is a limited number of agents that can undergo transdermal delivery. Hydrophilic macromolecules like peptides, proteins, or vaccines encountered some problems in the transportation to the skin(5). Approaches like mechanical abrasion (rubbing, vibration, etc.) and chemical enhancers (surfactants) can improve drug delivery, but effects lead to skin irritation and rupture after treatment. Several efforts have been made for the enhancement of transdermal drug delivery techniques. They can be generally divided into technologies, i.e., passive and active. While the utilization of chemicals, as skin enhancers (e.g.,azone, peptides, and more lately ionic liquids) has permitted in few studies to proliferate passive dispersion of small therapeutics agents. In recent years, researchers made several efforts to fabricate particle-based active technologies to alter the barrier characteristics of the Sub-Cutaneous (6).

There are some challenges due to the robust blockade property of skin which has become challenging. There is some specification for the drug delivered via a transdermal route, i.e., the MW should be less than 500 Da, the molecule must have satisfactory lipophilicity, and possess low melting point. Still, delivering hydrophilic drugs and large molecules like proteins, peptides, DNA, and siRNA is a challenging aim.The microscale dimension can upsurge skin permeability which leads to effective and efficient dermal delivery(7).

Recent years have seen a surge in interest in microneedle-mediated delivery, with an initial concentration on transporting macromolecules derived from biological sources. Microneedles are microscaled systems bypassing the stratum corneum barrier, contributing to the improvement of cutaneous drug administration (8). In 1998, the microneedle was introduced for cutaneous drug administration at the very first moment (9). Microneedle is a hard or cave-like cannula with a proper distance of 500-900 µm and a maximum diameter of 300 µm(10). For transdermal delivery, microneedle arrays are developed in a patch (11). Across the skin, the transfer of varied series of therapeutic with the support of MNs (microneedles) has been attained by four main approaches, i.e.,‘Poke’-and-‘patch’, ‘Coat’-and-‘poke’, ‘Poke’-and-‘flow’, and ‘Poke’-and-‘release’. Poke-with-patch mechanism-based complex MN grouping is applied to produce micro-pores accompanied by the elimination of the array and the administration of drug preparation in the form of transdermal patch, hydrogel, or solution.Coat-and-poke is done on the micro projection coating of the drug followed by the insertion of MN arrangement into the skin layer. Poke-and-flow is performed using hollow MN’s by injecting a liquid drug into the skin, and in poke and release, drug particles are incorporated into the assembly of polymeric and biodegradable MN’s and following insertion into the skin layer(10). Pain-less application of the drug, sidestepping the liver's first-pass metabolic process, quicker therapeutic action at the injection area compared to hypo-dermic needle(12–14), no fear of needles, reduced bacterial infiltration are some of the advantages. It is all because the MN only ruptures the epidermis, enhances API efficiency may be resulting in the decrease of dose, good acceptability with long-term oedema and erythema and quick medication delivery are the major advantages of these systems(11).

This review highlights the relevance of the transdermal route for the delivery of macromolecules, i.e., insulin, vaccines, protein and peptides, and antibodies by a microneedle assisted approach. The goals are to draw attention to

the limitations of macromolecule transdermal administration, the benefits of microneedles for such distribution, and the new empirical data that supports these claims.

Challenges in microneedle-based drug delivery

Table 1 comprises different routes of administration along with their limitations and approaches to overcome. Much more research is being done on the effect of microneedle on transdermic API transport, and there is a large potential for employing such microlength needles for efficient transdermal medicine delivery.The biodistribution of biotech macromolecule products has complications since these compounds have a large molecular weight and are also very hydrophilic, causing distribution through the dermis challenge (15). The 500 da law expresses that a pharmaceutical API should have a molecular mass less than 500 da and be hydrophobic to be delivered through human skin(16). Studies claimed that without the aid of any external support, it is difficult for a macromolecule to penetrate the skin layer. It can be improved either through the physical methods or the chemical methods. The most popular physical method is the use of microneedles to create micropores in the skin and release the active agent in different sections(17). This review will show how a microneedle may create a micropore in the skin, allowing macromolecules to be administered through the skin.

Table 1.Different routes, have their limitations, and approaches to overcome

Delivery route	Challenges	Approaches
Oral	- Enzyme attack by intestinal proteases and peptidases - Poor intrinsic permeability across biological membrane - Rapid post absorbance clearance - Physical instability like aggregation	- Using Enzyme inhibitor - Using Penetration enhancers - Using Carrier system Synthesizing prodrug
Buccal	- Mucus layer - Epithelial layer - Peptidases in saliva and microbial flora	- Adhesive tablets - Adhesive patches - Adhesive gels - Adsorption promoters
Nasal	- Alteration in absorption in diseased condition e.g., Allergic condition, Chronic Arthritis, URTI - Ciliotoxicity (due to use of preservatives and penetration enhancers) - Absorption varies with mucus secretion and mucus turnover - Peptidases and Proteinases in mucus serves as an enzymatic barrier	- Viscosity modification - pH modification - Permeation enhancers - Drug delivery design - Increasing nasal blood flow
Occular	- Tear dilution - Lachrymal drainage - Protein binding	- Ocular inserts - Absorbable gelatine sponge
Pulmonary	- Reproducibility in dose deposition. - Aerodynamics of aerosolized particles. - Site of dose deposition to the deep lung. - Delivery should be precise and consistent with every inspiration.	- Provides direct route to circulation - Fast absorption - Increased patient compliance - Decreased dose - Cost-effective - No triggering of immune functions
Parenteral	- Major route of choice but painful - IV, IM, SC - Intraperitoneal route	- Particulates - Soluble carrier - Miscellaneous system

Transdermal	Less permeation - High molecular weight. - Hydrophilicity and lipophilicity of drug - Stratum corneum	- Iontophoresis - Phonophoresis - Penetration enhancers - Prodrug - Microneedles

2. Recent advances in macromolecules delivery through microneedle

In regards to macromolecules, it becomes difficult to deliver these agents viaa transdermal route due to their very high molecular weight and high hydrophilic nature, making them hard to deliver through the skin. Recently**,** MNs have been extended to various aspects, including vaccines, genes, insulin, antibodies, protein, and peptides and with the help of MN, many of the macromolecules have been delivered in various diseases (**Table 2**). MNs make their delivery easy because of the creation of microspores

Table 2: List of formulations that have been delivered successfully by microneedle in various indications

Formulation	Title	Goal	Disease	Nct no.	Status	Pha
Microneedle	Insulin delivery using microneedle in type 1 diabetes	To determine if microneedles can effectively and painlessly deliver insulin to children and young adults with type 1 DM	Type 1 DM	00837512	Accomplished	2
Microneedle	Glucose measurement using microneedle patches (gump)	The MN patch will collect interstitial fluid to be tested for a glucose level. The microneedles are made from biocompatible polymer or metal	DM	02682056	Accomplished	N/a
Microneedle (vaccine)	Inactivated influenza vaccine delivered by microneedle patch or by a hypodermic needle	To investigate the safety, reactogenicity, acceptability and immunogenicity of an inactivated influenza vaccine delivered by mn patch	Influenza	02438423	Accomplished	1
Microneedle (vaccine)	Microneedle patch study in healthy infants/young children	To evaluate the safety, reactogenicity, and acceptability of placement of a placebo microneedle patch to the skin of children.	Vaccination	03207763	Accomplished	N/a
Microneedle (cancer)	The use of microneedle to expedite treatment in photodynamic therapy	To investigate how varying incubation periods of topical aminolaevulinic acid after pretreatment with microneedle application can facilitate the penetration and efficacy of photodynamic therapy.	Keratosis, actinic	02594644	Accomplished	N/a
Microneedle (cancer)	The use of microneedle in photodynamic therapy	To investigate how microneedle can facilitate the penetration and efficacy of photodynamic therapy in the treatment of actinic keratosis	Actinic keratosis	01812837	Accomplished	N/a
Microneedle (cancer)	Micro needle array-doxorubicin (mna-d) in patients with cutaneous t-cell lymphoma (ctcl)	The study hypothesis is that in situ mna-directed chemo-immunotherapy using doxorubicin will kill tumor cells locally and alter the tumor microenvironment to induce durable systemic tumor-specific immunity.	Cutaneous T cell lymphoma	02192021	Employing	1
Microneedle (biologics)	Adalimumab microneedle in	Pain perception is compared in the study and Adalimubab was generally given sc (40 mg dose) but here it was given intradermally	Pain	03607903	Unidentified	1

healthy volunteers through Microneedle.

3.1 Insulin delivery through microneedle

Insulin treatment is important for patients with diabetes by maintaining BGL, and it is also widely used in people with severe type 2 diabetes. While hypodermic insulin management via injection is the most conventional means of delivering insulin, it has been linked to pain, needle anxiousness, reduced adherence, and infection risk. Transdermal insulin delivery has been studied extensively in recent decades as a feasible alternative to dermal insulin transport for blood sugar control (18). Percutaneous devices are intended to stop insulin degradation as well as provide controlled and sustained insulin secretion, which may be useful for patients and lead to improved adherence and blood glucose results. Large molecular weight is considered the hurdle for dermal insulin delivery(18). Because of the natural protective characteristics of healthy skin, the dermis's thickness is considered a major challenge in insulin delivery. Small molecular weight (500 Da) therapies can easily infiltrate the epidermis, whereas the passive movement of high molecular weight protein medicines, such as 'insulin', is consciously hindered. Numerous approaches to physically and chemically accelerate the transport efficiency of the molecule over the dermis have been found to surpass barricades in trans-dermal insulin administration. Chemical enhancer-assisted, electrically enabled, machine-driven force-triggering, and microneedle aiding techniques are among the most substantial progress in percutaneous delivery of insulin. We will emphasize the MN-mediated methodology in this discussion (18). Microneedle technologies have now become a better solution for percutaneous peptides and protein administration (19–24).The microscaled spikes can breach the SC without inflicting pain and penetrate the skin to discharge the medication (25,26). The micro channels created by MN are only present for a short time, allowing the medicine to be transported, but they quickly disappear after MN is removed, preventing lengthy skin tissue damage (27,28).The Microneedle method is categorized into numerous types that depend on the materials and mode of drug administration. Solid and Dense MNs are often engineered to enter the dermis to promote drug absorption; hollow MNs are employed for injecting fluid drug formulations through skin openings created by spikes, and dissolved and biodegradable MNs are built from polymer encapsulating pharmaceuticals. Bio responsive MNs, which can react to physiological blood glucose for on-demand insulin delivery, are constantly being implemented (18).

Poke and patch technique is an old method that includes MN-assisted insulin diffusion into the skin, which is purely dependent on skin perforation(19). In this procedure, MNs pierce the skin to produce microchannels via which insulin can be delivered when a transdermal patch or any topical solution is applied later. Several reports have been conducted in recent decades that show efficient insulin delivery through the epidermis (18). For example, Martanto *et al.* confirmed the hypoglycaemic effect of insulin in diabetic rats using MNs(27). A stainless steel built an array of microneedle and lodged into the epidermis of diabetic induced rats, following which the solution of insulin was delivered in contact with the dermis for four hours. These hard metallic micro-sized needles provided enhanced percutaneous insulin delivery and a reduction in BGLs of up to 80% *in vivo*. In a model, Zhou *et al.* assessed the practicability of consuming an available commercially. The stainless steel-made Microneedles were tested in three different length ranges, viz, 250, 500, and 1000 µm (28). The introduction of MN rollers resulted in a quick fall in blood glucose level (BGL) in 1 hr, while the healing of the dermis pores created by MNs had a weakened effect. MN rollers of 500 µm or less have been proven harmless and the tendancy of enhancing transdermal insulin administration *in vivo*. It was stated that by changing the MNs' treated area, BGL might be reduced for an extended course of time (29).

Hollow shaped MNs are prepared in such a way that medication can be delivered into the epidermis via the use of needles. McAllister *et al.*administered insulin via hollow-glass MNs by micro infusion in diabetic induced rat skin, yielding a continuous reduction of up to 70% of pre-infusion BGLs over five hr (30). There was also design and fabrication of hollow shaped metal MN arrays for insulin delivery subcutaneously(31). According to the mechanical investigation, these MNs were presented to be robust enough to puncture the topmost layer without breaking. The MNs were attached to an insulin pump, and then put on the belly skin to regulate the insulin infusion rate. Results like lower Bgl and fast insulin absorption was seen in mice when the MNs were implanted in the skin at a depth of 1 mm.Supplementaryreadings have been performed to evaluate the effectiveness of hollow-shaped MNs for insulin delivery in mammals (32).

Several studies are performed on biocompatible polymeric microneedles for the metal and silicon materials used for the fabrication of microneedles (33).The soluble polymers create dissolved MNs, which enclose the drug in the atmospheric matrix and dissolve entirely when vaccinated into the dermis, releasing the drug. The therapy time is determined by the polymer material's dissolved rate, which can be modified from min to hrs to reach the treatment goal line(34). Moreover, by using biocompatible polymers evade any manufacture of sharp biohazardous remaining(35,36). Several dissolved MNs fabricated of sugar-glass materials such as polymers have been reported, such as maltose(37–40), trehalose(41–43)and(44,45). Sugar glass MNs are dissolved rapidly in human skin after insertion(46,47). Nonetheless, the production of these MNs necessitates a maximum temp of above 100 °C to cause the rubber to glass transitions in sugar glasses, which may compromise the biocompatibility of biomolecules such as insulin (48). New modeling techniques have been developed to solve the thermal problems of the melting manufacturing process. Martin *et al.*, for example, used a low-temperature manufacturing approach to make dissolved MNs (44). Alternative strategy can be implemented by using other polymers which have excellent soluble properties which help in The preparation of microneedles, such as hyaluronic acid (HA)(49,53) Carboxymethylcellulose (CMC), (41,54) Chitosan(5,55), Polyvinylpyrrolidone (PVP) (58–61), and Polyvinyl alcohol (PVA) (62–64). Associated manufacturing procedure can prevent high temperatures, allowing drug-containing MNs to store more drugs. Liu *et al.* used micromolding technology to create hyaluronic acid microneedles and characterized its use in the percutaneous transfer of insulin(65). Even after 30 days of storage at several temperature (-40, 4, 20, and 40 °C), the bioactivity was found to be greater than 90%. Furthermore, the HA MNs were more resistant to humidity distortion than the sugar glass MNs when the insulin-loaded hyaluronic acid microneedle was given to the diabetic induced rats; *in vivo* investigations revealed a dose dependant hypoglycaemic effect. After being organized with insulin-stacked HA MNs, in vivo concentrations in diabetic rodents showed a fraction subordinate hypoglycaemic effect. In addition, the temporary microchannel created by the implantation of MNs vanished after 24 hr.

Chen and co-workers fabricated a microneedle that can be easily dissolvable and made up of starch and gelatin for the delivery of insulin transdermally(66).The tough and solid composited MNs were prepared using gelatin with starch, and it further exhibited excellent skin penetration properties because of its film-forming capacity. Porcine skin with a thickness of 200 µm was taken, and *invivo* and *invitro* penetration tests were performed to estimate the microneedle's mechanical strength.The solvent casting method helped preserve the biological activity of insulin, which further reduces the blood glucose levels in diabetic rats on application.Moreover, the general accessibility and activity of the hormone were as yetmore significant than ninety percent after keeping it for 4 weeks at 25 or 37°C, recommending the application of starch with gelatin is considered a promising method for the transport of insulin. Although numerous kinds of dissolved MNs have been shown to distribute insulin efficiently and lower plasma sugar levels *invivo*, insufficient implantation of polymeric MNs due to dermis flexibility restricts transport proficiency, and results in medication waste (67).Finally,scientists planned a completely inserted MNs with a supportive assembly that gave a

stretched length for balancing skin compressive twisting during administration (68). Insulin was initially loaded on the needles of 600 µm-high poly-glutamic acid MNs, and then a secondary layer of 'PVA/PVP' was packed in the MNs mould to frame the 600 µm-high supporting structures in this research. Both the MNs and the supporting layer broke down within 4 min of being embedded in the skin, allowing the medication load to be delivered entirely. Diabetic-induced mice treated with an equal quantity of insulin (0.2 IU) via MN patches versus subcutaneous infusion had an equivalent hypoglycemic effect, confirming the practicality and precision of using this projected MN plan for insulin delivery(69).The endpoint of microneedles had high mechanical strength and could penetrate the dermis and break down rapidly to distribute encapsulated insulin. The experiment was performed in an obese mouse model that exhibiting fast insulin absorption into the bloodstream through the cutaneous, with the most dramatic serum insulin fixation occurring 2 hr after MN injection. The storage condition and insulin stability in the microneedle patch were observed to be 99.4% when the patch was kept for 20 days at the same room temperature. The multilayered manufacturing process used in this MN design can combine components to reduce waste while also meeting various mechanical performance requirements for tips and pedestals.

Theoretically speaking, two-layered MNs made from different substances in variable amounts were tested for optimal insulin delivery efficacy (70,71). Lee *et al.*examined the penetrating efficiency of MNs made up of a few different proportions of PVP with different molecular weights (PVP10/PVP360) and discovered that the 1:3 ratio was the most effective in vivo insulin administration(70). Other than unadulterated PVP360 supporting, the PVP360/CMC backing layer was chosen for improved elasticity for dermis fitting. Liu *et al.*produced a composite MNs coupled PVP matrix with insulin-loaded $CaCO_3$ microparticles to boost the properties of microneedles(72). In comparison to unadulterated PVP MNs, the manufactured MNs had improved hardness and slower dissolution. Typically, dissolved MNs are made using microsize moulds and a phased projection approach. Kim *et al.*established a new manufacturing approach that used droplet-born air blowing (DAB) to simply shape polymer drops to solidify MNs, resulting in generous (4-25 °C) and rapid (10 min) process conditions with no drug loss (73). In this technique, biopolymer beads were initially applied to the level surface for MN base creation, and another coating of API-containing beads for MN tip formation. From there, a sketch was used to expand the beads using atop plate. The MNs were toughened by air blowing during the procedure, and the amount and size can be fabricated according to the requirementof the droplet dispenser's pressure and time. The viability of insulin delivery related to this fabrication approach was proven by a fall in plasma sugar concentration and relative bioavailability (96.6 ± 2.4 percent).This technology might also provide a wide range of materials for the fabrication of dissolved MNs, such as HA, CMC, and PVP, while the surface morphology allowed for minimal therapeutic waste. Yang *et al.* recently demonstrated an electrospun pillar-array MN delivery that allowed for quick installation of MNs into the dermis to overcome fragmented drug delivery(74). After covering the pillar array with an electrospun fibrous PLGA (Polylactic-co-glycolicacid) sheet, dissolved HA beads were distributed on each support to form MNs using DAB. Because of the tensile breakdown of the fibrous sheet during compression, the succeeding MNs on the electrospun pillar array were quickly detached from the permeable fibrous substrate once wholly embedded in the pigskin. A fit mouse model was taken to demonstrate the hypoglycemic action of insulin-filled microneedle patch *in vivo*. Apart from using multilayered MNs to improve delivery productivity, Garland *et al.* investigated the application of polymeric MNs in blends with iontophoresis to increase the the bioavailability of insulin. They found that when ITP (iontophoresis) was used in conjunction with the soluble poly (methyl vinyl ether-co-maleic acid) (PMVE/MA) MN array, a synergistic increase in insulin discharge was achieved (75). Furthermore, their findings revealed that using electric flow could promote protein penetration throughout the entire MN patch rather than just the MN, hence increasing delivery productivity.

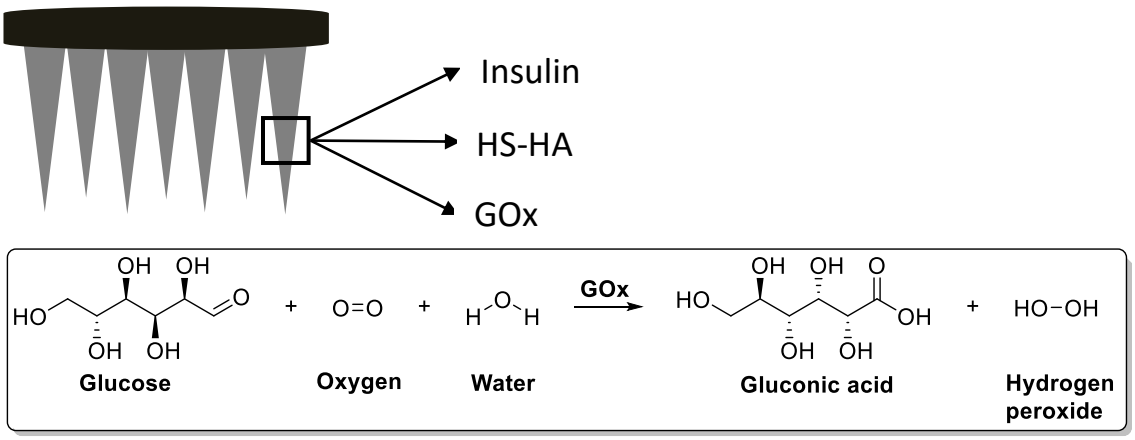
In the case of a degradable microneedle, the release dynamics of payloads from dissolved MNs are closely linked to the polymer dissolution rate, which is often fast. MNs with a long disintegration period are preferred as delivery devices for protein medicines that require a continuous therapeutic dosage(35). During the degradation process, the drug is gradually released from biodegradable MNs by passive diffusion, although swelling of MNs may further speed up drug diffusion. For example, calcium particle cross-linked alginate/maltose composite MNs for insulin delivery were explored, with maltose added to increase the MN's mechanical strength(76).The microneedle poses some physical characteristics such as mechanical strength of 0.41 N/needle and is swelled in not more than 5 min with the dissolution profile of 40 min. The difference between a subcutaneous hypodermic needle and a microneedle patch is that the MNs patch exhibited a rapid decline in plasma sugar levels in diabetics induced rats with controlled insulin release. Yang and coworkers devised a ‘swellable’ MN patch that could be self-adhered to the skin and used to delay insulin release (77).The dual laminated MN patch has swellable PS-PAA (Polystyrene-block-poly (acrylic acid) MN needles that may swell after implantation into the epidermis by storing bodily liquids, as well as a non-swellable PS layer. *In vitro* delivery ofinsulin from MNs was more consistent throughout a 12-hr period, with no bursts. The coated MNs, on the other hand, released more than >90% of the insulin. The release pattern of this swellable Microneedle showed a sustained low blood sugar effect in normal mice that lasted for 8 hr. The Jinfirmestablished a phase transition MN fix from PVA for transdermal insulin administration(78). When the microneedle was injected into the skin, the microcrystalline cross-linking made it inflate but not disintegrate, resulting in a continuous insulin release from the patch. Transdermal bioavailability in a diabetic pig model was shown to be greater than 20% in *in vivo* investigations. In new research, Di *et al.* included a stretchy MN patch for tensile straining activated subcutaneous insulin administration(79).The insulin-loaded microgel reservoirs inside the elastomer patch distorted under mechanical strain, allowing payloads to permeate into the cross-linked HA microneedle for transdermal delivery. In diabetic mice given this stretchy device, BGLs were successfully reduced in in vivo trials. Because of their appealing biocompatibility and robust mechanical capabilities, bio-ceramics have recently attracted increased attention in the area of subcutaneous drug transport. The elastic porous structure of bioceramics and the electrostatic interaction between the ceramic surface and bio-therapeutics, suggests that bioceramics can transfer biomolecules. Yu *et al.* designed organic-inorganic bioceramics composite microneedles MNs for expanded transdermal insulin administration utilizing gelatin and hydroxyapatite (80).Hydroxyapatite, which has a chemical makeup comparable to that of solidmammal tissues, is a biodegradable ceramic that has remainedextensivelyapplied in biomedical applications. When compared to hypodermicdose in diabetic induced rats, MNs manufactured of cross-linked gelatin &hydroxyapatite had adequate mechanical properties to permeate mammalian epidermis and had a successful antidiabetic action with the expanded the release of insulin in the blood. The scientists also studied calcium sulphate, and gelatine-linked composite MNs, which exhibited comparable behavior in insulin administration transdermally(81). Bio-responsive MNs that can react with physiological indications have been highlighted as a favorable methodology for glucose-controlled delivery of insulin(82–84).Glucose-responsive components are frequently combined with a polymeric MN matrix in this platform. Yu *et al.* described a glucose-responsive MN patch consisting of cross-linked HA matrix and glucose-responsive vesicles (GRVs) as a "smart insulin patch" in 2015, describing it as a painless, self-regulating method(85).

The ‘hypoxia-sensitive’‘hyaluronic acid’ derivatives (HS-HA), which included a hypoxia-sensitive group, 2-nitroimidazole, self-assembled the GRVs (NI). The hydrophobic NI of HS-HA is reduced to the hydrophilic 2-aminoimidazole under reductive circumstances, causing the nanovesicles to disassemble. The MNs were then implanted with GRVs encapsulating insulin and glucoseoxidase (GOx) to identify higher plasma sugar levels in the epidermis. GOx, a glucose-to-gluconic-acid-converting enzyme, has been extensively used as a sugar-sensing

component (86,87). During the GOx-catalysed oxidation of glucose, oxygen in the body fluid was consumed, driving a restricted hypoxic environment (88) (Figure 1).

HS-HA; Hypoxia sensitive hyaluronic acid that contains hypoxia sensitive group,*i.e.* 2-nitromidazole, GOx; Enzyme,*i.e.* used for the conversion of glucose to gluconic acid. Because of the bio-reduction of HS-HA, the enzyme-induced hypoxic microenvironment further triggered the disconnection of GRVs, resulting in insulin discharge. In the presence of glucose, the hypoxia-responsive GRVs are responsible for transporting insulin, and the plasma blood sugar level is drastically reduced to 200 mg/dL with 0.5 hr in type 1 diabetic mice and maintaining them in a regular array for up to 4 hr. In addition, with GRV (1/2E + I)-loaded MNs or GRV(I)-loaded MNs, an extra patch was able to extend the therapy period while minimizing the problem of hypoglycaemia. Adjusted with authorization from Ref [86], in addition to an enzyme-made hypoxic or acid atmosphere, the group of H_2O_2 during the reaction can likewise act as a trigger to promote insulin release from MNs (89). Hu *et al.* stateda bioresponsive MN fused with polymeric vacuoles (PVs) (90). PVs were shaped by self-assembling a block copolymer of polyethylene glycol (PEG) and phenyl-boronic ester (PBE)-conjugated polyserine and loading it into an MN patch. The enzymatically generated H_2O_2 oxidized the PBE pendant in high sugar levels, causing PV disassociation and insulin administration through the polymeric MN matrix. Modifying the quantity of GOx could modify the release patterns responding to glucose in this framework. In type 1 diabetic mouse model, the pairing of H_2O_2-responsive PVs and MNs demonstrated the ability of BGLs to self-regulate.

Fig. 1. Representation of the elements of bio responsive microneedle patch in a brief



An added ex. Tong *et al.* incorporated glucose-and H_2O_2-responsive PVs by manufacturing phenylboronic acid (glucose-sensitive) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl acrylate (H_2O_2-sensitive) functional group in the triblock copolymer (91). In a diabetic induced rat model, PVs-loaded MNs had a dominant hypoglycemic effect when compared to hypodermic injection or simply insulin-loaded MNs. Xu and colleagues created H2O2-responsive MSNs, which were subsequently overloaded with insulin and encapsulated in MNs for subcutaneous delivery (92).In hyper-glycemic state, the porous MSNs serves as an insulin reservoir, as well as a source of GOx for H_2O_2 production. The MSNs were replaced with the 4- (imidazoyl carbamate) phenylboronic acid pinacol ester to form a host-guest complexation with -cyclodextrin and thus keep insulin inside the MSNs. The phenylboronic ester on the membrane of the MSNs was oxidized when exposed to H_2O_2, ensuing in the disintegration of the host-guest complexes and the discharge of the preloaded insulin. In a recent study, mesoporous bioactive glasses were used as an insulin transporter and were combined with the MN device to dispense glucose-responsive insulin (93).The porous bioactive glasses were filled with insulin and 2 enzymes, GOx-CAT (Glucose oxidase/Chloramphenicol acetyltransferase) under hyperglycemiccircumstances, these ZnO QDs dissolved in the enzyme-regulated acid atmosphere, opening the voids on the bio-active glasses and releasing the enclosed insulin.

However, the objectionable by-product H_2O_2developed during the enzymatic oxidation of glucose may inhibit the concentration of GOx, slowing down the reaction rate. With prolonged use, the production of H_2O_2 may cause free radical-induced damage to skin tissue. Yu *et al.*developed hypoxia and H_2O_2 dual sensitive system based on polymersome-incorporated MNs for optimal insulin delivery to further improve glucose-responsive capacity (94).The dual sensitive polymersomes d-GRPs (diblock copolymer, glucose-responsive polymersome-based vesicles) were created using an amphiphilic diblock copolymer consisting of PEG and polyserine, in which the hypoxia-sensitive NI group was modifiedby an H_2O_2-responsive thioether moiety. Under highersugar levels, rapid oxygen usage and H_2O_2 synthesis by enzymatic processes increased the copolymer's water solubility, prompting the separation and discharge of insulin from the d-GRPs.*In vivo*experimentreveals that this film adequately controlled BGLs for 10 hr after administration with negligible skin inflammation. In another study, Wang *et al.* made core-shell organized MNs straightforwardly from H_2O_2-degradable polymeric gels(95).PVA network was cross-linked by an H_2O_2-cleavable linker, tetramethylpropane-1,3-diaminium (TSPBA), with an insulin chemical fastened to PVA through an H_2O_2-sensitive linkage, made up the central of MNs. GOx was enclosed in an acrylated nanogel (GOx-NG) to provide a massive size for covalent immobilization on PVA, limiting GOx spilling while maintaining insulin release simple. H_2O_2 was created locally by GOx under hyperglycaemic circumstances, causing oxidation and hydrolysis of both the PVA crosslinkers and insulin conjugates, promoting the rapid arrival of free insulin from the MNs.

Researchers confirmed that this H_2O_2-responsive insulin patch had a quick glucose response and could manage BGLs for 40 hours without severe hypoglycaemia when administered continuously with MNs. The MNs were also coated with a thin layer of nanogel incorporating H_2O_2scavenging enzyme (catalase) to facilitate H_2O_2 removal and reduce the physical threat of oxidative stress to normal tissues. This core-shell gelated MN patch successfully reduced oedema in dermis tissue treated with coated MNs in vivo when paralleled to noncoated MNs. A comparable research group recently developed a sheath-structured MN-based $H_2O_2$and pH cascade-activated insulin delivery system. (96).Insulin was entrapped in nanosized complex micelles by H_2O_2-sensitive and positively charged diblock copolymers. When incubated in hyperglycaemic conditions, this extremely positively charged polymer can be oxidized by $H_2O_2$and hydrolyzed into weakly positive-charged molecules. The decreased pH during glucose oxidation also reduced the density of negative charges on insulin, weakening the interaction between insulin and polymers and encouraging even more insulin release. Insulin was only discharged in both the oxidative and acidic environments created by glucose oxidation in the presence of GOx, thanks to a trigger mechanism that was dependent on both pH and H_2O_2.

Outside of synthetic insulin transporters, Ye *et al.* portrayed an inventive technique for transdermal insulin delivery, consolidating insulin-secreted pancreatic β-cells with MN patch for thetreatment of diabetes (97).Insulin was only discharged in both the oxidative and acidic environments created by glucose oxidation in the presence of GOx, thanks to a trigger mechanism that was dependent on both pH and H_2O_2.GOx was chosen to cause nanovesicle separation in hyperglycaemic circumstances. The released AM hydrolyzed the -amylose in the MNs to produce disaccharides and trisaccharide’s, which GA then converted to glucose.The "amplified" local concentrated glucose diffused effectively into the superficially located -cell capsules, boosting insulin release and diffusion through microneedles into the skin. When compared to MNs in the absence of GSAs, this design suggested prolonged bioavailability, as one such MN patch was observed to reduce plasma sugar levels in type 1 diabetic mice quickly and sustain the fall in sugar levels for more than 6 hours. The microneedle-based approach hasslightly greater delivery efficiency than other approaches because microneedles not only infiltrate the dermis to boost skin penetrability, but also convey insulin directly into the dermis layer.It is considered reasonable for persons with diabetes to use at home because of the valuable, straightforward,invasive, and painless application. It also shows outstanding promise for constant and successful

blood glucose management with approachable glucose moieties. However, any potential for breakage, irritation, or contamination should be methodicallyexamined before clinical application.

3.2.Vaccine delivery through microneedle

Moreover, successfullyaiming skin MNPs provides numerous benefits for vaccination, such as addressing logistical challenges to vaccine delivery. Several vaccines have been distributed with the assistance of MNs. (**Table 3**).

Table 3: Different types of vaccines delivered by microneedles

Vaccine type	Types of microneedle	References
Adenovirus	Coated and Dissolved microneedle	(98,99)
Amyloid β peptide	Dissolved microneedle	(100)
Botulism	Hollow microneedle	(101,102)
Chikungunya virus	Coated microneedle	(103)
Influenza	Hollow and Dissolved microneedle	(104)
Hepatitis C	Coated microneedle	(105)
Malaria	Dissolved microneedle	(106–108)
Japanese encephalitis	Hollow microneedle	(109)
Rotavirus	Coated microneedle	(110)
Poliovirus	Dissolved microneedle	(111)
Herpes simplex virus	Coated microneedle	(112,113)
Measles	Dissolved and Coated microneedle	(114,115)
Tetanus	Dissolved microneedle	(116)
Rabies virus	Hollow microneedle	(108)
Modified Vaccinia Ankara	Coated microneedle	(99,117)

Benefits of MN patches for vaccination. 1. Enhanced vaccine efficiency; 2. reduced demand for trained personnel; 3. Easy supply chain, 4. Decreased sharps risk, 5. Decreased vaccine wastage, 6. Vaccine reconstitutionwas not needed, 7. Lower vaccine/vaccination costs(118)vaccines are pharmacological formulations that contain the disease-causing antigen and can induce an immune response in a healthy human while not causing the disease (109). MNPs (microneedle patches) with an array of micron-sized needles with an adhesive backing were developed for vaccine delivery to the skin. These MNPs can be applied effectively and efficiently by pushing against the skin and, in some designs, do not produce sharps waste. The patches are simple to administer and do not require reconstitution because single-dose. Their small size simplifies storing, conveyance, and waste disposal, and they have the potential for upgrading vaccine immunogenicity,thermostability, and dose sparing.MNPs provide a prevailing new kind of technology that will allow for more effective vaccination(109).

MNPs are designed inthe form of solid metal, silicon, or polymer microneedles that is covered with a vaccine which delivers the vaccine when theouter covering dissolves into the skin, and solid and dissolved microneedles are made up

of water-soluble materials, which helps in the encapsulation ofthe vaccine and delivers the vaccines when the microneedles dissolve into the skin. Solid microneedleshave been used for the vaccination post skin pretreatment, followed by the application of topical vaccine formulation for delivery through the left opening of the skin and for delivery of liquid vaccine formulations into the skin hollow microneedles are being used.

MNPs contain a vaccine in a dried solid form that dissolves into the skin when administered, instead of hypodermic needles, which deliver the vaccine in a liquid form. Each MNP contains a single dose of vaccine and is simple to apply by pressing it on the skin with thehelp of thumb or using an applicator. The microneedles penetrate the skin when the MNP is applied on the skin, after that the patch is left behind on the skin for a few min to make them dissolved and deliver the payload within it. In coated MNPs, the coating dissolves but microneedles do not. Afterdissolved of MNP, the microneedles get dissolved into the skin and leave the backing only. MNPs targetvaccine delivery to the skin, the body's largest immunological organ andcompactly populated by antigen-presenting cells, which play a severe role in initiating an immune response.However, the Mantoux technique can be challenging to repeatusing intradermal injection with a hypodermic needle(119). MNPs providesa very straightforward and dependable method for targeting the skin and have been studied to deliver many vaccines (20,120–122).

MNPs have the potential to improvise the vaccination,but more work is desired to notice this potential. Overall, the translation of preclinical studies into clinical trials of MNP vaccination is critical, as is commercial manufacturing capable of mass-producing MNPs at a reasonable cost. There are some other things to think about.1. Increased vaccine effectiveness has been demonstrated in animal models, it has yet to be recognized in human subjects, and further investigation is required for the mechanisms linked with improved immunogenicity,2. Early research showed that MNPs could be used consistently by untrained personnel, including patients, but moreextensive testing and possibly improved MNP designs are required to ensure reliable vaccine delivery, 3. Reduced product size and increased vaccine thermostability are expected to simplify the supply chain; however, the true extent of thermostability and the actual impact on healthcare systems have yet to be determined,4. Sharps risk is expected to be reduced, particularly for dissolved MNPs, While MNPs reduce the risk associated with hypodermic needles, they may introduce new, unanticipated risks that become apparent only after they are placed in the hands of diverse users in diverse scenarios and cultures, 5. Although decreased vaccine waste and elimination of vaccine reconstitution appear to be inherent capabilities of MNP vaccines, the unintended consequences of these changes may present new challenges (118).

Small drugs (e.g., doxorubicin), genetic materials (e.g., pDNA and siRNA), proteins (e.g., ovalbumin), and even nanomedicines can be delivered using microneedle devices (123–125). The vaccine (immune or gene-based) is encapsulated in microneedles in a dried solid form, increasing its thermostability and making it easier to apply to the target area (119,126).

The goal of cancer immune-based vaccination is to activate the host's systemic immune response to eliminate tumour tissue (119). Antigen-presenting cells (APCs), such as dendritic cells, macrophages, and Langerhans cells, are densely populated in the skin, the body's biggest immune organ, therefore vaccine administration to the skin is typically investigated (127).These APCs can stimulate T (CD4+ and CD8+) and B cells, producing a systemic antitumoral immune reaction (68,128). Researchers have examined microneedle patches with immunostimulatory adjuvants and/or antigens as anticancer therapeutics (129).

To induce an antigen-specific immune response towards ovalbumin-expressing B16 melanoma tumours, Zaric *et al.*(130) used methylvinylether and maleic anhydride microneedles loaded with ovalbumin-loaded poly-D, L-lactide-coglycolide (PLGA) nanoparticles. To produce the MNs, the researchers employed a water-in-oil double emulsion process to make ovalbumin-loaded PLGA nanoparticles, which were then mixed with a methylvinylether and maleic

anhydride solution and placed onto silicone templates (19 by 19 arrays). The findings revealed that the microneedles could pierce the mouse skin and reach the dermal layer and local dendritic cells in *ex vivo* experiments. They also observed that the dendritic cells that had been transfected could travel to the proximal lymph nodes, activate CD8+ T cells, and generate cytokines like IFN-γ.Moreover, the immune system stimulation mediated by nanoparticles delivered by microneedles inhibited ovalbumin-expressing B16 melanoma tumours from developing for 13 days.

Microneedles can be designed to reverse or bypass tumour cells immune suppressive signals to deliver antibody-based immune treatments. Anti-PD1 antibody (aPD1) and 1-methyl-DL-tryptophan (1-MT) administration to B16F10 melanoma tumours was mediated by a hyaluronic acid-based microneedle array created by Ye and colleagues (131). The aPD1 receptor targets the PD-1 receptor expressed by T cells, permitting it to evade the inhibitory signalling of cancer cells, which limits T cell activation (51). 1-MT, but on the other side, it inhibits the immunosuppressive enzyme indoleamine 2,3-dioxygenase, causing an increased immunological response (132).The conical-shaped microneedle arrays were made using silicone moulds (15 by 15). The authors did this by chemically grafting the 1-MT to hyaluronic acid chains, which were then used to make micelles carrying aPD1microneedles, the micelles were loaded on silicone moulds, dried, covered with extra 1-MT modified hyaluronic acid, and dried at room temperature under vacuum. The researchers found that 1-MT and aPD1 were released in reaction after the breakdown of microneedles and micelles(131). Additionally, the microneedles possessed a breakage force of 0.41 N, which allowed them to pierce the dorsum skin of the mice and deliver their content at a depth of 200 μm. Furthermore, microneedle injection to mice bearing B16F10 melanoma cells boosted 1-MT and aPD1 retention in tumour tissue. The aggregation of 1-MT in melanoma was significantly 3-fold higher in the microneedle group on day 1 and 5-fold higher on days 2 and 3 than in the free 1-MT group. However, combined delivery of aPD1 and 1-MT through microneedles decreased tumour growth (tumour area less than 50 mm^2, compared to more than 300 mm^2 in the control group) and enhanced mouse survival.

Microneedles have also been examined for the administration of antitumoral gene treatments, in addition to their utility in triggering immune system activation through the introduction of antigens, adjuvants, or even antibodies. For the treatment of cervical cancer, Ali and colleagues developed a polyvinylpyrrolidone microneedle patch comprising E6/E7 pDNA RALA nanoparticles (133). By micromolding, the microneedles were created with the help of a solution of PVA containing E6/E7 pDNA/RALA nanoparticles in a negative mould. Moreover, the authors discovered that 90 % of the needle length could be introduced in the pig skin with the manual application, and within 15 min, the microneedle tips were completely dissolved inside the skin layers. Besides that, after the application of the microneedle, the concentrations of antibodies were two times higher when compared with those of the control group, and more responsiveness was shown by the T cells to HPV-16 oncogenic antigen-expressing cells (TC-1) (in the control group IFN- γ levels were ≈ 250 pg/mL, and ≈ 530 pg/mL for the microneedle immunized group). This increased immune response inhibited the formation of cervical tumours in 4 of the 9 mice treated with microneedles. Additionally, providing microneedles to mice with cervical tumours showed in tumour growth, 246 mm^2 in MN-treated mice, and 503.13 mm^2 in RALA-E6/E7 nanoparticle-vaccinated mice.

Contrarily, Pan and colleagues used microneedle patches to deliver polyethyleneimine/STAT3 siRNA complexes to skin melanoma tumours made up of dextran, hyaluronic acid, and polyvinylpyrrolidone (134). In a variety of tumours (including melanoma, breast cancer, and prostate cancer), STAT3 is one of the proteins that is overexpressed and has the capability to prompt the cancer cell proliferation, angiogenesis, survival, and immune evasion (135). Polyethyleneimine/STAT3 siRNA complexes were combined with a dextran/hyaluronic acid/polyvinylpyrrolidone solution, and then pyramidal needles were cast in PDMS moulds (12 by 12 arrays). When a greater force is applied, more than 20 N, almost 100 % of the microneedle tips pierce the skin of the rat; the microneedles have a failure force

of 86 N, allowing polyethyleneimine/STAT3 siRNA complexes to be delivered at a depth of 330 μm (136). The authors showed that topical injection of STAT3 siRNA using microneedles might cause necrosis in 40 % of tumour cells and lower STAT3 mRNA expression by 30 %. This action inhibited the growth of the melanoma tumour, resulting in a tumour weight that was 5 times lesser than the control.

3.1. Gene delivery through microneedle

Gene therapy involves the insertion of healthy foreign genes into target cells to treat the diseases caused by gene defects and abnormalities (137–139). Gene therapy development has been delayed by the target gene metabolizing in the circulation or being inactive beforereaching the target cells. MNPs paved the path for gene therapy by transferring the gene to the specific cells on the skin's surface (140). Numerous physical mechanisms for delivering DNA therapies through the SC barrier have also been examined, with varying levels of success, viz, electroporation(141), liquid jet injection (142), gene gun delivery (143), and MN delivery. Electroporation and gene gun delivery necessitate costly equipment and specialized skills for health care workers to be used clinically. A more uncomplicated, less expensive, and patient-friendly technique of delivering DNA over skin barriers will be clinically advantageous in the area of nucleic acid therapeutics (140). To deliver plasmid DNA and siRNA for cancer therapy, Wei *et al.*employed a flexible microneedle array. This microneedle array demonstrated efficient DNA delivery capability and proved to be a promising method for gene therapy (143). Furthermore, siRNA therapy has much promise for treating skin cancer. Nonetheless, the lack of appropriate tools for delivering siRNA limits its applicability. Chong *et al.* developed a coated steel microneedle for siRNA delivery into the skin for gene silencing. The siRNA-coated microneedles silenced the gene in vivo while also providing a convenient and patient-friendly siRNA delivery method for siRNA therapy (144).

Solid MN arrays can be used to disrupt the SC barrier either before or after applying topical DNA to the skin. The DNA can be mixed into a controlled release formulation or used as a solution. When MNs are administered before the DNA, the MNs transport the DNA into the pores, allowing it to travel via the skin. Otherwise, if the MNs are applied to the skin before applying the DNA formulation, the DNA will gradually diffuse into the previously created openings in the skin. Both approaches have been used in the delivery of genes (145). Pearton *et al.* (2008) investigated DNA delivery methods. A controlled release component was induced by the delivery method by mixing the DNA carrier with a hydrogel formulation. Using silicon MNs, the rupture was done of the SC barrier of donor human breast tissue which was pre-coated with a PLGA-PEG-PLGA hydrogel formulation and loaded with plasmid DNA (100 μg) containing the -galactosidase reporter gene. After that, the skin was cultured up overnight, and the reporter gene expression was evaluated 48 hr later. When the treated skin was examined, it was discovered that gene expression was observed only at the site of SC disruption by the MNs, probably because of better DNA interaction with epidermal cells. As a result, whereas the delivery system in this work successfully triggered areas of gene expression, further evaluation and optimization are needed before delivering a therapeutically relevant plasmid DNA. Several researchers have modified this method by using solid MNs which were coated with DNA in the form of a film on the surface of MN to create micropores in the SC (106,145,146). By inserting the device in the skin, the film coating gets dissolved by the skin, and to enter the dermal layer for cellular absorption is done by allowing the DNA and DNA- containing combinations.

Early research on using coated MNs concentrates on delivering naked DNA for vaccination against various therapeutically essential diseases. Gill *et al.* (2010) performed a DNA vaccination study to compare the efficacy of several delivery methods inducing an antigen-specific cytotoxic T cell response.The researchers evaluated immunological responses to plasmid DNA encoding hepatitis C virus nonstructural 3/4A protein administration through coated MN array, i.m. injection, and cutaneous delivery via a gene gun. The study discovered that one

application of DNA-coated MNs and a gene gun mediated immunotherapy, delivering 8 and 4 µg of DNA, respectively. This resulted in comparable antigen-specific CD8+ T cell activation. Moreover, a 30-fold increase in the dose of DNA administered through the IM route was necessary to produce a comparable level of immunological response. These findings demonstrate that DNA administration via coated MN array devices is a feasible delivery technique for CD8+ T cell priming.

It is a considerably superior delivery strategy in this situation when compared to traditional IM immunization, with the potential to be a dose-saving alternative (146).Since these findings are encouraging, it is impossible to say whether this delivery mechanism is capable of successful immunization because no challenge studies were performed to establish disease protection. The origin of dissolved MNs is one of the most recent and promising breakthroughs in the field of MN delivery.

There has been a shift in focus in the field of MN gene delivery away from the use of solid MN arrays and toward the development of dissolved delivery platforms, as demonstrated by an increase in the proportion of publications on the development of these dissolved delivery platforms. The idea of polymeric MNs encapsulating drugs within their matrix was offered as a way to overcome some of the limitations associated with solid MN arrays, such as portability, cost-effectiveness, and sharp disposal after usage (145).Dissolved MNs when coming in touch with the interstitial fluid after the application, they disintegrate by releasing the encapsulated medicine for local or systemic distribution. Gonzalez-Gonzalez *et al.*(2010) created a loadable, soluble protrusion array device (PAD) made up of polyvinyl alcohol (PVA), with 12 MN projections and each of them were roughly loaded with 12 ng DNA to allow SC penetration and DNA delivery (147). Therefore, when PVA MNs are injected into the skin, they breakdown and create a gel-like plug, releasing the nucleic acid payload, combining the delivery features of MNs with the drug release of a biocompatible polymer. In the ear of the mouse paw and flank skin, the *in vivo* delivery of a plasmid DNA encoding the luciferase reporter gene was tested, and bioluminescence was found in all animals after treatment of 24 hr, showing DNA delivery and monitoring gene expression at these sites of delivery (147).

The comparison was done between the PAD delivery system and the coated metal MN array for the delivery of DNA encoding the luciferase reporter gene(148). The researchers applied *in vivo* bioluminescence imaging to track reporter gene expression in living animals across time.Initially, the footpads of mice treated with different MN arrays were equivalent; however, those treated with coated metal MN arrays later showed greater gene expression levels. Up to 25 days after MN treatment, luciferase expression was seen in both of these delivery strategies. The PAD device's limited loading capability, 0.1 µg, compared to 3 µg coated onto the metal MN array was the main contributory explanation for the difference between the two delivery techniques. Concluding that the dissolving MN arrays have the potential for DNA delivery that would result in *in situ* sustained gene expression; nevertheless, formulation improvement is required to boost DNA carrying capacity.

3.4. Protein, peptide and antibody-based therapeutics delivery by microneedle

Proteins and peptides are highly specific and potent antimicrobial agents. They have shown tremendous promise as novel therapeutics in the treatment of a variety of diseases. Large molecule drugs have higher potency, activity, low unspecific binding, lower toxicity, less drug-drug interaction, and greater biological and chemical diversity compared to small molecule drugs(149).

Insulin delivery is not the only application for solid MN research. Li *et al.* (2010), for example, explored the properties of solid MN (metal DermaRoller™ and maltose) pretreatment on the *in vivo* transdermal delivery of human immunoglobulin G (IgG) (5 mg/mL applied concentration). The DermaRoller™ ability to makebroaderMN channels was attributed to increased flux and Cmax [158]. Cui *et al.* (2011) investigated the extent to which pretreatment with MNs (DermaRoller™, 250 µm, 500 µm, and 1000 µm) could improve ovalbumin-conjugated nanoparticle skin

permeation *in vitro* and *in vivo*. In *in vitro* studies, MN pretreatment significantly increased ovalbumin permeation compared to the control.Furthermore, ovalbumin in solution delivered 28.3± 6.5 % ofovalbumin transdermally, whereas ovalbumin nanoparticles delivered 13.6 ± 2.4 % of ovalbumin. The larger size of the ovalbumin nanoparticles hampered diffusion. Transcutaneous immunization with ovalbumin nanoparticles following MN pretreatment was greater when administered as a 70 µg/mouse dose (40).In 2013, Han and Das improved BSA distribution over pig ear skin by using sonophoresis and MNs together. When MNs and sonophoresis were not employed together then the BSA permeability was 0.43 and 0.40 µm/s, respectively; however, by combining these two physical strategies for the enhancement of permeation (1.5 mm MNs and 15-W ultrasound) were employed together, then it was observed that the permeability increased to 1 µm/s.It was reported to be around three times higher than the passive BSA diffusion (150). In 2014, Zhang *et al.* investigated the permeability transdermally of all four model peptides over the ear skin of a pig right after solid silicon MN pretreatment of 150 µm. Likewise in Cui *et al.* (2011), the transdermal permeability was altered due to the molecular weights of the peptides. While increasing the molecular weight of peptides, the amount of delivery was transdermally, and MN pretreatment showed remarkable enhancement in the penetration of all peptides (151).

The skin contains a high concentration of Langerhans and dendritic cells, so due to this even a minimal dose of vaccine given through the skin can elicit the necessary immunological response (152). A small amount of vaccine is adequate, the claimed drawback of limited drug loading on coated MNs does not apply, which explains the popularity of coated MNs for vaccine delivery. Saurer *et al.* (2010) used a layer-by-layer approach to coat stainless steel MNs with DNA and protein-containing polyelectrolyte films.The authors cited five key benefits of using these types of films: precise control over film thickness and thus drug concentration; no organic solvents are required in the fabrication process, improving MN safety; fabrication of films provides control over the release of defined amounts of multiple different agents;auxiliary agents (e.g., cationic polymers) can be incorporated into the films, and the fabrication process can coat objects with irregular shapes, such as medical devices or implantable materials. Fluorescence and optical microscopy were used to characterize the release of protein and DNA from the coated MNs after 2 hr of insertion into porcine cadaver skin in this study. The ability of the coated layer to be released almost entirely from the solid MNs and delivered into the epidermal and dermal layers of the skin is demonstrated by post-insertion fluorescence images (153). Recognizing that MN-mediated drug delivery has primarily focused on hydrophilic molecules, Zhao *et al.*(2017) For antigen-specific immunotherapy of type 1 diabetes, a novel formulation was made for the delivery of autoantigen peptides by a coating of MNs. For dissolved both hydrophilic and hydrophobic peptide auto-antigens at clinically relevant concentrations, three co-solvents (water, 2-methyl-2-butanol, and acetic acid) and PVA 2000 were used. Both *in vitro* (human skin) and *in vivo* (mouse skin) investigations were conducted to establish the potential of hydrophobic peptides to be administered via coated MNs. Delivery was enhanced when the metal surface of the MN array was electropolished, the thickness of the peptide coating was lowered, and peptides with better aqueous solubility were used (154).

Caudill *et al.* (2018) used PEG MNs to deliver BSA both *in vitro* and *in vivo*. MNs were placed in a solution-filled coating mask device, then removed and left for drying beforebeing pierced through the skin. Following 5 min of MN insertion, *in vitro* permeation of FITC-BSA loaded MNs (1000 µm, 64 needles/cm^2) across full-thickness porcine skin was found to be 45% at 24 hr. The concentration of FITC-BSA appeared to be concentrated in the epidermis, upper layers of the dermis, and around the microneedle penetration sites, with little fluorescent signal noticed in the lower dermis. The needle density and needle length were found to be important factors in the effectiveness of MNs (155). The *in vitro* data was followed by an *in vivo* study, according to the authors. MNs (700 µm in height) were coated in 7% BSA solution and placed on the backs of BALB/c mice for 2 min. MN-treated mice retained BSA at the

administration sitefor a longer period of time compared to a control subcutaneous dose.Moreover, BSA was reserved in the skin for a prolonged period of time than it was in the subcutaneous dose. At 6 hr and 72 hr, MN-treated mice had 79 % and 19 % fluorescence signal remaining, respectively. In comparison, the subcutaneous dose resulted in 14 % and 4 % fluorescence signal retention at 6 hr and 72 hr, respectively. The presence of high molecular weight methylcellulose (MW 17,000 Da) within the MN formulation was attributed to the depot effect, which kept the coated BSA near the administration site for a longer period of time.

Li *et al.*(2018) coated the surfaces of individual metal MNs with a variety of compounds (immiscible molecules, proteins, and nanoparticles) to allow the delivery of multiple therapies within the same MN patch. Compounds of various sizes and shapes, particles and free drugs were used to depict virtually any type of therapy that could be used in MN systems. MNs were applied for 5 sec to full-thickness porcine skin and taken away after 2 min. In the *in vitro* experiment, the protein employed was FITC-BSA, which came with free fluorescein sodium dye and fluorescently labeled nanoparticles. All three chemicals were successfully supplied, although at variable rates, according to the data. The transport of FITC-BSA was in the middle of the three compounds, with fluorescein sodium dye diffusing the fastest and fluorescently labelled nanoparticles diffusing the slowest (156). Dissolved MNs were researched for vaccine delivery (157–159), and with some advantages, actively support their investigation for protein and peptide drug delivery. Mönkäre*et al.* (2015) developed monoclonal IgG-loaded hyaluronan-based dissolved MNs for intradermal administration *invitro*.After 10 min ofadministration of 280 µm length microneedle, the bulk of the initial tip length (65 %) was dissolved, while IgG and hyaluronan were co-deposited to a depth of 150–200 µm in the skin. According to the authors, the low molecular weight of hyaluronan probably enhanced the dissolution rate when compared to earlier research that used hyaluronan as the basis of their dissolved MNs (159). Chen *et al.*(2016) developed an interferon-α-2b formulation containing dissolved MNs (680 µm in length) for transdermal drug delivery. The drug release efficiency *in vitro* was 49.2 %. At 40 min, *in vivo* studies revealed a C_{max} and T_{max} of 11.58 ng/mL. For two months, the dissolved MNs were stable enough. The bioequivalence of dissolved MNs and intramuscular (IM) injection control was closely related, implying that IM injections of interferon-α-2b could be replaced with MNs for self-administration and enhanced patient compliance(160).

Dillon *et al.* developed a dissolved MN system of PVA and trehalose to encapsulate active pharmaceutical peptides within the MN matrix (2017). Polymyxin B-loaded MNs were applied to the skin of the porcine ear for 30 s.The rate of drug delivery was found to be higher than the control (drug-loaded disc without MNs) for the first 4 hr following MN application, after which the rate of permeation was equal to the control, but the percentage of drug delivered transdermally was significantly higher. 66.9 ± 11.59 % of polymyxin B was delivered transdermally at the end of the 22-hr Franz cell experiment, compared to 54.14 ± 3.01 % for the control (161). It is evident that many parameters can be adjusted to improve the stability and activity of drugs encapsulated within a dissolved MN system. The impacts of polymer type, concentration, drying conditions, and storage temperature on the activity of lysozymes (model protein) loaded in dissolved MNs were studied by Lahiji, Jang, Huh, *et al.* (2018) and Lahiji, Jang, Ma, *et al.* (2018).When manufactured at 4 °C, allowed to dry naturally, and manufactured in the presence of stabilizing agents such as trehalose, lysozyme activity was preserved up to 99.8 ± 3.8 % for 12 weeks (135,162). Vora *et al.* (2020) acknowledged that the number of water-soluble, biodegradable polymersthat can be used to make dissolved MNs is limited. For the first time, they used a carbohydrate biopolymer (pullulan) to facilitate the delivery of FITC-BSA across the dermatome of neonatal porcine skin. *In vitro* studies were used to evaluate the transdermal delivery of FITCBSA from the novel dissolved MNs (600 µm needle length) after ensuring that the FITC-BSA remained intact in the formulation. FITC-BSA was detectable as early as 15 min after MN insertion, and at 28 hr, the dissolved MNs delivered 1105 123 g/cm^2. As a result, the authors demonstrated for the first time the ability of the carbohydrate

biopolymer pullulan to fabricate dissolved MNs for the successful delivery of high molecular weight compounds like FITC-BSA (163).

The majority of researches on hollow MN arrays has concentrated on fabrication aspects, such as design and characterization studies. As a result, they have received less attention in terms of their actual efficiency in delivering drug molecules across the skin (164). Again, the intradermal delivery of vaccines has been emphasized, particularly those loaded with nanoparticles that could not be delivered by other means, such as coated MNs (165,166). There has also been a comparison with dissolved MNs (167).

Chen *et al.* questioned the delivery of high molecular weight compounds into the skin (2010). The authors hypothesized that a combination of sonophoresis, a technique that uses low-frequency ultrasound to induce acoustic cavitation in the lipid layers of the SC, and MNs could answer this question. Calcine and BSA transdermal delivery were measured passively using sonophoresis or hollow MNs (300 µm length) alone or when the two methods were combined (SEMA). SEMA > sonophoresis alone > hollow MN alone > passive diffusion was the order of transdermal delivery for both compounds(168).

Although this study successfully proved that the two physical methods of permeation enhancement could enhance the transdermal delivery of macromolecules, the addition of sonophoresis to MNs eliminates some critical benefits of MNs, namely the ability for self-administration and the accessibility associated with the small array. Sonophoresis, like the use of solid MNs, returns the device to a two-step process. Torrisi *et al.* (2013) developed a “pocketed” MN device designed for intradermal delivery of botulinum toxin A to reduce pain, improve therapeutic targeting, and streamline the administration procedure. For liquid drug reservoir loading, pockets were cut into stainless steel MN shafts. Microneedle-mediated intradermal delivery of β-galactosidase and formaldehyde-inactivated botulinum toxoids achieved impressive deposition and subsequent dermal diffusion (103). Golombek *et al.* demonstrated *in vitro* intradermal delivery of synthetic mRNA using hollow MNs (2018). Following hollow MN penetrating, high levels of humanized Guassia luciferase (hGLuc) protein were detected. Levels after 24 and 48 hr were significantly higher than the control "naked mRNA."(169).

Hydrogel-forming MNs are a newer type of MNs than those discussed previously (170). As a result, many studies have focused on altering parameters that may affect their swelling capabilities and, as a result, their ability to deliver drugs transdermally. Polymer content (171), cross-linking agent molecular weight (172), cross-linking agent concentration (173), and foaming agent presence (174) are all factors that influence transdermal drug delivery from hydrogel-forming MNs.Courtenay *et al.* (2018) used this method to compare dissolved and hydrogel-forming MNs (500 µm needle length) for *in vivo* bevacizumab transdermal delivery. The dissolved MNs achieved a higher C_{max} at a faster rate (488.7 ng/mL at 6 hr) than the hydrogel-forming MNs (81.2 ng/mL and 358.2 ng/mL at 48 hr for hydrogel-forming MNs containing 5 mg and 10 mg of bevacizumab, respectively). The differences in the pharmacokinetic profile were attributed to bevacizumab's molecular weight (149,000 Da). It was proposed that the diffusion of the large macromolecule caused the delayed C_{max}through the tortuous hydrogel network instead of dissolved MNs (175). Because PVA is a hydrophilic polymer, incorporating bevacizumab into PVA dissolved MNs would allow for immediate dissolution and drug release following MN insertion. Because dissolved MNs appeared to release the drug as a bolus, but hydrogel-forming MNs showed a sustained release profile, the MN type could be tailored to the desired pharmacokinetic profile for the delivery of high molecular weight macromolecules.

3. Conclusion

Despite the fact that the intravenous route provides 100% bioavailability, patients often report discomfort and needle anxiety, which might lead them to refuse therapy. A lengthy history of skin-to-drug medication administration is associated with transdermal patches. The many benefits of TDDSs over other drug delivery methods, such as oral and

parenteral, have led to their widespread acceptance.Because of its tremendous value in transdermal administration of extremely large molecules with ionic and hydrophilic characteristics, the microneedle array has drawn the attention of researchers. The transdermal medication delivery system makes extensive use of microneedles due to its efficacy, safety, convenience, and lack of discomfort. Without irritating the skin's nerve endings, microneedles introduce the medicine to the dermis. Incredible therapeutic outcomes have resulted from the widespread use of microneedles for the delivery of medications, genes, proteins, RNA, and vaccinations in the last few years. Consequently, a microneedle-based medication delivery system may be studied as a possible remedy for the poor delivery of several macromolecules by traditional transdermal methods. Products derived from microneedles are eagerly awaited for market release because of the profound influence they may soon have on clinical care.

Abbreviations: kDa- kilo Dalton, UV- Ultraviolet, wt- weight, SC- Subcutaneous, Da- Dalton, DNA- Deoxyribonucleic acid, RNA- Ribonucleic acid, µm- Micrometres, MN- Microneedle, API- Active pharmaceutical ingredients, GUMP- Glucose measurement using microneedle patches, MNA-D- Microneedle array- Doxorubicin, CTCL-Cutaneous T-cell Lymphoma, ID- Intradermally, BLG- Blood glucose level, °C- Degree Celsius, HA- Hyaluronic acid, CMC- Carboxymethylcellulose, PVP- Polyvinylpyrrolidone, PVA-Polyvinyl alcohol, IU- International unit, DAB- Droplet-born air blowing, PLGA- Polylactic-co-glycolicacid, ITP- Iontophoresis, PMVE/MA- Poly(methyl vinyl ether-co-maleic acid), PS-PAA- Polystyrene-*block*-poly(acrylic acid), GRVs- Glucose responsive vesicles, NI- Nitromidazole, HS-HA- Hypoxia-sensitive hyaluronic acid derivatives, GOx- Glucose oxidase, dL- Decilitre, PVs- Polymeric vesicles, PEG- Polyethylene glycol, PBE- Phenylboronic ester, MSNs- Mesoporous silica nanoparticles, GOx/CAT- (Glucose oxidase/Chloramphenicol acetyltransferase), d-GRPs- diblock copolymer, glucose-responsive polymersome- based vesicles, TSPBA- Tetramethylpropane-1,3-diaminium, GOx-NG- Glucose oxidase nanogel, GSAs- Glucose-signal amplifiers, AM- Amylase, GA- Glucoamylase, MNPs- Microneedle patches, siRNA- Small interfering RNA, APCs- Antigen-presenting cells, CD4+- Cluster of differentiation 4+, CD8+- Cluster of differentiation 8+, IFN-γ- Interferon- γ, aPD1- Anti-PD1 antibody, 1-MT- 1-methyl-DL-tryptophan, RALA- Ras-related protein Ral-A, STAT3- Signal transducer and activity of transcription 3, PAD- Protrusion array device, URTI- Upper respiratory infection, IM- Intramuscular, IV- Intravenous, SC- Subcutaneous, IgG- Immunoglobulin, BSA- Bovine serum albumin, FITC-BSA- Fluorescein isothiocyanate labelled bovine serum albumin, MW- Molecular weight, SEMA- Sonophoretic enhanced microneedle array, hGLuc- Humanised Guassia luciferase, ITP- Iontophoresis, TDDS- Transdermal drug delivery system.

STATEMENTS AND DECLARATIONS

Author Contributions: Investigation, original draft, writing review.; formal analysis, data curation, validation, editing.; conceptualization, resources, supervision, All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of this work, ensuring integrity and accuracy. All authors have read and agreed to the published version of the manuscript.

Ethical Approval and Consent to Participate (Applicable if animals/human or cell lines are used)

Not applicable.

Human and Animal Rights Participate (Applicable if animals/human are used)

Not applicable.

Format for Availability of Data and Materials

Not applicable.

Format for Consent for Publication

Not applicable.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgments

The author also expresses his gratitude to Chairman, S. GURDEV SINGH BRAR, IAS (RETD.) and Director, Dr. Gursevak Singh, Principal Dr. Sukhpreet kaur Dasmesh College of Pharmacy, Faridkot (Punjab), India, for their excellent vision and support.

References

1. Duarah S, Sharma M, Wen J. Recent advances in microneedle-based drug delivery: Special emphasis on its use in paediatric population. Eur J Pharm Biopharm. 2019 Mar 1;136:48–69.
2. Singh N, Kalluri H, Herwadkar A, Badkar A, Banga AK Transcending the skin barrier to deliver peptides and proteins using active technologies. Crit Rev Ther Drug Carrier Syst. 2012 Jun ;29(4):265–98.
3. McCrudden MT, McAlister E, Courtenay AJ, González-Vázquez P, Raj Singh TR, Donnelly RF. Microneedle applications in improving skin appearance. Exp Dermatol. 2015 Aug 1 ;24(8):561–6.
4. Hampton T. Breaking barriers in transdermal drug delivery. JAMA. 2005 May 4 ;293(17):2083.
5. Chen MC, Ling MH, Lai KY, Pramudityo E. Chitosan microneedle patches for sustained transdermal delivery of macromolecules. Biomacromolecules. 2012 Dec 10 ;13(12):4022–31.
6. Jijie R, Barras A, Boukherroub R, Szunerits S. Nanomaterials for transdermal drug delivery: beyond the state of the art of liposomal structures. J Mater Chem B. 2017 ;5(44):8653–75.
7. Dharadhar S, Majumdar A, Dhoble S, Patravale V. Microneedles for transdermal drug delivery: a systematic review. Drug Dev Ind Pharm. 2019 Feb 1;45(2):188–201.
8. Migalska K, Morrow DI, Garland MJ, Thakur R, Woolfson AD, Donnelly RF. Laser-engineered dissolving microneedles for active transdermal delivery of nadroparin calcium. Eur J Pharm Biopharm. 2012 Oct ;82(2):299–307.
9. Wang Q, Jaimes-Lizcano YA, Lawson LB, John VT, Papadopoulos KD. Improved dermal delivery of FITC-BSA using a combination of passive and active methods. J Pharm Sci. 2011 ;100(11):4804–14.
10. Donnelly RF, Majithiya R, Singh TR, Morrow DI, Garland MJ, Demir YK, Migalska K, Ryan E, Gillen D, Scott CJ, Woolfson AD. Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. Pharm Res. 2011 Jan ;28(1):41–57.
11. Bariya SH, Gohel MC, Mehta TA, Sharma OP Microneedles: an emerging transdermal drug delivery system. J Pharm Pharmacol. 2012 Jan ;64(1):11–29.
12. Gupta J, Gill HS, Andrews SN, Prausnitz MR. Kinetics of skin resealing after insertion of microneedles in human subjects. J Control Release. 2011 Sep 5 ;154(2):148–55.
13. Haq MI, Smith E, John DN, Kalavala M, Edwards C, Anstey A, Morrissey A, Birchall JC. Clinical administration of microneedles: skin puncture, pain and sensation. Biomed Microdevices. 2009 ;11(1):35–47.
14. Burton SA, Ng CY, Simmers R, Moeckly C, Brandwein D, Gilbert T, Johnson N, Brown K, Alston T, Prochnow G, Siebenaler K. Rapid intradermal delivery of liquid formulations using a hollow microstructured array. Pharm Res. 2011 Jan ;28(1):31–40.
15. Ita K. Transdermal Delivery of Drugs with Microneedles-Potential and Challenges. Pharmaceutics. 2015 Jun 29 ;7(3):90–105.
16. Polat BE, Blankschtein D, Langer R Low-frequency sonophoresis: application to the transdermal delivery of macromolecules and hydrophilic drugs. Expert Opin Drug Deliv. 2010 Dec ;7(12):1415–32.
17. Münch S, Wohlrab J, Neubert RH. Dermal and transdermal delivery of pharmaceutically relevant macromolecules. Eur J Pharm Biopharm. 2017 Oct 1 ;119:235–42.
18. Zhang Y, Yu J, Kahkoska AR, Wang J, Buse JB, Gu Z. Advances in transdermal insulin delivery. Adv Drug Deliv Rev. 2019 Jan 15 ;139:51–70.
19. Prausnitz MR.. Microneedles for transdermal drug delivery. Adv Drug Deliv Rev. 2004 Mar 27 ;56(5):581–7.
20. Kim YC, Park JH, Prausnitz MR. Microneedles for drug and vaccine delivery. Adv Drug Deliv Rev. 2012 Nov ;64(14):1547–68.
21. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2013;131(3):636–45.
22. Jin X, Zhu DD, Chen BZ, Ashfaq M, Guo XD Insulin delivery systems combined with microneedle technology. Adv Drug Deliv Rev. 2018 Mar 1 ;127:119–37.
23. Rzhevskiy AS, Singh TR, Donnelly RF, Anissimov YG. Microneedles as the technique of drug delivery enhancement in diverse organs and tissues. J Control Release. 2018 Jan 28 ;270:184–202.
24. Donnelly RF, Larrañeta E. Microarray patches: potentially useful delivery systems for long-acting nanosuspensions. Drug Discov Today. 2018 May 1 ;23(5):1026–33.
25. Henry S, McAllister D V., Allen MG, Prausnitz MR. Microfabricated microneedles: A novel approach to transdermal drug delivery. J Pharm Sci. 1998;87(8):922–5.
26. Larrañeta E, McCrudden MT, Courtenay AJ, Donnelly RF. Microneedles: A New Frontier in Nanomedicine Delivery. Pharm Res. 2016 May 1

;33(5):1055–73.

27. Martanto W, Davis SP, Holiday NR, Wang J, Gill HS, Prausnitz MR. v Transdermal delivery of insulin using microneedles in vivo. Pharm Res. 2004 Jun ;21(6):947–52.

28. Zhou CP, Liu YL, Wang HL, Zhang PX, Zhang JL Transdermal delivery of insulin using microneedle rollers in vivo. Int J Pharm. 2010 Jun ;392(1–2):127–33.

29. Wu Y, Gao Y, Qin G, Zhang S, Qiu Y, Li F, Xu B. Sustained release of insulin through skin by intradermal microdelivery system. Biomed Microdevices. 2010 Aug ;12(4):665–71.

30. McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, Allen MG, Prausnitz MR. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. Proc Natl Acad Sci U S A. 2003 Nov 25 ;100(24):13755–60.

31. Davis SP, Martanto W, Allen MG, Prausnitz MR. Hollow metal microneedles for insulin delivery to diabetic rats. IEEE Trans Biomed Eng. 2005 May;52(5):909–15.

32. Kochba E, Levin Y, Raz I, Cahn A Improved Insulin Pharmacokinetics Using a Novel Microneedle Device for Intradermal Delivery in Patients with Type 2 Diabetes. Diabetes Technol Ther. 2016 Sep 1 ;18(9):525–31.

33. Ye Y, Yu J, Wen D, Kahkoska AR, Gu Z Polymeric microneedles for transdermal protein delivery. Adv Drug Deliv Rev. 2018 Mar 1 ;127:106–18.

34. Quinn HL, Larrañeta E, Donnelly RF. Dissolving microneedles: safety considerations and future perspectives. Ther Deliv. 2016 May 1 ;7(5):283–5.

35. Hong X, Wei L, Wu F, Wu Z, Chen L, Liu Z, et al. Dissolving and biodegradable microneedle technologies for transdermal sustained delivery of drug and vaccine. Drug Des Devel Ther. 2013;7:945–52.

36. An M, Liu H. Dissolving Microneedle Arrays for Transdermal Delivery of Amphiphilic Vaccines. Small. 2017 Jul 12 ;13(26).

37. Li G, Badkar A, Nema S, Kolli CS, Banga AK. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. Int J Pharm. 2009 Feb 23 ;368(1–2):109–15.

38. Lee K, Lee CY, Jung H. Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose. Biomaterials. 2011 Apr ;32(11):3134–40.

39. Kalluri H, Banga AK. Formation and closure of microchannels in skin following microporation. Pharm Res. 2011 Jan ;28(1):82–94.

40. Li G, Badkar A, Kalluri H, Banga AK. Microchannels created by sugar and metal microneedles: characterization by microscopy, macromolecular flux and other techniques. J Pharm Sci. 2010 ;99(4):1931–41.

41. Lee JW, Choi SO, Felner EI, Prausnitz MR. Dissolving microneedle patch for transdermal delivery of human growth hormone. Small. 2011 Feb 18 ;7(4):531–9.

42. Quan FS, Kim YC, Vunnava A, Yoo DG, Song JM, Prausnitz MR, Compans RW, Kang SM. Intradermal vaccination with influenza virus-like particles by using microneedles induces protection superior to that with intramuscular immunization. J Virol. 2010 Aug ;84(15):7760–9.

43. Kommareddy S, Baudner BC, Oh S, Kwon SY, Singh M, O'Hagan DT. Dissolvable microneedle patches for the delivery of cell-culture-derived influenza vaccine antigens. J Pharm Sci. 2012;101(3):1021–7.

44. Mistilis MJ, Joyce JC, Esser ES, Skountzou I, Compans RW, Bommarius AS, Prausnitz MR Long-term stability of influenza vaccine in a dissolving microneedle patch. Drug Deliv Transl Res. 2017 Apr 1 ;7(2):195–205.

45. Martin CJ, Allender CJ, Brain KR, Morrissey A, Birchall JC. Low temperature fabrication of biodegradable sugar glass microneedles for transdermal drug delivery applications. J Control Release. 2012 Feb 28 ;158(1):93–101.

46. Raphael AP, Crichton ML, Falconer RJ, Meliga S, Chen X, Fernando GJ, Huang H, Kendall MA. Formulations for microprojection/microneedle vaccine delivery: Structure, strength and release profiles. J Control Release. 2016 Mar 10 ;225:40–52.

47. Ito Y, Hagiwara E, Saeki A, Sugioka N, Takada K Feasibility of microneedles for percutaneous absorption of insulin. Eur J Pharm Sci. 2006 Sep ;29(1):82–8.

48. Ito Y, Nakahigashi T, Yoshimoto N, Ueda Y, Hamasaki N, Takada K. Transdermal insulin application system with dissolving microneedles. Diabetes Technol Ther. 2012 Oct 1 ;14(10):891–9.

49. Miyano T, Tobinaga Y, Kanno T, Matsuzaki Y, Takeda H, Wakui M, Hanada K. Sugar micro needles as transdermic drug delivery system. Biomed Microdevices. 2005 Sep ;7(3):185–8.

50. Hirobe S, Azukizawa H, Hanafusa T, Matsuo K, Quan YS, Kamiyama F, Katayama I, Okada N, Nakagawa S. Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch. Biomaterials. 2015 Jul 1 ;57:50–8.

51. Ye Y, Wang J, Hu Q, Hochu GM, Xin H, Wang C, Gu Z. Synergistic Transcutaneous Immunotherapy Enhances Antitumor Immune Responses through Delivery of Checkpoint Inhibitors. ACS Nano. 2016 Sep 27 ;10(9):8956–63.

52. Wang C, Ye Y, Hochu GM, Sadeghifar H, Gu Z. Enhanced Cancer Immunotherapy by Microneedle Patch-Assisted Delivery of Anti-PD1 Antibody. Nano Lett. 2016 Apr 13 ;16(4):2334–40.

53. Ye Y, Wang C, Zhang X, Hu Q, Zhang Y, Liu Q, Wen D, Milligan J, Bellotti A, Huang L, Dotti G. A melanin-mediated cancer immunotherapy patch. Sci Immunol. 2017 ;2(17).

54. Zhang Y, Liu Q, Yu J, Yu S, Wang J, Qiang L, Gu Z. Locally Induced Adipose Tissue Browning by Microneedle Patch for Obesity Treatment. ACS Nano. 2017 Sep 26 ;11(9):9223–30.

55. Lee JW, Park JH, Prausnitz MR. Dissolving microneedles for transdermal drug delivery. Biomaterials. 2008 May 3;29(13):2113–24.

56. Chen MC, Huang SF, Lai KY, Ling MH . Fully embeddable chitosan microneedles as a sustained release depot for intradermal vaccination. Biomaterials. 2013 Apr ;34(12):3077–86.

57. Chen W, Tian R, Xu C, Yung BC, Wang G, Liu Y, Ni Q, Zhang F, Zhou Z, Wang J, Niu G. Microneedle-array patches loaded with dual mineralized

protein/peptide particles for type 2 diabetes therapy. Nat Commun. 2017 Dec 1 ;8(1).

58. Yu W, Jiang G, Zhang Y, Liu D, Xu B, Zhou J Polymer microneedles fabricated from alginate and hyaluronate for transdermal delivery of insulin. Mater Sci Eng C Mater Biol Appl. 2017 Nov 1 ;80:187–96.

59. Ke CJ, Lin YJ, Hu YC, Chiang WL, Chen KJ, Yang WC, Liu HL, Fu CC, Sung HW. Multidrug release based on microneedle arrays filled with pH-responsive PLGA hollow microspheres. Biomaterials. 2012 Jul ;33(20):5156–65.

60. Guo L, Chen J, Qiu Y, Zhang S, Xu B, Gao Y. Enhanced transcutaneous immunization via dissolving microneedle array loaded with liposome encapsulated antigen and adjuvant. Int J Pharm. 2013 Apr 15 ;447(1–2):22–30.

61. Chen MC, Lin ZW, Ling MH. Near-Infrared Light-Activatable Microneedle System for Treating Superficial Tumors by Combination of Chemotherapy and Photothermal Therapy. ACS Nano. 2016 Jan 26 ;10(1):93–101.

62. Sun W, Araci Z, Inayathullah M, Manickam S, Zhang X, Bruce MA, Marinkovich MP, Lane AT, Milla C, Rajadas J, Butte MJ. Polyvinylpyrrolidone microneedles enable delivery of intact proteins for diagnostic and therapeutic applications. Acta Biomater. 2013 ;9(8):7767–74.

63. Pérennès F, Marmiroli B, Matteucci M, Tormen M, Vaccari L, Di Fabrizio E. Sharp beveled tip hollow microneedle arrays fabricated by LIGA and 3D soft lithography with polyvinyl alcohol. J Micromechanics Microengineering. 2006 Mar 1;16(3):473–9.

64. Lee IC, He JS, Tsai MT, Lin KC. Fabrication of a novel partially dissolving polymer microneedle patch for transdermal drug delivery. J Mater Chem B. 2015 Jan 14 ;3(2):276–85.

65. Zhang Y, Feng P, Yu J, Yang J, Zhao J, Wang J, Shen Q, Gu Z ROS-Responsive Microneedle Patch for Acne Vulgaris Treatment. Adv Ther. 2018 Jul 1;1(3).

66. Liu S, Jin MN, Quan YS, Kamiyama F, Katsumi H, Sakane T, Yamamoto A. The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. J Control Release. 2012 Aug 10 ;161(3):933–41

67. Ling MH, Chen MC. Dissolving polymer microneedle patches for rapid and efficient transdermal delivery of insulin to diabetic rats. Acta Biomater. 2013 Nov ;9(11):8952–61.

68. Leone M, Mönkäre J, Bouwstra JA, Kersten G. Dissolving Microneedle Patches for Dermal Vaccination. Pharm Res. 2017 Nov 1;34(11):2223–40.

69. Chen MC, Ling MH, Kusuma SJ. Poly-γ-glutamic acid microneedles with a supporting structure design as a potential tool for transdermal delivery of insulin. Acta Biomater. 2015 Sep 15 ;24:106–16.

70. Lau S, Fei J, Liu H, Chen W, Liu R. Multilayered pyramidal dissolving microneedle patches with flexible pedestals for improving effective drug delivery. J Control Release. 2017 Nov 10 ;265:113–9.

71. Lee IC, Wu YC, Tsai SW, Chen CH, Wu MH. Fabrication of two-layer dissolving polyvinylpyrrolidone microneedles with different molecular weights for in vivo insulin transdermal delivery. RSC Adv. 2017 Jan 17 ;7(9):5067–75.

72. Lee IC, Lin WM, Shu JC, Tsai SW, Chen CH, Tsai MT. Formulation of two-layer dissolving polymeric microneedle patches for insulin transdermal delivery in diabetic mice. J Biomed Mater Res A. 2017 Jan 1 ;105(1):84–93.

73. Liu D, Yu B, Jiang G, Yu W, Zhang Y, Xu B. Fabrication of composite microneedles integrated with insulin-loaded CaCO 3 microparticles and PVP for transdermal delivery in diabetic rats. Mater Sci Eng C Mater Biol Appl. 2018 Sep 1 ;90:180–8.

74. Kim JD, Kim M, Yang H, Lee K, Jung H. Droplet-born air blowing: novel dissolving microneedle fabrication. J Control Release. 2013 ;170(3):430–6.

75. Yang H, Kim S, Huh I, Kim S, Lahiji SF, Kim M, Jung H. Rapid implantation of dissolving microneedles on an electrospun pillar array. Biomaterials. 2015 Sep 1 ;64:70–7.

76. Garland MJ, Caffarel–Salvador E, Migalska K, Woolfson AD, Donnelly RF. Dissolving polymeric microneedle arrays for electrically assisted transdermal drug delivery. J Control Release. 2012 Apr 10 ;159(1):52–9.

77. Zhang Y, Jiang G, Yu W, Liu D, Xu B. Microneedles fabricated from alginate and maltose for transdermal delivery of insulin on diabetic rats. Mater Sci Eng C. 2018 Apr 1;85:18–26.

78. Seong KY, Seo MS, Hwang DY, O'Cearbhaill ED, Sreenan S, Karp JM, Yang SY. A A self-adherent, bullet-shaped microneedle patch for controlled transdermal delivery of insulin. J Control Release. 2017 Nov 10 ;265:48–56.

79. Yang S, Wu F, Liu J, Fan G, Welsh W, Zhu H, Jin T. Phase-Transition Microneedle Patches for Efficient and Accurate Transdermal Delivery of Insulin. Adv Funct Mater. 2015 Aug 1;25(29):4633–41.

80. Di J, Yao S, Ye Y, Cui Z, Yu J, Ghosh TK, et al. Stretch-Triggered Drug Delivery from Wearable Elastomer Films Containing Therapeutic Depots. ACS Nano. 2015 Sep 22;9(9):9407–15.

81. Yu W, Jiang G, Liu D, Li L, Tong Z, Yao J, Kong X. Transdermal delivery of insulin with bioceramic composite microneedles fabricated by gelatin and hydroxyapatite. Mater Sci Eng C Mater Biol Appl. 2017 Apr 1 ;73:425–8.

82. Yu W, Jiang G, Liu D, Li L, Chen H, Liu Y, Huang Q, Tong Z, Yao J, Kong X. Fabrication of biodegradable composite microneedles based on calcium sulfate and gelatin for transdermal delivery of insulin. Mater Sci Eng C Mater Biol Appl. 2017 Feb 1 ;71:725–34.

83. Yu J, Zhang Y, Kahkoska AR, Gu Z. Bioresponsive transcutaneous patches. Curr Opin Biotechnol. 2017 Dec 1 ;48:28–32.

84. Ma G, Wu C. Microneedle, bio-microneedle and bio-inspired microneedle: A review. J Control Release. 2017 Apr 10;251:11–23.

85. Chen G, Yu J, Gu Z. Glucose-Responsive Microneedle Patches for Diabetes Treatment. J Diabetes Sci Technol. 2019 Jan 1 ;13(1):41–8.

86. Yu J, Zhang Y, Ye Y, DiSanto R, Sun W, Ranson D, et al. Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery. Proc Natl Acad Sci. 2015 Jul 7 ;112(27):8260–5.

87. Wu Q, Wang L, Yu H, Wang J, Chen Z. Organization of glucose-responsive systems and their properties. Chem Rev. 2011 Dec 14 ;111(12):7855–75.

88. Bankar SB, Bule MV, Singhal RS, Ananthanarayan L Glucose oxidase--an overview. Biotechnol Adv. 2009 Jul ;27(4):489–501.

89. Yu J, Zhang Y, Hu X, Wright G, Gu Z. Hypoxia-Sensitive Materials for Biomedical Applications. Ann Biomed Eng. 2016 Jun 1 ;44(6):1931–45.

90. Wang J, Zhang Y, Archibong E, Ligler FS, Gu Z. Leveraging H 2 O 2 Levels for Biomedical Applications. Adv Biosyst. 2017 Sep 1 ;1(9).

91. Hu X, Yu J, Qian C, Lu Y, Kahkoska AR, Xie Z, Jing X, Buse JB, Gu Z. H 2 O 2-Responsive Vesicles Integrated with Transcutaneous Patches for Glucose-Mediated Insulin Delivery. ACS Nano. 2017 Jan 24 ;11(1):613–20.

92. Tong Z, Zhou J, Zhong J, Tang Q, Lei Z, Luo H, Ma P, Liu X. Glucose- and H 2 O 2-Responsive Polymeric Vesicles Integrated with Microneedle Patches for Glucose-Sensitive Transcutaneous Delivery of Insulin in Diabetic Rats. ACS Appl Mater Interfaces. 2018 Jun 13 ;10(23):20014–24.

93. Xu B, Jiang G, Yu W, Liu D, Zhang Y, Zhou J, Sun S, Liu Y H 2 O 2-Responsive mesoporous silica nanoparticles integrated with microneedle patches for the glucose-monitored transdermal delivery of insulin. J Mater Chem B. 2017 ;5(41):8200–8.

94. Xu B, Cao Q, Zhang Y, Yu W, Zhu J, Liu D, Jiang G. Microneedles Integrated with ZnO Quantum-Dot-Capped Mesoporous Bioactive Glasses for Glucose-Mediated Insulin Delivery. ACS Biomater Sci Eng. 2018 Jul 9 ;4(7):2473–83.

95. Yu J, Qian C, Zhang Y, Cui Z, Zhu Y, Shen Q, Ligler FS, Buse JB, Gu Z. Hypoxia and H 2 O 2 Dual-Sensitive Vesicles for Enhanced Glucose-Responsive Insulin Delivery. Nano Lett. 2017 Feb 8 ;17(2):733–9.

96. Wang J, Ye Y, Yu J, Kahkoska AR, Zhang X, Wang C, Sun W, Corder RD, Chen Z, Khan SA, Buse JB. Core-Shell Microneedle Gel for Self-Regulated Insulin Delivery. ACS Nano. 2018 Mar 27 ;12(3):2466–73.

97. Zhang Y, Wang J, Yu J, Wen D, Kahkoska AR, Lu Y, Zhang X, Buse JB, Gu Z. Bioresponsive Microneedles with a Sheath Structure for H 2 O 2 and pH Cascade-Triggered Insulin Delivery. Small. 2018 Apr 5 ;14(14).

98. Ye Y, Yu J, Wang C, Nguyen N-Y, Walker GM, Buse JB, et al. Drug Delivery: Microneedles Integrated with Pancreatic Cells and Synthetic Glucose-Signal Amplifiers for Smart Insulin Delivery (Adv. Mater. 16/2016). Adv Mater. 2016 Apr;28(16):3223–3223.

99. Vrdoljak A, McGrath MG, Carey JB, Draper SJ, Hill AV, O'Mahony C, Crean AM, Moore AC.. Coated microneedle arrays for transcutaneous delivery of live virus vaccines. J Control Release. 2012 Apr 10;159(1):34–42.

100. Erdos G. Dissolvable microneedle arrays deliver live adenovirus to the skin for genetic immunization. Vol. 188, The journal of immunology. Bethesda, Md.: American Association of Immunologists; p. 58.

101. Matsuo K, Okamoto H, Kawai Y, Quan YS, Kamiyama F, Hirobe S, Okada N, Nakagawa S. Vaccine efficacy of transcutaneous immunization with amyloid β using a dissolving microneedle array in a mouse model of Alzheimer's disease. J Neuroimmunol. 2014 Jan 15 ;266(1–2):1–11.

102. Morefield GL, Tammariello RF, Purcell BK, Worsham PL, Chapman J, Smith LA, Alarcon JB, Mikszta JA, Ulrich RG. An alternative approach to combination vaccines: intradermal administration of isolated components for control of anthrax, botulism, plague and staphylococcal toxic shock. J Immune Based Ther Vaccines. 2008 Sep 3 ;6.

103. Torrisi BM, Zarnitsyn V, Prausnitz MR, Anstey A, Gateley C, Birchall JC, Coulman SA. Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin. J Control Release. 2013 Jan 28 ;165(2):146–52.

104. Prow TW, Chen X, Prow NA, Fernando GJ, Tan CS, Raphael AP, Chang D, Ruutu MP, Jenkins DW, Pyke A, Crichton ML. Nanopatch-targeted skin vaccination against West Nile Virus and Chikungunya virus in mice. Small. 2010 Aug 16 ;6(16):1776–84.

105. Ding Z, Verbaan FJ, Bivas-Benita M, Bungener L, Huckriede A, van den Berg DJ, Kersten G, Bouwstra JA. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. J Control Release. 2009 May 21 ;136(1):71–8.

106. Gill HS, Söderholm J, Prausnitz MR, Sällberg M. Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine. Gene Ther. 2010 Jun ;17(6):811–4.

107. Carey JB, Vrdoljak A, O'mahony C, Hill AV, Draper SJ, Moore AC Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route. Sci Rep. 2014 Aug 21 ;4.

108. Matsuo K, Hirobe S, Yokota Y, Ayabe Y, Seto M, Quan YS, Kamiyama F, Tougan T, Horii T, Mukai Y, Okada N. Transcutaneous immunization using a dissolving microneedle array protects against tetanus, diphtheria, malaria, and influenza. J Control Release. 2012 Jun 28 ;160(3):495–501.

109. Carey JB, Pearson FE, Vrdoljak A, McGrath MG, Crean AM, Walsh PT, Doody T, O'Mahony C, Hill AV, Moore AC. Microneedle array design determines the induction of protective memory CD8+ T cell responses induced by a recombinant live malaria vaccine in mice. PLoS One. 2011 ;6(7).

110. Dean CH, Alarcon JB, Waterston AM, Draper K, Early R, Guirakhoo F, Monath TP, Mikszta JA. Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax)-JE) in non-human primates. Hum Vaccin. 2005 ;1(3):106–11.

111. Moon S, Wang Y, Edens C, Gentsch JR, Prausnitz MR, Jiang B. Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch. Vaccine. 2013 Jul 25 ;31(34):3396–402.

112. van der Maaden K, Trietsch SJ, Kraan H, Varypataki EM, Romeijn S, Zwier R, van der Linden HJ, Kersten G, Hankemeier T, Jiskoot W, Bouwstra J. Novel hollow microneedle technology for depth-controlled microinjection-mediated dermal vaccination: a study with polio vaccine in rats. Pharm Res. 2014 ;31(7):1846–54.

113. Chen X, Kask AS, Crichton ML, McNeilly C, Yukiko S, Dong L, Marshak JO, Jarrahian C, Fernando GJ, Chen D, Koelle DM. Improved DNA vaccination by skin-targeted delivery using dry-coated densely-packed microprojection arrays. J Control Release. 2010 Dec 20 ;148(3):327–33.

114. Kask AS, Chen X, Marshak JO, Dong L, Saracino M, Chen D, Jarrahian C, Kendall MA, Koelle DM. DNA vaccine delivery by densely-packed and short microprojection arrays to skin protects against vaginal HSV-2 challenge. Vaccine. 2010 Nov 3 ;28(47):7483–91.

115. Edens C, Collins ML, Goodson JL, Rota PA, Prausnitz MR. A microneedle patch containing measles vaccine is immunogenic in non-human primates. Vaccine. 2015 Sep 8 ;33(37):4712–8.

116. Edens C, Collins ML, Ayers J, Rota PA, Prausnitz MR. Measles vaccination using a microneedle patch. Vaccine. 2013 Jul 25 ;31(34):3403–9.

117. Laurent PE, Bourhy H, Fantino M, Alchas P, Mikszta JA. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. Vaccine. 2010 Aug ;28(36):5850–6.

118. Suh H, Shin J, Kim YC.. Microneedle patches for vaccine delivery. Clin Exp Vaccine Res. 2014 ;3(1):42.

119. Arya J, Prausnitz MR. Microneedle patches for vaccination in developing countries. J Control Release. 2016 Oct 28 ;240:135–41.

120. Zehrung D, Jarrahian C, Wales A Intradermal delivery for vaccine dose sparing: overview of current issues. Vaccine. 2013 Jul 25 ;31(34):3392–5. 23176978/

121. Pettis RJ, Harvey AJ. Microneedle delivery: clinical studies and emerging medical applications. Ther Deliv. 2012 Mar ;3(3):357–71.

122. van der Maaden K, Jiskoot W, Bouwstra J. Microneedle technologies for (trans)dermal drug and vaccine delivery. J Control Release. 2012 Jul 20 ;161(2):645–55.

123. Vrdoljak A. Review of recent literature on microneedle vaccine delivery technologies. Vaccine Dev Ther. 2013 Aug 22 ;3:47–55.

124. Bhatnagar S, Dave K, Venuganti VVK. Microneedles in the clinic. J Control Release. 2017 Aug 28;260:164–82.

125. Cheung K, Das DB. Microneedles for drug delivery: trends and progress. Drug Deliv. 2016 Sep 1 ;23(7):2338–54.

126. Chen W, Li H, Shi D, Liu Z, Yuan W. Microneedles As a Delivery System for Gene Therapy. Front Pharmacol. 2016 ;7(MAY).

127. Makkouk A, Weiner GJ. Cancer immunotherapy and breaking immune tolerance: new approaches to an old challenge. Cancer Res. 2015 Jan 1 ;75(1):5–10.

128. Irvine DJ, Hanson MC, Rakhra K, Tokatlian T. Synthetic Nanoparticles for Vaccines and Immunotherapy. Chem Rev. 2015 Oct 14 ;115(19):11109–46.

129. Wang D, Wang T, Liu J, Yu H, Jiao S, Feng B, Zhou F, Fu Y, Yin Q, Zhang P, Zhang Z. Acid-Activatable Versatile Micelleplexes for PD-L1 Blockade-Enhanced Cancer Photodynamic Immunotherapy. Nano Lett. 2016 Sep 14 ;16(9):5503–13.

130. Moreira AF, Rodrigues CF, Jacinto TA, Miguel SP, Costa EC, Correia IJ. Microneedle-based delivery devices for cancer therapy: A review. Pharmacol Res. 2019 Oct 1 ;148.

131. Zaric M, Lyubomska O, Touzelet O, Poux C, Al-Zahrani S, Fay F, Wallace L, Terhorst D, Malissen B, Henri S, Power UF. Skin dendritic cell targeting via microneedle arrays laden with antigen-encapsulated poly-D,L-lactide-co-glycolide nanoparticles induces efficient antitumor and antiviral immune responses. ACS Nano. 2013 Mar 26 ;7(3):2042–55.

132. Seton-Rogers S. Anticancer drugs: Switching off immune suppression. Nat Rev Drug Discov. 2016 Dec 29 ;16(1):16–7.

133. Salvadori ML, da Cunha Bianchi PK, Gebrim LH, Silva RS, Kfoury JR. Effect of the association of 1-methyl-DL-tryptophan with paclitaxel on the expression of indoleamine 2,3-dioxygenase in cultured cancer cells from patients with breast cancer. Med Oncol. 2015 Nov 1 ;32(11).

134. Ali AA, McCrudden CM, McCaffrey J, McBride JW, Cole G, Dunne NJ, Robson T, Kissenpfennig A, Donnelly RF, McCarthy HO. DNA vaccination for cervical cancer; a novel technology platform of RALA mediated gene delivery via polymeric microneedles. Nanomedicine. 2017 Apr 1;13(3):921–32.

135. Lahiji SF, Jang Y, Ma Y, Dangol M, Yang H, Jang M, Jung H. Effects of dissolving microneedle fabrication parameters on the activity of encapsulated lysozyme. Eur J Pharm Sci. 2018 May 30 ;117:290–6.

136. Pan J, Ruan W, Qin M, Long Y, Wan T, Yu K, Zhai Y, Wu C, Xu Y. Intradermal delivery of STAT3 siRNA to treat melanoma via dissolving microneedles. Sci Rep. 2018 Dec 1 ;8(1).

137. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer. 2014 Oct 27 ;14(11):736–46.

138. de Garibay AP, Solinís MA, del Pozo-Rodríguez A, Apaolaza PS, Shen JS, Rodríguez-Gascón A. Solid Lipid Nanoparticles as Non-Viral Vectors for Gene Transfection in a Cell Model of Fabry Disease. J Biomed Nanotechnol. 2015 Mar 1 ;11(3):500–11.

139. Kim DY, Kwon JS, Lee JH, Jin LM, Kim JH, Kim MS. Effects of the Surface Charge of Stem Cell Membranes and DNA/Polyethyleneimine Nanocomplexes on Gene Transfection Efficiency. J Biomed Nanotechnol. 2015 Mar 1 ;11(3):522–30.

140. Peng LH, Wei W, Shan YH, Zhang TY, Zhang CZ, Wu JH, Yu L, Lin J, Liang WQ, Khang G, Gao JQ. β-Cyclodextrin-Linked Polyethylenimine Nanoparticles Facilitate Gene Transfer and Enhance the Angiogenic Capacity of Mesenchymal Stem Cells for Wound Repair and Regeneration. J Biomed Nanotechnol. 2015 Apr 1 ;11(4):680–90. 26310074/

141. Hao Y, Li W, Zhou X, Yang F, Qian Z. Microneedles-Based Transdermal Drug Delivery Systems: A Review. J Biomed Nanotechnol. 2017 Dec 1 ;13(12):1581–97.

142. Samaranayake H, Määttä AM, Pikkarainen J, Ylä-Herttuala S. Future prospects and challenges of antiangiogenic cancer gene therapy. Hum Gene Ther. 2010 Apr 1 ;21(4):381–96.

143. Gorres JP, Lager KM, Kong WP, Royals M, Todd JP, Vincent AL, Wei CJ, Loving CL, Zanella EL, Janke B, Kehrli ME. DNA vaccination elicits protective immune responses against pandemic and classic swine influenza viruses in pigs. Clin Vaccine Immunol. 2011 Nov ;18(11):1987–95.

144. Wei Z, Zheng S, Wang R, Bu X, Ma H, Wu Y, Zhu L, Hu Z, Liang Z, Li Z. A flexible microneedle array as low-voltage electroporation electrodes for in vivo DNA and siRNA delivery. Lab Chip. 2014 Oct 21 ;14(20):4093–102.

145. Chong RH, Gonzalez-Gonzalez E, Lara MF, Speaker TJ, Contag CH, Kaspar RL, Coulman SA, Hargest R, Birchall JC. Gene silencing following siRNA delivery to skin via coated steel microneedles: In vitro and in vivo proof-of-concept. J Control Release. 2013 Mar 28 ;166(3):211–9.

146. McCaffrey J, Donnelly RF, McCarthy HO. Microneedles: an innovative platform for gene delivery. Drug Deliv Transl Res. 2015 Aug 10 ;5(4):424–37.

147. Versypt AN, Pack DW, Braatz RD. Mathematical modeling of drug delivery from autocatalytically degradable PLGA microspheres--a review. J Control Release. 2013 Jan 10 ;165(1):29–37.

148. Gonzalez-Gonzalez E, Speaker TJ, Hickerson RP, Spitler R, Flores MA, Leake D, Contag CH, Kaspar RL Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD). Mol Ther. 2010 ;18(9):1667–74.

149. González-González E, Kim YC, Speaker TJ, Hickerson RP, Spitler R, Birchall JC, Lara MF, Hu RH, Liang Y, Kirkiles-Smith N, Prausnitz MR. Visualization of plasmid delivery to keratinocytes in mouse and human epidermis. Sci Rep. 2011 ;1.

150. Kumar A, Li X, Sandoval MA, Rodriguez BL, Sloat BR, Cui Z. Permeation of antigen protein-conjugated nanoparticles and live bacteria through microneedle-treated mouse skin. Int J Nanomedicine. 2011 Jun ;6:1253.

151. Han T, Das DB. Permeability enhancement for transdermal delivery of large molecule using low-frequency sonophoresis combined with microneedles. J Pharm Sci. 2013 ;102(10):3614–22.

152. Zhang S, Qiu Y, Gao Y. Enhanced delivery of hydrophilic peptides in vitro by transdermal microneedle pretreatment. Acta Pharm Sin B. 2014 Feb ;4(1):100–4.

153. Saurer EM, Flessner RM, Sullivan SP, Prausnitz MR, Lynn DM. Layer-by-layer assembly of DNA- and protein-containing films on microneedles for drug delivery to the skin. Biomacromolecules. 2010 Nov 8 ;11(11):3136–43.

154. Zhao X, Coulman SA, Hanna SJ, Wong FS, Dayan CM, Birchall JC. Formulation of hydrophobic peptides for skin delivery via coated microneedles. J Control Release. 2017 Nov 10 ;265:2–13.

155. Caudill CL, Perry JL, Tian S, Luft JC, DeSimone JM. Spatially controlled coating of continuous liquid interface production microneedles for transdermal protein delivery. J Control Release. 2018 Aug 28 ;284:122–32.

156. Li S, Li W, Prausnitz M. Individually coated microneedles for co-delivery of multiple compounds with different properties. Drug Deliv Transl Res. 2018 Oct 1 ;8(5):1043–52.

157. Leone, M., Priester, M.I., Romeijn, S., Nejadnik, M.R., Mönkäre, J., O'Mahony, C., Jiskoot, W., Kersten, G. and Bouwstra, J.A. Hyaluronan-based dissolving microneedles with high antigen content for intradermal vaccination: Formulation, physicochemical characterization and immunogenicity assessment. Eur J Pharm Biopharm. 2019 Jan 1 ;134:49–59.

158. Liu S, Zhang S, Duan Y, Niu Y, Gu H, Zhao Z, Zhang S, Yang Y, Wang X, Gao Y, Yang P. Transcutaneous immunization of recombinant Staphylococcal enterotoxin B protein using a dissolving microneedle provides potent protection against lethal enterotoxin challenge. Vaccine. 2019 Jun 27 ;37(29):3810–9.

159. Mönkäre J, Nejadnik MR, Baccouche K, Romeijn S, Jiskoot W, Bouwstra JA. IgG-loaded hyaluronan-based dissolving microneedles for intradermal protein delivery. J Control Release. 2015 Nov 28 ;218:53–62.

160. Chen J, Qiu Y, Zhang S, Gao Y. Dissolving microneedle-based intradermal delivery of interferon-α-2b. Drug Dev Ind Pharm. 2016 ;42(6):890–6.

161. Dillon C, Hughes H, O'Reilly NJ, McLoughlin P. Formulation and characterisation of dissolving microneedles for the transdermal delivery of therapeutic peptides. Int J Pharm. 2017 Jun 30 ;526(1–2):125–36.

162. Lahiji SF, Jang Y, Huh I, Yang H, Jang M, Jung H. Exendin-4-encapsulated dissolving microneedle arrays for efficient treatment of type 2 diabetes. Sci Rep. 2018 Dec 1 ;8(1).

163. Vora LK, Courtenay AJ, Tekko IA, Larrañeta E, Donnelly RF. Pullulan-based dissolving microneedle arrays for enhanced transdermal delivery of small and large biomolecules. Int J Biol Macromol. 2020 Mar 1 ;146:290–8.

164. Tuan-Mahmood TM, McCrudden MT, Torrisi BM, McAlister E, Garland MJ, Singh TR, Donnelly RF. Microneedles for intradermal and transdermal drug delivery. Eur J Pharm Sci. 2013 ;50(5):623–37.

165. de Groot AM, Du G, Mönkäre J, Platteel AC, Broere F, Bouwstra JA, Sijts AJ. Hollow microneedle-mediated intradermal delivery of model vaccine antigen-loaded PLGA nanoparticles elicits protective T cell-mediated immunity to an intracellular bacterium. J Control Release. 2017 Nov 28 ;266:27–35.

166. Du G, Hathout RM, Nasr M, Nejadnik MR, Tu J, Koning RI, Koster AJ, Slütter B, Kros A, Jiskoot W, Bouwstra JA. Intradermal vaccination with hollow microneedles: A comparative study of various protein antigen and adjuvant encapsulated nanoparticles. J Control Release. 2017 Nov 28 ;266:109–18.

167. Mönkäre J, Pontier M, van Kampen EE, Du G, Leone M, Romeijn S, et al. Development of PLGA nanoparticle loaded dissolving microneedles and comparison with hollow microneedles in ntradermal vaccine delivery. Eur J Pharm Biopharm. 2018 Aug;129:111-121.

168. Chen B, Wei J, Iliescu C. Sonophoretic enhanced microneedles array (SEMA)—Improving the efficiency of transdermal drug delivery. Sensors Actuators B Chem. 2010 Mar 4 ;1(145):54–60.

169. Golombek S, Pilz M, Steinle H, Kochba E, Levin Y, Lunter D, Schlensak C, Wendel HP, Avci-Adali M. Intradermal Delivery of Synthetic mRNA Using Hollow Microneedles for Efficient and Rapid Production of Exogenous Proteins in Skin. Mol Ther Nucleic Acids. 2018 Jun 1 ;11:382–92.

170. Hardy JG, Larrañeta E, Donnelly RF, McGoldrick N, Migalska K, McCrudden MT, Irwin NJ, Donnelly L, McCoy CP. Hydrogel-Forming Microneedle Arrays Made from Light-Responsive Materials for On-Demand Transdermal Drug Delivery. Mol Pharm. 2016 Mar 7 ;13(3):907–14.

171. Raj Singh TR, Woolfson AD, Donnelly RF. Investigation of solute permeation across hydrogels composed of poly(methyl vinyl ether-co-maleic acid) and poly(ethylene glycol). J Pharm Pharmacol. 2010 Jun ;62(7):678–84.

172. Garland MJ, Singh TR, Woolfson AD, Donnelly RF. Electrically enhanced solute permeation across poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-co-maleic acid) hydrogels: effect of hydrogel crosslink density and ionic conductivity. Int J Pharm. 2011 Mar 15 ;406(1–2):91–8.

173. Wong RS, Ashton M, Dodou K. Effect of Crosslinking Agent Concentration on the Properties of Unmedicated Hydrogels. Pharmaceutics. 2015 Sep 9 ;7(3):305–19.

174. Singh TRR, Garland MJ, Migalska K, Salvador EC, Shaikh R, McCarthy HO, et al. Influence of a pore-forming agent on swelling, network parameters, and permeability of poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-co-maleic acid) hydrogels: Application in transdermal delivery systems. J Appl Polym Sci. 2012 Aug 15 ;125(4):2680–94.

175. Courtenay AJ, McCrudden MT, McAvoy KJ, McCarthy HO, Donnelly RF. Microneedle-Mediated Transdermal Delivery of Bevacizumab. Mol Pharm. 2018 Aug 6 ;15(8):3545–56.