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Progress and Perspective of Microneedle System for Anti-cancer Drug Delivery

Dongdong Li[1#], Doudou Hu[1#], Hongxia Xu[1], Hirak K Patra[2, 3]*, Xiangrui Liu[1], Zhuxian Zhou[1], Jianbin Tang[1]*, Nigel Slater[3], Youqing Shen[1]

[1]Key Laboratory of Biomass Chemical Engineering of Ministry of Education and Center for Bionanoengineering, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China

[2]Wolfson College, University of Cambridge, Cambridge CB3 9BB, United Kingdom

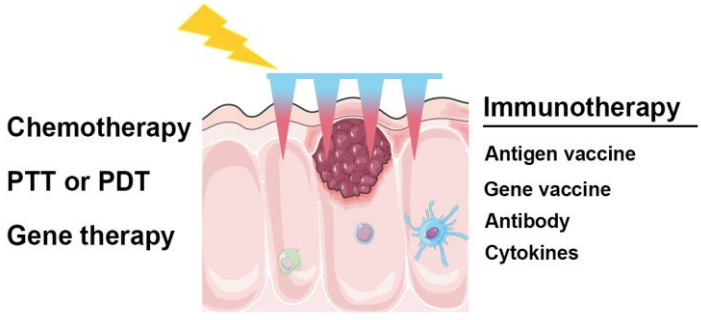
[3]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB3 0AS, United Kingdom.

*Email: jianbin@zju.edu.cn

#Equal contributor

Abstract： Transdermal drug delivery exhibited encouraging prospects, especially through superficial drug administration routes. However, only a few limited lipophilic drug molecules could cross the skin barrier, those are with low molecular weight and rational Log *P* value. Microneedles (MNs) can overcome these limitations to deliver numerous drugs into the dermal layer by piercing the outermost skin layer of the body. In the case of superficial cancer treatments, topical drug administration faces severely low transfer efficiency, and systemic treatments are always associated with side effects and premature drug degradation. MN-based systems have achieved excellent technical capabilities and been tested for pre-clinical chemotherapy, photothermal therapy, photodynamic therapy, and immunotherapy. In this review, we will focus on the features, progress, and opportunities of MNs in the anticancer drug delivery system. Then, we will discuss the strategies and advantages in these works and summarize challenges, perspectives, and translational potential for future applications.

Graphical abstract:



Keyword: Microneedle; Transdermal drug delivery; Cancer therapy; Immunotherapy; Phototherapy; Gene therapy

1 Introduction

Historically skin has been perceived as a “wall” to separate all the exogenous materials. However, with the finding of the new organic and inorganic substances that could directly be absorbed by the skin, we understood skin is not a completely impermeable barrier [1, 2]. The first-ever transdermal therapy to treat motion sickness was approved in 1979 in the US [3]. Since then, many classes of transcutaneous medicines were assessed in the clinical trials, such as for Vaccination (Clinical trial no - NCT03207763) and Topical Anaesthesia (Clinical trial no - NCT03629041). Some of them are already approved for the market [4-6] and are profoundly indicating that an alternative drug delivery strategy is widely emerging for the future healthcare sector. In recent times, the transdermal drug administration strategy becomes an essential therapeutic arrangement in complement with oral, hypodermic and intravenous injection, etc [3]. The transdermal strategies possess several advantages over others [6]. It is painless in comparison to the injection and as it is needle-less equipment, there is almost no risk of spreading diseases by reusing needles and can thus reduce an unsafe source that usually caused by medical waste, especially practices in developing countries [7].

All over again, the transcutaneous technique can easily overcome the limitations associated with the oral route. It can avoid the first-pass or pre-systemic metabolism where the dose of the drug is greatly reduced before even it enters the systemic circulation, and also can avoid gastrointestinal degradation. Hence it can improve the bioavailability of the active pharmaceutical ingredients (APIs). The transdermal patches can deliver drugs in much well-controlled fashion over a prolonged period, and the non-invasive feature makes it a self-administered device with improved patient compliance [3]. However, the widespread use of transdermal patches is limited because of the skin's excellent barrier function [6, 8].

2 The structure of the skin

Skin plays a very significant role in the human body by acting as a “wall” to prevent the substance outside from entering through the skin and interpreting the normal metabolism inside [9]. Thus, skin provides a suitable environment for internal organs with optimum temperature, humidity, etc. Moreover, it’s an organ to sense outside conditions like heat, cold, touch, and pressure, and transmit information to the central nervous system (**Fig. 1**) [10]. The skin has long been considered as an impermeable fence to the exogenous chemicals. However, numerous studies confirmed that skin is not completely impermeable [6]. During the early 20th century understanding, lipophilic agents were perceived more easily to permeate through the skin [6]. Later on, the skin was better understood and modeled as a multi-layered multifunctional structure than a mere simple barrier. The stratum corneum that makes up the outermost layer, is the actual barrier for the absorption of drugs [11]. The epidermis layer is next to the stratum corneum, and the deepest layer is classified as dermis [12, 13].

The drug has to cross the stratum corneum and viable epidermis before it makes passage

through the capillaries and the circulatory system [12, 14]. There are three well-accepted routes for passive diffusion of the drugs, namely through hair follicles, sweat ducts, and through the stratum corneum. (**Fig. 1**) The stratum corneum is primarily formed by dead keratinocytes and can prevent water loss from the inner tissues and the penetration of substances from outside [14], which leads to the limited efficacy of transdermal patches. On the other hand, the efficacies of the other two routes are too low, and their contributions are nearly negligible [14]. Thus, it needs to diffuse across the stratum corneum for a successful transcutaneous delivery [15]. To do so, the drug must be of lower molecular weight (less than 600Da) to obtain a higher diffusion coefficient and acceptable lipophilicity with Log *P* value between 1 and 3. This feature confirms that the drug molecules could effectively permeate into the stratum corneum and could reach to more hydrophilic dermis environment instead of being trapped within the lipid layers [16]. There is a constant effort going on to find alternative enrichment strategies to increase the delivery efficacy such as chemical enhancers [17], iontophoresis [18], ultrasound [19], electroporation [20], etc. However, the practicality and ease of use of these approaches are still very limited due to their poor ability to deliver [21]. Hence, new delivery devices are highly desired to competently cross the barriers of the stratum corneum for transdermal drug delivery and therapy [22].

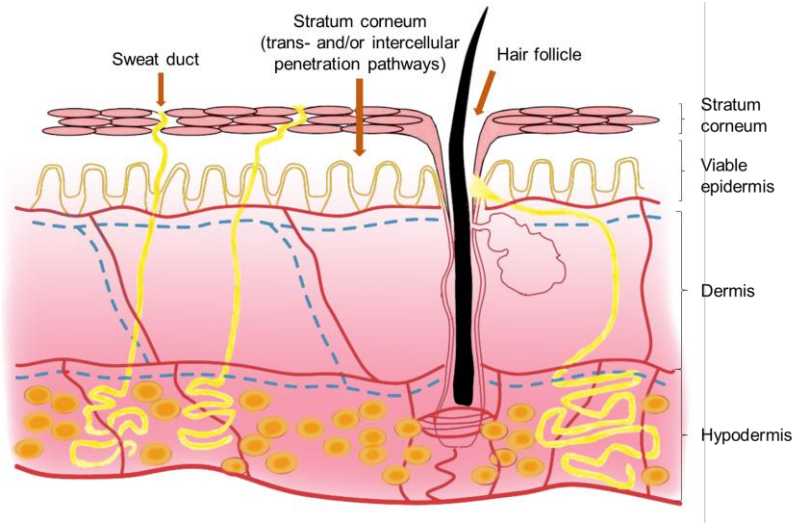


Fig. 1. Schematic illustration of the structure of the skin [12].

3 Microneedles

MNs are minimally invasive devices, composed of micron-size needle arrays of about 50-900 μm in height. The MNs can be manufactured through microfabrication techniques with various materials, geometries and they can deliver drugs into the subcutaneous layer, circumventing the stratum corneum barrier [23, 24]. The needles of MNs pierce the stratum corneum and the epidermis to create micropores in the skin, through which the drug molecules could passively permeate into the dermal layer. Interestingly, the individual needle is long enough to overcome the stratum corneum barrier but short enough to stimulate the nerve ends [14, 25, 26]. Therefore, this equipment is easy-to-use and painless compared to traditional invasive injections and/or oral based

strategies with functional superiority [27]. The MNs can not only help the drug molecules to circumvent the first-pass metabolism and gastrointestinal degradation but also broaden their application scope of drug types, irrespective of their molecular weight and hydrophilicity [6, 14]. There is no confirmed report whatsoever that indicates MNs can cause or increase the chance of skin infection [28] as a side effect, neither being reported that they might affect normal skin functions [29, 30]. Hence, numerous investigations carried out with MNs to deliver diverse hydrophilic or hydrophobic small molecules [31-34], oligonucleotides [35], peptides [36, 37], DNA [38, 39], and macromolecules such as antibody [40, 41], insulin [42], ovalbumin [43-45], including nanoparticles and others [46-52]. A large number of MNs have been entered into clinical trials for the treatment of various diseases, showing their universal effectiveness irrespective of the type of disease (summarized in **Table 1** for clinical trials in non-cancer diseases) (clinicaltrials.gov).

A wide range of materials and technology are used to fabricate MNs. To date, there are five major different types of MNs being reported, namely solid, coated, hollow, dissolvable, and the hydrogel-forming MNs (**Fig 2**) [53]. The application of solid MNs for drug delivery involves a two-step process; firstly, applied on the skin to create the micro-channels, and then the MNs are removed. In the second step, the drugs are layered on the surface to diffuse through [54, 55]. The coated MNs are achieved from the solid MNs through coating drugs on the surface of the needles [56, 57]. The dissolving MNs are fabricated with the biodegradable materials and the active ingredients. Once the MNs are injected into the skin, they will be dissolved and release their cargoes [13, 58, 59]. The hollow MNs consist of hollow channels through which the medications are injected into the skin after insertion [60, 61]. The hydrogel-forming MNs are made of swelling materials in which the drugs are loaded. Once the MNs are inserted into the skin, the needles will absorb interstitial fluid and release the drugs into the tissues subsequently [62, 63].

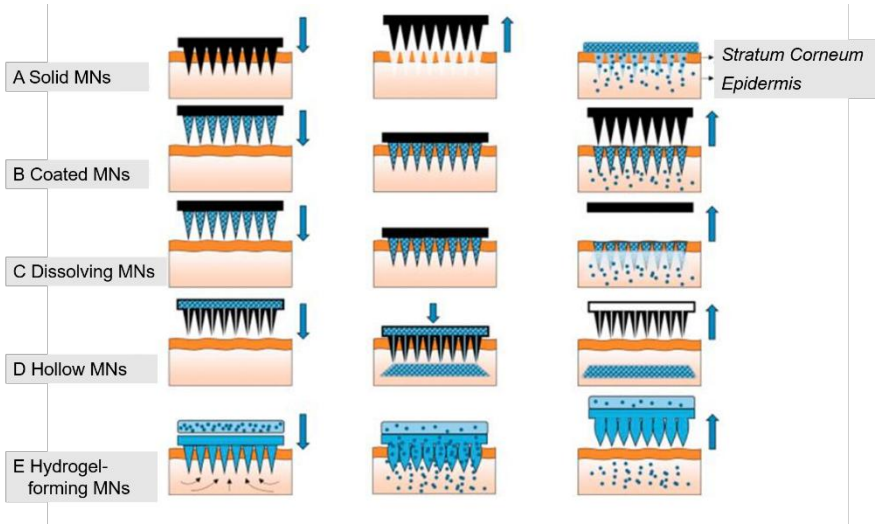


Fig. 2. Schematic representation of the different types of microneedles (MNs). (A) Solid MNs, (B) Coated MNs, (C) Dissolving MNs, (D) Hollow MNs, (E) Hydrogel-forming MNs [6].

Table. 1. Clinical trials with MNs (clinicaltrials.gov)

MNs type	Conditions	Phase	Status	NCT Identifier
Solid	Primary Axillary Hyperhidrosis	-	Completed	NCT03054480
	Primary Axillary Hyperhidrosis	-	Unknown	NCT02823340
	Hyperhidrosis	Phase 1	Completed	NCT03203174
	Pain	-	Completed	NCT02596750
	Dental Pain	-	Completed	NCT02966067
	Actinic Keratosis	-	Completed	NCT01812837
	Actinic Keratosis	Phase 2	Completed	NCT02632110
	Keratosis, Actinic	-	Completed	NCT02594644
	Wrinkle	-	Recruiting	NCT03739398
	Aging	-	Unknown	NCT02368626
	Aging, Wrinkle	-	Active, not recruiting	NCT03426098
	Topical Anaesthesia	Phase 1	Completed	NCT03629041
	Melasma	-	Not yet recruiting	NCT03472235
	Postmenopausal Osteoporosis	Phase 3	Recruiting	NCT04064411
Hollow	Type 1 Diabetes	Phase 2/3	Completed	NCT00837512
	Type 1 Diabetes	Phase 1	Active, not recruiting	NCT02837094
	Type 1 Diabetes	Phase 2	Unknown	NCT01684956
	Diabetes	Early Phase 1	Completed	NCT00602914
	Diabetic Macular Edema	Phase 2	Completed	NCT03126786
	Diabetic Macular Edema	Phase 1/2	Completed	NCT02949024
	Macular Edema, Retinal Vein Occlusion	Phase 3	Terminated	NCT03203447
	Uveitis	Phase 3	Completed	NCT03097315
	Uveitis	-	Completed	NCT02952001
	Uveitis	Phase 3	Completed	NCT02595398
	Uveitis	Phase 2	Completed	NCT02255032

	Uveitis	Phase 1/2	Completed	NCT01789320
	Influenza	-	Completed	NCT01304563
	Influenza	-	Completed	NCT00558649
	Influenza	-	Completed	NCT01767337
	Influenza	Phase 1/2	Completed	NCT01707602
	Influenza Infection	-	Completed	NCT01049490
	Intradermal Injection	-	Completed	NCT03415373
	Intracutaneous Drug Delivery	-	Completed	NCT01767324
	Local Anesthesia	-	Completed	NCT00539084
	Pain		Active, not recruiting	NCT03607903
	Tuberculosis Test	-	Completed	NCT01611844
	Gingival Recession	-	Completed	NCT03274674
Coated	Allergic Reaction to Nickel	-	Completed	NCT02995057
	Acute Migraine	Phase 2/3	Completed	NCT02745392
	Cluster Headache	Phase 2/3	Not yet recruiting	NCT04066023
Dissolving	Vaccination	-	Completed	NCT03207763
	Psoriasis	-	Unknown	NCT02955576
	Influenza	Phase 1	Completed	NCT02438423
	Basal Cell Carcinoma	Phase 1	Not yet recruiting	NCT03646188
	Cutaneous T Cell Lymphoma	Phase 1	Recruiting	NCT02192021
-	Oral Cavity Disease	-	Completed	NCT03855397
-	Diabetes	-	Completed	NCT02682056
-	Skin Barrier recovery	-	Recruiting	NCT03332628
-	Psoriasis Vulgaris	-	Recruiting	NCT03795402
-	Acne Scars	-	Not yet recruiting	NCT03380845
-	influenza	-	Unknown	NCT01039623
-	Sensing of Beta-lactam Antibiotic Concentrations	Phase 1	Completed	NCT03847610
-	Migraine	Phase 3	Completed	NCT03282227
-	Penicillin Delivery	Phase 1	Not yet recruiting	NCT04053140

4 Microneedles for cancer therapy

Almost for the last four decades, MNs have been widely operated for the transdermal delivery of substances, showing improvement in the drug delivery efficacy. For cancer therapy, MNs have been reported for delivery of primarily photothermal and photodynamic agents, chemotherapy drugs, therapeutic genes, and agents for immunotherapy.

4.1 Chemotherapy

Chemotherapy is one of the leading arsenals against cancers. However, anti-cancer chemotherapeutics are struggling with severe side effects caused during traveling through systemic route, poor tumor-targeting capability, inability to cope up with disease spreading (metastasis), and high off-target toxicity [64, 65]. The MNs provide a unique opportunity to deliver anticancer drugs through the transdermal route [31, 33, 34, 66-71]. *Banga et al*. reported many such investigations for the assessment of transdermal delivery efficacy for both hydrophobic [31, 72, 73] and hydrophilic drugs [74]. For example, they developed solid MNs for the delivery of Methotrexate (MTX) [72]. Maltose-made solid MNs created the holes, and then the mixture of MTX and poloxamer applied on the skin surface. The poloxamer can easily be transformed into a gel by changing the temperature from 32 ^{o}C to 37 ^{o}C (skin temperature). The acquired hydrogel could sustainably release the MTX at a steady rate [72]. They further compared MNs efficacy with skin laser-ablated technique and found more uniform distribution of MTX by MNs than ablation method, despite the lower delivery capacity of MNs [31]. Similarly, they also used MNs to deliver hydrophilic drugs like diclofenac sodium [74]. Again, despite the MNs hold the better ability to deliver the drug with a faster diffusion rate, the amount of drug delivered into the skin was lower than the laser-ablated method, which is primarily because of contact area difference [74]. They went on further to deliver DOX and Vismodegib and found that MNs significantly increased the drug permeability [73, 75]. In another context, *Lan et al*. delivered cisplatin loaded pH-responsive nanoparticles using MNs (**Fig 3**) [70]. The high loading capacity of the drugs and topical application provided strong support for *in vivo* therapeutic outcomes while minimizing the side effects [70].

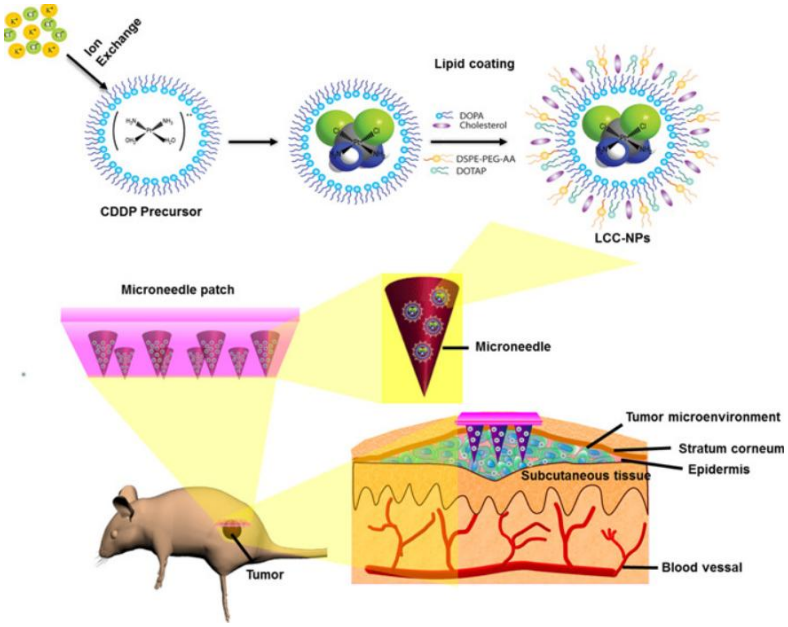


Fig. 3. Schematic diagram for transdermal delivery of the lipid-coated cisplatin nanoparticles for cancer therapy [70].

All-trans retinoic acid (ATRA) can promote basal keratinocyte proliferation and can increase the transformation speed of the stratum corneum, leading to the weakening of the lesion from the skin. But poor water solubility limiting its application in the treatment of Seborrheic keratosis (a benign skin tumor). *Hiraishi et al*. fabricated MNs with sodium hyaluronate for transdermal delivery of ATRA. They evaluated the stability of ATRA in MNs and found that after 24 weeks almost 85% of the ATRA remain available [76]. Following the treatment, MNs promoted epidermal hyperplasia and the respective mRNA and protein expression profile supported, and it showed that MNs could be a useful tool for topical use of ATRA [77]. *Gill et al*. loaded DOX into Poly (lactic-co-glycolic) acid (PLGA) NPs and coated the NPs on solid MNs. The DOX diffusion was found in a steady process with a uniform distribution in the porcine skin tissue model. Compared to the hypodermic injection, the MNs found significantly reduced the leakage of the DOX [78]. They further estimated the effect of salivary washout on the drug delivered by the MNs. Even though the salivary flow could be the cause of this drug loss, still the drug could diffuse into the deep dermal when provided with enough insertion time [79]. *Lee et al*. built an MN system with double-stranded salmon DNA (sDNA) for its biocompatibility and strong interactions between DNA molecule and DOX, which made it a stable vehicle for drug delivery [80]. There are various materials used to develop MNs to explore release kinetics and skin diffusion of anticancer drugs such as dacarbazine [81], doxorubicin [73, 78, 82] and MTX [31, 72]. However, successful anticancer reports have been hardly published and MNs still hold the prospect of delivering chemotherapeutics as they are not only appropriate for carrying different kinds of molecules irrespective of their molecular weight, charge and the hydrophobicity, also they can enhance the delivery efficacy and promote the uniform distribution of drugs into the tissue.

4.2 Photothermal therapy (PTT) and photodynamic therapy (PDT)

PPT and PDT are two promising alternative approaches for cancer therapy. PTT and PDT use the light (laser) energy to generate heat and/or cytotoxic singlet oxygen to destroy the cancer cells. The controlled manner of PTT and PDT are thought to be able to reduce the side effects and toxicity, because of their minimal invasion and ease of use applicable features [83-86]. Near-infrared PTT has been reported with improved anticancer effects synergistically with chemotherapy [87]. However, limited efficiency to deliver PTT agents into tumors, restricted their applications [87-89]. *Ying et al.* coated MNs with PEGylated gold nano-rods and coalesced with docetaxel-loaded micelles for intravenous delivery [87]. This combined therapy showed excellent anticancer effect than either PTT or chemotherapy only and found to cure without relapse. *Chen et al.* also made several advancements in transdermal drug delivery through MNs, especially for anti-cancer PTT [32, 90, 91]. They developed reproducible, low-leakage, and non-invasive triggering of MNs to transfer photosensitive agents into the skin [90]. Besides, they set a dissolvable MNs system by poly(vinyl alcohol)/polyvinylpyrrolidone with a photosensitive nanomaterial (lanthanum hexaboride; LaB_6) platform where DOX embedded within the MNs (**Fig 4**) [92]. LaB_6 nanomaterials can convert NIR radiation into heat for the thermal ablation of cancer cells, while MNs helped to prevent rapid clearance of LaB_6 NPs from the body and thus enhanced the therapeutic efficacy. After inserting MNs into tumor tissues, LaB_6 NPs first absorbed the NIR laser irradiation, then the MNs were melted and released the loaded DOX into the tumor. The DOX release can be controlled through switching the light source 'on' or 'off' in line of the concept of switchable anti-cancer therapy [93]. The MNs system has provided a synergistic effect of the PTT and chemotherapy in the treatment of the superficial tumors after tuning the irradiation parameters. As a result, tumors were found to be completely eradicated after a single patch with three cycles of laser irradiations within 1 week. There is no significant body weight loss and tumor recurrence found after the treatment, which confirmed that the combination of chemotherapy and PPT through MNs could effectively enrich the anticancer treatment efficacy against superficial tumors [92].

Likewise, *Dong et al*. built a dissolvable synergistic MNs for chemotherapy and PPT with DOX and gold nanocages (AuNC) [94]. Apart from its photothermal effect, the loaded AuNCs also helped to overcome the drawbacks of low mechanical strength for the dissolving MNs. *Pei et al.* exploited mesoporous silica NPs (MSNs) coated with photothermal agent indocyanine green (ICG) and loaded with DOX, through which they achieved a collegial effect of PTT and chemotherapy [95].

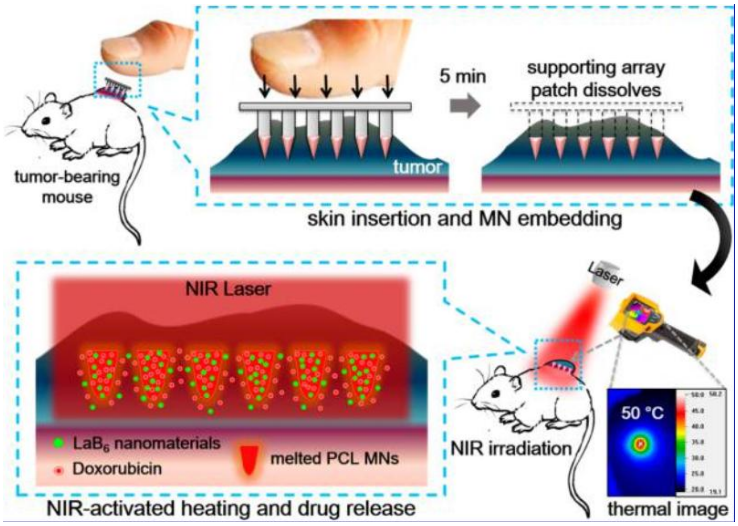


Fig. 4. Schematic illustrations of the MNs system for the combined tumor therapy [92].

Tham et al. developed an MN system for PDT using photosensitizers-loaded mesoporous nanovehicle [86]. The reported system found with enhanced quantum yield and photostability of the photosensitizers by linking them to organo-silica matrix. After co-loading with small-molecule inhibitors, they achieved higher anticancer effects in the xenograft melanoma mouse model. Similarly, *Donnelly et al.* reported many works with MNs for 5-aminolevulinic acid (5-ALA) transdermal delivery [62, 96-100]. The 5-ALA is a widely used photodynamic agent for cancer therapy that can be transformed into protoporphyrin IX (PPIX) inside the mitochondria and can kill the cancer cells under irradiation of light [101, 102]. The 5-ALA is a zwitterionic molecule, which makes it harder to penetrate into the stratum corneum through the topical application [101]. They employed silicon MNs to overcome the limitation of the poor tissue penetration of 5-ALA. Both *in vitro* and *in vivo* results revealed that the MNs increased the skin penetration of 5-ALA and enhanced the PPIX production [98]. *Zhao et al*. have used sodium hyaluronate (HA) to build fast-dissolving MNs patches [102]. Even though the injection dose was relatively lower, the transdermal pathway achieved much better tumor inhibition rate (66 % to 97 %) compared to injection. *Jain et al.* coated 5-ALA on solid needles and evaluated the obtained MNs with a porcine cadaver skin model. In comparison with conventional cream formulation, the delivery efficacy of the MNs was found to 3.2-fold higher and MNs can deliver PPIX at least 3 times deeper (~ 480 μm) than the cream formulation (~ 150 μm) with better anti-tumor effects [103].

4.3 Gene therapy

Abnormal gene expression causes many kinds of diseases including cancers, and small interfering RNA (siRNA) has provided a useful tool for gene therapy for such diseases [104]. *Deng et al*. used solid silicon MNs to deliver siRNA into the dermal layer to treat skin cancer; the siRNA was modified by cholesterol to facilitate the uptake and for prolonged circulation in the blood. The siRNA was mostly found to be accumulated within the treated area instead of in the

liver compared to what observed in systemic injection [105]. Additionally, they also locally injected the siRNA by an injectable microneedle device to evaluate its anticancer ability (**Fig 5**) [106]. The siRNA was only detected in the tumor instead of other organs and it showed excellent anticancer effect against human cervical carcinoma (SiHa) xenografts mice model without any major adverse effect.

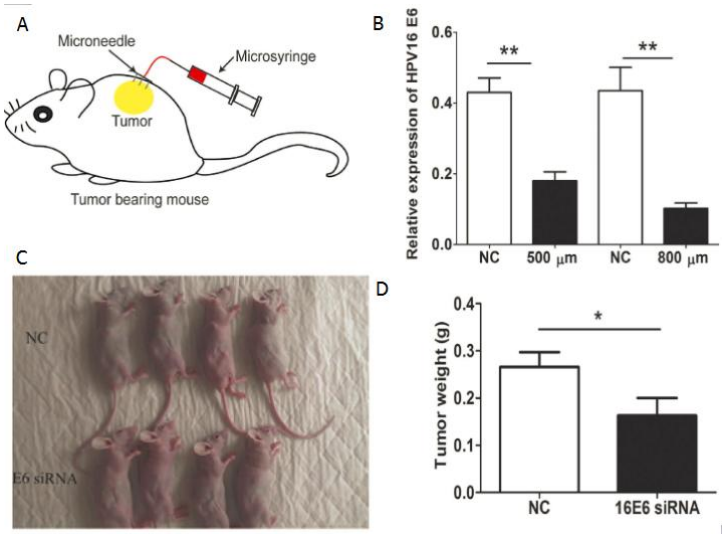


Fig. 5. Microneedle-based siRNA local administration for cancer therapy. (A) The delivery of the siRNA with the injectable microneedle; (B) The expression of HPV16 E6 gene in the tumor when the siRNA was delivered by 500 μm or 800 μm MNs 24 h after administration. (C) SiHa xenografts model was photographed ten days after the first treatment. (D) The statistical analysis of the tumor weight in SiHa xenografts model [106].

Haigh et al. applied cationic liposomes to complex siRNA to prevent degradation and found after coated on the solid MNs instead of modifying siRNA with cholesterol, loaded siRNA had excellent gene silence ability [107]. *Chong et al.* coated respective siRNA on stainless steel solid MNs and 50 % to 85 % of the siRNA was delivered into the skin after topical application, resulted in successful gene silencing *in vivo* [108].

5. Microneedles for the cancer immunotherapy

Cancer immunotherapy is considered as a breakthrough of the Year 2013, because of its great achievement in cancer management [109]. It marks an entirely different trail for the treatment of cancers that aims to target the immune system instead of the tumor directly. The immunotherapy strategy was first proposed for delivering the exogenous antigen, called therapeutic vaccine, to activate the immune response [110]. However, until now only a few successes have been shown and reproduced, and thus the vaccination strategy still needs further consideration and optimization [110]. Recently, there are two greatest promising treatment pathways in cancer immunotherapy been unfolded [111]. The first one is an attempt to transfer the engineered T cells, expressing the chimeric antigen receptors (CARs), into the body to improve the anti-tumor immune response [112, 113]. The other one is through checkpoint blockade; the

antibodies used to block the inhibitory effect enforced by the tumor microenvironment, such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1, and its two ligands PD-L1/2) [114-116].

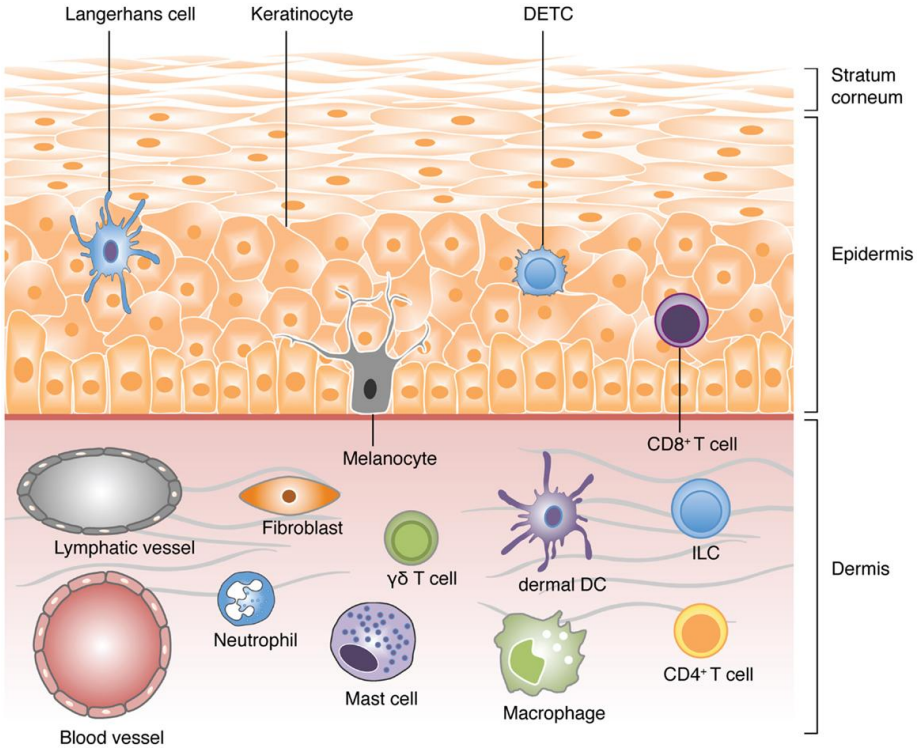


Fig. 6. Schematic diagram of the different cell types beneath the skin [6].

Skin is an extremely active organ for immune defense and plays an important role in immunomodulation as there are plenty of Langerhans cells (LCs) and dermal dendritic cells (DCs) present under the stratum corneum (**Fig 6**) [117]. DCs are the most dominant antigen-presenting cells (APC), which are responsible for immune surveillance [117-119]. They could recognize and endocytose the antigens from the local environment and then become activated. After degradation in proteasomes within the DCs, the antigens will be presented onto major histocompatibility complex (MHC) as antigen peptides. The activated DCs could migrate to the draining lymph nodes and interact with the T cells to form the immune synapse, which indicates the activation of the immune system [118, 120]. Thus, immunotherapy conducted by MNs has shown countless prospects, especially for overcoming the skin barrier standing in the way of the transdermal delivery for macromolecules like antigen, antibody, and DNA [5, 117, 121].

5.1 Antigen vaccine

Successful transdermal vaccination always means the effective activation of DCs, following presenting the antigen information to the T-cell and causing long-lasting immune memory [122-125]. Now cancer vaccine has shown great potential for cancer immunotherapy [126-128] and MN-mediated transdermal vaccination has been proved to be a more effective approach. Therefore, it seems to be a promising strategy to conquer cancer by MN-based vaccination. As for

the transcutaneous vaccine, the ideal antigen model was ovalbumin (OVA) and many researchers have been devoted to evaluating the efficacy conducted by the MNs with OVA [129].

The MNs can smoothly deliver OVA into the skin, noticeably the peak concentration in plasma comes at 1 h after the MNs application and the plasma level concentration keeps constant for more than 6 h [130, 131]. Importantly, the formed antigen depot of the MNs could sustainably release the macromolecule for days [43-45, 132], which led to comparable, even better OVA-specific antibody responses than the intramuscular injection of free OVA[133-136]. As the immune responses can be influenced by the different types of MNs and skin locations, further consideration should carefully be evaluated to optimize the MNs-based immunization strategies [137, 138]. Additionally, co-delivering of antigens and adjuvants [56, 127, 139-141] or utilizing functional MNs [142-144] have also been explored to improve the transdermal vaccination efficacy.

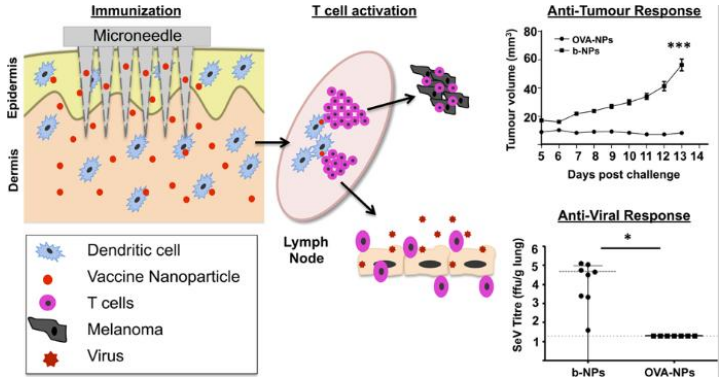


Fig. 7. Schematic illustration of the dissolving MNs system for cancer immunotherapy [134].

Fujiyama et al. percutaneously immunized 59 patients with advanced malignant melanoma by several peptides derived from the human lymphocyte antigen typing in individuals [145]. The prolonged survival time demonstrated that effectively activating DCs and tumor-specific cytotoxic T lymphocytes could indeed improve the anticancer effects. Even though the vaccination was not conducted by MNs, it is confirmed that the transdermal pathway was a valid strategy for invoking immune response and curing cancer [145]. *Zaric et al.* used OVA as a model antigen to develop a dissolving MNs system (**Fig 7**) [134], where the antigen was encapsulated in poly-D, L-lactide-co-glycolide (PGLA) NPs and loaded into the MNs made by a copolymer of methylvinylether and maleic anhydride (PMVE/MA). The antigen-loaded NPs delivered transdermally by the MNs to successfully induce the T cells immunization and efficiently inhibited the proliferation of the OVA-expressing B16 melanoma tumors. Furthermore, NP-based formulations are not only prolonged the retention time in the skin, but also improved the stability of the antigens. They further investigated the immune behavior after the antigen-loaded NPs were delivered into the skin and found that the antigens in NPs were favorably processed and cross-presented by Langerhans cells (LCs) [146]. *Bhowmik et al*. has introduced an MN system for the delivery of

melanoma cancer cells antigens microparticles [147] and confirmed the ability to inhibit the development of melanoma after the vaccination with a solid MN equipment Dermaroller®.

Apart from single antigen vaccination, *Tawde et al.* developed a NP-based metallic MN for transdermal delivery of whole cell lysate [148]. The murine ovarian cancer cells were lysed as the antigen and loaded into the NPs. After immunization with NP-based MNs, increased $CD8^+$ T cells, $CD4^+$ T cells, and natural killer (NK) cells were observed in the spleen in a mice model. The anticancer efficacy was significantly improved after combining with interleukins. *Maaden et al.* has developed hollow MNs for transdermal injection of a therapeutic cancer vaccine [149]. They used synthesized peptides derived from human papilloma virus (HPV) as the vaccine, which were loaded inside cationic liposomes. Compared with the classical intradermal vaccination, the MN induced stronger immune response with considerably lower peptide dosage, showing its potential for anti-cancer vaccination.

Apart from antigens, adjuvants are another component of the vaccine to improve the immune response [150]. *Zeng et al*. combined the human melanoma antigens tyrosinase-related protein 2 (Trp2) with toll-like receptor agonists (TLRa) and CPG (a potent vaccine adjuvant), in their MN system [151]. They deposited the vaccine and the adjuvant on the MNs and applied them on the ear immunization sites. They observed that the MN system evoked much more tumor-specific T cell expansion than classical intradermal immunization. Given many adjuvants are hydrophobic and therefore difficult to co-load with hydrophilic antigens, especially in degradable MNs, *Kim et al.* investigated the capacity of an amphiphilic copolymer, rather than completely water-soluble materials, as an MN matrix to co-load OVA and R848 [150]. Their MNs can form into nanomicelles after dissolved and the OVA and R848 containing nanomicelles could target lymph nodes and can activate the APCs. After topical usage, the system successfully evoked the immune defense and showed significant anti-tumor effects, while minimizing the potential toxicity by reducing the systemic exposure of R848. *Gu et al*. packed whole tumor lysate into a vaccine MN patch and utilized the contained melanin to transfer the NIR radiation into heat (**Fig 8**) [152]. After the injection with the MN patch, the loaded antigens could be sustainably released into the dermal layer. At the same time, NIR light induced local heat could trigger the release of the inflammatory cytokines and generating immunogenic molecules. Also, the elevated local temperature could surge the blood and lymphatic flow which may further improve the migration of APCs and T cells to enhance the immune response [152]. Moreover, a widely used adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) was also added into the MN patch [153]. The MN system could prevent tumor development and inhibit the growth of distant B16F10 tumors. Even for other tumor models, like $BRAF^{V600E}$-mutated BP melanoma and triple-negative breast cancer carcinoma, the MN patches also showed improved anticancer effect than intradermal injections.

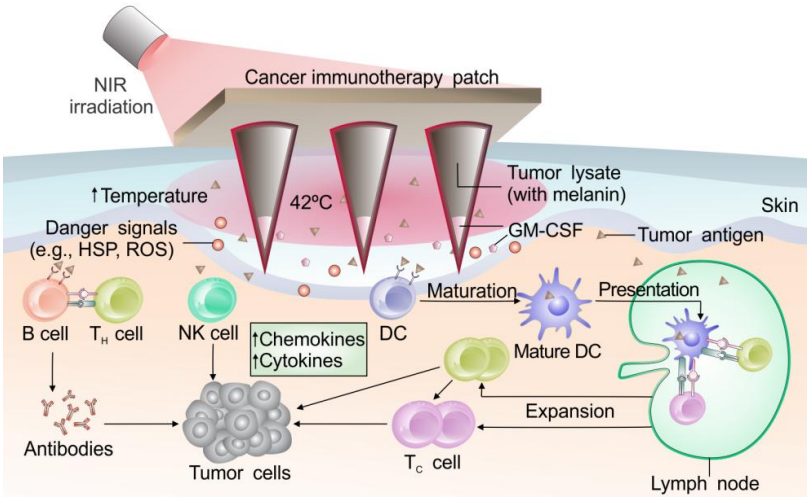


Fig. 8. Melanin-based MN patch for cancer immunotherapy [152].

5.2 Gene vaccine

Gene therapy has made several achievements for the prevention and treatment of various diseases in recent years [154-156]. As for gene vaccine function, gene delivered into cells should remain active and express the respective antigen to induce immune response [157]. However, their clinical translation has been limited due to their degradation and fast clearance during the delivery process [154, 155]. The transcutaneous gene immunization with MNs is a suitable answer to overcome such limitations [158-161]. *Pearton et al.* found that coated MNs could efficiently load plasmid DNA (pDNA) and increase its stability [162], and the reporter genes were transfected successfully both in vitro and in vivo [162-166]. Many MN systems have also been developed to investigate the immune behavior genes using MNs, including different material-based MNs [167], NP-based pDNA vaccine MNs [168], and environmental-sensitive functional MNs [169, 170]. For example, *Hu et al.* designed a NP-based DNA vaccine using PEI-modified MNs with cell-penetrating peptide (CPP) and the mannose (CPP-PEI1800-Man/DNA NPs) [171]. The CPP could synergize with the mannose to enhance the receptor-mediated endocytosis and to increase the efficiency of the intracellular delivery. The gene encoded Trp2, which was overexpressed on the surface of the melanocytes and melanomas. The in vivo results showed that the transdermal delivery of the CPP-PEI1800-Man/DNA NPs could significantly improve the anti-tumor immunity and can prolong the survival time of B16-xenografted BALB/c mice [172]. Furthermore, they have improved the MNs system by utilizing the mannosylated N,N,N-trimethyl chitosan (mTMC) as the gene vector and low-dose paclitaxel (PTX) as the adjuvant, the newly formed MNs significantly promoted the proliferation of CD4+ and CD8+ T cells and enhanced the anti-tumor efficacy [173, 174].

In case of cervical cancer, HPV infection is the leading cause of the disease. *Ali et al.* delivered transdermal DNA (encoding oncoproteins E6 and E7) with MNs for the treatment of cervical cancer [175, 176]. Their MN system not only evoked a strong immune response, but also clearly inhibited the progress of cervical cancer and prolonged the survival time of the mice with

TC-1 tumor. Apart from DNA vaccine, messenger RNA (mRNA) can also serve as the gene vaccine. *Koh et al*. evaluated stability and delivery efficacy of the mRNA in MN systems under the ambient condition for weeks, and found that the delivery depth instead of the contact surface area determined the transfection efficiency of the mRNA in MNs [58]. Although the RNA patch did not show a better anticancer effect compared with the subcutaneous injection, it still induced cellular and humoral immune response. Undoubtedly, the higher delivery efficacy and strong immune response proved the promising future of gene vaccines with MNs for cancer treatment.

5.3 Antibody

Monoclonal antibodies (mAbs) have high specificity and potency and a huge effort has been devoted to developing mAbs for cancer immunotherapy especially as the immune checkpoint inhibitors. Even though the checkpoint inhibitors have achieved much exciting clinical progress, it still faces some shortcomings with serious side effects and low objective response [116, 177, 178]. To overcome the limitations, *Gu et al.* loaded anti-PD1 antibody (aPD1) into the MNs to achieve the local delivery [179-182]. The MNs were designed as a pH-responsive platform to precisely control the drug release, and the aPD1 and glucose oxidase were loaded in the pH-responsive carrier. After the MN-mediated transdermal delivery, the glucose oxidase could convert blood glucose into gluconic acid to reduce the pH, and led to the dissociation of the NPs that can induce the release of the cargo under low pH values. The local delivery strategy not only improved the stability of the protein drugs, but also enhanced the retention time of the aPD1 in the tumor tissues. Compared with the intravenous injection of the aPD1, the MN system showed much better tumor inhibition with lower side effects (**Fig 9**) [182].

The Indoleamine 2, 3-dioxygenase (IDO) is one of the ranges of immunosuppressive molecules that is over-expressed in the tumor microenvironment and can degrade the tryptophan and inactivate the T cells to cause the immune evasion. *Gu et al.* combined aPD-1 with an IDO inhibitor 1-methyl-dl-tryptophan (1-MT) in an MN system and reported potent anti-tumor synergistic effect in the B16F10 mouse melanoma model [181].

Courtenay et al. have used the dissolving and hydrogel-forming MNs to deliver a high dose of bevacizumab [183]. They confirmed the delivery ability of the MN platform in the lymph and systemic circulation system and highlighted the continuous delivery features, which is critical for the treatment of metastatic cancers. Also, encapsulation of the protein drugs into the MN was in favor of maintaining the biological activity after months' storage at room temperature [184, 185].

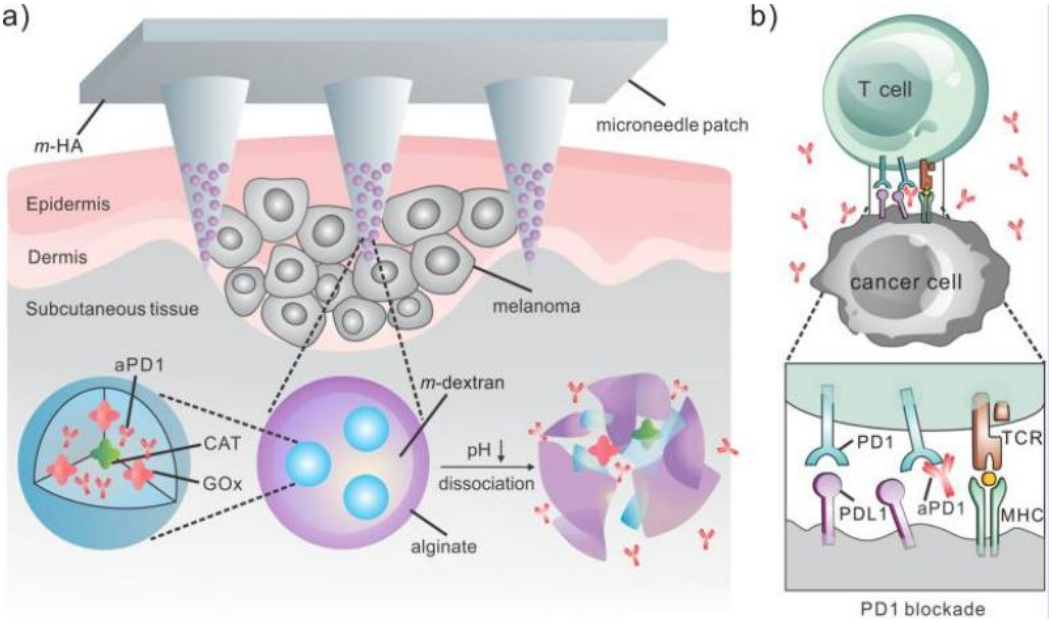


Fig. 9. The MNs patch for the delivery of aPD1 and treatment of the tumor [182].

5.4 Cytokines

Cytokines can regulate cell growth and differentiation, mediate inflammatory signals between cells, and modulate tumor immune microenvironment, which is beneficial for cancer immunotherapy [186, 187]. For example, interferon-alpha (IFNα) is widely used for the treatment of various types of human malignancy, because of the high biological activity, which is mainly administered by subcutaneous or intramuscular injection. However, the frequent application is necessary for the short half-life (4–10 h) in the body, which may compromise patient compliance. *Chen et al*. employed the degradable MNs to deliver and compared the pharmacokinetic characteristics with the intramuscular injection of IFNα-2b [188]. Although there is no significant difference observed between these two methods in pharmacokinetics, the MNs could improve the stability of the IFNα-2b and thus provide an alternative administration way for IFNα-2b.

Interleukin-12 (IL-12) can improve the efficacy of vaccines for anti-cancer treatment through regulating T cells and natural killer cell differentiation and IFN-γ expression [186, 189]. *Mahato et al*. constructed an MN system loaded with a plasmid vector (p2CMVmIL-12) which can express IL-12 and injected it into the tumor to evaluate the anticancer effect [190]. The p2CMVmIL-12 was not only successfully expressed, but also significantly inhibited the tumor growth. Furthermore, *Lee et al.* delivered the p2CMVmIL-12 with a hybrid electro microneedle (HEM) transcutaneously [189]. They confirmed the HEM as a useful tool for cutaneous permeation, release, and intracellular transfection of genes. HEM further exhibited excellent tumor inhibition ability in a B16F10 melanoma mice model. Taken together, cytokines can be efficiently delivered by a transdermal route with enhanced stability and reduced pains.

6. Summary and future perspectives

MNs have broadened the strategic delivery views for cancer therapy and can reform drug administration patterns that can bring an alternative way for drug delivery. MNs could transfer the drugs through painlessly piercing the epidermis and creating micro-channels on stratum corneum. This allows a broad spectrum of drugs with unsuitable features that could penetrate through the skin and reach the region of the lesion. Because of the relieved restrictions, the completely hydrophilic or hydrophobic drugs, macromolecules can be delivered through a transcutaneous pathway. Additionally, MNs increase the stability of the drugs and reduce the necessity for well-trained operators, thus making them a promising tool.

However, selections of MNs have been designed for several kinds of applications, the clinical research is still at its preliminary stage. Currently, besides its applications for cosmetic purposes, MN-based influenza immunization is the most promising application in the therapeutic aspect. Compared to the muscle injection, vaccines delivered through a transcutaneous pathway mediated by MNs could evoke a stronger immune response. Presently, there are two hollow MN-based vaccines delivery system have been approved [16, 52] and 54 clinical trials have been evaluated for treating many diseases, including diabetes, actinic keratosis, and vaccination (ClinicalTrials.gov).

As far anticancer therapy is concern, there are some necessary guidance we should take into consideration before making the new research effort. Firstly, as skin is a potent immune defense organ, how to activate the immune cells beneath the skin (e.g., DCs, at a maximum extent) is of great significance. Furthermore, the focus should be shifted to metastatic cancers instead of superficial cancer, and the immunotherapy effect should be strong enough to kill and eradicate the localized and moving cancer cells. However, the potential risk of allergic response should not be ignored. *Secondly*, the topical application of the checkpoint blockade antibody or cytokines loaded in MNs is an alternative strategy to reduce the side effects, although deeper diffusion and accuracy in organ distribution are needed to be investigated, especially for the macromolecular drugs like antibodies, cytokines or vaccines. Evaluation of the bioavailability and pharmacokinetic information is also required for clinical translation. Thirdly, there is still an unmet need to cautiously assess the safety profiles of MNs. Even though the minimally invasive feature of MNs makes them safer devices, whether the frequency of use will affect the normal skin function and whether the administered polymers induce any long-term side effects should also be further evaluated. Hence, it is important to consider the biocompatibility of the MNs, especially the long-term safety risks should be deeply studied. Finally, the manufacturing process and the financial cost involved in scaling up should also be taken into consideration, including the structure defects, drug loading capacity and mechanical strength as these indexes could directly influence the clinical translation. Although the clinical successes are still limited, the recent pre-clinical work is aiming to discover the potential of MNs for cancer therapy.

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***Declaration of Interest Statement**

Declaration of competing interest

There are no conflicts to declare.