



Microneedle Transdermal Drug Delivery Systems for Allergen-Specific Immunotherapy, Skin Disease Treatment, and Vaccine Development

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Transdermal drug delivery systems (TDDSs) overcome the hurdle of an intact skin barrier by penetrating the skin to allow molecules through. These systems reduce side effects associated with conventional hypodermic needles. Here, we introduce novel microneedle (MN) TDDSs that enhance drug delivery by creating micron-sized pores across the skin. Many MN TDDSs designed to deliver a diverse array of therapeutics, including allergen-specific immunotherapy, skin disease treatments, and vaccines, are under pre-clinical and clinical trials. Although epicutaneous approaches are emerging as new options for treating food allergy in many clinical trials, MN TDDSs could provide a more efficient and convenient route to deliver macromolecules. Furthermore, MN TDDSs may allow for safe vaccine delivery without permanent scars. MN TDDSs are a major emerging strategy for delivering novel vaccines and treatments for diseases, including skin diseases, allergic diseases, and so on.

Key Words: Microneedle, transdermal drug delivery system, allergen-specific immunotherapy, skin diseases, vaccines

INTRODUCTION

Most bio-therapeutics and vaccines are injected using a hypodermic approach (subcutaneous route). These injections are cost-effective and a direct way to deliver virtually any type of molecule into the body. However, subcutaneous injections are not easily administered by patients themselves, and patient compliance can be limited by needle phobia, pain, and injection-related adverse reactions. Although oral drug delivery overcomes some of the problems associated with patient compliance, inefficient absorption and degradation of drugs in the digestive system make this route unsuitable for many drugs. Sublingual administration partially solves these issues by per-

mitting drugs to bypass the gastrointestinal digestive system, resulting in easy and rapid absorption. Although other routes for administration have been investigated, none are considered as effective as direct subcutaneous needle injection. Recently, transdermal drug delivery systems (TDDSs) have emerged as attractive administration methods for many drugs.^{1,2}

TDDSs overcome many problems associated with drug administration by oral delivery or hypodermic injection. TDDSs are less invasive, painless, and can be administered by the patient to them self. Furthermore, this method allows for sustained drug release to improve the pharmacokinetic profile. TDDSs are more convenient and cost-effective than other methods. However, the impenetrable skin barrier is a major obstacle to the effectiveness of TDDSs and their ability to be widely used clinically. In particular, the intact stratum corneum presents a challenge to transdermal drug diffusion.^{3,4}

Microneedles (MNs) are one of the most popular TDDSs. MNs can be fabricated at a depth of 200 μm without penetrating across the dermis; as a result, this method of transdermal delivery is still pain free. In this article, we introduce the basic concept of MN-based TDDS and summarize the recent pre-clinical and clinical progress made in several fields, including allergen-specific immunotherapy, skin disease treatment, and novel vaccine development.

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MICRONEEDLES

MN based TDDS consists of hundreds of micrometer-sized needles in arrays on a backing. This method enhances drug delivery by creating micron-sized pores across the skin. A MN patch (MNP) comprises a MN array with a bandage, allowing for simplified application to the skin. As shown in Fig. 1, MNs are categorized into four types according to drug delivery method: solid, coated, dissolving, and hollow.¹

Solid MNs

Solid MNs are used as for skin pretreatment to improve permeability prior to drug delivery. Solid MNs are inserted and removed to create micro-channels on the skin surface according to the poke-and-patch principle. Solid MNs can be applied once or several times as part of a roller that punctures the stratum corneum as it moves over the skin.⁵ After solid MN application, drugs are delivered above the pores using drug-loading patches commonly used for conventional transdermal drug delivery or with semi-solid topical formulations (e.g., ointment, gel, or lotion).^{1,3,5}

Coated MNs

The coated MNs approach consists of MNs coated with a drug solution or dispersion and employs the coat-and-poke principle

in which the coated drug allows diffusion to the epidermal layers after MN insertion. Various methods for coating therapeutic agents onto MNs have been studied: the most commonly used method involves dip coating MNs in a coating solution.⁶ When the coated MNs are applied to the skin, the coated layer is released. By penetrating vertically into the epidermal layer of the skin in a minimally invasive manner, the desired drug dose is rapidly delivered to the tissue. However, this approach is used less frequently than other MNs because it provides a relatively small surface area for drug absorption.⁷

Dissolving MNs

Dissolving MNs operate on the poke-and-release principle. The release of drugs or vaccines is controlled using biodegradable materials, such as various polymers and sugars, loaded with therapeutic agents. These MNs are easier to manufacture than conventional hollow MNs and involve only a single-step application. Various methods to deliver vaccines by dissolving MNs have been extensively studied. For example, carboxymethyl cellulose and hyaluronan MNs made via droplet-born air blowing method have been found to completely dissolve in mice skin without any drug loss.^{8,9}

Hollow MNs

Hollow MNs, which deliver drugs through a poke-and-flow ap-

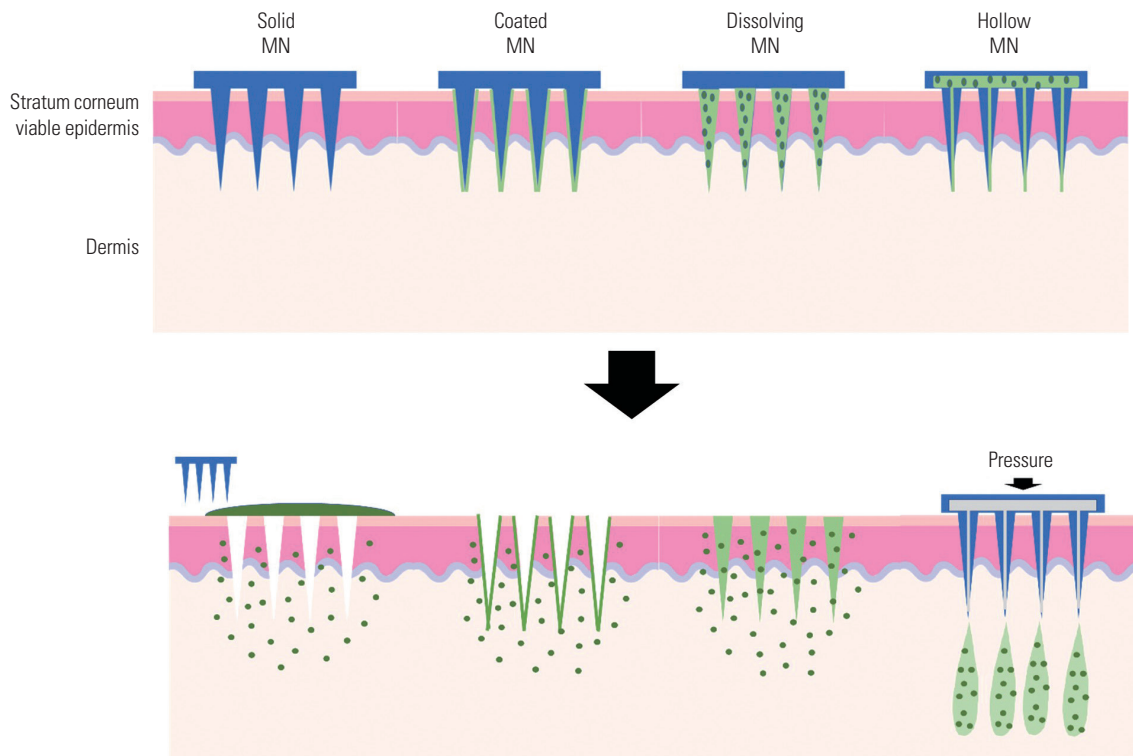


Fig. 1. Transdermal drug delivery methods using MNs. MNs are applied to the skin. Solid MNs method: skin is pretreated with solid MNs. Then, a drug-loaded patch is applied to the pretreated skin, and the drug is absorbed through the pores. Coated MNs method: after injecting the drug-coated MNs, the drug coating melts away from the MNs with the aqueous environment of the skin. Dissolving MNs method: drug-loaded MNs are made of a water-soluble or biodegradable material and encapsulate the target drug. As the MNs dissolve, the drug is released together. Hollow MNs method: the liquid formulation drugs are injected by applied pressure and flow through the hollow pathway in the MNs. MNs, microneedles.

proach, are miniature versions of conventional hypodermic needles. These MNs comprise a hollow pathway through which liquid formulation drugs are injected by applied pressure. Applying a force to the patch surface speeds up drug delivery by accelerating the fluid release, resulting in the delivery of significant amounts to the dermal layer. In addition, the desired drug dosage in the solution can be more easily adjusted according to the needs of the patient. However, hollow MNs are difficult to make due to their complex structure and are fragile due to insufficient mechanical strength.⁸

APPLICATIONS OF MNs

MN-based TDDS techniques have been studied in various fields, including allergen-specific immunotherapy, cosmetics, skin disease treatments, and novel vaccine development. A summary of applications utilizing MNs is presented in Table 1.

Epicutaneous/MNP-based transdermal immunotherapy for allergic diseases

Allergen-specific immunotherapy is an effective treatment for

IgE-mediated diseases, including allergic rhinoconjunctivitis, asthma, and atopic dermatitis. Allergen-specific immunotherapy seeks to induce allergen-specific peripheral immune tolerance via repeated injections of a sensitized allergen.¹⁰

Epicutaneous immunotherapy

Interest in epicutaneous immunotherapy (EPIT) has increased with the growing need for novel delivery routes to improve the safety and efficacy of allergen-specific immunotherapy.¹¹ EPIT involves transdermal administration of food allergens (e.g., peanut, cashew, cow's milk) via an adhesive transdermal patch to induce peripheral immune tolerance.¹² EPIT takes advantage of the high-density of Langerhans cells in the epidermis. EPIT shows a safer profile by applying allergens to the epidermis, which are then delivered to the dermis with minimal invasiveness. Additionally, this method is more convenient for patients because it is non-invasive (needle-free) and can be self-administered. However, delivering a sufficient dose of allergens through the epidermis without incurring too great of a Th₂ immune response remains a major challenge for EPIT. Furthermore, the majority of allergens are macromolecules that cannot penetrate through the stratum corneum.¹³ As a result,

Table 1. Summary of Applications of MNs in Allergic Diseases, Skin Diseases, and so on

| Application of MNs | Disease | Description |
|--------------------|----------------------------|--|
| Allergic diseases | Asthma/AD | Lyophilized extracts of allergens loaded PLD-MNA ²³ HDM-loaded MNPs ^{25,26} |
| Cosmetics | Wrinkle improvement | HA-based dissolving MNPs ³⁰⁻³² Ascorbic acid-loaded dissolving MNPs ³⁴ |
| | Skin whitening | HA-dissolving MNPs with whitening agents ³⁷ |
| | Scar | MNs can destroy thickened collagen and induce wound healing ^{40,41} |
| Skin diseases | Alopecia | Disc MNs dermaroller ⁴⁴ Topical electrical MNs+5% topical minoxidil ⁴⁵ |
| | Psoriasis | MTX-loaded MNs ⁴⁹ CyA-loaded MNs ⁵⁰ Anti-TNF- α loaded MNs ⁵⁰ |
| | Prurigo nodularis | Biodegradable MNPs to increase penetration of topical steroids ⁵⁴ |
| | Acne | Fractional radiofrequency MNs ⁵⁵ Drug-loaded ROS responsive MNPs using a polyvinyl alcohol matrix ⁵⁵ Polyionic liquid-based MNPs containing salicylic acid ⁵⁶ |
| | Cancer | OVA-loaded dissolving MNPs ⁶⁰ anti-PD1 MNPs ⁶¹ |
| | Herpes virus infection | Acyclovir-loaded MNs ⁶⁵ |
| | Vaccine | Influenza vaccine |
| BCG vaccine | | BCG vaccine loaded MNPs ^{78,80} |
| HBV vaccine | | Adjuvant-free monovalent HBV vaccine using dissolving MNPs ^{82,83} HBN MNs vaccine formulated with polylactic acid and carboxymethyl cellulose in a dual release pattern ⁸⁴ |
| Others | Neurodegenerative diseases | 95% DPH encapsulated dissolving MNs ⁸⁷ |
| | Obesity | β 3-adrenoceptor agonist and thyroid hormone T3 loaded dissolving MNs ⁸⁹ Rosiglitazone encapsulated dissolving MNs ⁹⁰ |

AD, atopic dermatitis; BCG, Bacillus Calmette-Guerin; CyA, cyclosporine A; DPH, donepezil hydrochloride; HA, hyaluronic acid; HBV, Hepatitis B virus; HDM, house dust mite; MNs, microneedles; MNPs, microneedle patches; MTX, methotrexate; OVA, ovalbumin; PLD-MNA, powder-laden dissolvable microneedle array; ROS, reactive oxygen species; TNF, tumor necrosis factor.

allergens must be applied for a long time to break through the skin barrier.

In the field of EPIT, the Epicutaneous Viaskin Patch (EVP) (DBV Technologies, Paris, France) is the leading technology. EVPs are under development to treat patients with allergy to peanut and cow's milk. Furthermore, researchers are using animal models to elucidate the efficacy and the exact mechanisms of EPIT. In a murine model of peanut allergy, repeated EVP application for 8 weeks exhibited comparable efficacy to subcutaneous allergen-specific immunotherapy.¹⁴ Additionally, EPIT has been found to decrease levels of peanut-specific IgE, increase specific IgG₂a, and decrease Th₂ cytokine production by peanut-stimulated splenocytes.^{11,15} EVP application on intact skin targets antigen-presenting cells in the superficial layers of the skin. In a mouse model of peanut allergy treated with EVP, allergens were captured by Langerhans cells and then migrated to the draining regional lymph nodes. Here, the allergens activated the adaptive immune system and induced T cell polarization or tolerance.¹⁶ Repeated application of EVP also leads to a decrease in the systemic allergen-specific immune response via the induction of regulatory T (Treg) cells. Furthermore, EPIT induces both effector/memory and naïve (long-lived) Treg cells. Currently, EVP is under development for the treatment of food allergy. In the future, EVP could be developed for other allergens, including house dust mites (HDMs), pet dander, and pollen.¹⁷

As of yet, EVP has not been approved for clinical use. EVP has completed a phase 3 clinical trial for peanut allergy patients who are 4–11 years of age for which the primary endpoint was success rate after 52 weeks of treatment. Although EVP showed high patient compliance and moderate beneficial effects, the treatment did not meet the preset primary end point in the % difference of good responders.¹⁸ Therefore, it is necessary to develop a new MN-based TDDS for safe and convenient allergen delivery.^{19,20}

MNP-based transdermal immunotherapy

To overcome the weaknesses of EPIT, MNP-based transdermal immunotherapy (TDIT) was developed. Compared to conventional skin patches for allergen delivery, TDIT provides easy

dose control and improves treatment response consistency.³ The advantages and limitations for EPIT and MNP-based TDIT are shown in Table 2.^{21,22}

The use of powdered allergen-based TDIT is trending in allergen-specific immunotherapy development. Powder-laden dissolvable microneedle arrays (PLD-MNA) can sufficiently carry allergens into the epidermis without laser-mediated microporation and with minimal skin reaction. The powdered allergens are retained within the epidermis for a prolonged period, creating an "antigen-depot" effect.^{13,23} Moreover, in contrast to aqueous allergens that spread quickly into circulation, powdered allergens are secured in the epidermis with minimal leakage to the circulatory system. Various lyophilized extracts of allergens, which are currently available for skin prick testing and allergen-specific immunotherapy, can be directly loaded into PLD-MNAs.²⁴ These innovative delivery technologies fully preserve the allergenicity and/or adjuvant, programming tolerogenic microenvironment that rewires the immunological response to induce tolerance.²³

In two recent studies, the efficacy of MNs-based TDIT was examined using HDM-loaded MNPs. In murine models of asthma and atopic dermatitis, TDIT using allergen-loaded MNPs decreased Th₂-related inflammation more effectively than conventional subcutaneous allergen-specific immunotherapy with the same allergen dosage. The stability, delivery rate, and safety of HDM-loaded MNPs have been determined in HDM-induced atopic dermatitis and asthma mouse models.^{25,26} These experiments revealed two other important advantages of MNP-based TDIT: First, the size of MNs is much smaller than conventional needles used in subcutaneous allergen-specific immunotherapy, making treatment painless. Second, allergen delivery through MNPs is more effectively absorbed than EPIT. Because MNPs directly target the dendritic cell-rich dermal layer, treatment induces similar immunogenicity to EPIT with a lower allergen dosage.²⁶

Cosmetics and skin diseases

Conventional drugs do not satisfactorily penetrate the stratum corneum of the skin. This weak point limits their therapeutic efficacy. As a result, transdermal drug delivery is widely used in

Table 2. Advantages and Limitations of EPIT and MN-Based TDIT for Allergen-Specific Immunotherapy

| | EPIT | MNs-based TDIT |
|-------------|---|---|
| Advantages | <ul style="list-style-type: none"> - High safety profile - Increased convenience - Painless and free from needle phobia - Self-administrable application method - No additional irritant constituents - No visible, long-lasting damage to skin | <ul style="list-style-type: none"> - Low side effects - Painless and free from needle phobia - Self-administrable application method - Room temperature storage - No visible, long-lasting damage to skin - Large molecules can be administered |
| Limitations | <ul style="list-style-type: none"> - More data are needed regarding patients with aeroallergen sensitized allergic rhinoconjunctivitis - Limited to small molecule drugs | <ul style="list-style-type: none"> - Limited drug dose due to the size of the MNs - Can cause local and systemic allergic reactions |

EPIT, epicutaneous immunotherapy; MN, microneedle; TDIT, transdermal immunotherapy.

the treatment of various skin diseases, and the use of MNs is receiving great attention in the treatment of various skin diseases, including hair loss, psoriasis, and acne.^{27,28} Presently, MNs have been approved for clinical trials related to cosmetic medicine.

Cosmetic purposes

Wrinkle improvement

Transdermal MNPs can be used efficiently to decrease wrinkles. In a study evaluating the efficacy of MNs to reduce the appearance of wrinkles in Korean women aged 45–65 years, participants were divided into three treatment groups: soluble MNP alone, soluble MNP plus anti-wrinkle cream, and anti-wrinkle cream alone. The treatments were performed on crow's feet and nasolabial folds. The combination treatment with anti-wrinkle cream and MNPs showed significant improvement in crow's feet and nasolabial folds. Interestingly, treatment with MNP alone was sufficient to induce improvement in crow's feet.²⁹ In another study, the application of hyaluronic acid (HA)-based dissolving MNPs exhibited significant anti-aging benefits, including improved skin structure, function, and appearance.^{30,31} Previous data also indicate that HA-dissolving MNs can induce collagen synthesis, resulting in rejuvenation and improvement of skin appearance. Finally, HA gel in a dissolving MNP has been found to penetrate into deep layers of the skin and acts as an active carrier for drug transportation.^{29,32}

Another study evaluated whether a dissolving MNP loaded with ascorbic acid could effectively eliminate reactive oxygen species (ROS) accumulation, which induces wrinkles.^{33,34} The double-blind study found that ascorbic acid-loaded dissolving MNP had a wrinkle-improving effect and did not cause skin irritation and hypersensitivity; therefore, this MNP could be effectively used in cosmetics.³⁴

Skin whitening

Skin whitening products are part of a growing cosmetic sector. Whitening or lightening agents interfere with melanin production. Although melanin is essential for protecting skin from ultraviolet (UV) rays, excessive melanin production is a result of UV damage and aging. These processes induce pigmentation disorders, such as freckles and senile lentigo.³⁵ Tyrosinase inhibition is the most popular strategy employed to achieve skin whitening. Several approaches have been used to inhibit the catalytic activity of tyrosinase and then interfere with the synthesis and release of melanin.³⁶ Minimally invasive MNs improve the absorption of topical compounds and dissolving MNs effectively deliver the skin pigmentation remover to the melanocytes. Clinical studies on the efficacy and safety of HA-dissolving MNPs with whitening agents have been reported.^{36,37} HA-dissolving MNs are biocompatible with skin, because HA is an endogenous component of the skin's extracellular matrix. HA is important for tissue regeneration and decreases in the skin with age. HA-based gels are the most widely-used ingredi-

ent for aged skin therapy. Intriguingly, safety and efficacy data for MNPs with sodium hyaluronate, a salt form of HA, are promising.³⁵ HA-dissolving MNPs contain various active ingredients to prevent skin aging, including melatonin, arbutin, niacinamide, and tranexamic acid. These ingredients are helpful in skin depigmentation and do not cause skin irritation.³⁸ HA-dissolving MNPs improve the contrast brightness and perform better than topical application formulation.³⁵

Scarring

Scars form as a result of tissue damage due to various traumatic events (e.g., burns, scalds, acne) and involve morphological and histopathological changes in normal skin.³⁹ Currently, MNs have emerged as an alternative strategy for scar treatment. Animal models and in vitro experiments of human tissues indicate that MNs can destroy thickened collagen and induce a wound healing cascade by creating micro-channels and micro-wounds.⁴⁰ Because micro-channels cause little epidermal damage, MNs are an ideal treatment for those with darker skin phototypes who are highly vulnerable to post-inflammatory hyperpigmentation. Gene expression profiles before and after MNs treatment demonstrate upregulation in the expression of important signaling molecules for collagen production and neovascularization (e.g., type I collagen, glycosaminoglycans, vascular endothelial growth factor, fibroblast growth factor-7, epidermal growth factor, transforming growth factor- β).^{41,42} Histologically, skin tissue following the application of MNs exhibit thickening of the epidermis, as well as an increase in dermal collagen and elastic fiber deposition. Over a period of weeks to months, newly formed type III collagen is replaced by mature type I collagen, causing skin tightening and a decrease in the appearance of scars or rhytides.⁴²

Skin diseases

Alopecia

Hair loss is caused by various factors, including age, disease, hormones, and drugs. Those with alopecia suffer from decreased confidence and increased inferiority due to psychological and physiological stress.⁴³ In a murine study, researchers observed hair growth following repetitive treatment with a disc MN roller. Additionally, researchers observed increased expression levels of hair growth-related genes, including β -catenin, Wnt3a, Wnt10b and vascular endothelial growth factor.⁴⁴ Another randomized clinical study investigated the therapeutic effect of electric MNs in combination with 5% minoxidil on androgenetic alopecia. Subjects were randomly divided into three groups who were treated with either topical 5% minoxidil, topical electrical MNs, or topical electrical MNs plus 5% minoxidil. MNs plus 5% minoxidil treatment group showed the best hair growth effect. Pretreatment with electro-MNs may increase the transdermal absorption of minoxidil and further stimulate hair growth.⁴⁵ Currently, hair transplantation is considered an

effective treatment for alopecia. However, hair transplantation is extremely expensive and highly invasive. The induction of intrinsic hair follicles is a promising non-invasive treatment for alopecia patients. A previous study has shown that MNPs loaded with the small molecule material UK5099 and follicular stem cell activators from mesenchymal stem cell-derived exosomes had a beneficial effect for hair regrowth and pigmentation. The MNs consisted of keratin extracted from hair and supported by a base of HA. Due to this structure, MNs were easily separated from HA base during the application process. MNs induced hair follicle regeneration by direct and continuous delivery to hair follicle stem cells. Furthermore, the MNs system promoted pigmentation and hair regeneration at lower doses, compared to the subcutaneous injection, in a mouse model.⁴⁶

Psoriasis

In recent years, biologics have opened new avenues for psoriasis therapy. However, recurrence and treatment resistance make it difficult to treat psoriasis completely. In systemic therapy, the first-line drugs for psoriasis include methotrexate (MTX), cyclosporine A (CyA), and retinoic acid.⁴⁷ These drugs are effective, but they do pose harmful side effects, such as hepatotoxicity, impaired renal function, and hypertension. Biologic agents permit targeted attack on pathogenic pathways and have better efficacy with fewer adverse reactions than those of conventional drugs. However, high cost is an obstacle to established biologics as a basic treatment for psoriasis.⁴⁸

Due to the limitations of existing topical agents for treating psoriasis, including relatively poor penetration and side effects, researchers are committed to developing innovative drug delivery methods. An accurate dose could be achieved by controlling the encapsulation of MTX in each MN. MTX-loaded MNs have been found to successfully alleviate the thickened epidermis in an imiquimod-induced murine model of psoriasis. MTX-loaded MNs have also been shown to be more effective than oral MTX at reducing skin thickness and Ki67 gene expression levels.⁴⁹

Transdermal delivery of CyA, an effective immunosuppressive agent, is difficult due to its large molecular size (1202 Da) and hydrophobic properties. Recent studies have investigated several new transdermal therapies to enable topical application of CyA. MNPs containing CyA mixed with methanol-based hydroxypropyl cellulose were applied to a psoriasis animal model. This study showed that the dissolving MNPs reduced systemic toxicity with compatible efficacy to systemic ingestion of CyA.⁵⁰

Anti-tumor necrosis factor (TNF)- α antibody biological agents effectively treat inflammatory diseases. Therefore, a dissolving MN loaded with anti-TNF- α antibody was developed and applied to a psoriasis mouse model. The treatment resolved inflammation by reducing epidermal thickness, inflammatory cell infiltration into the epidermis, and interleukin (IL)-1 β mRNA expression.⁵¹

Prurigo nodularis

Prurigo nodularis is a chronic inflammatory skin disease characterized by severe itching, burning, and stinging distributed symmetrically along the arms, legs, upper back, and/or abdomen.⁵² The standard treatment for prurigo nodularis is topical drugs, including corticosteroids, calcineurin inhibitors, and capsaicin.⁵³ However, the efficacy of these agents is limited by the thickness of the lesion. A study investigated whether biodegradable MNPs designed to be three times longer than the typical epidermal thickness could penetrate into the dermis to improve the efficacy of topically applied steroids. A 3D skin model confirmed that drug penetration of topical steroids upon applying biodegradable MNPs increased compared to the penetration observed with topical steroids alone. In clinical trials, MNs-assisted drug delivery also showed significantly improved efficacy of topical steroids.⁵⁴

Acne

Retinoic acid effectively reduces existing acne but has limited clinical application due to side effects such as skin irritation. Recently developed as an acne treatment, fractional radiofrequency microneedles (FRMNs) directly target the sebaceous glands without side effects. FRMNs reduce sebum production and induce long-term dermal remodeling of the reticular dermis. In addition, researchers have prepared a drug-loaded ROS responsive MNP using a polyvinyl alcohol matrix to increase transdermal drug penetration and treatment efficacy.⁵⁵ Compared to existing anti-acne creams, the MN system greatly promotes the transdermal penetration of drugs and improves the therapeutic efficacy for skin lesions. This system also reduced skin edema and inhibited bacterial growth in an acne mouse model.⁴

Polyionic liquid-based MNPs containing salicylic acid have been developed for the treatment of *Cutibacterium acnes* infection. These MNPs create high mechanical strength microchannels in the skin, allowing salicylic acid to penetrate into the skin. When applied to a mouse model of *C. acnes* infection, the MNPs containing salicylic acid inhibited *C. acnes* growth and reduced the number of inflammatory cells, leading to the suppression of inflammatory factors.⁵⁶

Cancer

Cancer immunotherapy is a therapeutic strategy that involves activating the immune response with several tumor antigens.⁵⁷ The skin is a very active organ for immune defense. Specifically, Langerhans cells and dendritic cells in the skin capture and present antigens under the stratum corneum as part of the antigen-specific immune response.⁵⁸

Studies have investigated antigen-based immunity using antigen-coated MNPs because MNPs are effective in delivering vaccines through the skin. The ideal antigen model for conventional transdermal therapeutic vaccines is ovalbumin (OVA).⁵⁹ One study investigated whether OVA-loaded dissolv-

ing MNPs could induce or enhance antigen-specific immune responses. Activating dendritic cells and OVA-specific cytotoxic T lymphocytes (CTL)-mediated immune response were effectively induced in mice immunized via dissolving MNPs. The researchers suggested that OVA-loaded dissolving MNPs induced an OVA-specific CTL response targeting OVA-expressing EG7 tumor cells, potentially killing tumors. Further studies are needed whether the TDDS using dissolving MNPs loaded with OVA can be used as a therapeutic or prophylactic vaccine for cancer immunotherapy.⁶⁰

Researchers have also designed MNPs that inject promising immunotherapeutic nanoparticle therapeutics directly into melanoma. According to a previous study, a nanoparticle drug formulated with an anti-programmed cell death protein 1 (PD1) antibody effectively suppressed tumors in mice, compared to conventional intravenous injection.⁶¹ Anti-PD1 MNPs inhibit the ability of melanoma to avoid the body's immune surveillance system. Ligands on melanoma cells bind to PD1 on the surface of T cells, effectively short-circuiting immune responses. If anti-PD1 binds to PD1 first, however, it disables the cancer's defense. However, anti-PD1 can also stimulate assault on healthy cells, which can lead to autoimmune diseases, such as type 1 diabetes, and severe adverse reactions.⁶² These MNPs allow topical drug administration to melanoma-affected tissues, and the nanoparticle formulation allows for sustained drug release. In the particles, anti-PD1 is encased in a matrix of modified dextran. The matrix encapsulates glucose oxidase enzymes, which convert blood glucose into gluconic acid. The resulting acid degrades the modified dextran particles, releasing the anti-PD1 continuously over several days.⁶¹ Furthermore, recent studies have reported the anti-cancer effects using MNs for breast cancer and ovarian cancer in a mouse model. The tumor suppression rates were better when the cancer vaccines were delivered through MNs.⁶³ Although the MNs approaches for all cancers have not been introduced in this paper, the research on cancer immunotherapy through MNs predict the future prospects for targeting various cancers more effectively.

Herpes virus infection

Herpes virus mainly invades mucous membranes, skin, and nerve tissues. It causes a variety of diseases and reverts to latent infection, which seriously threatens human health. Treatment of herpes virus infection with systemic (or oral) antiviral agents is effective, but gastrointestinal side effects or headache sometimes may occur. Local recurrences are very common, and new topical antiviral patches have been developed recently. Acyclovir is widely used to treat cold sores infected with herpes simplex virus type 1 (HSV-1). Because HSV-1 is generally located in the basal epithelium of the skin, a study was conducted to evaluate the penetrating ability of MNs to deliver acyclovir. MNs with a long needle length (>600 μm) and low density (<2000 needles/cm²) effectively facilitated penetration of acyclovir. In addition, these MNs significantly increased the rate of transepider-

mal water loss in in vivo percutaneous penetration experiments performed in hairless rats.⁶⁴

Another study showed that the penetration of acyclovir-loaded MNs was approximately 45 times greater than that of commercial creams in porcine skin. In vivo studies also showed that transdermal administration of acyclovir retained significantly more drug than the cream formulation.⁶⁵

Vaccine

Vaccine delivery via a dermal or intradermal route has existed since vaccines were first invented centuries ago.⁸ MNs are designed to facilitate intradermal delivery of vaccines and improve patient compliance. Moreover, the route of vaccine administration can potentially affect the performance of the vaccine formulation.⁶⁶ Although most current vaccines are administered intramuscularly or subcutaneously, previous studies have shown that MN delivery has similar immune effects at a much lower dose than subcutaneous injection.⁶⁷ Since this discovery, MNs have been extensively studied for vaccination against various viral and bacterial infections.⁸

Influenza vaccine

Influenza causes approximately 5 million serious illnesses and 250000 to 500000 deaths annually worldwide. If a highly contagious pandemic strain emerges in today's hyper-connected world, it has the potential to kill 60 million people. Although many factors influence vaccination decisions, a simple and effective influenza vaccine delivery method could increase vaccination rates and achieve good health outcomes.⁶⁸

Solid coated MNs and dissolving polymer MNs are being developed for influenza vaccines. First, pre-clinical studies suggest that MNs offer several advantages over standard intramuscular injections for influenza vaccines. In a mouse experiment, vaccination with coated MNs protected against H3N2 seasonal influenza.⁶⁹ The inclusion of trehalose in the coating formulation as a stabilizer prevented the loss of antigenicity of the vaccine. MNs coated with the stabilized vaccine induced an antibody IgG response, increased hemagglutination inhibitory antibody titers, and neutralized antibody activity. These responses are similar to those of conventional intramuscular vaccines.^{70,71} Vaccine stability was improved by using dissolving MNs made of polyvinylpyrrolidone, which encapsulates the lyophilized inactivated influenza vaccine. Since then, dissolving MN vaccines using various water-soluble polymer formulations and sugars have been introduced. A trivalent influenza vaccine administered using carboxymethyl cellulose-based dissolving MNs induced a robust immune response in mice. Together, these experiments in mice suggest that transdermal delivery of influenza vaccine by MNs could induce a long lasting and stronger antibody response than intramuscular vaccine.^{72,73}

Clinical studies of MNs vaccines have been conducted on healthy adults and older adults. MN vaccination of 20% and 40% of the standard intramuscular dose produced an immune

response in healthy adults similar to that induced by full-dose intramuscular vaccination. Intriguingly, MN vaccines induced a superior immune response in the older adults, compared to intramuscular vaccination. Studies are ongoing to determine whether MNs can offer reproducible advantages over intramuscular injection.⁷⁴

Bacillus Calmette-Guerin vaccine

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. There is an urgent need for vaccination against TB, which continues to cause more deaths than any other bacterial disease. The Bacillus Calmette-Guerin (BCG) vaccine is a live, attenuated vaccine derived from *Mycobacterium bovis* and has been used extensively to prevent TB since its introduction nearly 90 years ago.⁷⁵

According to current World Health Organization (WHO) recommendations, the BCG vaccine is administered to newborns and infants by intradermal injection with a 26- or 25-gauge half-inch needle in a 1-mL syringe. Accurate intradermal injection requires a skilled medical professional. Inappropriate injection such as accidental intramuscular injection can deposit the BCG vaccine beyond the dermal layer into less immunogenic spaces. Therefore, effective and technically correct dosing is necessary for optimal immune induction.^{76,77}

MNPs for BCG vaccine delivery have been developed to overcome issues associated with injections. Dissolving powder-laden MNP have been found to efficiently deliver BCG vaccine with little skin irritation and to induce innate, humoral, and cellular immunity similar to conventional intradermal vaccines. In addition, the MNP can be stored at room temperature for more than 60 days without detrimental effects on the penetrability or viability of the vaccine. Unlike intradermal BCG vaccine delivery, which causes severe inflammation at the injection site for several weeks, BCG-MNP have a similar vaccination efficacy without skin irritation and post-vaccination scarring.⁷⁸

BCG vaccines in coated MN formulations were optimized to preserve viability and shelf life based on previous influenza vaccines.⁷⁹ Trehalose confers better stabilizing effect compared to other cryoprotectants following freeze drying. Therefore, maintenance of vaccine activity was significantly improved with 15% trehalose in the coating solution. BCG-MN vaccination induced both TNF- α and IFN- γ secretion from dual-functional CD4⁺ T cells with similar frequencies in addition to antigen-specific IgG in a guinea pig model. This vaccine showed no safety problems in this model, which is particularly important because of the high potential for the role of BCG-induced multifunctional CD4⁺ T cells in anti-mycobacterial immunity. These studies provide evidence that MNP can be used to administer live attenuated BCG vaccine without detrimental effects on immunogenicity.⁸⁰

Hepatitis B vaccine

Hepatitis B virus (HBV) infection and subsequent chronic se-

quelae are still huge burdens to global public health due to perinatal HBV infection. The WHO recommends HBV vaccine administration at birth, followed by the regular childhood HBV vaccine series.⁸¹ Previous studies on the influenza vaccine have shown that vaccine delivery through MNP can induce a strong immune response. Critically, transmission of adjuvant-free monovalent HBV vaccine using dissolving MNP was found to induce humoral and cellular immune responses in a rhesus monkey model.^{82,83} Furthermore, HBV MN vaccine formulated in a dual release pattern using polylactic acid and carboxymethyl cellulose generated an immune response comparable to that of two doses using the conventional administration method.⁸⁴

Other applications

MNP are being used to develop novel treatment modalities in various other diseases, such as neurodegenerative disorders and obesity.

Neurodegenerative diseases

Alzheimer's disease is the most common form of dementia. The benefits of transdermal products for older adults and those with dementia have been confirmed by the commercial success of the rivastigmine patch, which administers a cholinesterase inhibitor.⁸⁵ Recently, researchers formulated donepezil hydrochloride (DPH) as a transdermal patch similar to rivastigmine. DPH is an acetylcholinesterase inhibitor, which improves neurotransmission by inhibiting the hydrolysis of acetylcholine and increasing the acetylcholine concentration in the synaptic cleft. DPH is a safe and long half-life drug that has been approved by the FDA as an effective treatment for Alzheimer's disease.⁸⁶ In a pig skin model, which is similar to human skin in terms of stratum corneum thickness and hair distribution, dissolving MNP encapsulating 95% DPH applied to skin for 5 minutes were more effective than oral administration.⁸⁷

Obesity

Several MN-based treatments are in development for obesity. Caffeine is reported to have anti-obesity activity, and caffeine-loaded dissolving MNP have been shown to enable significant weight loss in high-fat diet-induced obese mice.⁸⁸ Additionally MN-based treatments have been developed to convert white adipose tissue (WAT) into brown adipose tissue (BAT) because BAT increases the body's energy expenditure by generating heat. The novel transdermal dissolving MNP contain β_3 -adrenoceptor agonist and thyroid hormone T₃. This transdermal delivery method suppresses weight gain and enables long-term management for obesity.⁸⁹ A patch encapsulated with rosiglitazone has also been developed to inhibit WAT gain, enhance BAT, and improve insulin sensitivity. The HA-based dissolving MNP device is embedded with nanoparticles encapsulating rosiglitazone as a browning agent, glucose oxidase to provide acid environment, and catalase to consume undesired H₂O₂. The device delivers the browning agents to convert WAT to BAT, thus

reducing adipose tissue.⁹⁰

CONCLUSIONS

For many centuries, hypodermic needles were the only usable tool for transdermal drug delivery to treat various disorders. Now, we have reached a turning point as the field transitions from conventional drug delivery with intramuscular or subcutaneous administration to novel MN-based TDDS. These systems overcome the tough skin barrier efficiently and conveniently. Furthermore, MN-based TDDSs can generate immune reactions even with small doses of allergens or vaccines because the skin is more abundant in immune cells, including Langerhans cells, dendritic cells, macrophages, and T cells. Painless and patient friendly, MNs could permit more people to easily receive treatments. We hope that a new era of MN-based TDDSs may soon be welcomed in various fields.

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