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EMERGING DEVELOPMENTS IN TRANSDERMAL DELIVERY VIA MICRONEEDLE

[1]Dhruv Bhavsar,
[1]Assistant Professor,
[1] Department of Pharmaceutics, Arihant School of Pharmacy and Bio-research institute, Adalaj, Gandhinagar, India – 382421
*CORRESPONDING ADDRESS:
DHRUV BHAVSAR*
Department of Pharmaceutics,
Arihant School of Pharmacy and Bio-research institute,
Adalaj, Gandhinagar,
India – 382421

ABSTRACT

The effectiveness of transdermal drug delivery has been limited due to the inability of most drugs to penetrate the skin at therapeutic rates. However, the use of micron-scale needles has been proposed as a solution to increase skin permeability and improve transdermal delivery, particularly for macromolecules. Microneedles have been fabricated with various sizes, shapes, and materials using microelectronics industry tools. Solid microneedles have been the focus of most drug delivery studies, as they have been shown to enhance skin permeability to a wide range of molecules and nanoparticles in vitro. In vivo studies have demonstrated successful delivery of oligonucleotides, insulin-induced reduction of blood glucose levels, and immune response induction from protein and DNA vaccines. Proteins and peptides have become a significant therapeutic modality for various diseases due to their high potency and specificity. However, their large molecular weight, poor stability, and conformational flexibility make them challenging to formulate and deliver. Injection is the primary route for clinical administration of protein and peptide drugs, but it often results in poor patient compliance. Microneedles offer a portable, minimally invasive solution that can overcome the skin barrier and create reversible microchannels for effective macromolecule permeation. This review highlights recent advances in microneedle-mediated transdermal delivery of protein and peptide drugs, with a focus on representative microneedle design and fabrication. The current application status of microneedle-mediated transdermal protein and peptide delivery, particularly in the fields of infectious disease, diabetes, cancer, and other disease therapies, is also summarized. Finally, the current status of clinical translation and future development perspectives are provided.

Keywords

Transdermal, Microneedle, Protein, Peptide, nanoparticle, Hydrogel, cancer

1. INTRODUCTION

Proteins and peptides play a crucial role in the human body, contributing to various functions including molecular transportation, cellular regulation, biological scaffold formation, and enzymatic catalysis. These biomolecules have made significant contributions to almost every medical field [1-4]. The approval of insulin as the first therapeutic protein in 1982 marked a milestone in the development of protein and peptide therapeutics, leading to remarkable progress in their clinical application [5,6]. However, the utilization of protein and peptide drugs is often limited by certain factors. Their large molecular weight significantly hampers their ability to permeate biological barriers like the skin and mucous membranes. Additionally, the susceptibility of these drugs to loss of biological activity due to external conditions (such as moisture and temperature) and endogenous proteolytic enzymes poses significant challenges in formulation and delivery technologies [7]. Currently, injection remains the primary route for the clinical administration of protein and peptide drugs, with intravenous, subcutaneous, and intramuscular injections being the most commonly employed methods [2,8-12]. Irrespective of the method of injection, the majority of protein and peptide drugs undergo degradation by various metabolic enzymes in the body, resulting in a short half-life in vivo. This necessitates frequent injections. Moreover, injection therapy is inconvenient and not user-friendly, particularly for patients with chronic ailments like rheumatoid arthritis and diabetes. Additionally, injection safety is a concern as needle contamination during administration can lead to the transmission of infectious diseases such as Hepatitis B and C. Consequently, there is a significant demand for an alternative drug delivery system that offers improved therapeutic efficacy, patient compliance, and safety for the delivery of protein and peptide drugs. Transdermal drug delivery presents itself as a viable option, allowing the delivery of biologically active substances through the skin for local or systemic effects. This approach is noninvasive and can be self-administered [13]. There are specific criteria that must be met for drugs intended for transdermal administration. These include a maximum molecular weight of 1000 Da and a balance between hydrophobicity and polarity to overcome the stratum corneum barrier[14]. However, most protein and peptide drugs are hydrophilic and macromolecular in nature, making it difficult for them to penetrate the skin. In recent decades, several chemical and physical methods have been developed as potential strategies to enhance transdermal drug permeation, such as penetration enhancers [15], microjet [16], laser [17], electroporation [18], sonophoresis [19], and iontophoresis [20]. Despite these advancements, these techniques are often costly and cumbersome to use, and their efficiency in delivering macromolecular drugs transdermally remains limited.

In recent times, microneedles (MNs) have emerged as a novel method for delivering drugs. The applications of MNs have expanded to various areas, encompassing small chemical molecules [21,22], vaccines [23,24], genes [25], proteins [4,26], and nanoparticles [27]. Notably, MNs offer a promising avenue for transdermal delivery of proteins and peptides [28,29]. MNs are a minimally invasive device consisting of orderly arranged needles (<1 mm) on a base. These needles can create reversible microchannels in the skin, allowing direct penetration of the stratum corneum. These microchannels enable drugs to reach the dermal microcirculation in the deeper layers of the skin (Fig. 1). Compared to injections, MNs do not come into contact with blood vessels and nerves in the deep dermis, resulting in improved patient compliance and a safer profile. Furthermore, the gentle fabrication conditions of MNs do not compromise the biological activity of proteins and peptides. This review presents comprehensive updates on the transdermal delivery of protein and peptide drugs mediated by MNs. The focus is on the latest advancements and developments in MNs design and fabrication. Additionally, we have summarized recent studies on the applications of MNs in protein and peptide delivery, with a particular emphasis on infectious diseases, diabetes, cancer, and other therapeutic areas. Finally, the current status of clinical translation and future prospects for development are also discussed.

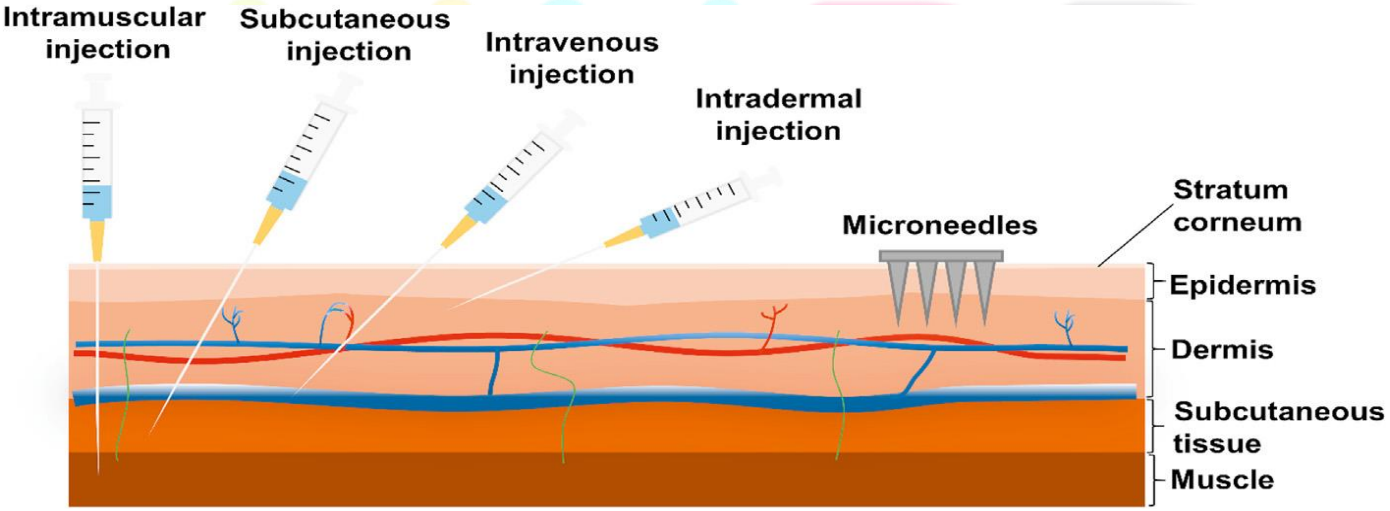

Fig. 1 Schematic illustration of protein and peptide drug delivery by conventional injections and microneedles.[28]

2. Representative types of MNs

In 1971, Gerstel et al. introduced the concept of MNs, while Henry et al.[30] was the first to report the use of MNs for transdermal drug delivery in vivo in 1998 [30,31]. Since then, a variety of MNs have been successfully developed [32]. MNs can be broadly classified into five categories based on different drug delivery strategies: solid MNs, coated MNs, hollow MNs, dissolving MNs, and hydrogel-forming MNs (Fig. 2). Each type of MNs has been extensively studied for transdermal drug delivery. However, protein and peptide drugs are often sensitive to high temperature, pH value, and organic solvents compared to inert small molecules [33]. To preserve their biological activity, it is crucial to understand the properties of each type of MNs and select the appropriate MNs for formulation. This section provides a detailed description of the typical applications associated with different MNs-mediated delivery approaches.

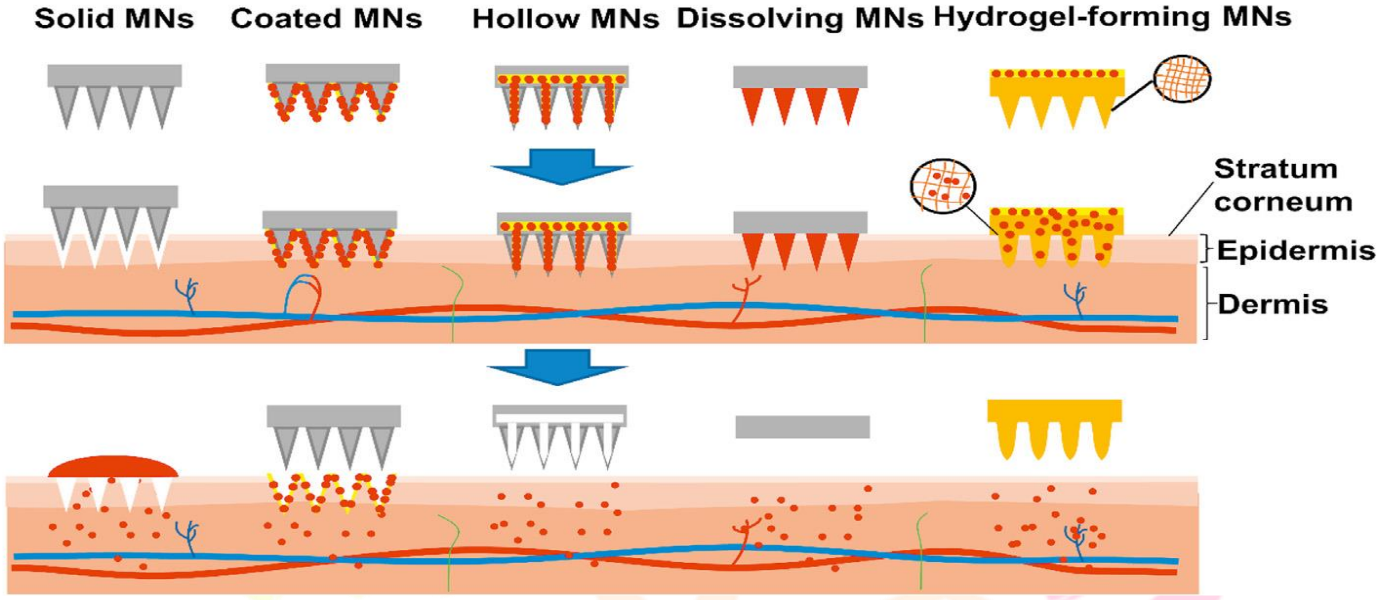

Fig 2. Representative types of MNs for transdermal drug delivery.

2.1. Solid MNs

Solid microneedles (MNs) typically require a two-step process for drug delivery. Initially, the solid MNs are inserted into the skin and then removed to create temporary microchannels. Subsequently, a suitable pharmaceutical dosage form, such as gel, cream, or ointment, is applied to these microchannels that were formed earlier [23,34]. The mechanical strength of solid MNs should be adequate to ensure successful skin pretreatment, which can be achieved by carefully selecting the materials for MN fabrication [23]. Silicon [35] and metal [36,37] are commonly used materials for solid MN fabrication due to their favorable properties. However, it is important to note that these materials may not be suitable for transdermal drug delivery as they are non-biodegradable and can pose safety concerns once inserted into the skin. On the other hand, polymeric materials are often preferred due to their good biocompatibility. Various polymeric materials, including polylactic acid (PLA), polymethylmethacrylate, polycarbonate, and carboxymethylcellulose (CMC), have been developed as alternatives to non-biodegradable metal or silicon for the preparation of solid MNs [38-40].

Solid microneedles (MNs) facilitate drug delivery through passive diffusion via microchannels created in the skin. Consequently, the design of the length and density of solid MNs utilized for skin pretreatment plays a crucial role in determining the extent of drug penetration [41,42]. Additionally, the characteristics of the drugs themselves also influence the efficiency of delivery. In contrast to conventional transdermal delivery methods, the microchannels formed by pretreating the skin with solid MNs enhance the permeation of hydrophilic compounds [43]. McAllister et al. [44] demonstrated that the permeability of bovine serum albumin (BSA) and insulin increased following skin pretreatment using solid silicon MNs. Furthermore, the molecular weight of drugs can impact passive transport when employing solid MNs [45,46]. Verbaan et al. [46] observed that the transport rate of a compound with a larger molecular weight (72 kDa) was significantly lower compared to compounds with molecular weights of 10 kDa and 538 Da. Despite their advantages, solid MNs do have inherent limitations. The two-step administration process involving MNs array pretreatment and subsequent application of pharmaceutical preparations is considered inconvenient and may lead to imprecise dosage [47]. Consequently, alternative MN-based drug delivery strategies have gained prominence due to their ability to address these concerns and improve patient compliance.

2.2. Coated MNs

In order to streamline the application process, drugs are applied to the surface of solid MNs, resulting in coated MNs. These coated MNs offer a more convenient and controlled method for transdermal drug delivery. Once inserted, the drug coating layer on the MNs dissolves, releasing the active pharmaceutical ingredients into the skin [48]. Subsequently, the MNs can be removed. Coated MNs are commonly made from metal or silicon, but there has also been extensive research on polymeric coated MNs to avoid the use of less biocompatible materials. The solid microstructure transdermal system (sMTS) is created using a robust polymer that maintains its structural integrity when inserted into the skin [49-51].

Kapoor and colleagues [51] devised a method to deliver Peptide A by coating it on a patch with 316 needles, known as sMTS. The researchers successfully achieved transdermal delivery of Peptide A, with the bioavailability comparable to subcutaneous injection. Additionally, they observed a significant improvement in the stability of Peptide A when coated on the sMTS [51]. Various techniques, including spray coating, dip coating, and piezoelectric inkjet printing, were employed for the coating of MNs [52]. Spray coating and dip coating are the most commonly used methods, utilizing an aqueous drug solution with high viscosity to ensure a higher retention of drugs on the surface of MNs. The main challenge lies in achieving a uniform coating of sufficient therapeutic agents. Therefore, it is crucial to optimize the coating process and formulation composition. Formulations typically require surfactants, viscosity enhancers, and peptide stabilizers to ensure coating stability and uniformity of the drugs [53]. Since most biomolecules are hydrophilic, the coating solution is usually aqueous. Zhao and colleagues [54] developed a coating formulation that incorporated ternary co-solvents and polyvinyl alcohol 2000, enabling loading of both hydrophilic and hydrophobic peptides while maintaining their bioactivity. Other methods, such as the layer-by-layer technique, have also proven effective in coating MNs. In this method, MNs can have drug molecules applied to them by immersing them alternately in two solutions that contain solutes with opposite charges. This process results in the formation of a polyelectrolyte multilayer [55]. While the mechanical strength of the coated MNs is generally maintained, their sharpness at the tip is reduced due to the drug loading. This reduction in sharpness can potentially affect the ability of the MNs to penetrate the skin [56]. Consequently, the amount of drug that can be loaded onto the coated MNs is compromised. This suggests that proteins and peptides with high potency, such as desmopressin [56], human growth hormone [57], and interferon alpha [58], are suitable for this approach.

2.3. Hollow MNs

Sub-millimeter hollow MNs act as micron-scale syringes, penetrating the stratum corneum to deliver liquid formulations into the epidermis or dermis. Passive diffusion is the simplest method of drug delivery, but dense tissues have a low diffusion rate. Pressure-driven flow or diffusion can achieve faster transport rates, making hollow MNs more effective than solid MNs for administering larger doses with precise transport rates. [59, 21, 47, 60-62]

The digitally controlled hollow MNs injection system, known as the DChMN-iSystem, has the capability to administer precise amounts of therapeutic vaccine. A study conducted on mice to evaluate immunization showed that the delivery of HPV peptide vaccine through the DC-hMN-iSystem resulted in a robust cytotoxic and T helper response [63]. Utilizing hollow MNs for intradermal delivery of nanoparticles has proven to be an effective strategy in enhancing the efficacy of vaccines. When antigen-loaded poly(D,L-lactide-glycolide) nanoparticles were delivered via hollow MNs, it elicited a significantly higher antibody response and increased lymphocyte count compared to intramuscular injection and soluble antigen delivered via hollow MNs [64]. However, the fabrication process for hollow MNs is typically more complex. Apart from creating a needle with appropriate inner holes, hollow MNs also need to be combined with a drug reservoir. It is worth noting that hollow MNs, which are typically made from metal or silicon with varying inner hole diameters, are inherently weaker than solid MNs and carry a higher risk of breakage [65].

2.4. Dissolving MNs

Dissolvable microneedles (MNs) are commonly made from materials that can dissolve and contain therapeutic agents within the needles. This allows for effective drug delivery into the skin through the dissolution of the needle matrix [66-68]. Various materials have been utilized to create dissolving MNs, ranging from low molecular weight carbohydrates to high molecular weight biodegradable polymers. These materials include dextran, CMC sodium, hyaluronic acid (HA), chondroitin sulfate, polyvinylpyrrolidone (PVP), and polyvinylalcohol (PVA). The use of dissolving MNs offers the advantage of being a one-step administration method, which is highly convenient for patients. Furthermore, dissolving MNs have unique benefits such as leaving no harmful residue and not generating biohazardous sharp waste after application [69-71]. Additionally, the mild preparation conditions of dissolving MNs make it easier to achieve industrialization, which is particularly advantageous for protein and peptide drugs. Moreover, the solid state of encapsulated biomolecules within dissolving MNs provides protection during cold chain storage and transport [72]. Various techniques have been developed for the fabrication of dissolving MNs, including micromolding [73], drawing lithography [74], droplet-borne air blowing [75], electro-drawing [76], and photolithography [77]. Among these methods, micromolding is the most commonly used. In this process, micromolds are filled with a polymer melt or solvent casting, sometimes with the assistance of vacuum and/or centrifugal force [23]. The molds are then allowed to solidify or undergo in situ polymerization of the liquid within the microcavities. It is important to note that these methods are primarily suitable for small-scale production of MNs in the academic field [78]. For larger-scale fabrication, several innovative techniques have been developed to achieve a highly efficient, controllable, and scalable production of dissolving MNs. Our group has also developed the double-penetration female mold-based positive-pressure microperfusion technique for the scale-up fabrication of dissolving MNs [79].

Proteins and peptides that are sensitive to heat should be enclosed within micromolds and solidified under gentle conditions that do not compromise their functionality. In a study conducted by Park et al.[80], poly-lactide-co-glycolide (PLGA) MNs were created using the micromolding technique to encapsulate microparticles containing BSA and calcein. The researchers successfully demonstrated the controlled release of calcein and BSA using these polymeric MNs80. However, the use of high temperatures during the process resulted in a slight decrease in protein activity. To tackle this issue, Lee et al.[69] employed a milder preparation approach to fabricate dissolving MNs from ultra-low viscosity CMC, while maintaining the full enzymatic activity. Similarly, dissolving MNs loaded with erythropoietin were prepared at room temperature using a thread-forming polymer as a base [81].

Despite the numerous advantages of dissolving MNs in transdermal drug delivery, controlling the amount and localization of drugs within the needles is challenging due to drug diffusion from the needles to the base during the micromolding process. This

diffusion can result in imprecise dosing and limited drug delivery efficiency [82]. To address this issue, Prausnitz's group [83,84] concentrated drugs in the tips of the MNs by incorporating an air bubble at the base, effectively preventing drug diffusion. Multilayered dissolving MNs have also been found to be beneficial in achieving controlled drug delivery [85-87]. Li et al. [88] developed a multilayered MNs patch with an effervescent backing to facilitate rapid separation. Our group [85] also developed rapidly separating dissolving MNs that enable precise drug delivery and rapid separation. In this approach, the drugs were concentrated in the needle tip, while the blank separating part allowed for separation within 30 seconds in simulated skin [85]. The choice of materials used as the matrix for dissolving MNs is crucial as it can impact the preparation process and the efficacy of the drug. Additionally, it is important to note that long-term use of dissolving MNs may pose safety concerns regarding polymer accumulation in the skin [89].

2.5. Hydrogel-forming MNs

Hydrogel-forming microneedles (MNs) are typically made from crosslinked polymeric materials. These materials can penetrate the outermost layer of the skin, known as the stratum corneum, and absorb fluid from the surrounding tissue, causing the polymeric matrix to swell [22]. This swollen matrix allows for the diffusion of drugs into the dermal tissue, enabling effective delivery. One advantage of hydrogel-forming MNs is that they can be easily removed from the skin without leaving behind much residue [22]. Additionally, unlike hollow MNs, the drug diffusion of hydrogel-forming MNs is not hindered by compressed skin tissue. In the case of hydrogel-forming MNs, the drug is typically not directly loaded into the needles themselves. Instead, drugs are loaded into a separate reservoir, such as a polymeric film [92]. This approach allows for a larger amount of drug to be loaded and delivered into the skin. However, there are also newer variations of hydrogel-forming MNs where the drug is loaded directly into the needles. Another development in this field is the use of biocompatible thermosensitive copolymers to create in situ hydrogel-forming MNs. These MNs undergo a transition from a solution at room temperature to a gel at skin temperature, allowing for the formation of a hydrogel in situ. Regardless of the drug location, the degree of swelling in the hydrogel matrix is crucial for drug delivery. By adjusting the crosslink density of the matrix, the release rate of the drug can be controlled [93].

Hydrogel-forming microneedles (MNs) can also serve as a diagnostic tool by analyzing the interstitial fluid absorbed by the MNs when inserted into the skin. These MNs are made from swellable materials formed by chemically or physically cross-linking polymers [95], such as crosslinked poly(methylvinylether/maleic acid) (PMVE/MA)-poly(ethylene glycol) [97] (PEG) 10,000, and PVA-dextran [96]. Hydrogel-forming MNs can be considered a subtype of polymeric MNs that exhibit the physicochemical properties of hydrogels. The micromolding method is commonly used to prepare hydrogel-forming MNs. In a study conducted by Donnelly et al., an aqueous blend containing PMVE/MA and PEG10,000 was used to produce hydrogel-forming MNs using a silicone micromold. The drug reservoir patch was prepared beforehand and then attached to the needles with moderate pressure, creating an integrated hydrogel MNs system. This system successfully delivered various drugs with different molecular weights, including large molecular weight proteins and peptides like insulin and BSA. Yang et al [96]. designed a phase-transition MNs system that facilitated highly efficient transdermal delivery of insulin by utilizing polyvinyl alcohol as the microneedle material through a microcrystalline cross-linking strategy. Lutton et al [73]. also developed a scalable manufacturing process for hydrogel-forming MNs, which was carried out at ambient conditions using a combination of injection molding and roller casting. As hydrogel-forming MNs are typically made from polymeric materials, it is important to consider their mechanical strength and physical stability during application and storage.

3. Application of MNs-mediated protein and peptide delivery

Proteins and peptides have emerged as important therapeutic options for a range of diseases, consistently making their way into the market [99-101]. This can be attributed to their ability to specifically target certain areas, their high effectiveness, and their favorable safety profile compared to traditional small-molecule drugs. Microneedles (MNs), as a minimally invasive tool, can enhance patient compliance and serve as a versatile platform to overcome the skin barrier for hydrophilic and macromolecular drugs [32]. Additionally, the gentle manufacturing conditions and solid state nature of MNs offer a significant advantage over traditional injection methods using aqueous solutions, as they can improve drug stability and reduce the need for cold chain storage [80]. With advancements in material science and microfabrication technology, numerous strategies for delivering proteins and peptides using MNs have been developed. MNs have been successfully employed to deliver various types of cargoes, ranging from native drugs to nanoparticle or microparticle-based formulations [27]. In this section, we provide an overview of the recent progress in MNs-mediated protein and peptide delivery, with a particular focus on their application in infectious disease therapy, diabetes therapy, and cancer therapy.

3.1. Infectious disease therapy

Influenza, measles, and hepatitis B are infectious diseases that contribute significantly to human mortality rates, posing a significant global public health concern [47,102]. Vaccination has emerged as the most successful and cost-effective strategy to combat these diseases [103]. Among various antigen molecules, proteins have the unique ability to stimulate both cellular and humoral immunity. This makes protein-based vaccines highly effective in inducing artificial immunity due to their versatility and customizability 104. However, the current methods of vaccine administration, such as subcutaneous or intramuscular injection, often result in discomfort and low patient compliance [59,105-107]. The skin, with its abundance of antigen-presenting cells like

macrophages, dermal dendritic cells, and Langerhans cells, presents a unique opportunity for immunomodulation. Transcutaneous immunization using microneedles offers a promising approach to enhance vaccine efficacy while minimizing pain and inconvenience for patients (Fig. 3) [108-110].

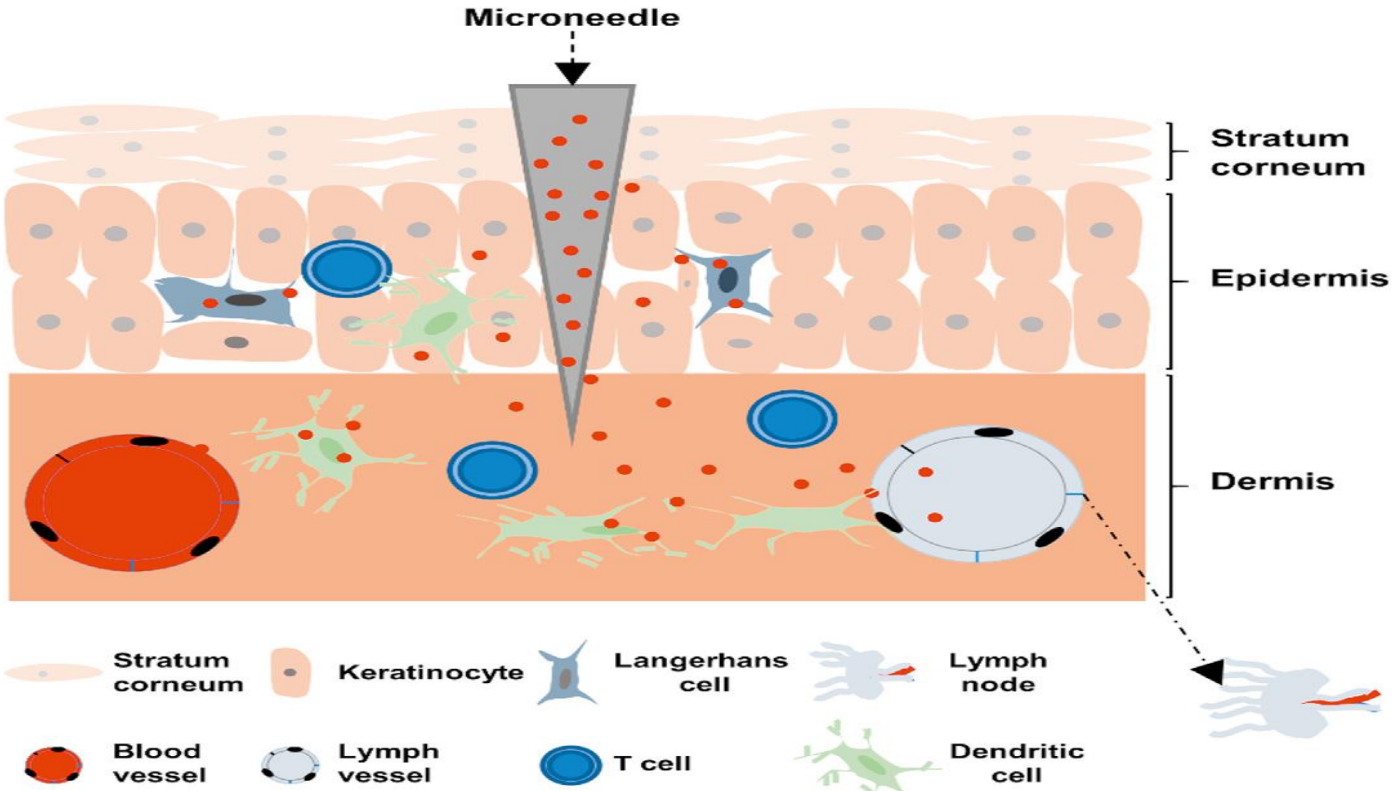

Fig 3 Mechanisms of MNs-mediated transdermal immunomodulation.

In recent years, MNs have proven to be a successful experimental delivery system for a variety of protein and peptide vaccines. Through transcutaneous vaccination mediated by MNs, antigens can be effectively presented to immunocytes residing in the skin, resulting in a stronger topical immunization and the potential for lower dosage requirements [143,144]. Matriano et al.[37] conducted a study comparing different routes of OVA (model antigen) administration, and found that the immune response was most efficient when using coated MNs and intradermal administration, as opposed to subcutaneous or intramuscular administration. Additionally, mice that received 0.5 mg of antigen with MNs exhibited comparable or higher levels of IgG titers compared to those that received 5 mg of antigen through intramuscular administration [137]. Furthermore, the use of dissolving MNs for influenza vaccine delivery has shown promise in improving virus clearance efficiency and enhancing cellular recall response, when compared to conventional intramuscular injection [72,129]. One crucial aspect in the formulation of protein and peptide vaccines is maintaining the stability of the vaccine component throughout the fabrication, transportation, and storage processes. By employing appropriate formulation techniques using MNs, the long-term immunogenicity of the antigen can be preserved, allowing for flexible storage conditions [145,146]. DeMuth et al.[127] discovered that the sucrose-coated MNs effectively transported adenovirus into the skin and could be stored at room temperature for several months without compromising the biological activity of adenovirus vectors. Mistilis et al.[130] conducted a study to evaluate different combinations of dissolving MNs formulations for stabilizing a trivalent subunit influenza vaccine. Even after being stored at 25°C for 24 months, dissolving MNs formulated with arginine/heptagluconate, sucrose/arginine, and trehalose/sucrose combinations maintained the vaccine's immunogenicity. The mice immunization experiment also demonstrated that the antibody titer was comparable to that of the fresh liquid vaccine administered through intradermal injection130. Many vaccines available in the market contain adjuvants [112,119,121-123]. Balmert et al.[121] utilized dissolving MNs to deliver OVA and Poly(I:C) adjuvant. While the addition of Poly(I:C) had minimal impact on the IgG1 response, it did contribute to a moderate increase in the IgG2c response. Additionally, several polymeric matrix materials used in MNs can also function as adjuvants, enhancing the immune response due to their intrinsic immunogenicity. For instance, poly[di(carboxylatophenoxy)phosphazene] can serve as both a vaccine adjuvant and a fabrication material. When incorporated into coated MNs for antigen delivery, it exhibited superior activity in pigs and showed significant potential for antigen sparing compared to intramuscular administration138. It is anticipated that this will further drive the utilization of polymeric MNs in immunization. OVA, a model protein known for its unique lymph node-targeting ability, is commonly employed to evaluate the performance of MNs in immunization[37,111-119,121,124]. Zaric and colleagues [118] utilized PLGA nanoparticles to encapsulate OVA, which were then delivered to the skin using dissolving MNs. This delivery method allowed skin-derived DCs to transport the nanoparticles to the skin draining lymph nodes via afferent lymphatic vessels, resulting in a strong antigen-specific immune response. Additionally, the PLGA nanoencapsulation ensured the stability of the antigen within the dissolving MNs, promoting its retention in the skin [118]. On the other hand, He et al. [114] developed layer-by-layer coated MNs using a synthetic pH-induced charge-invertible polymer. This innovative approach significantly reduced the implantation time, as the layer-by-layer films could be implanted in vivo within just 60 seconds during the insertion process Fig. 4.

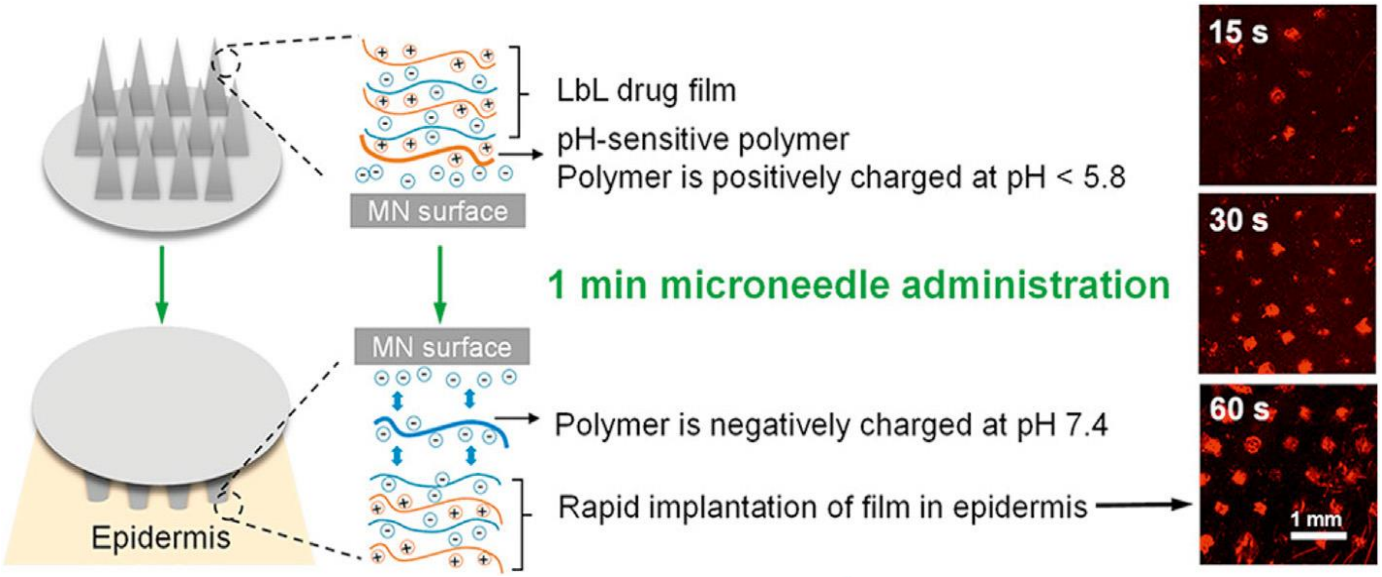

Fig 4 The implantation of layer-by-layer drug films using coated MNs for enhanced transdermal vaccination.[114]

The MNs coated with a layer triggered a robust immune response, resulting in significantly higher levels of serum OVA-specific IgG1 compared to the subcutaneous and intramuscular injection groups [114]. The coated MNs group exhibited 160 times and 9 times higher IgG1 levels, respectively [112]. The field of nanotechnology has witnessed rapid advancements, leading to the utilization of MNs for efficient delivery of macromolecules and nanoparticle-based therapies [112]. By harnessing the advantages of both nanoparticles and MNs, the transdermal delivery efficiency of proteins and peptides [72,126-133] can be enhanced. In a study conducted by Du et al.[126], the intradermal delivery efficiency of four nanoparticulate vaccines was compared using hollow MNs. Both nanoparticles and solution elicited strong total IgG and IgG1 responses, but the nanoparticles notably increased the IgG2a response. MNs-mediated transdermal immunomodulation has primarily been explored in the context of influenza. Zhu et al [126]. coated virus protein on stainless MNs and successfully immunized mice. After four weeks, all mice immunized with virus-coated MNs survived, similar to those receiving intramuscular injection, while the control group mice succumbed to the challenge on Day 5-8. Littauer and colleagues [133] demonstrated that by incorporating the thermolabile granulocyte-macrophage colony stimulating factor into the H1N1 vaccine-loaded dissolving MNs, there was an improvement in vaccine-induced immunity. This finding opens up possibilities for using other active recombinant molecules as adjuvants to enhance vaccination efficacy against influenza. MNs-mediated transdermal immunomodulation has also been extensively studied in the context of combating other infectious diseases, including HIV [134,135], diarrhea [137], hepatitis B [138], plague [139], tuberculosis [140], measles [141], and leishmaniasis [142]. Of particular concern is the recent COVID-19 pandemic, which poses a significant threat to public health. Vaccines targeting the coronaviruses-S1 subunit have shown promise in preventing coronavirus infections. Kim and colleagues [136,147] developed dissolving MNs using carboxymethyl cellulose and incorporated the protein at room temperature. All dissolving MNs vaccines induced higher levels of neutralizing antibodies compared to subcutaneous injection of monophosphoryl lipid A adjuvanted vaccine. While further research is needed to determine their efficacy and safety, transdermal delivery of proteins and peptides through MNs holds great potential in combating various infectious diseases. In particular, for vaccines that require multiple administrations, transdermal MNs vaccination offers a more convenient option [1].

3.2. Diabetes therapy

Diabetes is a chronic condition characterized by an abnormal accumulation of glucose in the bloodstream due to a disorder in glucose metabolism [148]. This disorder can be caused by either reduced insulin secretion (type 1 diabetes) or the body's impaired responsiveness to insulin (type 2 diabetes) [149,150]. To effectively manage diabetes, the administration of exogenous insulin is essential. Insulin, a peptide consisting of 51 amino acids, is a hormone that helps regulate blood glucose levels [151,152]. However, the frequent and repeated subcutaneous injections required for insulin delivery can cause significant pain and negatively impact treatment compliance. In contrast, transdermal delivery of insulin [153], which involves delivering insulin through the skin, is an appealing alternative method. By incorporating microneedles (MNs) [105,154] into insulin delivery, a large number of diabetic patients can benefit from a minimally painful and easy-to-administer treatment. Solid MNs, made from various materials such as silicon, metal, and polymer [26,155,156], have successfully improved insulin permeability through skin pretreatment, leading to a reduction in blood glucose levels. Researchers have also explored the use of stainless steel MNs with different needle lengths to evaluate the effectiveness of insulin delivery to diabetic rats.[158] The results demonstrated an increase in the skin's permeability to insulin and a rapid decrease in blood glucose levels within one hour [162]. Additionally, the integration of solid MNs with techniques like iontophoresis can further enhance the efficiency of transdermal insulin delivery [44]. Hollow MNs, on the other hand, enable intradermal insulin delivery with faster onset, which can be achieved through passive diffusion, pressure, or electricity. Studies have shown that hollow MNs allow microliters of solutions to enter the skin, and higher pressure leads to a more rapid decrease in blood glucose levels. Roxhed and colleagues [162] developed a patch system utilizing microneedles (MNs) and an electronically controlled liquid dispenser. The electrically driven active administration resulted in a plasma insulin concentration approximately five times greater than the passive diffusion group at three hours after dosing [162].

Insulin delivery through drug-free MNs, whether solid or hollow, typically requires multiple steps, which can be inconvenient for patients. However, drug-loaded MNs, such as coated MNs, dissolving MNs, and hydrogel-forming MNs, offer a solution to these challenges. In a study by Ross et al.[163], insulin polymeric layers were coated onto metal MNs. These thin and uniform layers effectively preserved the integrity of insulin, allowing for rapid release within 20 minutes. This suggests that solid-

state insulin delivery through coated MNs is a viable option. However, further research on insulin coated MNs is limited, possibly due to the insufficient dosage of coated insulin. On the other hand, dissolving MNs that encapsulate insulin within their matrix show more promise. They offer favorable biocompatibility, a relatively simple manufacturing process, and low cost. To incorporate insulin into dissolving MNs, various water-soluble polymers such as HA164, chondroitin sulfate [12], poly-gammaglutamic acid [165], and a mixture of starch and gelatin [166] have been used. These polymers allow for the preparation of insulin-loaded dissolving MNs at room temperature using the micromold casting method. In a study by Liu et al.[164], the ability of dissolving MNs prepared with HA to deliver insulin to diabetic rats was evaluated. The results demonstrated that insulin administered through dissolving MNs effectively entered the systemic circulation, and its hypoglycemic effect was comparable to subcutaneous injection [164].

The traditional method of treating diabetes through subcutaneous injection is often linked to poor control of blood glucose levels. However, the closed-loop drug delivery technique has shown great potential in diabetes treatment by precisely regulating insulin release in response to changes in blood glucose levels. To achieve this, glucose-responsive microneedles (MNs) have been developed using glucose-sensing elements such as glucose oxidase (GOx) [167-173] and phenylboronic [175]. Yu et al [174]. utilized a non-degradable glucose-responsive polymer to design an MNs patch loaded with insulin [174]. The polymeric matrix swelled under hyperglycemic conditions, weakening the electrostatic interaction between negatively charged polymers and insulin, thereby promoting insulin release. Conversely, under euglycemic conditions, the inhibited volume change and restoration of electrostatic interaction slowed down the insulin release rate [173-175,176]. Another potential approach to treating diabetes is through the use of glucagon-like peptide-1 receptor agonists. Chen et al [173]. constructed a smart exendin-4 delivery platform based on MNs incorporated with dual mineralized microparticles containing GOx and exendin-4. The closed-loop MNs system demonstrated excellent glucose regulation ability by rapidly responding to hyperglycemia, significantly improving the therapeutic performance of exendin-4 173.

3.3. Cancer therapy

The widespread prevalence, high morbidity, and mortality of cancer make it the primary concern of public health. While surgery, radiotherapy, and chemotherapy have been the traditional methods of cancer treatment, immunotherapy has emerged as an effective strategy [178]. Unlike other treatments, immunotherapeutic drugs activate the body's immune system to attack cancer cells instead of directly killing them. This makes immunotherapy a promising approach to treating and potentially curing certain types of cancer. The number of approved immunotherapeutic drugs is increasing, and there are many treatments in preclinical and clinical stages [179.] Immunotherapeutic agents are mainly divided into five categories, including cancer vaccines, checkpoint inhibitors, engineered T cells, lymphocyte-promoting cytokines, and agonistic antibodies against co-stimulatory receptors [180]. Many of these agents are composed of proteins and peptides, and preclinical studies have shown promising efficacy in cancer immunotherapy. Therapeutic cancer vaccines, which use a patient's own immune system, represent a viable option for active immunotherapy of cancers. These vaccines include cell vaccines (tumor or immune cell), genetic (DNA, RNA, and viral) vaccines, and protein/peptide-based vaccines [195]. Vaccination using antigens administered by MNs has the ability to induce a strong immune response that is specific to the antigen. This response is characterized by the activation of CD8 cytotoxic T-lymphocytes, which effectively target and eliminate tumors. This process is similar to the overall protection provided by vaccination against infectious diseases. Additionally, an immune adjuvant can be employed alongside the antigen, either simultaneously or beforehand, to enhance the body's immune response in a non-specific manner. In a study conducted by Kim et al.[196], dissolving MNs were utilized to transport a model antigen (OVA) and an immunostimulatory adjuvant (resiquimod) to the lymph nodes, where they facilitated the maturation and activation of antigen-presenting cells Fig. 5.

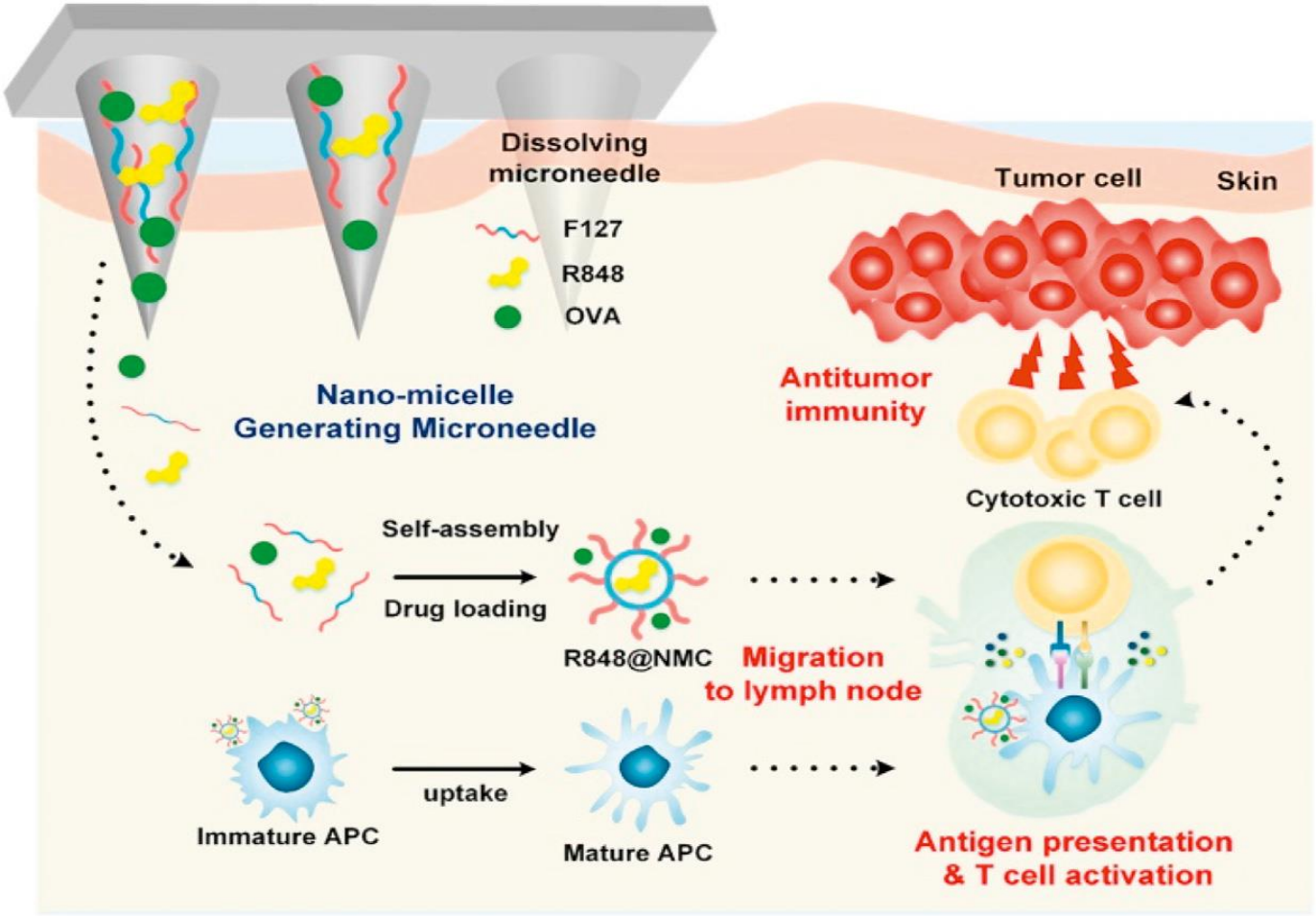

Fig 5 Enhanced cancer vaccination by in situ nanomicelle-generating dissolving MNs containing OVA and resiquimod (R848).[181]

The amphiphilic triblock copolymer-based dissolving MNs have the ability to form nanomicelles in the skin upon dissolution [196]. This property facilitates the delivery of poorly water-soluble resiquimod. In a study conducted on tumor-bearing mice, the application of dissolving MNs containing OVA and resiquimod resulted in a significant level of antigen-specific cellular and humoral immunity, indicating an antitumor immune response. Additionally, proteins and peptides with catalytic abilities can serve as adjuvant agents for other therapeutic modalities or as anticancer drugs themselves [187]. Moreover, certain proteins and peptides can function as drug delivery carriers due to their biocompatibility and bioresorbable properties. Some cell-penetrating peptides can be combined with vaccines for immunotherapy. For instance, Ruan et al. developed a targeted anti-melanoma treatment by combining a siBraf delivery system based on cell-penetrating peptide octaarginine nanocomplexes with coated MNs. The results demonstrated that octaarginine exhibited lower cytotoxicity compared to polyethyleneimine, while maintaining comparable gene transfection and silencing efficacy. The octaarginine/siBraf coated MNs successfully penetrated the melanoma site and effectively inhibited tumor growth [187]. Duong and colleagues [188] developed a polypeptide cocktail based on dissolving microneedles (MNs) to enhance cancer immunotherapy. In comparison to subcutaneous vaccination, the dissolving MNs demonstrated a higher OVA-specific antibody titer and effectively inhibited the growth of OVA-expressing metastatic tumors. These immunomodulatory antibodies have the ability to stimulate a robust anti-tumor immune response. However, they often lead to significant autoimmunity, resulting in adverse effects [197]. To address this issue, targeted and controlled release of antibodies specifically to desired cell types can minimize off-target effects and reduce toxicity. By directly delivering immunotherapies to the site of the disease using MNs, it becomes possible to effectively target the desired tumor and immune cells. Therefore, the integration of MNs with immunomodulatory antibodies holds great promise in the fight against malignant tumors. Notably, MNs encapsulated with nanoparticles have been designed to enable the controlled release of immune checkpoint inhibitors, such as aPD-1/aPD-L1 [189,190], aCTLA-4 [191,194], and 1-methyl-D,L-tryptophan [192,194]. Wang and colleagues [189] developed self-degradable MNs for the sustained delivery of aPD-1. These MNs were formulated using hyaluronic acid integrated with pH-sensitive dextran nanoparticles containing aPD-1 and GOx. The acidic microenvironment of the tumor facilitated the sustained release of aPD-1. In an in vivo study using a mouse melanoma model, the application of self-degradable MNs induced a strong immune response compared to MNs without a degradation trigger or intratumor injection of free aPD-1 [189]. Furthermore, the co-loading of different checkpoint inhibitors into MNs resulted in a synergistic treatment approach for tumors [189,192]. In their study, Ye et al. [192] developed the MNs platform to simultaneously deliver aPD-1 and 1-methyl-D,L-tryptophan. The findings of their research revealed that this combined treatment significantly improved the immune response of T cells in a B16F10 melanoma model [192]. Typically, drug delivery using MNs relies on passive diffusion, which may pose limitations on the distribution and depth of penetration of therapeutic agents. To overcome this challenge, Lopez-Ramirez et al. [191] incorporated magnesium particles into the MNs, acting as an internal engine to facilitate faster and deeper intradermal drug delivery SSSSFig. 6.

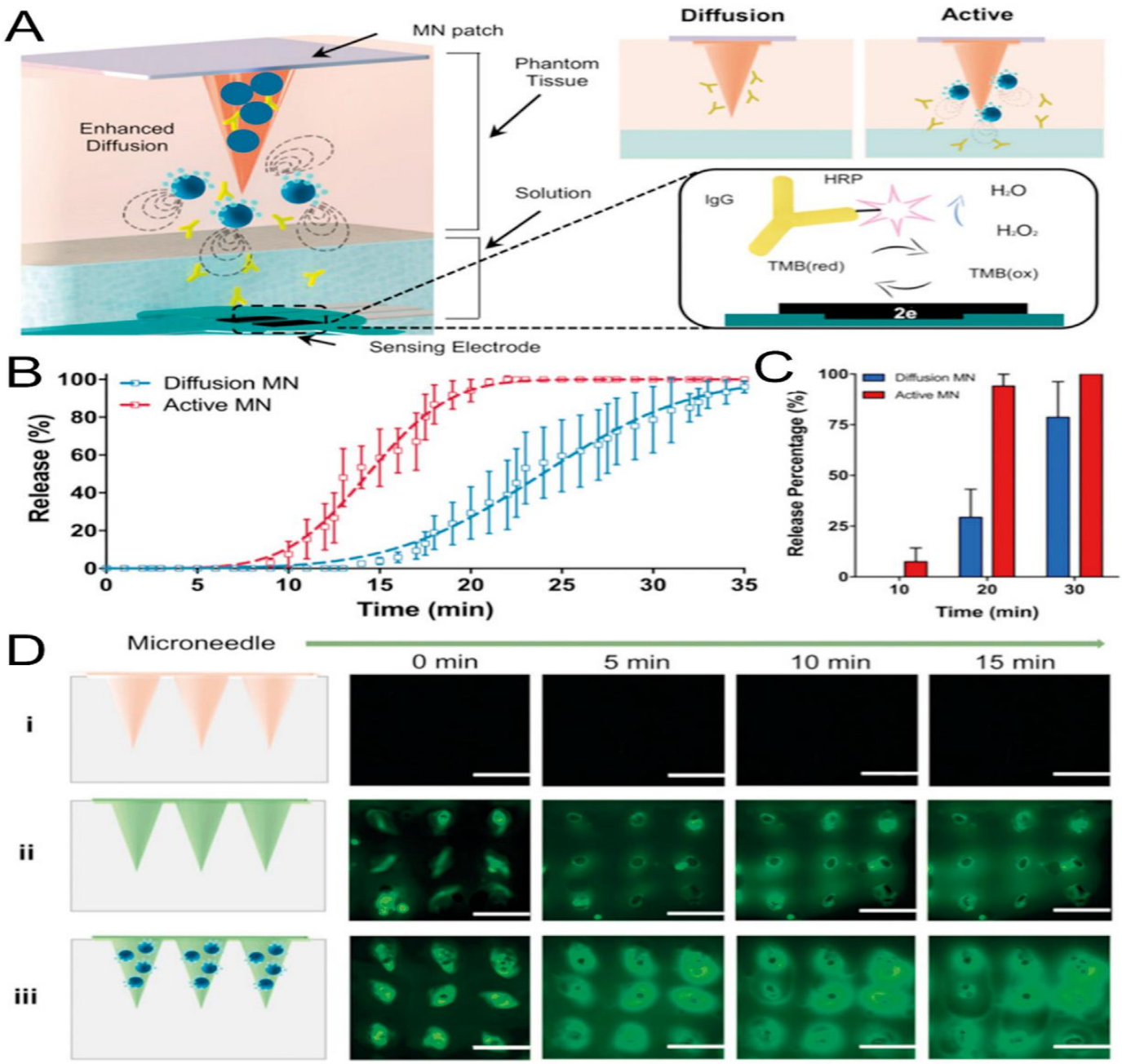

Fig 6. Built-in active MNs patch with enhanced drug delivery. (A) Schematic illustration of the design and mechanism of the active MNs patch. (B) Drug release kinetics of different MNs at pH 6.0. (C) Corresponding release percentage of aCTLA-4. (D) The fluorescence images of MNs patch obtained from top view. (i) Blank MNs, (ii) FITC-loaded MNs, and (iii) FITC-loaded active MNs. Scale bar, 1 mm [191].

The interstitial fluid can react with the magnesium particles to rapidly produce H2 bubbles, resulting in a localized increase in fluid flow [191]. This can help penetrate the dermal barrier and improve the delivery of local treatments. In experiments conducted on living organisms, passive MNs carrying aCTLA-4 initially slowed down the growth of B16F10 melanoma tumors [190,194]. However, by day [46], all mice in this group had tumors exceeding 1500 mm3. In contrast, 60% of the mice treated with active MNs showed no signs of tumors. MNs-based immune checkpoint blockade therapy can be combined with other cancer treatments. Additionally, activating the skin's immune system can enhance anti-cancer immunity both locally and throughout the body. Chen et al. developed hollow MNs that combined a checkpoint inhibitor with cold atmospheric plasma. The plasma induced tumor cell death, leading to an immune response triggered by the released tumor-associated antigens. Furthermore, the hollow MNs patch released aPD-L1, which further boosted the anti-tumor immune response [190]. Combining immunotherapy with phototherapy is another approach to enhance the anti-cancer effect. Chen et al. designed a MNs-assisted platform that combines photodynamic therapy and immunotherapy. This platform encapsulates hydrophobic zinc phthalocyanine and hydrophilic aCTLA-4 [193].

The initial step in this method involved using photodynamic therapy to eliminate the tumor and stimulate the immune response [193]. This was followed by the administration of aCTLA-4, which further enhanced the immunotherapy [194]. Our research team also developed MNs with a core-shell structure to enhance the immune response by combining photothermal therapy and immunotherapy [194]. This system proved to be highly effective in eradicating primary melanoma tumors and inhibiting the spread of metastasized tumors. Apart from immunotherapy, proteins can also play a role in combating cancer through alternative treatment methods. One such example is the use of bevacizumab to inhibit tumor angiogenesis and treat various types of cancer. Courtenay et al. 198 demonstrated the potential of MNs in delivering high doses of bevacizumab transdermally, thereby enabling sustained drug delivery to the systemic and lymph circulation. Overall, the utilization of MNs to assist in the delivery of proteins and peptides for cancer treatment is a valuable strategy.

3.4. Other disease therapy

MNs-mediated transdermal delivery of proteins and peptides has shown potential in various disease therapies, including hypoglycemia [199], osteoporosis [200], cosmeceuticals [45], and wound healing [201]. Hypoglycemia is a dangerous condition characterized by abnormally low blood glucose levels, often caused by insulin administration. To address this issue, GhavamiNejad et al. 199 developed a smart MNs patch that releases glucagon specifically in the presence of hypoglycemia [202]. This patch was created using photocrosslinked methacrylated hyaluronic acid embedded multifunctional microgels, allowing for hypoglycemia-triggered release. In a rat model of type 1 diabetes, this MNs patch effectively prevented hypoglycemia caused by insulin overdose. In the treatment of osteoporosis, Naito et al. 200 designed a dissolving MNs patch loaded with human parathyroid hormone [199]. The MNs patch significantly improved the stability of the hormone compared to a solution. In vivo studies demonstrated that the bioavailability of parathyroid hormone-loaded MNs was 100 ± 4% relative to subcutaneous injection [45]. In a rat model of osteoporosis, the parathyroid hormone-loaded MNs successfully inhibited the decrease in bone density. Furthermore, proteins and peptides have important applications in cosmetics. Mohammed et al. investigated the effect of stainless steel MNs on the skin penetration of peptides with different chain lengths, such as melanostatin, rigin, and palmitoyl-pentapeptide. Their findings revealed that peptides with smaller molecular weights exhibited enhanced local delivery when using MNs [45]. Chi et al. [201] formulated chitosan MNs loaded with vascular endothelial growth factor to enhance the healing process of wounds. The release of the drug was regulated by the increase in temperature caused by the inflammatory response at the site of the wound. The results of the antibacterial test conducted in vitro and the study on wound healing conducted in vivo indicated that the application of the MNs patch facilitated the deposition of collagen, inhibited inflammation, and promoted tissue regeneration during the closure of the wound.

4. MNs-mediated protein and peptide delivery in the clinic

As previously stated, MNs-mediated protein and peptide delivery has been proven advantageous and feasible through fundamental research. Currently, numerous therapies utilizing MNs-mediated transdermal delivery of protein and peptide drugs have entered clinical use, with a focus on vaccination for infectious diseases and insulin delivery for diabetes treatment. The majority of active clinical trials utilize the hollow MNs infusion system, with only a few investigating dissolving or coated MNs. This is due to the fact that research on coated, dissolving, or hydrogel-forming MNs began later and requires more sophisticated MNs design and manufacturing techniques. The interdisciplinary divide between microfabrication and pharmaceutical research has also delayed drug delivery development. However, the field is currently at an important transitional point, with more MNs products expected to be translated into clinical and medical practice in the near future.

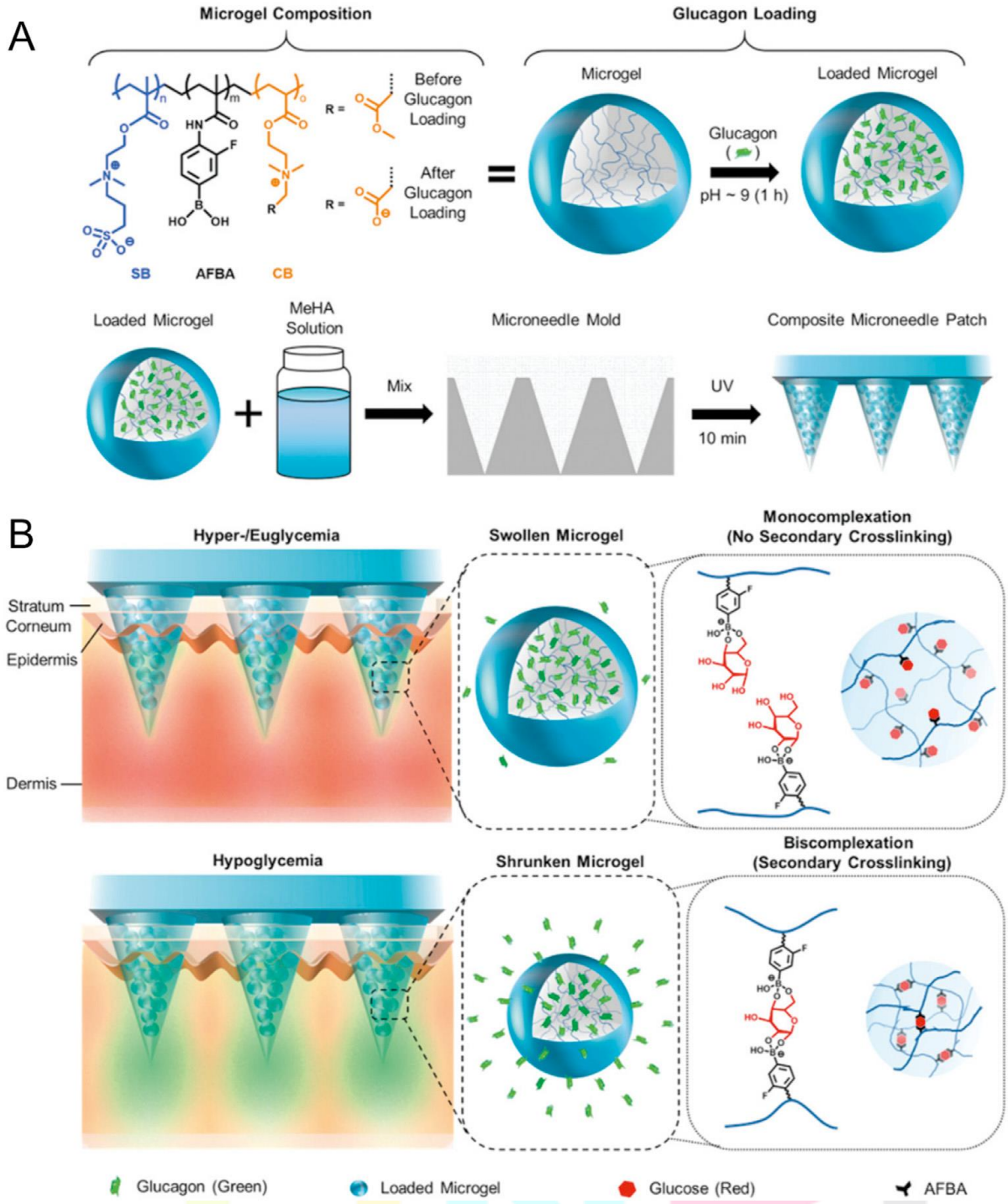

Fig 7. Schematic illustration of the controlled glucagon release from the MNs patch. (A) The fabrication process of MNs patch. (B) The mechanism of glucagon release from the MNs patch. [200]

5. Conclusions and prospects

Compared to small molecules, proteins and peptides exhibit high specificity and potency in treating various diseases. However, their large molecular weight, poor stability, and conformational flexibility make them inconvenient to administer through injection. To address this issue, MNs have been developed with different delivery strategies, including solid, coated, hollow, dissolving, and hydrogel-forming MNs, which can improve patient compliance and overcome the skin barrier for protein and peptide drugs. Additionally, MNs-mediated vaccine delivery can synergize with the active immune environment in the skin to fight infectious diseases and treat cancers. MNs also have important applications in diabetes treatment and make safer closed-loop glucoseresponsive therapies possible. Furthermore, MNs-mediated transdermal delivery of checkpoint inhibitors has reduced their off-target effect and achieved local targeted delivery to treat superficial cancers. In summary, MNs offer great potential as a strategy for delivering proteins and peptides to treat various diseases. To successfully formulate and handle these substances, it is crucial to have a deep understanding of their physical, chemical, and biological characteristics. Special attention must be given to optimizing their stability and effectiveness. Research efforts focused on key aspects such as drug loading, pharmacokinetics, pharmacodynamics, safety, and storage of MNs will advance the field of transdermal protein and peptide drug delivery. The progress made in microfabrication technologies will lead to the development of more intelligent MNs systems. Proteins and peptides, as potent active pharmaceutical ingredients, have the potential to overcome the limitations of low drug loading in MNs. Comprehensive characterization methods, both in vitro and in vivo, are being used to assess the safe and effective delivery of drugs into the skin

using MNs. The current approaches being employed will contribute to the establishment of standardized protocols for evaluating MNs in the future. It is anticipated that a combination of extensive academic research and collaboration with the pharmaceutical industry will expedite the clinical translation of MN-mediated transdermal delivery of protein and peptide drugs.

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