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REVIEW ARTICLES

Enhancing Skin Penetration: The Role of Microneedles

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Enhancing Skin Penetration: The Role of Microneedles

SUMMARY

Since transdermal delivery systems provide some important advantages over oral delivery systems and parenteral delivery systems, they have attracted the attention of researchers. Degradation of the drug in the gastrointestinal (GI) system, irritation of the GI system tract, and the first-pass effect of the drug are some of the disadvantages of oral administration, while the need for medical staff to administer it and creating phobia in the patient are among the disadvantages of parenteral administration. To overcome these drawbacks, researchers have developed formulations for the transdermal delivery of drugs. The most important handicap of transdermal drug administration is the Stratum Corneum layer (St. Corneum), which forms the enormous barrier layer of the skin. Some techniques have been developed to overcome this serious barrier problem of the skin. Microneedles are one of the physical methods to increase the penetration of therapeutic agents through the skin. Microneedles consist of needle arrays long enough to deliver the drug to the dermis layer and micron-sized enough to not reach the nerve cells and not cause pain. Microneedles can be classified into five different types as solid microneedles, dissolving microneedles, hollow microneedles, coated microneedles, and hydrogel microneedles according to the properties of the materials used in the fabrication and the mechanisms of release of the therapeutic agent. Microneedles can be used in the application of vaccines, proteins, nucleotides, drug delivery systems, cosmetic, and for diagnostic purposes. Although important technological developments have been experienced for microneedle in many areas such as drug delivery systems, disease diagnosis, and cosmetics in the last two decades, there are many working areas that need to be developed. Especially in long-term treatments, studies should be done to develop them as smart devices.

Key Words: *Drug Delivery, Intradermal, Microfabricated device, Microneedle, Skin Penetration, Transdermal.*

Cilt Penetrasyonunun Artırılması: Mikroiğnelerin Rolü

ÖZ

Transdermal taşıyıcı sistemler, oral taşıyıc sistemler ve parenteral taşıyıcı sistemlere göre bazı önemli avantajlar sağladığından araştırmacıların dikkatini çekmiştir. İlacın gastrointestinal (GI) sistemde bozunmaya uğraması, ilacın GI sistem yolağında oluşturduğu tahriş ve ilacın ilk geçiş etkisine uğraması, oral uygulamanın bazı dezavantajlarındandır. İlacın parenteral uygulanması için bir tıbbi personele ihtiyaç duyulması ve parenteral uygulamada kullanılan iğnenin hastalarda korku yaratması parenteral uygulamanın dezavantajları arasındadır. Bu olumsuzlukların üstesinden gelmek için, araştırmacılar transdermal ilaç taşıyıcı sistemler geliştirdiler. Transdermal ilaç uygulamasındaki en büyük engel, derinin muazzam bariyer tabakasını oluşturan Stratum Corneum (St. Corneum) tabakasıdır. Derinin bu ciddi bariyer probleminin üstesinden gelmek için bazı teknikler geliştirilmiştir. Mikroiğneler, terapötik ajanların deriye nüfuz etmesini arttırmak için geliştirilen fiziksel yöntemlerden biridir. Mikroiğneler, ilacı dermis tabakasına iletecek kadar uzun ve sinir hücrelerine ulaşmayacak ve ağrıya neden olmayacak kadar mikron boyutlu iğne dizilerinden oluşmaktadır. Mikroiğneler, imalatta kullanılan malzemelerin özelliklerine ve terapötik maddenin salınım mekanizmalarına göre katı mikroiğneler, çözünebilen mikroiğneler, içi boş mikro iğneler, kaplı mikro iğneler ve hidrojel mikro iğneler olarak 5 farklı tipte sınıflandırılabilir. Mikroiğneler aşıların, proteinlerin, nükleotidlerin, ilaç taşıyıcı sistemlerinin, kozmetiklerin uygulanmasında ve teşhis amaçlı kullanılabilir. Son yirmi yılda ilaç taşıyıcı sistemleri, hastalık teşhisi ve kozmetik gibi birçok alanda mikroiğneler için önemli teknolojik gelişmeler yaşanmış olsa da, geliştirilmesi gereken birçok çalışma alanı vardır. Özellikle uzun süreli tedavilerde, mikroiğneleri akıllı cihaz olarak geliştirmek amacıyla araştırmalar yapılmalıdır.

Anahtar kelimeler: *İlaç taşıyıcı, intradermal Mikrofabrikasyon cihaz, Mikroiğne, Deri penetrasyonu, Transdermal.*

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INTRODUCTION

Although the transdermal administration of drugs for systemic effect is a new administration way, the drugs have been applied to the skins for years for both cosmetic and therapeutic purposes. Previously, they were administering drugs to the skin to act locally. Subsequently, studies have shown that drugs can penetrate through the skin and they can reach systemic circulation. Poor drug absorption or enzymatic degradation in the gastrointestinal tract or liver restricts the use of some drugs orally (W. Liu et al., 2016). Also, the administration of hypodermic needles is limited due to the pain and psychological distress (Subramony, 2013). Such problems further increase the interest in the administration of drugs to the skin. Some advantages of the application of drugs to the skin can be listed as follows (Zhou et al., 2018):

- To prevent the degradation of drugs in the digestive tract and the low absorption of food-related.
- Avoiding the first pass effect of liver
- Improving bioavailability
- Providing constant plasma concentration up to 7 days with the same patch
- Elimination of disorders related to hypodermic needle-related pain, fear, and infection

In spite of that, affections of drugs in creams, patches, solutions, and other traditional transdermal and intradermal dosage forms are limited because of the skin's impressive barrier function to the transport of ingredients into the body (Y. Park, Kim, Chung, Sung, & Kim, 2016). The *Stratum Corneum* (St. Corneum) is the main barrier in the skin and the outermost layer of the skin (Cortes et al., 2019). Restricted drug delivery through the skin is one of the disadvantages of topical creams and transdermal patches (Waghule et al., 2019). Due to the drawbacks of hypodermic needles such as pain and fear, researchers have developed various methods to increase penetration through the skin. Chemical and natural penetration enhancers (J. Chen et al., 2016), iontophoresis (Dixit, Bali, Baboota, Ahuja, & Ali, 2007), electroporation (Escobar-Chavez, Bonilla-Martinez, Villegas-Gonzalez, & Revilla-Vazquez, 2009), ultrasound-mediated systems (Polat, Hart, Langer, & Blankschtein, 2011), local hypobaric pressure (Inacio et al., 2016) and Microneedles (MNs) (Ita, 2015b) increase the penetration of molecules by creating tiny holes in the skin. For the development of effective and innovative skin penetration enhancing systems, the structure of the skin, its components, and the mechanisms through the skin should be well known.

GENERAL INFORMATION

Skin's structure and permeability

In an adult, an average weight of skin is about 9 kg, and an average surface area of 2 m2 makes the skin the largest organ (Degim, 2006). The skin completely covers the body and forms a flexible barrier between the organism and the external environment (Degim, 2006). In addition to being the organ of the sense of touch in our body (Tsakovska et al., 2017), adjusting body temperature is one of the functions of the skin (Sawasaki, Iwase, & Mano, 2001). We can list some of the functions of the skin as follows (Tsakovska et al., 2017);

- Providing protection against unwanted toxic substances and pathogenic microorganisms
- Protects the body against mechanical forces
- Prevents body from losing water
- It has an immunological activity by creating an inflammatory response against foreign substances entering the skin.

Skin consists of three basic layers shown in Figure 1. (Lai-Cheong & McGrath, 2013).

- Epidermis
- Dermis
- Subcutis

The epidermis consists of a multilayered epithelium layer, interfollicular epidermis associated hair follicles, and sebaceous and sweat glands (Niemann & Watt, 2002). The epidermis consists of basic four layers. The deepest part of the epidermis is the basal layer where the keratinocytes are single layers. Keratinocytes differentiate towards the skin surface and form the *St. Corneum* layer at the outermost part of the skin (Lai-Cheong & McGrath, 2013).

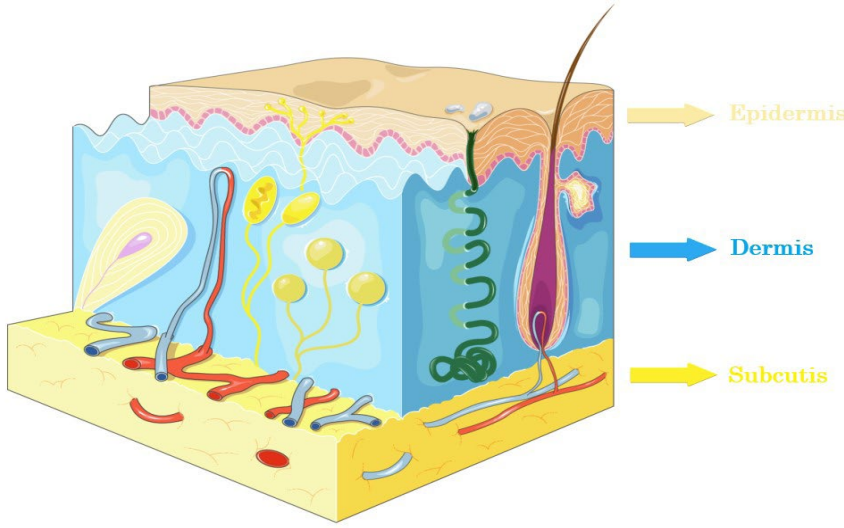

Figure 1. Structure of skin (https://smart.servier.com/smart_image/skin/)

The *St. Corneum* consists of a structure known as the "brick and mortar" model (Larraneta, McCrudden, Courtenay, & Donnelly, 2016). Bricks consist of terminally differentiated keratinocyte consisting of keratin filaments and filaggrin. The mortar consists of intercellular lipids (Meckfessel & Brandt, 2014). The lipid structure consists of a combination of ceramide, cholesterol, and free fatty acids in a roughly 1: 1: 1 ratio (Lundborg et al., 2018). *St. Corneum* lipids prevent transepidermal water loss (TEWL) and form the main barrier function of the skin (Meckfessel & Brandt, 2014). A deformation in the *St. Corneum* structure causes TEWL, leading to dysfunction of the barrier function of the skin. There is increasing interest in drug delivery via the skin to alleviate the drawbacks of parenteral administration and oral administration of drugs. However, The *St. Corneum* provides the body with tremendous protection against external influences and is a major obstacle to drug administration to the skin. The structure of the skin in the area where the drug is administered and the physicochemical properties of the drug are important factors affecting the penetration of the drug through the skin (Kamble, Sadarani, Majumdar, & Bhullar, 2017). In order for a drug to penetrate easily through the skin, its partition coefficient must be high (Log P range of 1-3), its molecular weight should be less than 500 Da, and its melting point should be less than 200 Co.(Prausnitz & Langer, 2008). The drug can reach the lower layers of the skin in different three pathways such as intracellular route, intercellular lipid route, and transappendageal route (Figure 2) (Marwah, Garg, Goyal, & Rath, 2016). To increase drug penetration through the skin, chemical methods such as chemical penetration enhancers, salt formation, ion pairs, and prodrugs, or physical methods such as iontophoresis, electroporation, sonophoresis, and MNs are applied (Marwah et al., 2016).

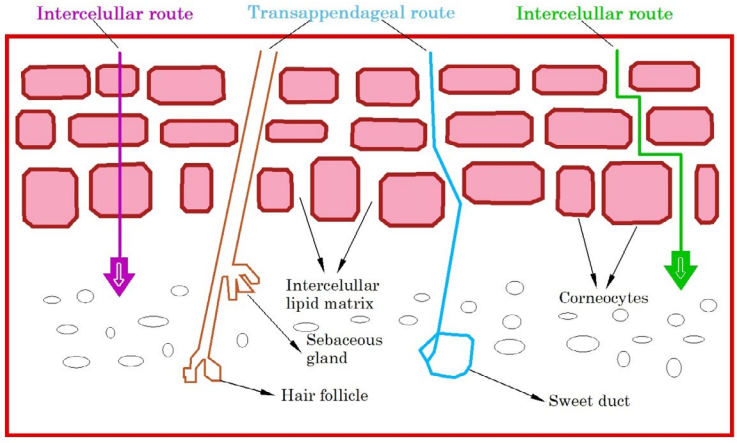

Figure 2. Drug permeation pathways through skin (Marwah, Garg, Goyal, & Rath, 2016)

Historical development and physical properties of MNs

The term MN was first mentioned in a research paper by author Robert Chambers in 1921 (Chambers, 1921). The first patent invention for the delivery of drugs by MN design was obtained in the United States in 1971 by Martin S Gerstel and Virgil A Place (Bhatnagar, Dave, & Venuganti, 2017; Gerstel & Place, 1976). The patent describing solid and hollow MNs. However, the definition of MN was first made in 1998 by Henry et al. (Henry, McAllister, Allen, & Prausnitz, 1999). In this study, it was shown that the penetration amount of calcein from excised human skin was four times higher with MN applications than passive topical application (Henry et al., 1999). The first invention of the coated MN was made in 1975 by Pistor Michel Louis Paul (Bhatnagar et al., 2017). The application of genetic materials through the skin with metallic solid MNs was first developed in 2001 by Alza Corporation (Bhatnagar et al., 2017). The first study on the use of MNs in the immune system was conducted in 2002 by Mikszta et al. with silicone MNs on mice (Mikszta et al., 2002). In an article published in 2003 by McAllister et al., It was demonstrated by a study on human cadaver skin where transdermal applications of macromolecules and nanoparticles can be made with solid and hollow MNs (McAllister et al., 2003). Miyano et al. first tested ascorbate-2-glycoside-containing maltose MNs on healthy volunteers in 2005, and it was observed that soluble MNs were tolerated by the skin and that the skin exceeded the *St. Corneum* barrier (Miyano et al., 2005). Glass hollow MNs were first used in 2005 to detect glucose by using dermal interstitial fluid extraction (Wang, Cornwell, & Prausnitz, 2005). In 2005, Fernandes demonstrated its effectiveness on human skin as skin tightening and wrinkle remover for cosmetic purposes (Fernandes, 2005).

Figure 3 shows a chronological timeline of the events in MN development (Bhatnagar et al., 2017). Mark Praustnizt, one of the leading researchers in the field with MNs studies, defines MNs as third-generation transdermal systems (Prausnitz & Langer, 2008). MNs make micro-size holes in the skin, allowing the drug to cross the skin's *St. Corneum* barrier. MNs are produced using microfabrication technology in different sizes (50-900 micrometers height, 2000 MNs/cm2, 1-25 micron thickness) and using different materials such as silicon, metal, glass, polymer (Larraneta, Lutton, Woolfson, & Donnelly, 2016). MNs are long enough to reach the dermis and short enough not to reach the skin nerve cells and blood vessels (Larraneta, Lutton, et al., 2016). There is no report that MNs cause skin infection and patients can apply MNs to the skin without the need for an additional applicator (Donnelly et al., 2014).

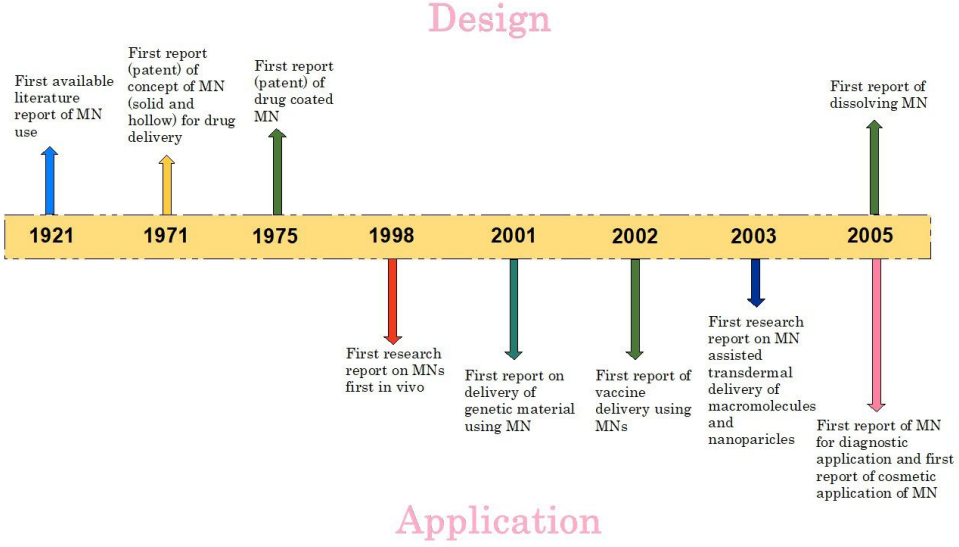

Figure 3. Chronological timeline of MN development with important design and application milestones (Bhatnagar et al., 2017)

Types of MNs

According to the materials used in formulations and the different drug delivery systems, MNs can be classified into five different groups as solid MNs, dissolving MNs, coated MNs, and hollow MNs. Arrangements on the shapes and components of MNs can provide targeted and controlled release of drugs to various tissues and organs (Duarah, Sharma, & Wen, 2019). The main function of MNs is to overcome the barrier role of the tissue mechanically and to ensure that the drug reaches the target area (Figure 4) (Rzhevskiy, Singh, Donnelly, & Anissimov, 2018).

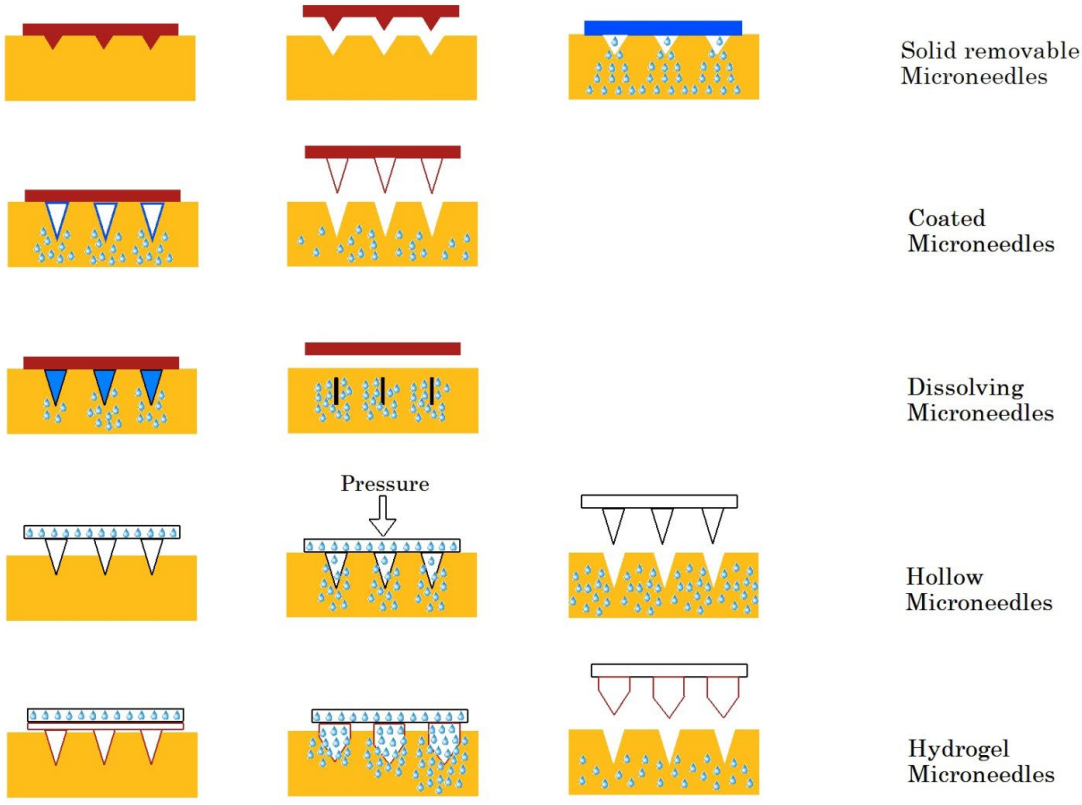

Figure 4. Types of MNs (Rzhevskiy, Singh, Donnelly, & Anissimov, 2018)

Solid MNs

Solid MNs mechanically puncture the barrier tissue of the skin to form micron-sized pores so that the skin is ready to be applied to topical preparations, transdermal patches. The microchannels formed on the skin are closed immediately after the MN patches are removed from the skin surface to prevent undesired toxic substances and pathogenic substances entrance to the body (Gupta, Gill, Andrews, & Prausnitz, 2011). In the fabrication of solid MNs, non-biodegradable materials such as silicon, polymers such as polycarbonate, biodegradable polymers such as maltose, metals such as stainless steel, titanium, and nickel, and a wide variety of materials are used (Duarah et al., 2019). Different factors such as the sharpness of the MN tips, the density of the MNs, the insertion force of the MNs affect the penetration of therapeutic agents through the skin (Duarah et al., 2019). A combination of solid MNs with a penetration enhancing method such as iontophoresis has shown that to produce an increased effect on the penetration of some drugs through the skin (Donnelly, Raj Singh, & Woolfson, 2010). Solid MNs were first manufactured in 1998 from silicone (Henry et al., 1999). Narayanan et al. successfully manufactured long, sharp solid silicon MNs with an average length of 158 μm and a base

width of 110.5 μm using a tetramethylammonium hydroxide etching process (S. P. Narayanan & Raghavan, 2017). In his next study, Narayanan manufactured 250 μm long, 52.8 μm base width, gold-coated silicon MNs with an aspect ratio of 4.73, a tip angle of 24.5°, and a diameter of 45 μm. In this study, mechanical strength and bioavailability of MNs were improved (S Pradeep Narayanan & Raghavan, 2019). Li et al. have successfully fabricated polylactic acid MNs with a length of 800 μm and 256 microns per cm2 to prove that biodegradable polymers have the mechanical strength to penetrate the *St. Corneum* layer and can be used in solid MN fabrication (Q. Y. Li, Zhang, Chen, Wang, & Guo, 2017).

Hollow MNs

Hollow MNs are in the form of an array of hypodermic needles that are sized to micron sizes. There is an empty storage area for loading the therapeutic agent and a hole at the end of the needles for drug release. Hollow MNs can be developed using a variety of materials such as silicon, metal, glass, ceramics, polymers, and carbohydrates (Duarah et al., 2019). There are hollow MNs developed from 30 commercially available hypodermic needles, each with a length of 300 μm and a diameter of 300 μm (Verbaan et al., 2008). Hollow MNs are mainly used for therapeutic agents such as large molecular weight proteins, vaccines, and oligonucleotides (Ita, 2015a). The storage space inside the needle allows for a greater amount of therapeutic agent loading (Waghule et al., 2019). It is important that the flow rate through the holes at the tip of the needle has a constant value (Cheung, Han, & Das, 2014). The flow from the hollow MNs depends on the length of the MN and the hole diameter at the MN tip (Bodhale, Nisar, & Afzulpurkar, 2010). By increasing the hole size in the hollow MNs, the drug can accelerate the flow through these holes. However, this change reduces the sharpness and strength required for the MN to enter the skin. Sometimes, the needles are covered with metal to increase the strength of the needle. However, this application can make the needles sharp (Ita, 2015a). In one study, Mishra et al. developed a hollow MN on a silicon substrate with a length of 500-600 μm and an outer diameter of 100 μm, with a flow rate of 0.93 μl s – 1 with a pressure difference of 2 K Pa at the inlet (Mishra, Maiti, & Bhattacharyya, 2018). Suzuki et al. fabricated MNs by three-dimensional laser lithography that mimicking mosquitoes, and exhibits a better penetration through the skin. In this method, the reduction in the number of MN makes both fabrication and drilling easier (Suzuki, Takahashi, & Aoyagi, 2018). Maaden et al. manufactured fused silica hollow MNs using hydrofluoric acid etching, which can inject a smaller amount of vaccine into the skin in order to eliminate the drawbacks of the administration of the vaccines with hypodermic needles (van der Maaden et al., 2018). Chen et al. investigated the penetration of hydrophilic large molecular weight compounds such as calcein and bovine serum albümine (BSA) from pigskin with a combination of silicone hollow MN and sonophoresis. In this study, hollow MNs broke the *St. Corneum* barrier and allowed the drug to reach the lower layers of the skin, while ultrasound increased the diffusion of both in the epidermis and MNs (B. T. Chen, Wei, & Iliescu, 2010; Mishra et al., 2018).

Dissolving MNs

Dissolving MNs are made of biodegradable materials. Polysaccharides such as carboxymethylcellulose, maltose, and sugar, biopolymers such as sodium hyaluronate, chondroitin sulfate, and polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), polyvinylpyrrolidone (PVP), poly (vinylpyrrolidone-methacrylic) acid (PVPMAA), and poly (methyl vinyl ether) -maleic anhydride) (PMVE-MA) are used to produce dissolving MNs (Duarah et al., 2019). The therapeutic agents stored in the MN tips are released as a result of the dissolution of the biodegradable material in the skin after entering the skin. The structure and physicochemical properties of the polymer affect the release of the drug from dissolving MNs. The biocompatible and biodegradable polymers used in the production of dissolving MNs make them more advantageous than other MN types (Duarah et al., 2019).

The use of bio-degrading substances prevents bio-harmful substances from entering the body and the accumulation of residues (J. H. Park, Allen, & Prausnitz, 2005). Since there is no medical waste left after dissolving MNs, the risk of infection is eliminated. Ease of administration with dissolving MNs increases patient compliance. Despite all these advantages, drug loading and fabrication processes adversely affect either the stability and strength of the MNs or the stability of the drug (Duarah et al., 2019). Donnelly et al. prepared galactose MNs loaded with 5-aminolevulinic acid and (ALA) and BSA by melting galactose powder at 160 °C by micro-molding technique (Donnelly, Morrow, Singh, et al., 2009). In this study, it is stated that temperature will cause both losses of active substance and degradation of the

active substance. In 2018, Pan and colleagues developed dissolving MNs of STAT3 siRNA to increase the penetration of siRNA from the skin and used polyethyleneimine (PEI, 25kDa) as a carrier to increase the cellular uptake of siRNA (Pan et al., 2018). In vitro experiments have shown that the STAT3 siRNA PEI complex increases cellular uptake and transfection, thereby inhibiting the growth of tumor cells. *In vivo* experiments, dose-dependent inhibition of melanoma growth by administration of STAT3 siRNA PEI complex with dissolving MNs was found. Du et al. developed hyaluronic acid-based methotrexate loaded dissolving MNs to eliminate the drawbacks of oral and parenteral administration of methotrexate (Du et al., 2019). These dissolving MNs loaded with methotrexate have been shown to be penetrated into mice skins with a thickness of the imiquimod-induced epidermis. Methotrexate-loaded MNs were found to be more effective in the treatment of psoriasis than oral dosing at the same dose and eliminate the risk of possible dose-dependent side effects.

Coated MNs

Coated MNs are the type prepared by adhering the therapeutic agent to the surface of the MN. The very small surface area of the MN limits the number of therapeutic agents to be loaded into the MN (Gill & Prausnitz, 2007b). The thickness of the coating layer affects the drug loading dose. The layer-by-layer technique is used to increase the amount of therapeutic agent to be loaded onto the coated MN. In this method, MNs are either immersed in high viscosity aqueous solution or spray (Yang, Liu, Fu, & Song, 2019). Physicochemical properties, stability, and coating processes of MN materials are factors affecting the effectiveness of MNs. It should be noted that there may be a loss of therapeutic agent from MN surfaces when MNs penetrate through the skin (Gill & Prausnitz, 2007a). Since antigens can easily target dendritic cells in the dermis and Langerhans cells in the epidermis, coated micro-needles are of particular interest for the transdermal administration of vaccines (Prausnitz, Mikszta, Cormier, & Andrianov, 2009). Jain et al. investigated the application of 5-ALA to the skin with coated MNs, which were naturally converted by tissue cells into a photosensitizer called protoporphyrin IX (PPIX) (Jain, Lee, & Gill, 2016). In this study, 5- ALA coated MNs reached lower layers of skin (~ 480 μm) compared to the topical cream formulation (~ 150 μm). *In vivo* experiments showed that as small as 1.75 mg of 5-ALA coated MNs suppressed the growth of subcutaneous tumors while topical cream formulation loaded with 5 mg of 5-ALA showed ineffective to suppress tumor growth and comparable to the untreated group.

Hydrogel MNs

One of the characteristics of a drug should be ease of administration. The ease of administration is among the advantages of the most innovative drugs. However, in solid MNs, first, the MN is applied to the skin, and then the topical formulation is applied to the area where MNs are removed. Since this application consists of two stages, it is not preferred. Donnelly and his colleagues developed hydrogel MNs to overcome this problem. Hydrogel MNs do not contain any drugs and swell by absorbing interstitial fluid after entering the skin (Donnelly et al., 2013).

Hydrophilic polymers are used to form hydrogel MNs that can absorb large amounts of water into their three-dimensional polymeric structure (Waghule et al., 2019). When these polymers penetrate into the skin, they swell in the presence of interstitial fluid to form channels between the capillary circulation and the drug-containing patch (Waghule et al., 2019). The drug is released by the swelling controlled release mechanism. Hydrogel MNs can be easily sterilized and removed from the skin without deterioration, making hydrogel MNs advantageous over other MNs (Donnelly et al., 2013). The most important advantage of hydrogel-forming MNs over dissolving MNs is that the loading dose is not very limited (Duarah et al., 2019). The storage of the therapeutic agent in a separate reservoir allows for greater and easier loading. Hydrogel MNs take advantage of the absorption of interstitial fluid into the body and can be used for research, diagnosis, and analysis (Tuan-Mahmood et al., 2013). Closure of internal wounds is clinically challenging due to air/fluid leakage, local ischemia, and deterioration of healing (Jeon et al., 2019). Recently, to overcome these drawbacks, Jeon and his colleagues developed a double-layer hydrogel MN, inspired by swollen endoparasites that attach their tails to the host's intestines (Jeon et al., 2019). The shell portion of these hydrogel MNs consists of swellable mussel adhesive protein (MAP), and the core portion consists of non-swollen silk fibroin. In ex vivo, hydrogel-forming MNs (139.7 ± 14.1 mmHg) great wound closure capacity against lumen leaks comparable to suture (151.0 ± 23.3 mmHg). In vivo, excellent results were obtained for wet and/or dynamic external internal tissues.

Application of MNs

Although the skin has a barrier property, it is advantageous to administer the therapeutic agents, bioactive agents, and cosmetic products via the skin. MNs provide an increase in penetration through the skin for the administration of therapeutic agents through the skin. Nowadays, in addition to drug delivery, research on MNs in diagnosis, cosmetic, and vaccine administration is being conducted.

Drug delivery

Since the first publication of MNs by Henry et al., various types of MNs have been developed and applied with various therapeutic agents over the last 20 years. Since then, small molecules such as caffeine, lidocaine, metronidazole (Garland, Caffarel–Salvador, Migalska, Woolfson, & Donnelly, 2012), ibuprofen sodium (J. W. Lee, Park, & Prausnitz, 2008; McCrudden et al., 2014), sulfordamine B (J. W. Lee et al., 2008), 5-aminolevulinic acid (Donnelly, Morrow, McCarron, et al., 2009) and macromolecules such as insulin (Ito, Hirono, Fukushima, Sugioka, & Takada, 2012; S. Liu et al., 2012), BSA (Duarah et al., 2019), low molecular weight heparin (Gomaa et al., 2012), ovalbumin (Matsuo et al., 2012; Naito et al., 2012), leuprolide acetate (Ito, Murano, Hamasaki, Fukushima, & Takada, 2011), erythropoietin (Ito, Yoshimitsu, Shiroyama, Sugioka, & Takada, 2006), and growth hormone (Jeong Woo Lee, Choi, Felner, & Prausnitz, 2011) have been investigated for MNs administration. Pre-treatment MNs have been also investigated for non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, paracetamol) (Stahl, Wohlert, & Kietzmann, 2012), naltrexone (NTX), dyclonine (X. Li et al., 2010; Wermeling et al., 2008) and some photo-sensitizer [5-aminolevulinic acid, 5-aminolevulinic acid methyl ester and mesotetra (N-methyl-4) -pyridyl) porphine tetratosylate] (Donnelly, Morrow, McCarron, et al., 2009; Mikolajewska et al., 2010). Some researchers have used MNs combined with microparticles and nanoparticles systems to further facilitate penetration through the skin of MNs. Recently, Yu et al. have developed a "smart insulin patch" consisting of MNs containing insülin loaded sensitive glucose-sensitive vesicles at the tips (Yu et al., 2015). This vesicular consists of hypoxia sensitive hyaluronic acid conjugated with the hydrophobic compound that is biodegradable to the hydrophilic structure under hypoxic conditions (2-nitroimidazole). During the enzymatic oxidation of glucose, a local hypoxia microenvironment occurs. The reduction of the hyaluronic acid / 2-nitroimidazole conjugate can occur by leading to the separation of vesicles and insulin release. In the *in vivo* study, it was tested on artificially induced rats with type-1 diabetes, and within a few hours, the glucose level was controlled by MNs.

Recently, Ronnander et al. have investigated the in vitro transdermal transmission of sumatriptan succinate using a combination of skin penetration enhancing techniques such as dissolving polymeric MN and iontophoresis (Ronnander, Simon, & Koch, 2019). Skin penetration experiments were performed to evaluate the effect of formulation parameters on drug release from polyvinylpyrrolidone systems under the low electric current (≤500 μA / cm2). They prepared preparations of hydrophilic, positively charged molecules encapsulated in a water-soluble and biocompatible polymeric material. Using silver/silver chloride electrodes, currents of 100, 300, and 500 μA / cm2 were applied for 6 hours. The MN patch was composed of 600 needles with an area size of 0.785 cm2. Tests with diffusion cells and the skin of mini-Göttingen pigs showed that small decreases in polymer concentration led to negligible lag times and in the cumulative amount of drug permeated in 6 h (Q6h) and in the flux (Jss).

In a recent study, Ita and Abiandu investigated the influence of MN rollers on the permeation of potassium chloride across porcine skin in order to eliminate the negative effects such as delayed peak plasma concentration in oral administration and pain swelling, trypanophobia and hyperkalemia in parenteral administration of potassium chloride (Abiandu & Ita, 2019). Permeation studies in vitro Franz diffusion cells showed a significant increase in the amount of potassium chloride transporting through the skin compared to passive diffusion (0.637 ± 0.02 mg / cm2 / h) with roller MNs (6.33 ± 18.70 mg / cm2 / h). For the treatment of hyperhidrosis, multiple injections of botulinum neurotoxin A (BoNT / A) into the palm with conventional hypodermic needles is a painful and frightening method, and Shim et al. designed a BoNT / A coated MN to address this drawback (Shim et al., 2019). Polylactic acid MN coated with BoNT / A formulations were successfully penetrated from thick skin in vitro experiments. Their stability was then tested at 4, 25, and 37 °C for 24 hours. BoNT-MN was more stable than liquid BoNT / A. In addition, *in vivo* study, the right paws of the mice were treated with BoNT-MNs, which showed a significant reduction in sweating in the right paws of the mice.

Recently, Donnelly et al. investigated the potential for transdermal administration of high-dose metformin hydrochloride (metformin HCL) with hydrogel MN patches (Migdadi et al., 2018). Patches (two layers) were assembled from a lyophilized drug reservoir layer, with the MN layer made from an aqueous blend of 20% w/w poly (methyl vinyl ether-co-maleic acid) crosslinked by esterification with 7.5% w/w poly (ethylene glycol) 10,000 Da. >90% of metformin was recovered from homogeneous drug reservoirs. They constantly penetrated MNs to Parafilm®, an approved skin model. Permeation of metformin HCL across the dermatomed skin of neonatal pig in vitro was increased using MNs. The combined MN and metformin HCL reservoir patch (containing 75 mg or 50 mg metformin HCl, respectively) delivered 9.71 ± 2.22 mg and 10.04 ± 1.92 mg at 6 h, respectively, and 28.15 ± 2.37 mg and 23.25 ± 3.58 mg at 24 h, respectively. Compared with a control system used only drug reservoirs, 0.34 ± 0.39 mg and 0.85 ± 0.68 mg was delivered at 6 h, respectively, and 0.39 ± 0.39 mg and 1.01 ± 0.84 mg was delivered at 24 h, respectively. The *in vivo* rat study, metformin HCL plasma concentration was 0.62 ± 0.51 µg / mL at 1 h after MNs and increased to 3.76 ± 2.58 µg / mL at 3 h. The maximum concentration of 3.77 ± 2.09 µg / ml was determined at 24 h. Bioavailability of transdermal administration of Metformin was determined by micro-needle around 30%.

Bhatnagar et al. have developed dissolving MNs consisting of a composite of polyvinyl pyrrolidone and polyvinyl alcohol for the combined administration of doxorubicin and docetaxel anti-cancer drugs (Bhatnagar, Bankar, Kulkarni, & Venuganti, 2019). In this study, maximum amounts of doxorubicin (533 ± 65 µg) and docetaxel (227 ± 23µg) loaded on a MN patch. Ex-vivo studies on excised mouse skin showed no delay in the administration of MNs and permeation of chemotherapeutics. MNs were dissolved in the excised skin within 1 hour. The efficacy of dissolving MNs on 4T1 breast cancer cells was investigated in a xenograft Balb / c mouse model. Intratumoral injection of doxorubicin and doxorubicin + docetaxel showed a severe toxicity problem resulting in an excessive decrease in body weight and 100% death after nine days and two doses. Compared with intratumoral injection, doxorubicin and docetaxel using MN combined or alone have significantly reduced toxicity (100% survival after 16-days and 4-dose administration). In addition, doxorubicin and docetaxel combined administration were found to be more effective in inhibiting tumor growth than the administration of single molecules.

Lee and colleagues developed non-invasive hollow MNs for local and extended-release of phenylephrine from the sol-gel formulation in the treatment of intermittent fecal incontinence (H. Lee, Park, & Park, 2017). They integrated the hollow MN with the low-temperature system to maintain the low temperature of the sol formulation. They prepared various sol-gel formulations using Pluronic F-127 (PF-127) and hydroxy-propyl-methyl-cellulose (HPMC). They observed gelling temperatures, flow properties, and diffusion retardation. They measured resting anal sphincter pressure in response to phenylephrine (PE) sol-gel formulation using an air-charged catheter. They evaluated the biocompatibility of the sol-gel PE formulation by observing the immunological response. *In vivo* study, the PF-127 25%, HPMC 1%, and PE formulation (PF25-HPMC1 PE) were injected through the peri-anal skin of the rat, the highest pressure on the anal sphincter muscle occurred at 6-8 h, and anal pressure increased and lasted twice as long as with the phosphate-buffered saline (PBS)-PE formulation. After the PF25-HPMC1-PE formulation and the PBS-PE formulation were administered to the rat *in vivo*, no significant difference was observed between the numbers of mast cells.

Table 1. A recent summary of active substance made with different types of MNs

Active substance	Type of MN	Reference
Insulin	Dissolving	(Yu et al., 2015)
Sumatriptan	Dissolving	(Ronnander, Simon, & Koch, 2019)
Potassium chloride	Solid	(Abiandu & Ita, 2019)
BoNT / A	Coated	(Shim et al., 2019)
Metformin HCL	Hydrogel	(Migdadi et al., 2018)
Doxorubicin and Docetaxel	Dissolving	(Bhatnagar, Bankar, Kulkarni, & Venuganti, 2019)
Phenylephrine	Hollow	(H. Lee, Park, & Park, 2017).

CONCLUSION

In this review, we have focused on the types of MNs, which are one of the physical methods to increase penetration through the skin, and current research as drug delivery systems.

Since MNs are painless, easy, and reliable, especially in elderly and pediatric patients, the drug can be improved as a drug delivery system. At first, only large

molecules were applied with MNs, and nowadays, small molecular weight drugs with high therapeutic doses are loaded into MNs, and more effective and reliable treatment is provided. Although important technological developments have been experienced for MNs in many areas such as drug delivery systems, disease diagnosis, and cosmetics in the last two decades, there are many working areas that need to be developed. Especially, in long-term treatments, studies should be done to develop them as smart devices.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Idea of the manuscript (Yener F. G.), literature research, data arrangement (Samancı B.), corrections and revisions (Değim İ. T.).

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