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Current and future technological advances in transdermal gene delivery

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Abstract

Transdermal gene delivery holds significant advantages as it is able to minimize the problems of systemic administration such as enzymatic degradation, systemic toxicity, and poor delivery to target tissues. This technology has the potential to transform the treatment and prevention of a range of diseases. However, the skin poses a great barrier for gene delivery because of the "bricks-and-mortar" structure of the stratum corneum and the tight junctions between keratinocytes in the epidermis. This review systematically summarizes the typical physical and chemical approaches to overcome these barriers and facilitate gene delivery via skin for applications in vaccination, wound healing, skin cancers and skin diseases. Next, the advantages and disadvantages of different approaches are discussed and the insights for future development are provided.

Keywords: Skin delivery; transdermal delivery; physical approaches; chemical approaches; gene therapy; skin vaccination

Abbreviations: AuPT, gold nanoparticles; CpG-ODN, cytosine–phosphate–guanosine oligodeoxynucleotide; CPPs, cell penetrating peptides; CTAB, cetyltrimethylammonium bromide; CTLs, cytotoxic T lymphocytes; DCs, dendritic cells; DC-Chol, 3β-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol; DEFB4, defensing beta 4; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE: dioleoylphosphatidylethanolamine; DOPyCl, pyrrolidinium lipid; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DSPC, 1,2-Distearoyl-sn-glycero-3-phosphocholine; EGFR, epidermal growth factor receptor; FTIR, Fourier-transform infrared

spectroscopy; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; GM3S, Ganglioside-monosialic acid 3 synthase; HEM, hybrid electro-microneedle; HSV-2-gD2, herpes simplex virus type 2 surface glycoprotein D2; IFNγ, interferon gamma; IL-4, interleukin-4; IL-10, interleukin-10; IL-12, interleukin-12; IL-36α, Interleukin-36 alpha; JEV, Japanese encephalitis virus; LMWP, low molecular weight protamine; MW, molecular weight; NaChol, sodium cholate; NF-κB, nuclear factor-κB; ODN, oligonucleotide; PEG, polyethylene glycol; PEI, polyethylenimine; PLGA, Poly(lactide-co-glycolide); PSA, prostate specific antigen; PVP, poly(vinylpyrrolidone); SC, stratum corneum; siRNA, small interfering RNA; SiRelA, a siRNA oligonucleotides for mouse RelA; SPACE, skin penetrating and cell entering; STAT3, signal transducer and activator of transcription 3; SWCNT, single-walled carbon nanotubes; TAT, twin-arginine translocation; TEM, transmission electron microscopy; TNFα, Tumor necrosis factor-α.

Contents

1. Introduction

Gene therapy holds great potential to treat or prevent many diseases that cannot be addressed with conventional approaches. By April 2017, over 2400 gene therapy clinical trials have been carried out, but only 93 and 3 of these entered phase III (3.8%) and phase IV (0.1%), respectively (http://www.wiley.com/legacy/wileychi/genmed/clinical/). The successful translation of gene therapy is significantly inhibited by many problems such as lack of targeted delivery of genes to diseases sites and cells, degradation of gene during delivery, and fast clearance in circulation [1, 2].

To address these issues, one great alternative solution is to deliver genes via skin. Skin delivery of drugs and genes offers tremendous advantages such as being pain free, avoiding the hepatic first-pass metabolism [3], digestion in the gastrointestinal tract, and enzymatic degradation and clearance from the blood circulation, preventing needle-stick injuries, as well as achieving high efficacy and low side effects.

To realize these potentials, significant research has been conducted. Fig. 1 summarizes the applications, challenges and technologies of gene delivery via skin. Fig. 2 and Fig. 3 show representative chemical [4-6] and physical [7-10] approaches for drug and gene delivery. These methods are capable of enhancing permeation of different pharmaceutical and biological molecules through cell membranes or the skin as long as working parameters are properly optimized. In this review, I highlight the physical and chemical approaches that efficiently deliver genes including plasmid DNA and siRNA into the skin and demonstrate their great effectiveness in different applications spanning from vaccination and wound healing to skin diseases and cancers. After comprehensive review of the progress of gene delivery via skin, discussion of the advantages and disadvantages of different approaches and insights into future development of the field are presented.

2. The skin anatomy

Skin is the body's natural barrier and protects us from water loss and microorganism invasion. The skin is mainly composed of three layers including the epidermis, dermis and subcutaneous layers

(Fig. 4a) [11, 12]. The epidermis contains the outmost layer of the skin called stratum corneum (SC) and the beneath layer – the viable epidermis. The barrier of the skin is largely due to the SC that has a “bricks-and-mortar” structure [13] and is consisting of corneocytes and the surrounded multiple layers of hydrophobic lipids [14]. With this structure, the permeation of materials through the skin must take place via the intercellular, transcellular, or appendageal routes [12, 15]. Intercellular route requires passive diffusion of materials through the tortuous lipid matrix in the SC and lipophilic drugs prefers this approach. Transcellular route involves substances passing through the cells. Therefore, the process requires the transport of materials through aqueous environment (inside the cells) and the hydrophobic sections (the cell membranes and the lipid matrix between cells). Under normal conditions, it is very difficult for substances to be delivered through this route. The third skin delivery pathway is appendageal route in which materials can be transported into the skin through hair follicles and glandular ducts. These routes provide a direct approach to transport substances to the dermal microcirculation. However, hair follicles and glandular ducts only account for approximately 0.1% and 0.01% of the total skin area [16, 17], respectively, so little amount of materials can be transported through this route. In general, the skin structure only allows permeation of drugs with molecular weight (MW) of below 500 Dalton [18] and lipid-soluble drugs into the skin. Besides the SC, the underlying viable epidermis serves another barrier due to the tight junctions between keratinocytes as illustrated in Fig. 4b [11, 19]. This leads to difficult transport of drug molecules in both vertical and horizontal direction in the epidermal layer. All of these characteristics pose a great challenge in delivering drugs and genes through the skin.

The dermis is the middle layer of the skin and contains different types of cells, connective tissues, blood and lymphatic vessels, glands, hair follicles and nerve endings. The subcutaneous layer, also called hypodermis, lies below the dermis and is the innermost layer of the skin, mainly containing adipose tissue (fat). The dermis and hypodermis are composed of complex capillary network and connect to the systemic circulation.

3. Approaches to facilitate gene delivery via skin for a range of applications

Owing to the great potential of gene delivery via skin, a broad range of approaches including biological, chemical, and physical approaches have been developed for different applications. In biological approaches, various viral vectors such as adenovirus serotype 5, and human immunodeficiency virus-1-based lentivirus were employed for gene therapy in skin [20-22]. Although very effective, these methods have the risk of inducing insertional oncogenesis and severe immunological responses [23, 24]. Therefore, a range of non-viral chemical and physical strategies have been developed for safe delivery of genes to the skin. This review will focus on the currently commonly used physical and chemical methods for transdermal delivery with applications in vaccination, would healing and treating skin diseases and cancers.

3.1. Chemical approaches

3.1.1. Peptides

Cell penetrating peptides (CPPs) are small peptides and have been broadly used to facilitate intracellular delivery of drugs and genes. Cargo loading can be done via covalent link such as disulphide bonding between CPPs and genes or forming nanoparticles through the interaction of CPPs and genes [4, 25-29], as illustrated in Fig. 2a. Since 2006, many peptides started to be developed for skin delivery [28, 30]. The SC layer of the skin is consisting of corneocytes and the surrounded multiple layers of hydrophobic lipids. The underlying viable epidermis is another barrier layer due to the tight junctions between keratinocytes. Therefore, various chemicals systems can be designed to interact with the lipids, proteins, and the tight junctions within the skin to facilitate drug and gene delivery. Table 1 summarize the recent examples of peptide based systems for gene delivery via skin. In application, peptides and DNA complex can be topically applied on the surface of skin for subsequent delivery. Chen et al. first reported to use a short synthetic peptide TD-1 for insulin delivery through intact rat skin. The study showed that TD-1 was able to create transient opening on the skin barrier to facilitate drug permeation [30]. Lin et al. later used TD-1 to deliver anti- glyceraldehyde-

3-phosphate-dehydrogenase (GAPDH) siRNA through non-follicle rat skin, which led to dramatically decreased level of GAPDH in 3 days [31]. Following the success of TD-1, Hsu and Mitragotri developed a peptide named skin penetrating and cell entering (SPACE) peptide and employed it to deliver interleukin-10 (IL-10) and GAPDH siRNA, enabling increased delivery to skin and knockdown of corresponding gene targets. SPACE peptide could deliver cargos to the epidermis and deep dermis of mouse skin and was also shown to permeate human skin [32]. When combine with cationic ethosomes such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), the system could deliver 18.5±3.3% (6.3±1.7 fold increase compared with aqueous solution of siRNA application) and 4.8±0.8% (~ 10 fold increase with reference to aqueous solution of siRNA application) of the applied total siRNA dose to whole porcine skin and the epidermis, respectively. It is noted that the majority of drug is within the SC layer of the skin after delivery [33]. Recently, Vij et al. created Mgpe9 for plasmid DNA delivery to uncompromised skin. Mgpe9 is an amphipathic peptide (CRRLRHLRHHYRRRWHRFRC, Fig. 5a) and forms an alpha-helical structure in hydrophobic environment. Fluorescence labelling the peptide demonstrated its ability of penetration to human skin (Fig. 5c) and efficient uptake by over 90% of cells (Fig. 5d). The peptide could form nanocomplexes with a 4.7 kb plasmid DNA (Fig. 5e). The nanoparticles were able to enter HaCaT cells (Fig. 5f) and reach the basal layer of the epidermis of integrated human skin at 24 hours after topical application (Fig. 5g). The peptide could effectively transfect the cells in human tissue and the efficiency was similar to that of commercial Lipofectamine 2000™. Attractively, when tested in SKH-1 mice without hair follicles, the peptide were still able to deliver plasmid DNA to the skin and achieve efficient transfection. Although the efficacy is similar with that of Lipofectamine 2000™, the safety profile of Mgpe9 is better with less disruption to skin integrity and lower cytotoxicity to cells [34]. Through Fourier-transform infrared spectroscopy (FTIR) and time dependent microscopy analysis, it was found that Mgpe9 entered the skin through transient alternation of lipid packaging and skin protein, and cause disruption to the tight junctions of skin.

Table 1 Schematic overview of recent peptide based systems for gene delivery via skin

Year and reference	Formulation	Gene	Test model	Application
2016, [34]	Peptide Mgpe9 Peptide Tat	EGFP DNA	SKH-1 hairless mice Human foreskin	Gene expression
2014, [33]	SPACE peptide + lipid DOTAP + cholesterol + SPACE-peptide-POPE + SPACE-siRNA	GAPDH-siRNA	BALB/c mice	Gene regulation
2012, [35]	PEI/DNA core + PGA/LMWP CCP (layer-by-layer coating)	EGFP DNA	SD rats	Gene expression
2011, [36]	Peptide TD1-R8	MITF-siRNA	BALB/c mice Human, clinical trial	Melasma
2011, [37, 38]	Peptide Tat + AT1002	Anti-RelA siRNA	ICR mice	Atopic dermatitis
2011, [32]	SPACE peptide	GAPDH siRNA IL-10 siRNA	BALB/c mice Human skin	Gene regulation Atopic dermatitis

In using this technology, it is advantageous to combine different peptides together to simultaneously enhance skin permeation and subsequent intracellular delivery for maximized therapeutic efficacy. For example, peptides AT1002 and Tat have been jointly employed for this purpose. AT1002 is a six-mer synthetic peptide and can reversibly open the tight junctions between cells. Tat is a cell penetrating peptide and can pass through the plasma membrane of cells. Therefore, these two peptides were combined for siRNA delivery and attempted to cure atopic dermatitis. This is a skin disease with disrupted SC (either broken or peeled off), but the epidermal layer still has tight junctions and these hinder the diffusion of siRNA. To simulate the disease condition, tape-stripping was used to partially remove the SC of mice ear skin. When siRelA (a siRNA oligonucleotides for mouse RelA) was conjugated with AT1002 and Tat, its intradermal permeation and RelA silencing function were demonstrated in NC/Nga atopic dermatitis model. The findings revealed that the combination of the peptides could open the tight junctions at the bottom of the epidermis (granular layer). As a result, compared with naked siRelA, the nanocomplex of siRelA with AT1002 and Tat

dramatically decreased local inflammatory tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4) and serum IgE level, indicators of the symptoms in atopic dermatitis mice [37, 38].

Besides using peptides alone, they can also be employed to coat onto the surface of nanoparticles for increased permeation to skin. Yang et al. built a type of quaternary nanoparticles for DNA delivery (Fig. 5h). The core of the nanoparticles was formed through the complexation between DNA and polyethylenimine (PEI). Then a layer of poly(γ-glutamic acid) (PGA) and another layer of low molecular weight protamine (LMWP) CPP were sequentially adsorbed to the surface of the core through electrostatic attraction. The outmost layer of CPP was to facilitate skin permeation and cellular uptake. The results indicate that the cellular uptake and transfection of these nanoparticles increased by 4-fold in comparison with conventional PEI/DNA nanostructures. When tested on full-thickness abdominal rat skin, 14% of the applied dose could fully permeate the skin, reflecting a 4-fold increase in comparison with that in PEI/DNA nanoparticles group. When co-applying these CPP modified nanoparticles with a chemical permeation enhancer sodium lauryl sulfate, the skin permeation amount could further increase to 23.8% of the total dose [35]. It seems that such soft-matter nanoparticles could penetrate deeper to the skin than rigid nanomaterials [39, 40].

3.1.2. Liposomes

Liposomes are spherical vesicles containing one or more lipid bilayers (Fig. 2b) [5]. The core of liposomes encloses an aqueous environment. Liposomes can load either hydrophilic or lipophilic drugs for delivery. Cationic liposomes can form complexes with DNA. The formation of liposome-DNA complexes is able to protect DNA from degradation and significantly enhance cell gene transfection. The transfection efficiency can be influenced by factors including liposome to DNA ratio, the size of complexes, and chemical nature of cationic liposomes. Cationic liposomes had been tested for passive cutaneous delivery of genes in 1990s. Cheng et al. reported using cationic liposomes, 3β -[N-(N, N-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol)/dioleoylphosphatidyl ethanolamine (DOPE) and DOTAP, for transdermal antigen DNA delivery. DOTAP and DC-Chol

are cationic lipids. DOPE is a neutral lipid and often used together with cationic lipids because it is able to aid endosome escape [41]. For maximal transfection efficiency, the weight ratio of liposomes to DNAs was set to be 5 as determined through BHK-21 cell transfection assays. The size, zeta potential, and transfection efficiency of both liposome/DNA complexes are provided in Table 2. When these liposome/DNA complexes were tested on the dorsal skin of hair-removed mice, gene expression was detected in epidermis and spleen for over 3 days. When tested in C3H/HeN mice via skin delivery of liposome-pCJ-3/ME (Japanese encephalitis virus (JEV) E-protein gene), the approach elicited strong antibodies and protective immune responses against 50×LD_{50} of JEV (Fig. 6) [42].

Table 2 Optimal ratios of lipoplexes and their characteristics [42]

	Transfection efficiency (%)	**Particle size (nm)**	**Zeta potential (mV)**
DC-Chol/DOPE	23.1±0.8	211.3±12.6	19.9±4.2
DOTAP	10.4±1.1	361.3±25.1	8.84±2.7

Permeation enhancers like surfactants can be introduced to increase vesicle elasticity and deformability for enhanced penetration in the skin [23]. Jin and Kim used lipid nanoparticles (cLNs) composed of 4 components including DOTAP, DOPE, Tween 20, and tricaprin, with a weight ratio of 1:1:1:1.67 for DNA delivery to the skin [43]. In the formulation, DOTAP is a positively charged lipid so it can allow the formation of complexes with DNA. DOPE and Tween 20 can enhance the stability of cLNs, improve DNA transfection efficiency, and increase SC permeation [44, 45]. Tricaprin is a solid lipid and can soften the nanoparticles to facilitate skin delivery because of its melting point at 32 ºC [46]. Through these synergistic effects, the designed cLNs could transfer plasmid DNA to the skin after topic application and the expression of mRNA in the skin and blood were able to be measured [43].

With the principle, elastic liposomes were developed with high flexibility and deformability for efficient penetration through much smaller pores than their own sizes (Fig. 7) [47, 48]. Geusens et al. designed nanosomes named "SECosomes" (surfactant-ethanolcholesterol-osomes) [49], composed of 4 components including a cationic lipid DOTAP, a helper lipid/stabilizer (cholesterol), a single chain surfactant (sodium cholate) and penetration enhancer (30% ethanol). This liposome system could deliver fluorescence labelled siRNA to the epidermis of intact human skin. Later in another study, the same group used these nanosomes to deliver defensing beta 4 (DEFB4) siRNA and down-regulate the psoriasis marker human beta-defensin 2 [50]. However, the cargos were only delivered to the upper epidermis of the skin. Based on these two studies, Dorrani et al. added different amount of edge activators sodium cholate (NaChol) to DOTAP [51]. It was then found that the liposomes penetrated the skin deeper when the DOTAP:NaChol ratio was 8. When these liposomes were mixed with siRNA at a ratio of 16:1, most cargo was deposited to the upper dermis, while siRNA could be delivered to the lower dermis when the ratio was 12:1 or 8:1. This work shows that the correct balance between liposome size, charge, and edge activator content has significant influence on permeation depth. Besides these, many other types of liposomes and liposome like structures have also been developed for transdermal delivery [52, 53]. Rattanapak et al. investigated using liposomes, transfersomes, ethosomes, and cubosomes for skin delivery of a peptide antigen in the absence or presence of adjuvants Quil A. It was found that the delivery efficiency was below approximately 6% with the order in the following: cubosomes > ethosomes > transfersomes > liposomes. After addition of Quil A, the delivery efficiency of all approaches increased [53]. The recent examples of using liposomes and liposome like structures for gene delivery are listed in Table 3.

Table 3 Schematic overview of recent liposome based systems for gene delivery via skin

Year and reference	**Formulation**	**Gene**	**Test model**	**Application**
2017, [54]	Pyrrolidinium lipid (DOPyCl) + 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) + cetyl trimethylammonium	IL36α siRNA	Human skin CD®(SD) hrBi hairless rat skin C57BL/6 mice	Psoriasis

	bromide (CTAB) + cholesterol			
2017, [55]	DOTAP + NaChol	Keap1 siRNA	57BL/6J mice	Wound healing
2016, [50]	DOTAP + DOPE + Chol + EtOH	siRNA against defensing beta 4	Human skin	Psoriasis
2016, [51]	DOTAP + NaChol	BRAF-siRNA	Human cadaver skin	Melanoma
2014, [43]	DOTAP + DOPE + Tween 20 + tricaprin	GFP plasmid DNA	Hairless mice BALB/c mice	Gene expression
2013, [56]	Poly(lactide-co-glycolide) (PLGA) + polyethylene glycol (PEG) + 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) + cyclic-head lipid	Anti-TNFα siRNA	C57BL/6 mice	Skin inflammation
2011, [57]	DOPE + cholesterol-3-sulfate	DNA oligonucleotide thymidine dinucleotide	Nude mice	P53 generation for skin cancers
2009, [42]	DOTAP liposome + DNA DC-Chol/DOPE liposome + DNA	Japanese encephalitis virus DNA vaccine	C3H/HeN mice	Vaccination

3.1.3. Nanomaterials

Various nanomaterials have been widely employed for improved intracellular delivery of genes [58-62] and recently the use starts to be extended to facilitate skin delivery (Table 4). Siu et al. applied succinated polyethylenimine functionalized single-walled carbon nanotubes (SWCNT) for siRNA delivery [63]. In the experiments, topical application of siRNA and succinated polyethylenimine could not lead to skin delivery, but with additional aid of SWCNT, siRNA was effectively delivered to the skin. In vivo delivery of Braf siRNA to a C57BL/6 mice melanoma model indicated that this method could transport siRNA to tumor and knockdown Braf which can lead to tumor growth, consequently resulting in significant tumor growth inhibition over the 25-day experimental period.

Table 4 Schematic overview of recent nanoparticle based systems for gene delivery via skin

Year and reference	**Nanoparticle type**	**Gene**	**Test model**	**Application**
2017, [64]	TAT Gold nanoparticles	pDNA encoded with miRNA-221 inhibitor gene	Nude Mice	Cutaneous melanoma
2015, [65]	Gold nanoparticles	Ganglioside-monosialic acid 3 synthase (GM3S) siRNA	C57BL/6 mice	Wound healing

2014, [63]	Carbon nanotube	Braf siRNA	CD-1 mice	Melanoma

Zheng et al. coated thiolated siRNA-duplexes to citrate-capped gold nanoparticles (SNA-NCs) (Fig. 8a) and topically applied them to SKH1-E hairless mice for gene regulation [66]. The results showed that the siRNA nanoparticles could penetrate to the epidermis and dermis of the mice skin at 3 h after a single treatment. In vivo topical application of 1.5 μM epidermal growth factor receptor (EGFR) siRNA for 3 weeks (3 times a week) to SKH1-E mice skin resulted in nearly complete knockdown of EGFR expression and almost 40% reduction of epidermal thickness. When 25 nM EGFR SNA-NCs was applied to human skin equivalents, the nanoparticles were detected in the basal layer of the epidermis (Fig. 8b) at only 2 h after treatment. The nanoparticle penetration to the skin and cellular uptake increased with time (Fig. 8c-d). This amount of nanoparticles knocked down EGFR mRNA expression by 52% (Fig. 8e) and EGFR protein expression by 75% (Fig. 8f) with reference to nonsense siRNA control group, although only 4.7% of the application dose was delivered to the epidermis.

Niu et al. reported using gold nanoparticles coated with both HIV-1 twin-arginine translocation (TAT) peptide and PEI for transdermal delivery of plasmid DNA to treat cutaneous melanoma [64]. Gold nanoparticles have been demonstrated to be able to modulate membrane lipid phase transitions and therefore improve the lipid fluidity, contributing to skin penetration [67]. TAT is a skin permeable protein and can further enhance the skin permeation of gold nanoparticles. Also importantly, TAT is non-immunogenic and does not lead to toxic responses in cells and animals. For application, TAT and PEI coated gold nanoparticles were mixed with plasmid DNA encoded with miRNA-221 inhibitor gene to form nanocomplex, followed by topical application to nude mice skin (Fig. 9). The transmission electron microscopy (TEM) image indicated that the nanoparticles were round in shape and the zeta potential was approximately 35 mV. The in vivo study demonstrated that these nanoparticles were able to penetrate deeply into melanoma tissues and induce a tumor inhibition trend.

3.2. Physical approaches

3.2.1. Electroporation

Electroporation uses a device containing an array of electrodes to apply high voltage pulses to create transient perturbation to the skin for enhanced permeation of materials (Fig. 3c) [68]. The size, shape, spacing and orientation of electrodes can be tuned. In use, electrodes can be applied on the surface of the skin or penetrated into a certain depth within the skin, following by application of voltage [69, 70]. Electroporation typically occurs when transmembrane voltage reaches between 0.3-1.0 V (equivalent to 30-100 V across the SC) [71, 72]. Once electroporation takes place, water is forced into the lipid environment and forms transient aqueous pathways in lipid bilayer membranes, leading to many orders of magnitude increase of molecule permeation to the skin [73]. The technique was first reported in 1993 in which it was demonstrated that electroporation could increases the flux of polar molecules including calcein (623 Da, -4 charge), Lucifer yellow (457 Da, -2 charge), erythrosine derivative (1025 Da, -1 charge) to human skin by up to 4 orders of magnitude [74]. Since then, electroporation has been widely employed to facilitate the delivery of many types of biological molecules including genes. Table 5 summarizes the recent studies of electroporation based gene delivery.

Table 5 Schematic overview of recent electroporation based gene delivery systems after intramuscular and intradermal injection

Year and reference	**Electroporation conditions (Voltage, pulse duration)**	**Gene**	**Test model**	**Application**
2017, [75]	25 V, 100 ms	Influenza plasmid DNA vaccine	Guinea pig	Vaccination
2017, [76]	700 V/cm, 100 µs + 200 V/cm, 400 ms 1600 V/cm, 100 µs + 170 V/cm, 150 ms	Plasmid DNA encoding ovalbumin, hCAP-18/LL-37	Mouse	Wound healing Vaccination
2016, [77]	570 V, 100 µs 60 V, 150 ms	Plasmid DNA encoding interleukin-12 (IL-12), shRNA against endoglin, shRNA against melanoma cell adhesion molecule	Mouse	Wound healing Melanoma tumor

2015, [78]	1600 V/cm, 100 µs	Plasmid DNA encoding IL-12 with collagen promoter	Mouse	Vaccination
2015, [79]	700 V/cm, 100 µs 200 V/cm, 400 ms 200 V/cm, 20 ms	Plasmid DNA encoding luciferase, ovalbumin, gp160 against HIV, P1A against P815 mastocytoma	Mouse	Vaccination Mastocytoma tumor
2015, [80]	1125 V/cm, 50 μs + 275 V/cm, 10 ms	Plasmid DNA encoding EGFP, Her-2/neu	Mouse	Vaccination Her2/neu tumor
2015, [81]	325 V, 100 ms	Puumala DNA vaccine, H5HA DNA vaccine	Hamster Guinea pig	Vaccination
2014, [82]	10-50 V, 20 ms	Plasmid DNA encoding RFP Cy5-siRNA	Mouse	Vaccination
2014, [83]	700 V/cm, 100 µs + 200 V/cm 400 ms 400 V/cm, 20 ms + 1,800 V/cm, 100 µs	Plasmid Encoding hCAP-18/LL-37	Mouse	Wound healing
2014, [84]	50 V, 150 ms	$VEGF_{165}$ plasmid	Rat	Ischemic skin flaps
2013, [85]	1125 V/cm, 50 µs + 275 V/cm, 10 ms	DNA vaccine coding for rhesus prostate spe-cific antigen (PSA)	Human	Vaccination Prostate cancer
2012, [86]	1125 V/cm, 50 µs + 275 V/cm, 10 ms	Semliki Forest virus replicon RNA	Mouse	Vaccination
2011, [69]	15 V, 100 ms 100 V, 60 ms	Influenza plasmid DNA vaccine	Guinea pig	Vaccination

Electroporation is often used immediately after injection of genes to the skin or muscle for significantly improved efficacy [80]. In one study, a multi-electrode array containing 4 × 4 2-mm-apart pins [87] was used to boost plasmid DNA delivery in guinea pig model. The applied voltage was 30 –70 V between the two pins and the pulse duration was 150 ms. In comparison with intradermal injection alone, the application of electric pulses could improve gene expression by approximately 2-4 logs and the expression could last 12-15 days. In another study, a multi-head intradermal electroporation device was employed to perform intradermal electroporation after injection of Puumala and H5HA DNA vaccines to hamsters and guinea pigs, respectively. It was found that the delivery dose and immune responses were dramatically enhanced [81]. When a gp160

plasmid DNA was injected to the muscle with subsequent electroporation, potent humoral response against gp160 was induced, with antibody titers of 1.6 and 16 fold greater than those elicited by intradermal injection of DNA to ear and abdominal skin, respectively, followed by electroporation [79]. These findings and also the results described in Table 5 indicate that electroporation is an effective method to deliver genes via skin for different uses including vaccination, wound healing, skin diseases and skin cancers [88].

Although intramuscular electroporation achieves better results, intradermal electroporation is probably more suitable in clinical applications due to increased safety and tolerability. To investigate this, a DNA vaccine coding for rhesus prostate specific antigen was tested on 15 patients with intradermal injection followed by skin electroporation [85]. This was the first clinical trial investigating the immune responses of intradermal injection of a DNA vaccine followed by electroporation. Although the immune response might not be as strong as those elicited through intramuscular injection in combination with electroporation, this method caused less patient discomfort, so the next goal is to increase its efficacy.

The delivery efficiency of electroporation is dependent on many parameters such as the duration, amplitude, shape, and frequency of electric pulses, as well as the size, shape, spacing, and orientation of electrodes [89-93], so it is essential to perform research to optimize the design. For luciferase and GFP plasmid DNA delivery to skin by intradermal injection and electroporation, it was observed that needle electrodes worked significantly better than plate ones [92]. However, it is worth noting that the distribution of the electric field produced by a needle electrode may lead to irreversible electroporation in the cells close the tips of the needles, but less electroporation in the cells which are far away [94]. In the work, the plate electrodes were placed on the surface of the skin while the needle electrodes were penetrated into the skin with 7 mm in depth. In the case of IL-12 plasmid DNA delivery in mouse skin, the use of high voltage pulses improved transfection in shallow skin depth like epidermal and dermal skin layers, while low voltage pulses were good for deep layer transfection including muscle cells [77]. Roos et al. demonstrated that electroporation following intradermal

injection of a prostate cancer DNA vaccine led to 100-1000 times increased T cell response with reference to intradermal delivery alone [95], but their subsequent study suggested that a reduction of 90% of the pulse delivery time could induce similar gene expression and immune responses. Apparently, the fast pulse operation will be more tolerable and suitable for clinical use [96]. Therefore, it is very important to optimize parameters in electroporation for transdermal delivery. Under non-optimal conditions, electroporation may lead to cell mortality, muscle contractions, pain, skin sensation, and transient impairment of the skin's barrier function [71, 72].

3.2.2. Microneedle/nanoneedle arrays

Microneedle technology for transdermal delivery usually involves using several to several thousand of needles with a size of micrometer range, packed on a substrate to pierce the SC for facilitated drug and gene delivery via skin [97-107]. Depending on the specific ways of drugs and genes being loaded and delivered, different types of microneedle arrays can be classified, including solid, coated, dissolving, and hollow microneedles as a few examples (Fig. 3d-e) [10]. Compared with other physical approaches, this technique is simple and cost-effective, without needing expensive devices [108-112]. This method also induces minimal pain and tissue damage and has the potential of self-administration. Therefore, this technology has attracted significant interest since its initial demonstration in 1990s and tremendous progress has been achieved in various applications. The application of using different types of microneedle arrays for gene delivery via skin is summarized in Table 6. From the table, it is apparent that the application has been predominantly focused on skin vaccination.

Table 6 Schematic overview of recent microneedle based gene delivery systems

Year and reference	**Microneedle type**	**Gene**	**Animal model**	**Application**
2016, [113]	Dissolving 10x10 array 680 µm high 300 µ wide at base	Rabies DNA vaccine Delivery efficiency 65-70%	Dog	Vaccination
2016, [114]	Coated	Influenza nucleoprotein DNA vaccine	Mouse	Vaccination

	4x4 mm (> 21,000 projections/cm^2) 110 µm high	Delivery efficiency 37.5%		
2015, [115]	Coated A row of 5 microneedles 750 µm high	Human papillomavirus pseudovirus-encapsidated plasmid DNA vaccine	Mouse	Vaccination
2013, [116]	Coated A row of 5 microneedles >500 µm high	Influenza hemagglutinin DNA vaccine	Mouse	Vaccination
2013, [117]	Coated 9 rows of 8-9 microneedles/row 650 µm high 250 µm wide at base	Luciferase plasmid DNA vaccine Plasmid encoding HIV antigen	Mouse	Gene expression
2012, [118]	Coated A row of 5 microneedles 750 µm high 200 µm wide at base	Influenza hemagglutinin DNA vaccine	Mouse	Vaccination
2012, [119]	Coated A row of 5 microneedles 750 µm high	EGFP plasmid DNA	Human skin	Gene expression
2012, [120]	Coated A row of 5 microneedles 700 µm high 160 µm wide at base	Avian H5 influenza hemagglutinin DNA vaccine	Mouse	Vaccination
2010, [121]	Coated A row of 5 microneedles 700 µm high 160 µm wide at base	Hepatitis C plasmid DNA vaccine Delivery efficiency > 90%	Mouse	Vaccination
2010, [122, 123]	Coated 4x4 mm (> 21,000 projections/cm^2) 110 µm high	herpes simplex virus type 2 surface glycoprotein D2 (HSV-2-gD2) DNA vaccine Delivery efficiency 8±1%	Mouse	Vaccination
2015, [124]	Nanoneedles 5 µm high 50 nm apical width 600 nm base diameter	Human VEGF165 plasmid DNA, glyceraldehyde-3-phosphate-dehydrogenase-siRNA	Mouse	Tissue regeneration, Gene regulation

In 2010, coated microneedle arrays started to be used for functional DNA vaccination. Gill et al. applied metal microneedles to deliver plasmid DNA encoding hepatitis C virus NS3/4A protein [121]. With this technique, 8 µg of DNA could induce NS3/4A-specific cytotoxic T lymphocytes (CTLs) response which is similar to that obtained through intramuscular injection of 100 µg of DNA. Chen

and Kask and co-workers used a much more densely packed silicon microneedle array to deliver herpes simplex virus type 2 surface glycoprotein D2 (HSV-2-gD2) DNA vaccine [122, 123]. The prototype and scanning electron microscopy (SEM) images of the patch are presented in Fig. 10a-c. These densely packed microneedles were able to deliver coated DNA vaccine to the vicinity of a large number of immune cells (Fig. 10d). Systematic study of the immune responses including antibody titer, seroconversion, and survival rate upon lethal viral challenge were performed. It was found that this method could deliver HSV-2-gD2 DNA vaccine and achieve comparable immune responses with intramuscular injection, but with less than 1/10th of the delivery dose. Following this, microneedle arrays have been also tested to deliver avian H5 influenza hemagglutinin DNA [120], influenza hemagglutinin DNA [116], human papillomavirus pseudovirus-encapsidated plasmid DNA [115], influenza nucleoprotein DNA [114], and rabies DNA vaccine [113]. When rabies DNA vaccine was tested in dogs, it was also demonstrated that the dose sparing was approximately 10 times in comparison with intramuscular injection [113]. Similarly, microneedle array delivery of 1 μg of influenza nucleoprotein DNA vaccine elicited antibody titers which were comparable with those by 10 μg of intramuscular injection. Apparently, microneedle array delivery of naked DNA is already able to greatly boost the immunogenicity of DNA vaccines. This is very important, because DNA vaccines have many advantages but the low immunogenicity remains a bottle neck for their clinical applications [125, 126]. When use microneedle arrays, gene delivery dose and location can be controlled by tuning microneedle design parameters and application conditions [127-129]. To achieve good control of patch application force and speed for reproducible and reliable operation, motorized microneedle patches were developed [130, 131].

In using microneedles for transdermal drug delivery, so far, over 60 clinical trials have been completed or are ongoing (https://clinicaltrials.gov). Although these clinical trials are not for gene delivery, the results have demonstrated the acceptability, safety and effectiveness of microneedle technology [110, 132-134]. These are very important for utilizing this technique for transdermal gene delivery in the future. As one example, dissolving microneedle arrays have recently been tested to

deliver influenza vaccine in a Phase 1 clinical trial [110]. In the study, microneedle patches were manually administered to the wrist by participants themselves without healthcare worker intervention. It was found that the participants strongly prefer these microneedle patches over conventional intramuscular injection. No safety issues were identified in the study and dissolving microneedle patches induced strong immune responses, comparable with those generated from intramuscular injection. Also attractively, these patches were stable at 40 °C for at least a year and generated little or even no medical waste. With these advantages, this technique has a great potential to be embraced by clinicians.

Beyond microneedle patches, nanoneedle arrays are a promising alternative for gene delivery. Compared with microneedles, nanoneedles are much smaller, generally with a diameter of below 1 micrometer (Fig. 11a). Due to the small diameter, nanoneedles can deliver genes into targeted cells without causing irreversible damage [135]. Chiappini et al. used biodegradable silicon nanoneedles for gene delivery [124]. The method could directly co-deliver GFP DNA and GAPDH siRNA to cells with a transfection efficiency of great than 90% and 80% silencing in GAPDH expression at 48 h, respectively. These nanoneedles could deliver fluorescent dyes to mouse ear skin with good uniformity. Additionally, these nanoneedles could deliver naked human VEGF165 plasmid DNA to the muscle of mouse (a small incision was made to expose the muscle before application). The muscles treated with nanoneedle injection displayed significantly more interconnected and structured vessels, ultimately resulted in much higher blood perfusion and number of nodes than those achieved by intramuscular injection (Fig. 11b-d). Besides silicon nanoneedles, Chen and co-workers developed diamond nanoneedle arrays for drug delivery [136-141]. Diamond is the hardest materials in nature with extremely high Young's modulus [142]. The excellent mechanical properties at even nanometer scale make diamond nanoneedles a great choice for gene delivery via skin [143, 144]. The extremely small diameter of diamond nanoneedles enable direct gene delivery to target cells without affecting their viability.

3.2.3. Iontophoresis

Iontophoresis is to apply a current to deliver charged drugs through the skin [145]. Different from electroporation which mainly acts on the skin structure, iontophoresis is primarily working on drugs themselves and drive their transport via an electric field (Fig. 12) [146]. When an electric potential difference is applied, drug ions are transported through the epidermis and dermis layers of the skin, ultimately diffusing into the blood stream. The drugs can permeate into the skin through sweat ducts, hair follicles, and imperfection sites. A small number of work was recently carried out using iontophoresis for gene delivery (Table 7). Kigasawa et al. demonstrated successful delivery of naked anti-IL-10 siRNA into the epidermis of an atopic dermatitis-like model rat, resulting in 73% reduction of the level of IL-10 mRNA [147]. In this work, the siRNA was accumulated within the epidermis of the skin, without passing through the basal layer into the dermis. Later on, the same group tested iontophoresis delivery of cytosine–phosphate–guanosine oligodeoxynucleotide (CpG-ODN) in a hairless mice model [148]. It was found that CpG-ODN could be delivered to both epidermis and dermis layers of the skin. Although not penetrating into the subcutaneous tumors, the delivery of CpG-ODN elicited antitumor immune responses against B16F1 melanoma.

Table 7 Schematic overview of recent iontophoresis based gene delivery systems

Year and reference	**Iontophoresis conditions**	**Gene**	**Animal model**	**Application**
2011, [148]	0.3 mA/cm^2	CpG-ODN	Hos:HR-1 hairless mice	Melanoma
2010, [147]	0.3 mA/cm^2	siRNA against rat IL-10 mRNA	Brown Norway (BN) rats	Atopic dermatitis
2010, [149]	0.38 mA/cm^2	nuclear factor-κB (NF-κB) decoy oligonucleotides	C57BL/6	Atopic dermatitis

4. Discussion

Gene therapy is a type of treatment to use genes to cure diseases. It is becoming increasingly attractive for treatment of hard-to-cure diseases such as skin diseases and cancers [150, 151]. However, the translation of this technique to the clinical application experiences many challenges such as enzymatic degradation in blood circulation in systemic administration and direct delivery to

target tissues [1, 152]. To address these issues, gene delivery via skin offers a great opportunity because it causes minimal pain and discomfort, avoids first-pass effect and enzymatic degradation and clearance in the blood stream, and reduces systemic toxicity [1, 153]; and more importantly, the skin itself is the target tissue for many applications. For example, the skin is a highly immunogenic site, containing a large population of immune cells such as keratinocytes, lymphocytes and dendritic cells, and T cells [154], making it an ideal target for highly efficient vaccination. Additionally, the skin is the natural target for treating skin diseases and skin cancers. Although promising, successful translation of gene therapies via skin delivery still requires to conquer a series of challenges: (1) highly efficient and controllable gene delivery via skin; (2) highly effective and targeted intracellular delivery and nuclear gene expression; (3) high stability of genes during migration and transport through the extracellular matrix within skin layers, endocytosis pathways, and the cytoplasm environment after entering cells.

To meet these challenges, many chemical and physical approaches have been designed and tested for a wide range of applications. Each method has its advantages and disadvantages. In general, chemical approaches have advantages in solving the latter 2 challenges. When nanoparticles, liposomes, peptides, polymers are used to deliver genes, it is very convenient to design numerous systems for highly efficient cellular uptake, cell targeting and improved stability. For example, PEI, gold nanoparticles, and liposomes have been broadly applied to enhance cellular uptake of DNA and siRNA and the subsequent transfection and gene expression [155-160]. During the journey, the systems have been continuously modified to improve the performance [161-163]. For instance, it was found, when PEI was modified with amino acids, the transfection efficiency and biocompatibility could be further improved. Glycolic acid-grafted PEI was able to achieve 23 times higher luciferase gene expression [164] than PEI alone. When PEI (MW 800 Dalton) was used to coat nanodiamond, the gene expression increased 70 times in comparison with PEI alone [165]. Peptide-capped gold nanoparticles were shown to have five-fold increase in cellular uptake comparing with nanoparticles alone [166]. The properties of nanomaterials also greatly affect gene expression and its outcome [167].

For instance, it was found that cationic DNA nanoparticle formulation could essentially lead to no immunogenicity when delivered to the skin. The reason might be that the extracellular matrix components in the skin have a negative charge [168] and this can block the migration and diffusion of the positively charged nanoparticles. To solve the issue, the nanoparticles were PEGylated with close to neutral charge and this enabled dramatically enhanced immune responses in both murine and human skin [169].

During intracellular transport, these gene delivery systems often enter cells through endosomal/lysosomal pathways that involve very low pH, so genes may be degraded. For this, chloroquine was created to raise endosomal pH and inhibit lysis [170]. Branched PEI could also promote endosome escape. Stabilizing DNA with PEG or PEG-PLL could effectively protect gene from degradation in cytosol [171, 172]. Another challenge of gene delivery is nuclear targeting. In a study, it was detected that cationic lipids could delivery only 0.3% of DNA to inside the nucleus at 24 h time point [173]. To improve this, nuclear-targeting peptides were developed [174]. Clearly, chemical approaches have high flexibility in drug delivery system design. More attractively, different designs can be conveniently integrate into one system to simultaneously solve a number of challenges in gene delivery [175].

Despite of the fact that various chemical systems can be designed for enhanced skin permeation, the efficiency is generally much lower and less controllable than that of physical methods. Up to now, a range of physical approaches have also been developed. The main advantages include: (1) these methods could be very effective in terms of delivery efficiency; for example, the delivery efficiency of microneedle technology is up to over 90%; (2) the methods can be universal to facilitate a great range of genes, drugs and other materials to the skin; (3) it is possible to precisely control gene delivery to desirable skin layers; (4) these approaches can achieve relatively constant and reproducible outcomes with minimal influences from the skin properties. However, physical approaches usually only partially solve the challenges of gene delivery as outlined above. For SC breaching methods such as microneedles, after gene being delivered to the skin epidermal/dermal

layers, the lateral diffusion of genes is still blocked by the tight cell-cell junction and also importantly, the problems of cellular uptake, cell targeting, nuclear targeting, and stability of genes often remain. Nonetheless, despite this, microneedle array delivery of many naked DNA vaccines is still able to elicit potent and protective immune responses, and even with approximately 10-fold dose sparing in comparison with intramuscular injection [121, 122]. The mechanism of successful DNA transport to cells and nucleus with strong gene expression needs to be thoroughly investigated.

Owing to the distinct advantages and disadvantages of different approaches, to effectively and collectively address the challenges of gene delivery via skin, it is natural that the most efficient way is to combine different methods. There is a clear trend that an increasing number of studies have been using combined skin delivery strategies. Table 8 summarizes an overview of the recent multiple approach gene delivery systems. Lee et al. used a drawing lithography to produce an integrated hybrid electro-microneedle (HEM) system as shown in Fig. 13. The device is composed of dissolving microneedle tip to pierce the SC for gene release within the skin, and electrode to generate electric field pulses for increased cellular uptake of the release genes [176]. When it was applied to deliver p2CMVmIL-12 for melanoma tumor, the gene transfection efficiency was significantly better than that induced by dissolving microneedle array delivery without electroporation. Correspondingly, the tumor control achieved by DNA delivery via the electro-microneedle device was also better than that via microneedle alone. Other types of microneedle-electroporation integrated delivery systems have also been designed for gene delivery [177]. Beside the combination with electroporation, microneedle technology has been used to combine with different chemical approaches for enhanced gene delivery. In 2010, Prow et al. reported using microneedle patches to deliver EGFP-PEI nanoparticles for gene expression [178]. In another work, a peptide RALA was used to form nanoparticles with DNA followed by incorporating into a poly(vinylpyrrolidone) (PVP) dissolving microneedle patch [179]. In the system, the nanoparticles were designed to protect DNA from degradation, increase cellular uptake, aid endosome escape and enter cell nucleus. The microneedles were employed to pierce the SC for highly efficient nanoparticle delivery to the skin. Beyond this, nanoparticles can also be further

designed with specific targeting capacity. Hu et al. reported a copolymer containing mannosylated grafted cell-penetrating peptide and PEI (MW 1800 Dalton) and the system is referred to as CPP-PEI_{1800}-Man. Subsequently, the copolymer was applied to form nanoparticles with DNA. Then microneedles were used to deliver these nanoparticles to the epidermal and dermal layers of mouse skin (Fig. 14). The nanoparticles could be specifically delivered to dendritic cells (DCs) because of their surface mannose receptors. The conjugated CPP and PEI were able to increase the nanoparticles' intracellular delivery. The experimental results reveal that 42.2% and 49.6% of the DCs in lymph nodes and splenocytes respectively were both GFP and CD11c positive. In contrast, only 1.59% and 5.9% of the DCs were double-positive if naked DNA was delivered under the same conditions. In mice model, protective immune responses were induced with this approach, demonstrated by 90% survival rate at 100 days after B16 melanoma cell challenge. Through this method, CD^{4+} and CD^{8+} T cells were probably recruited to the tumors and this significantly enhanced the mice's immune responses via generation of interferon gamma (IFN-γ) and IL-12 [180].

Other than microneedles, Labala et al. used iontophoresis to aid the delivery of chitosan coated gold nanoparticles with STAT3 siRNA to treat melanoma (Fig. 15) [181]. Signal transducer and activator of transcription 3 (STAT3) are found to be overexpressed in different cancers including melanoma. STAT3 protein can prevent apoptosis and boost proliferation of melanocytes. In the system, STAT3 siRNA was deposited on chitosan coated gold nanoparticles and subsequently another layer of chitosan was further coated on the surface in a layer-by-layer manner. The nanoparticle size was approximately 150±10 nm and the zeta potential was 35±6 mV. In the in vitro test, these nanoparticles could inhibit growth of B16F10 murine melanoma cells by 49.0±0.6% and 66.0±0.2% when the concentrations of siRNA were 0.25 and 0.5 nM, respectively. For in vivo skin application, iontophoresis was able to aid the delivery of these nanoparticles to the viable epidermis with 70 μm depth in comparison with 30 μm depth in the passive application (without using iontophoresis).

Table 8 Schematic overview of recent multiple approach based gene delivery systems

Year and reference	Combined technologies	Gene	Animal model	Application
2017, [182]	Microneedle + PLGA-PLL/γPGA nanoparticles + DNA	Ebola DNA vaccine	Mouse	Vaccination
2017, [183]	Microneedle + RALA peptide and DNA nanoparticles	HPV-16 E6/E7 DNA	Mouse	Cervical cancer
2017, [184]	Microneedle + polyplex of tripolyphosphate, branched PEI and DNA	Porcine circovirus Type 2 DNA vaccine	Mouse	Vaccination
2017, [185]	Microneedle + PLGA/PEI nanoparticles	H1N1 DNA vaccine	Mouse	Vaccination
2016, [186]	Microneedle + liposome encapsulated with DNA	Hepatitis B DNA vaccine	Mouse	Vaccination
2015, [187]	Microneedle + CPP-PEI-DNA nanoparticles	Melanoma DNA vaccine	Mouse	Melanoma
2014, [188]	Microneedle + polyplex with mannosylate PEI	Abeta DNA vaccine	Mouse	Alzheimer's disease
2012, [189]	Microneedle + cationic PLGA and DOTAP nanoparticles	pGPA plasmid DNA vaccine	Mouse	Anthrax
2017, [190, 191]	Iontophoresis + liposome	STAT3 siRNA	Porcine skin	Skin cancer
2016, [181, 192]	Iontophoresis + gold nanoparticles	STAT3 siRNA	Porcine skin	Melanoma
2015, [193]	Iontophoresis + dendrimer nanoparticles	Antisense oligonucleotide	Porcine skin CD1 mice	Skin cancer
2011, [176]	Microneedle + electroporation	p2CMVmIL-12 DNA	Mouse	Melanoma

Besides combination of physical and chemical approaches for more effective gene delivery via skin, there is also a large scope for each method to be further optimized. So far, a lot of work has been focused on applying a technique such as microneedle arrays or gold nanoparticles to deliver drug and gene molecules via the skin, followed by systematic investigation of drug delivery and distribution profiles as well as therapeutic or immunological functions. While these researches are very important in assessing the applicability of different techniques and their application range, significantly less

work has been done to combine the design and improvement of transdermal delivery techniques and their application assessment in drug delivery. In other words, major effort has been placed on extending the application range of existing technologies, instead of improving technology design itself. To bring the technologies forward with a faster pace and ultimately use them in clinical applications, researchers and engineers from a range of disciplines should work together to advance the design of skin delivery techniques and their mass production and then rigorously test their applications and safety profiles in cell lines, animal models and clinical trials. For example, for physical approaches, mechanical and electrical engineers should be invited to optimize the design and manufacturing of various devices. For chemical approaches, materials scientists and chemists should be invited to have rigorous design and characterization of different liposome/nanoparticles-gene systems. Equally importantly, researchers working on biology, pharmacology, vaccinology and immunology should focus on exploring the working mechanisms of each method so that these can be used as a guidance to engineers, materials scientists and chemists to improve the design. Last but not least, doctors, clinicians, and regulators are also important to contribute to the proper design and construction of gene delivery tools to steer the research toward clinical applications. With this concerted effort, the field of gene delivery via skin can achieve further success.

5. Conclusions

Gene delivery via skin offers great advantages over alternative routes. Although skin poses a significant barrier and there is a cascade of challenges including cellular uptake, cell/nucleus targeting, stability, tremendous progress has been achieved and many physical and chemical approaches such as peptides, liposomes, nanomaterials, microneedles, electroporation, and iontophoresis have been established for effective gene delivery. Translation of these techniques to clinical use will surely transform the treatment of a range of diseases and vaccination approaches to protect human being from infection diseases, and generate a huge impact. Despite the progress, future researches should focus on: (1) optimizing the design and manufacturing of physical devices and chemical systems for gene delivery; (2) exploring the mechanisms of gene delivery via skin and gene expression

enhancement of the technologies; and (3) investigating the long term safety profiles of different techniques.

References

[1] M. Zakrewsky, S. Kumar, S. Mitragotri, Nucleic acid delivery into skin for the treatment of skin disease: Proofs-of-concept, potential impact, and remaining challenges, J. Control. Release, 219 (2015) 445-456.

[2] C.V. Pecot, G.A. Calin, R.L. Coleman, G. Lopez-Berestein, A.K. Sood, RNA interference in the clinic: challenges and future directions, Nat. Rev. Cancer, 11 (2011) 59-67.

[3] M.R. Prausnitz, R. Langer, Transdermal drug delivery, Nat. Biotechnol., 26 (2008) 1261-1268.

[4] W.Y. Tai, X.H. Gao, Functional peptides for siRNA delivery, Adv. Drug Deliv. Rev., 110 (2017) 157-168.

[5] D. Landesman-Milo, S. Ramishetti, D. Peer, Nanomedicine as an emerging platform for metastatic lung cancer therapy, Cancer Metastasis Rev., 34 (2015) 291-301.

[6] C.A. Hong, Y.S. Nam, Functional nanostructures for effective delivery of small interfering RNA therapeutics, Theranostics, 4 (2014) 1211-1232.

[7] S. Mitragotri, Immunization without needles, Nat. Rev. Immunol., 5 (2005) 905-916.

[8] S.E. Cross, M.S. Roberts, Physical enhancement of transdermal drug application: Is delivery technology keeping up with pharmaceutical development?, Curr. Drug Deliv., 1 (2004) 81-92.

[9] J. Goldstein, T.R. Smith, N. Pugach, J. Griesser, T. Sebree, M. Pierce, A sumatriptan iontophoretic transdermal system for the acute treatment of migraine, Headache, 52 (2012) 1402-1410.

[10] Y.C. Kim, J.H. Park, M.R. Prausnitz, Microneedles for drug and vaccine delivery, Adv. Drug Deliv. Rev., 64 (2012) 1547-1568.

[11] P. Volz, A. Boreham, A. Wolf, T.Y. Kim, J. Balke, J. Frombach, S. Hadam, Z. Afraz, F. Rancan, U. Blume-Peytavi, A. Vogt, U. Alexiev, Application of single molecule fluorescence microscopy to characterize the penetration of a large amphiphilic molecule in the stratum corneum of human skin, Int. J. Mol. Sci., 16 (2015) 6960-6977.

[12] S. Jain, N. Patel, M.K. Shah, P. Khatri, N. Vora, Recent advances in lipid-based vesicles and particulate carriers for topical and transdermal application, J. Pharm. Sci., 106 (2017) 423-445.

[13] G.K. Menon, G.W. Cleary, M.E. Lane, The structure and function of the stratum corneum, Int. J. Pharm., 435 (2012) 3-9.

[14] M.R. Prausnitz, P.M. Elias, T.J. Franz, M. Schmuth, J.C. Tsai, G.K. Menon, W.M. Holleran, K.R. Feingold, Skin barrier and transdermal drug delivery, Dermatology, 3 (2012) 2065-2073.

[15] F. Erdo, N. Hashimoto, G. Karvaly, N. Nakamichi, Y. Kato, Critical evaluation and methodological positioning of the transdermal microdialysis technique. A review, J. Control. Release, 233 (2016) 147-161.

[16] R.J. Scheuplein, Mechanism of percutaneous absorption: II. Transient diffusion and relative importance o various routes of skin penetration, J. Invest. Dermatol., 48 (1967) 79-88.

[17] G. Szabo, The number of eccrine sweat glands in human skin, in: W. Montagna, R.A. Ellis, A.F. Silver (Eds.) Advances in biology of the skin, New York: Pergamon Press1962, pp. 1-5.

[18] J.D. Bos, M. Meinardi, The 500 Dalton rule for the skin penetration of chemical compounds and drugs, Experimental Dermatology, 9 (2000) 165-169.

[19] M. Furuse, M. Hata, K. Furuse, Y. Yoshida, A. Haratake, Y. Sugitani, T. Noda, A. Kubo, S. Tsukita, Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice, J. Cell Biol., 156 (2002) 1099-1111.

[20] E.H. Teo, K.J. Cross, E.D. Bomsztyk, D.C. Lyden, J.A. Spector, Gene therapy in skin choosing the optimal viral vector, Ann. Plast. Surg., 62 (2009) 576-580.

[21] E. Gorell, N. Nguyen, A. Lane, Z. Siprashvili, Gene therapy for skin diseases, Cold Spring Harb. Perspect. Med., 4 (2014) a015149.

[22] M. Braun-Falco, A. Doenecke, H. Smola, M. Hallek, Efficient gene transfer into human keratinocytes with recombinant adeno-associated virus vectors, Gene Ther., 6 (1999) 432-441.

[23] B. Geusens, T. Strobbe, S. Bracke, P. Dynoodt, N. Sanders, M. Van Gele, J. Lambert, Lipid-mediated gene delivery to the skin, Eur. J. Pharm. Sci., 43 (2011) 199-211.

[24] D. Almarza, G. Bussadori, M. Navarro, F. Mavilio, F. Larcher, R. Murillas, Risk assessment in skin gene therapy: viral-cellular fusion transcripts generated by proviral transcriptional read-through in keratinocytes transduced with self-inactivating lentiviral vectors, Gene Ther., 18 (2011) 674-681.

[25] P.A. Wender, D.J. Mitchell, K. Pattabiraman, E.T. Pelkey, L. Steinman, J.B. Rothbard, The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters, Proc. Natl. Acad. Sci. U. S. A., 97 (2000) 13003-13008.

[26] M. Pooga, C. Kut, M. Kihlmark, M. Hallbrink, S. Fernaeus, R. Raid, T. Land, E. Hallberg, T. Bartfai, U. Langel, Cellular translocation of proteins by transportan, Faseb J., 15 (2001) 1451-1453.

[27] M.C. Morris, J. Depollier, J. Mery, F. Heitz, G. Divita, A peptide carrier for the delivery of biologically active proteins into mammalian cells, Nat. Biotechnol., 19 (2001) 1173-1176.

[28] S.A. Nasrollahi, C. Taghibiglou, E. Azizi, E.S. Farboud, Cell-penetrating peptides as a novel transdermal drug delivery system, Chem. Biol. Drug Des., 80 (2012) 639-646.

[29] A. Bolhassani, Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery in cancer, Biochim. Biophys. Acta-Rev. Cancer, 1816 (2011) 232-246.

[30] Y.P. Chen, Y.Y. Shen, X. Guo, C.S. Zhang, W.J. Yang, M.L. Ma, S. Liu, M.B. Zhang, L.P. Wen, Transdermal protein delivery by a coadministered peptide identified via phage display, Nat. Biotechnol., 24 (2006) 455-460.

[31] C.M. Lin, K. Huang, Y. Zeng, X.C. Chen, S. Wang, Y. Li, A simple, noninvasive and efficient method for transdermal delivery of siRNA, Arch. Dermatol. Res., 304 (2012) 139-144.

[32] T. Hsu, S. Mitragotri, Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer, Proc. Natl. Acad. Sci. U. S. A., 108 (2011) 15816-15821.

[33] M. Chen, M. Zakrewsky, V. Gupta, A.C. Anselmo, D.H. Slee, J.A. Muraski, S. Mitragotri, Topical delivery of siRNA into skin using SPACE-peptide carriers, J. Control. Release, 179 (2014) 33-41.

[34] M. Vij, P. Natarajan, B.R. Pattnaik, S. Alam, N. Gupta, D. Santhiya, R. Sharma, A. Singh, K.M. Ansari, R.S. Gokhale, V.T. Natarajan, M. Ganguli, Non-invasive topical delivery of plasmid DNA to the skin using a peptide carrier, J. Control. Release, 222 (2016) 159-168.

[35] Y.X. Yang, Y.F. Jiang, Z. Wang, J.H. Liu, L. Yan, J.X. Ye, Y.Z. Huang, Skin-permeable quaternary nanoparticles with layer-by-layer structure enabling improved gene delivery, J. Mater. Chem., 22 (2012) 10029-10034.

[36] X. Yi, G. Zhao, H. Zhang, D. Guan, R. Meng, Y. Zhang, Q. Yang, H. Jia, K. Dou, C. Liu, F. Que, J.Q. Yin, MITF-siRNA formulation is a safe and effective therapy for human melasma, Mol. Ther., 19 (2011) 362-371.

[37] T. Uchida, T. Kanazawa, M. Kawai, Y. Takashima, H. Okada, Therapeutic effects on atopic dermatitis by anti-RelA short interfering RNA combined with functional peptides Tat and AT1002, J. Pharmacol. Exp. Ther., 338 (2011) 443-450.

[38] T. Uchida, T. Kanazawa, Y. Takashima, H. Okada, Development of an efficient transdermal delivery system of small interfering RNA using functional peptides, Tat and AT-1002, Chem. Pharm. Bull., 59 (2011) 196-201.

[39] Y.Z. Huang, F.Q. Yu, Y.S. Park, J.X. Wang, M.C. Shin, H.S. Chung, V.C. Yang, Co-administration of protein drugs with gold nanoparticles to enable percutaneous delivery, Biomaterials, 31 (2010) 9086-9091.

[40] X. Wu, K. Landfester, A. Musyanovych, R.H. Guy, Disposition of charged nanoparticles after their topical application to the skin, Skin Pharmacol. Physiol., 23 (2010) 117-123.

[41] H. Farhood, N. Serbina, L. Huang, The role of dioleoyl phosphatidylethanolamine in cationic liposome-mediated transfer, Biochim. Biophys. Acta-Biomembranes, 1235 (1995) 289-295.

[42] J.Y. Cheng, H.N. Huang, W.C. Tseng, T.L. Li, Y.L. Chan, K.C. Cheng, C.J. Wu, Transcutaneous immunization by lipoplex-patch based DNA vaccines is effective vaccination against Japanese encephalitis virus infection, J. Control. Release, 135 (2009) 242-249.

[43] S.E. Jin, C.K. Kim, Charge-mediated topical delivery of plasmid DNA with cationic lipid nanoparticles to the skin, Colloid Surf. B-Biointerfaces, 116 (2014) 582-590.

[44] S. Sun, M. Wang, K.A. Alberti, A. Choy, Q.B. Xu, DOPE facilitates quaternized lipidoids (QLDs) for in vitro DNA delivery, Nanomed.-Nanotechnol. Biol. Med., 9 (2013) 849-854.

[45] R.M. Hathout, S. Mansour, N.D. Mortada, A.S. Geneidi, R.H. Guy, Uptake of microemulsion components into the stratum corneum and their molecular effects on skin barrier function, Mol. Pharm., 7 (2010) 1266-1273.

[46] S.J. Lim, C.K. Kim, Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid, Int. J. Pharm., 243 (2002) 135-146.

[47] A. Kumar, K. Pathak, V. Bali, Ultra-adaptable nanovesicular systems: a carrier for systemic delivery of therapeutic agents, Drug Discov. Today, 17 (2012) 1233-1241.

[48] F. Yin, S. Guo, Y. Gan, X. Zhang, Preparation of redispersible liposomal dry powder using an ultrasonic spray freeze-drying technique for transdermal delivery of human epithelial growth factor, Int. J. Nanomed., 9 (2014) 1665-1675.

[49] B. Geusens, M. Van Gele, S. Braat, S.C. De Smedt, M.C.A. Stuart, T.W. Prow, W. Sanchez, M.S. Roberts, N.N. Sanders, J. Lambert, Flexible nanosomes (SECosomes) enable efficient siRNA delivery in cultured primary skin cells and in the viable epidermis of ex vivo human skin, Adv. Funct. Mater., 20 (2010) 4077-4090.

[50] E. Desmet, S. Bracke, K. Forier, L. Taevernier, M.C.A. Stuart, B. De Spiegeleer, K. Raemdonck, M. Van Gele, J. Lambert, An elastic liposomal formulation for RNAi-based topical treatment of skin disorders: Proof-of-concept in the treatment of psoriasis, Int. J. Pharm., 500 (2016) 268-274.

[51] M. Dorrani, O.B. Garbuzenko, T. Minko, B. Michniak-Kohn, Development of edge-activated liposomes for siRNA delivery to human basal epidermis for melanoma therapy, J. Control. Release, 228 (2016) 150-158.

[52] M. Ashtikar, K. Nagarsekar, A. Fahr, Transdermal delivery from liposomal formulations - Evolution of the technology over the last three decades, J. Control. Release, 242 (2016) 126-140.

[53] T. Rattanapak, K. Young, T. Rades, S. Hook, Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: characterisation and in vitro skin penetration, J. Pharm. Pharmacol., 64 (2012) 1560-1569.

[54] C.H.A. Boakye, K. Patel, R. Doddapaneni, A. Bagde, S. Marepally, M. Singh, Novel amphiphilic lipid augments the co-delivery of erlotinib and IL36 siRNA into the skin for psoriasis treatment, J. Control. Release, 246 (2017) 120-132.

[55] P.S. Rabbani, A. Zhou, Z.M. Borab, J.A. Frezzo, N. Srivastava, H.T. More, W.J. Rifkin, J.A. David, S.J. Berens, R. Chen, S. Hameedi, M.H. Junejo, C. Kim, R.A. Sartor, C.F. Liu, P.B. Saadeh, J.K. Montclare, D.J. Ceradini, Novel lipoproteoplex delivers Keap1 siRNA based gene therapy to accelerate diabetic wound healing, Biomaterials, 132 (2017) 1-15.

[56] P.R. Desai, S. Marepally, A.R. Patel, C. Voshavar, A. Chaudhuri, M. Singh, Topical delivery of anti-TNF alpha siRNA and capsaicin via novel lipid-polymer hybrid nanoparticles efficiently inhibits skin inflammation in vivo, J. Control. Release, 170 (2013) 51-63.

[57] Y.P. Fang, Topical delivery of DNA oligonucleotide to induce p53 generation in the skin via thymidine dinucleotide (pTT)-encapsulated liposomal carrier, Int. J. Nanomed., 6 (2011) 3373-3381.

[58] Y. Ding, Z.W. Jiang, K. Saha, C.S. Kim, S.T. Kim, R.F. Landis, V.M. Rotello, Gold nanoparticles for nucleic acid delivery, Mol. Ther., 22 (2014) 1075-1083.

[59] E.P. Thi, C.E. Mire, A.C.H. Lee, J.B. Geisbert, J.Z. Zhou, K.N. Agans, N.M. Snead, D.J. Deer, T.R. Barnard, K.A. Fenton, I. MacLachlan, T.W. Geisbert, Lipid nanoparticle siRNA treatment of Ebola-virus-Makona-infected nonhuman primates, Nature, 521 (2015) 362-365.

[60] K. Ladewig, M. Niebert, Z.P. Xu, P.P. Gray, G.Q.M. Lu, Efficient siRNA delivery to mammalian cells using layered double hydroxide nanoparticles, Biomaterials, 31 (2010) 1821-1829.

[61] Y. Wong, K. Markham, Z.P. Xu, M. Chen, G.Q. Lu, P.F. Bartlett, H.M. Cooper, Efficient delivery of siRNA to cortical neurons using layered double hydroxide nanoparticles, Biomaterials, 31 (2010) 8770-8779.

[62] N. Mitter, E.A. Worrall, K.E. Robinson, P. Li, R.G. Jain, C. Taochy, S.J. Fletcher, B.J. Carroll, G.Q. Lu, Z.P. Xu, Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses, Nat. Plants, 3 (2017) article no.: 16207.

[63] K.S. Siu, D. Chen, X.F. Zheng, X.S. Zhang, N. Johnston, Y.L. Liu, K. Yuan, J. Koropatnick, E.R. Gillies, W.P. Min, Non-covalently functionalized single-walled carbon nanotube for topical siRNA delivery into melanoma, Biomaterials, 35 (2014) 3435-3442.

[64] J. Niu, Y. Chu, Y.F. Huang, Y.S. Chong, Z.H. Jiang, Z.W. Mao, L.H. Peng, J.Q. Gao, Transdermal gene delivery by functional peptide-conjugated cationic gold nanoparticle reverses the progression and metastasis of cutaneous melanoma, ACS Appl. Mater. Interfaces, 9 (2017) 9388-9401.

[65] P.S. Randeria, M.A. Seeger, X.-Q. Wang, H. Wilson, D. Shipp, C.A. Mirkin, A.S. Paller, siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) 5573-5578.

[66] D. Zheng, D.A. Giljohann, D.L. Chen, M.D. Massich, X.Q. Wang, H. Iordanov, C.A. Mirkin, A.S. Paller, Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation, Proc. Natl. Acad. Sci. U. S. A., 109 (2012) 11975-11980.

[67] K.A. Dawson, A. Salvati, I. Lynch, Nanoparticles reconstruct lipids, Nature Nanotechnology, 4 (2009) 84-85.

[68] J.L. Vargas Luna, M. Krenn, J.A.C. Ramirez, W. Mayr, Dynamic Impedance Model of the Skin-Electrode Interface for Transcutaneous Electrical Stimulation, PLoS One, 10 (2015) e012560.

[69] F. Lin, X.F. Shen, J.R. McCoy, J.M. Mendoza, J. Yan, S.V. Kemmerrer, A.S. Khan, D.B. Weiner, K.E. Broderick, N.Y. Sardesai, A novel prototype device for electroporation-enhanced DNA vaccine delivery simultaneously to both skin and muscle, Vaccine, 29 (2011) 6771-6780.

[70] J. Glasspool-Malone, S. Somiari, J.J. Drabick, R.W. Malone, Efficient nonviral cutaneous transfection, Mol. Ther., 2 (2000) 140-146.

[71] A.R. Denet, R. Vanbever, V. Preat, Skin electroporation for transdermal and topical delivery, Adv. Drug Deliv. Rev., 56 (2004) 659-674.

[72] K. Ita, Perspectives on Transdermal Electroporation, Pharmaceutics, 8 (2016) Article No. 9.

[73] V. Preat, M.R. Prausnitz, Enhanced drug delivery using high-voltage pulses, Adv. Drug Deliv. Rev., 35 (1999) 1-2.

[74] M.R. Prausnitz, V.G. Bose, R. Langer, J.C. Weaver, Electroporation of mammalian skin - A mechanism to enhance transdermal drug delivery, Proc. Natl. Acad. Sci. U. S. A., 90 (1993) 10504-10508.

[75] K. Schultheis, H. Schaefer, B.S. Yung, J. Oh, K. Muthumani, L. Humeau, K.E. Broderick, T.R.F. Smith, Characterization of guinea pig T cell responses elicited after EP-assisted delivery of DNA vaccines to the skin, Vaccine, 35 (2017) 61-70.

[76] S. Kos, K. Vanvarenberg, T. Dolinsek, M. Cemazar, J. Jelenc, V. Preat, G. Sersa, G. Vandermeulen, Gene electrotransfer into skin using noninvasive multi-electrode array for vaccination and wound healing, Bioelectrochemistry, 114 (2017) 33-41.

[77] S. Kos, T. Blagus, M. Cemazar, U.L. Tratar, M. Stimac, L. Prosen, T. Dolinsek, U. Kamensek, S. Kranjc, L. Steinstraesser, G. Vandermeulen, V. Preat, G. Sersa, Electrotransfer parameters as a tool for controlled and targeted gene expression in skin, Mol. Ther. Nucleic. Acids, 5 (2016) article no. e356.

[78] S. Kos, N. Tesic, U. Kamensek, T. Blagus, M. Cemazar, S. Kranjc, J. Lavrencak, G. Sersa, Improved specificity of gene electrotransfer to skin using pDNA under the control of collagen tissue-specific promoter, J. Membr. Biol., 248 (2015) 919-928.

[79] G. Vandermeulen, K. Vanvarenberg, A. De Beuckelaer, S. De Koker, L. Lambricht, C. Uyttenhove, A. Reschner, A. Vanderplasschen, J. Grooten, V. Preat, The site of administration influences both the type and the magnitude of the immune response induced by DNA vaccine electroporation, Vaccine, 33 (2015) 3179-3185.

[80] A. Lamolinara, L. Stramucci, A. Hysi, M. Iezzi, C. Marchini, M. Mariotti, A. Amici, C. Curcio, Intradermal DNA electroporation induces cellular and humoral immune response and confers protection against HER2/neu tumor, J Immunol. Res., (2015) article no. 159145.

[81] J.R. McCoy, J.M. Mendoza, K.W. Spik, C. Badger, A.F. Gomez, C.S. Schmaljohn, N.Y. Sardesai, K.E. Broderick, A multi-head intradermal electroporation device allows for tailored and increased dose DNA vaccine delivery to the skin, Human Vaccines Immunother., 11 (2015) 746-754.

[82] Z.W. Wei, S.Q. Zheng, R.X. Wang, X.L. Bu, H.L. Ma, Y.D. Wu, L. Zhu, Z.Y. Hu, Z.C. Liang, Z.H. Li, A flexible microneedle array as low-voltage electroporation electrodes for in vivo DNA and siRNA delivery, Lab Chip, 14 (2014) 4093-4102.

[83] L. Steinstraesser, M.C. Lam, F. Jacobsen, P.E. Porporato, K.K. Chereddy, M. Becerikli, I. Stricker, R.E.W. Hancock, M. Lehnhardt, P. Sonveaux, V. Preat, G. Vandermeulen, Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide promotes wound healing, Mol. Ther., 22 (2014) 734-742.

[84] G. Basu, H. Downey, S.Q. Guo, A. Israel, A. Asmar, B. Hargrave, R. Heller, Prevention of distal flap necrosis in a rat random skin flap model by gene electrotransfer delivering VEGF(165) plasmid, J. Gene. Med., 16 (2014) 55-65.

[85] F. Eriksson, T. Totterman, A.K. Maltais, P. Pisa, J. Yachnin, DNA vaccine coding for the rhesus prostate specific antigen delivered by intradermal electroporation in patients with relapsed prostate cancer, Vaccine, 31 (2013) 3843-3848.

[86] D.X. Johansson, K. Ljungberg, M. Kakoulidou, P. Liljestrom, Intradermal electroporation of naked replicon RNA elicits strong immune responses, PLoS One, 7 (2012) artice no. e29732.

[87] R. Heller, Y. Cruz, L.C. Heller, R.A. Gilbert, M.J. Jaroszeski, Electrically mediated delivery of plasmid DNA to the skin, using a multielectrode array, Hum. Gene Ther., 21 (2010) 357-362.

[88] S.Q. Guo, A.L. Israel, G. Basu, A. Donate, R. Heller, Topical gene electrotransfer to the epidermis of hairless guinea pig by non-invasive multielectrode array, PLoS One, 8 (2013) article no. e73423.

[89] V. Todorovic, U. Kamensek, G. Sersa, M. Cemazar, Changing electrode orientation, but not pulse polarity, increases the efficacy of gene electrotransfer to tumors in vivo, Bioelectrochemistry, 100 (2014) 119-127.

[90] A. Donate, R. Heller, Assessment of delivery parameters with the multi-electrode array for development of a DNA vaccine against Bacillus anthracis, Bioelectrochemistry, 94 (2013) 1-6.

[91] B. Zorec, S. Becker, M. Rebersek, D. Miklavcic, N. Pavselj, Skin electroporation for transdermal drug delivery: The influence of the order of different square wave electric pulses, Int. J. Pharm., 457 (2013) 214-223.

[92] A. Gothelf, F. Mahmood, F. Dagnaes-Hansen, J. Gehl, Efficacy of transgene expression in porcine skin as a function of electrode choice, Bioelectrochemistry, 82 (2011) 95-102.

[93] B. Ferraro, L.C. Heller, Y.L. Cruz, S. Guo, A. Donate, R. Heller, Evaluation of delivery conditions for cutaneous plasmid electrotransfer using a multielectrode array, Gene Ther., 18 (2011) 496-500.

[94] L. Daugimont, N. Baron, G. Vandermeulen, N. Pavselj, D. Miklavcic, M.-C. Jullien, G. Cabodevila, L.M. Mir, V. Preat, Hollow microneedle arrays for intradermal drug delivery and DNA electroporation, J. Membr. Biol., 236 (2010) 117-125.

[95] A.K. Roos, S. Moreno, C. Leder, M. Pavlenko, A. King, P. Pisa, Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation, Mol. Ther., 13 (2006) 320-327.

[96] A.K. Roos, F. Eriksson, D.C. Walters, P. Pisa, A.D. King, Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation, Mol. Ther., 17 (2009) 1637-1642.

[97] L. Yan, A.P. Raphael, X.Y. Zhu, B.L. Wang, W. Chen, T. Tang, Y. Deng, H.J. Sant, G.Y. Zhu, K.W. Choy, B.K. Gale, T.W. Prow, X.F. Chen, Nanocomposite-Strengthened Dissolving

Microneedles for Improved Transdermal Delivery to Human Skin, Adv. Healthc. Mater., 3 (2014) 555-564.

[98] L. Yan, Y. Yang, W.J. Zhang, X.F. Chen, Advanced materials and nanotechnology for drug delivery, Adv. Mater., 26 (2014) 5533-5540.

[99] X.F. Chen, T.W. Prow, M.L. Crichton, D.W.K. Jenkins, M.S. Roberts, I.H. Frazer, G.J.P. Fernando, M.A.F. Kendall, Dry-coated microprojection array patches for targeted delivery of immunotherapeutics to the skin, J. Control. Release, 139 (2009) 212-220.

[100] T.M. Tuan-Mahmood, M.T.C. McCrudden, B.M. Torrisi, E. McAlister, M.J. Garland, T.R.R. Singh, R.F. Donnelly, Microneedles for intradermal and transdermal drug delivery, Eur. J. Pharm. Sci., 50 (2013) 623-637.

[101] M.J. Garland, K. Migalska, T. Mazlelaa, T. Mahmood, T. Raghu, R. Singh, A.D. Woolfson, R.F. Donnelly, Microneedle arrays as medical devices for enhanced transdermal drug delivery, Expert Rev. Med. Devices, 8 (2011) 459-482.

[102] H. Lee, T.K. Choi, Y.B. Lee, H.R. Cho, R. Ghaffari, L. Wang, H.J. Choi, T.D. Chung, N. Lu, T. Hyeon, S.H. Choi, D.-H. Kim, A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy, Nat. Nanotechnol., 11 (2016) 566-572.

[103] J. Yu, Y. Zhang, A.R. Kahkoska, Z. Gu, Bioresponsive transcutaneous patches, Curr. Opin. Biotechnol., 48 (2017) 28-32.

[104] J. Yu, C. Qian, Y. Zhang, Z. Cui, Y. Zhu, Q. Shen, F.S. Ligler, J.B. Buse, Z. Gu, Hypoxia and H2O2 dual-sensitive vesicles for enhanced glucose-responsive insulin delivery, Nano Lett., 17 (2017) 733-739.

[105] Y. Zhang, J. Yu, J. Wang, N.J. Hanne, Z. Cui, C. Qian, C. Wang, H. Xin, J.H. Cole, C.M. Gallippi, Y. Zhu, Z. Gu, Thrombin-responsive transcutaneous patch for auto-anticoagulant regulation, Adv. Mater., 29 (2017) 1604043.

[106] J. Yu, Y. Zhang, Y. Ye, R. DiSanto, W. Sun, D. Ranson, F.S. Ligler, J.B. Buse, Z. Gu, Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) 8260-8265.

[107] Y. Ye, J. Yu, C. Wang, N.-Y. Nguyen, G.M. Walker, J.B. Buse, Z. Gu, Microneedles integrated with pancreatic cells and synthetic glucose-signal amplifiers for smart insulin delivery, Adv. Mater., 28 (2016) 3115-3121.

[108] C. Wong, M. Jiang, J.H.S. You, Potential Cost-Effectiveness of an Influenza Vaccination Program Offering Microneedle Patch for Vaccine Delivery in Children, PLoS One, 11 (2016).

[109] B.B. Adhikari, J.L. Goodson, S.Y. Chu, P.A. Rota, M.I. Meltzer, Assessing the Potential Cost-Effectiveness of Microneedle Patches in Childhood Measles Vaccination Programs: The Case for Further Research and Development, Drugs in R&D, 16 (2016) 327-338.

[110] N.G. Rouphael, M. Paine, R. Mosley, S. Henry, D.V. McAllister, H. Kalluri, W. Pewin, P.M. Frew, T.W. Yu, N.J. Thornburg, S. Kabbani, L.L. Lai, E.V. Vassilieva, I. Skountzou, R.W. Compans, M.J. Mulligan, M.R. Prausnitz, T.M.S. Grp, The safety, immunogenicity, and acceptability of inactivated influenza vaccine delivered by microneedle patch (TIV-MNP 2015): a randomised, partly blinded, placebo-controlled, phase 1 trial, Lancet, 390 (2017) 649-658.

[111] X. Chen, G.J.P. Fernando, M.L. Crichton, C. Flaim, S.R. Yukiko, E.J. Fairmaid, H.J. Corbett, C.A. Primiero, A.B. Ansaldo, I.H. Frazer, L.E. Brown, M.A.F. Kendall, Improving the reach of vaccines to low-resource regions, with a needle-free vaccine delivery device and long-term thermostabilization, J. Control. Release, 152 (2011) 349-355.

[112] S. Bhatnagar, K. Dave, V.V.K. Venuganti, Microneedles in the clinic, J. Control. Release, 260 (2017) 164-182.

[113] J.M. Arya, K. Dewitt, M. Scott-Garrard, Y.-W. Chiang, M.R. Prausnitz, Rabies vaccination in dogs using a dissolving microneedle patch, J. Control. Release, 239 (2016) 19-26.

[114] G.J.P. Fernando, J. Zhang, H.I. Ng, O.L. Haigh, S.R. Yukiko, M.A.F. Kendall, Influenza nucleoprotein DNA vaccination by a skin targeted, dry coated, densely packed microprojection array (Nanopatch) induces potent antibody and CD8(+) T cell responses, J. Control. Release, 237 (2016) 35-41.

[115] R.C. Kines, V. Zarnitsyn, T.R. Johnson, Y.Y.S. Pang, K.S. Corbett, J.D. Nicewonger, A. Gangopadhyay, M. Chen, J. Liu, M.R. Prausnitz, J.T. Schiller, B.S. Graham, Vaccination with human papillomavirus pseudovirus-encapsidated plasmids targeted to skin using microneedles, PLoS One, 10 (2015) article no. e0120797.

[116] Y.C. Kim, D.G. Yoo, R.W. Compans, S.M. Kang, M.R. Prausnitz, Cross-protection by co-immunization with influenza hemagglutinin DNA and inactivated virus vaccine using coated microneedles, J. Control. Release, 172 (2013) 579-588.

[117] P.C. DeMuth, Y.J. Min, B. Huang, J.A. Kramer, A.D. Miller, D.H. Barouch, P.T. Hammond, D.J. Irvine, Polymer multilayer tattooing for enhanced DNA vaccination, Nat. Mater., 12 (2013) 367-376.

[118] J.M. Song, Y.C. Kim, E.J. O, R.W. Compans, M.R. Prausnitz, S.M. Kang, DNA vaccination in the skin using microneedles improves protection against influenza, Mol. Ther., 20 (2012) 1472-1480.

[119] M. Pearton, V. Saller, S.A. Coulman, C. Gateley, A.V. Anstey, V. Zarnitsyn, J.C. Birchall, Microneedle delivery of plasmid DNA to living human skin: Formulation coating, skin insertion and gene expression, J. Control. Release, 160 (2012) 561-569.

[120] Y.C. Kim, J.M. Song, A.S. Lipatov, S.O. Choi, J.W. Lee, R.O. Donis, R.W. Compans, S.M. Kang, M.R. Prausnitz, Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch, Eur. J. Pharm. Biopharm., 81 (2012) 239-247.

[121] H.S. Gill, J. Soderholm, M.R. Prausnitz, M. Sallberg, Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine, Gene Ther., 17 (2010) 811-814.

[122] X.F. Chen, A.S. Kask, M.L. Crichton, C. McNeilly, S. Yukiko, L.C. Dong, J.O. Marshak, C. Jarrahian, G.J.P. Fernando, D.X. Chen, D.M. Koelle, M.A.F. Kendall, Improved DNA vaccination by skin-targeted delivery using dry-coated densely-packed microprojection arrays, J. Control. Release, 148 (2010) 327-333.

[123] A.S. Kask, X.F. Chen, J.O. Marshak, L.C. Dong, M. Saracino, D. Chen, C. Jarrahian, M.A. Kendall, D.M. Koelle, DNA vaccine delivery by densely-packed and short microprojection arrays to skin protects against vaginal HSV-2 challenge, Vaccine, 28 (2010) 7483-7491.

[124] C. Chiappini, E. De Rosa, J.O. Martinez, X. Liu, J. Steele, M.M. Stevens, E. Tasciotti, Biodegradable silicon nanoneedles delivering nucleic acids intracellularly induce localized in vivo neovascularization, Nat. Mater., 14 (2015) 532-539.

[125] C. Coban, K. Kobiyama, T. Aoshi, F. Takeshita, T. Horii, S. Akira, K.J. Ishii, Novel strategies to improve DNA vaccine immunogenicity, Curr. Gene Ther., 11 (2011) 479-484.

[126] S. Lu, Immunogenicity of DNA vaccines in humans It takes two to tango, Hum. Vaccines, 4 (2008) 449-452.

[127] M.L. Crichton, A. Ansaldo, X.F. Chen, T.W. Prow, G.J.P. Fernando, M.A.F. Kendall, The effect of strain rate on the precision of penetration of short densely-packed microprojection array patches coated with vaccine, Biomaterials, 31 (2010) 4562-4572.

[128] M.L. Crichton, B.C. Donose, X.F. Chen, A.P. Raphael, H. Huang, M.A.F. Kendall, The viscoelastic, hyperelastic and scale dependent behaviour of freshly excised individual skin layers, Biomaterials, 32 (2011) 4670-4681.

[129] X.F. Chen, H.J. Corbett, S.R. Yukiko, A.P. Raphael, E.J. Fairmaid, T.W. Prow, L.E. Brown, G.J.P. Fernando, M.A.F. Kendall, Site-selectively coated, densely-packed microprojection array patches for targeted delivery of vaccines to skin, Adv. Funct. Mater., 21 (2011) 464-473.

[130] K.Y. Park, W.S. Jang, Y.Y. Lim, J.H. Ahn, S.J. Lee, C.W. Kim, S.E. Kim, B.J. Kim, M.N. Kim, Safety evaluation of stamp type digital microneedle devices in hairless mice, Ann. Dermatol., 25 (2013) 46-53.

[131] G. Yan, N. Arelly, N. Farhan, S. Lobo, H.N. Li, Enhancing DNA delivery into the skin with a motorized microneedle device, Eur. J. Pharm. Sci., 52 (2014) 215-222.

[132] J. Wise, Microneedle patch for flu vaccination proves successful in human clinical trial, BMJ-Br. Med. J., 357 (2017) j3120.

[133] S. Marshall, L.J. Sahm, A.C. Moore, The success of microneedle-mediated vaccine delivery into skin, Human Vaccines Immunother., 12 (2016) 2975-2983.

[134] J.J. Norman, J.M. Arya, M.A. McClain, P.M. Frew, M.I. Meltzer, M.R. Prausnitz, Microneedle patches: Usability and acceptability for self-vaccination against influenza, Vaccine, 32 (2014) 1856-1862.

[135] S.W. Han, C. Nakamura, I. Obataya, N. Nakamura, J. Miyake, Gene expression using an ultrathin needle enabling accurate displacement and low invasiveness, Biochem. Biophys. Res. Commun., 332 (2005) 633-639.

[136] X. Zhu, M.F. Yuen, L. Yan, Z. Zhang, F. Ai, Y. Yang, P.K.N. Yu, G. Zhu, W. Zhang, X. Chen, Diamond-Nanoneedle-Array-Facilitated Intracellular Delivery and the Potential Influence on Cell Physiology, Adv. Healthc. Mater., 5 (2016) 1157-1168.

[137] X. Zhu, S.Y. Kwok, M.F. Yuen, L. Yan, W. Chen, Y. Yang, Z. Wang, K.N. Yu, G. Zhu, W. Zhang, X. Chen, Dense diamond nanoneedle arrays for enhanced intracellular delivery of drug molecules to cell lines, J. Mater. Sci., 50 (2015) 7800-7807.

[138] Y. Yang, M.-F. Yuen, X. Chen, S. Xu, Y. Tang, W. Zhang, Fabrication of arrays of high-aspect-ratio diamond nanoneedles via maskless ECR-assisted microwave plasma etching, Crystengcomm, 17 (2015) 2791-2800.

[139] L. Yan, J. Zhang, C.-S. Lee, X. Chen, Micro- and nanotechnologies for intracellular delivery, Small, 10 (2014) 4487-4504.

[140] Y. Wang, Y. Yang, L. Yan, S.Y. Kwok, W. Li, Z. Wang, X. Zhu, G. Zhu, W. Zhang, X. Chen, P. Shi, Poking cells for efficient vector-free intracellular delivery, Nat. Commun., 5 (2014) article no. 4466.

[141] X. Chen, G. Zhu, Y. Yang, B. Wang, L. Yan, K.Y. Zhang, K.K.-W. Lo, W. Zhang, A diamond nanoneedle array for potential high-throughput intracellular delivery, Adv. Healthc. Mater., 2 (2013) 1103-1107.

[142] J. Guo, B. Wen, R. Melnik, S. Yao, T. Li, Molecular dynamics study on diamond nanowires mechanical properties: Strain rate, temperature and size dependent effects, Diam. Relat. Mater., 20 (2011) 551-555.

[143] X. Chen, W. Zhang, Diamond nanostructures for drug delivery, bioimaging, and biosensing, Chem. Soc. Rev., 46 (2017) 734-760.

[144] O. Shenderova, D. Brenner, R.S. Ruoff, Would diamond nanorods be stronger than fullerene nanotubes?, Nano Lett., 3 (2003) 805-809.

[145] M. Aldawsari, M.B. Chougule, R.J. Babu, Progress in Topical siRNA Delivery Approaches for Skin Disorders, Curr. Pharm. Des., 21 (2015) 4594-4605.

[146] S. Lakshmanan, G.K. Gupta, P. Avci, R. Chandran, M. Sadasivam, A.E.S. Jorge, M.R. Hamblin, Physical energy for drug delivery; poration, concentration and activation, Adv. Drug Deliv. Rev., 71 (2014) 98-114.

[147] K. Kigasawa, K. Kajimoto, S. Hama, A. Saito, K. Kanamura, K. Kogure, Noninvasive delivery of siRNA into the epidermis by iontophoresis using an atopic dermatitis-like model rat, Int. J. Pharm., 383 (2010) 157-160.

[148] K. Kigasawa, K. Kajimoto, T. Nakamura, S. Hama, K. Kanamura, H. Harashima, K. Kogure, Noninvasive and efficient transdermal delivery of CpG-oligodeoxynucleotide for cancer immunotherapy, J. Control. Release, 150 (2011) 256-265.

[149] Abu Hashim, II, K. Motoyama, A.E.H. Abd-ElGawad, M.H. El-Shabouri, T.M. Borg, H. Arima, Potential use of iontophoresis for transdermal delivery of NF-kappa B decoy oligonucleotides, Int. J. Pharm., 393 (2010) 127-134.

[150] R.W. Johnstone, Histone-deacetylase inhibitors: Novel drugs for the treatment of cancer, Nat. Rev. Drug Discov., 1 (2002) 287-299.

[151] A. Abdul-Wahab, W. Qasim, J.A. McGrath, Gene therapies for inherited skin disorders, Semin. Cutan. Med. Surg., 33 (2014) 83-90.

[152] F. Liu, L. Huang, Development of non-viral vectors for systemic gene delivery, J. Control. Release, 78 (2002) 259-266.

[153] M.B. Brown, G.P. Martin, S.A. Jones, F.K. Akomeah, Dermal and transdermal drug delivery systems: Current and future prospects, Drug Deliv., 13 (2006) 175-187.

[154] W.R. Heath, F.R. Carbone, The skin-resident and migratory immune system in steady state and memory: innate lymphocytes, dendritic cells and T cells, Nat. Immunol., 14 (2013) 978-985.

[155] U. Lungwitz, M. Breunig, T. Blunk, A. Gopferich, Polyethylenimine-based non-viral gene delivery systems, Eur. J. Pharm. Biopharm., 60 (2005) 247-266.

[156] X. Gao, L. Huang, Cationic liposome-mediated gene transfer, Gene Ther., 2 (1995) 710-722.

[157] E.R. Toke, O. Lorincz, Z. Csiszovszki, E. Somogyi, G. Felfoldi, L. Molnar, R. Szipocs, A. Kolonics, B. Malissen, F. Lori, J. Trocio, N. Bakare, F. Horkay, N. Romani, C.H. Tripp, P. Stoitzner, J. Lisziewicz, Exploitation of Langerhans cells for in vivo DNA vaccine delivery into the lymph nodes, Gene Ther., 21 (2014) 566-574.

[158] Y.C. Chen, S.R. Bathula, Q. Yang, L. Huang, Targeted nanoparticles deliver siRNA to melanoma, J. Invest. Dermatol., 130 (2010) 2790-2798.

[159] G. Lin, H. Zhang, L. Huang, Smart polymeric nanoparticles for cancer gene delivery, Mol. Pharm., 12 (2015) 314-321.

[160] Y. Wang, L. Miao, A. Satterlee, L. Huang, Delivery of oligonucleotides with lipid nanoparticles, Adv. Drug Deliv. Rev., 87 (2015) 68-80.

[161] T. Segura, M.J. Volk, L.D. Shea, Substrate-mediated DNA delivery: role of the cationic polymer structure and extent of modification, J. Control. Release, 93 (2003) 69-84.

[162] C. Angell, S. Xie, L. Zhang, Y. Chen, DNA nanotechnology for precise control over drug delivery and gene therapy, Small, 12 (2016) 1117-1132.

[163] J.C. Kaczmarek, A.K. Patel, K.J. Kauffman, O.S. Fenton, M.J. Webber, M.W. Heartlein, F. DeRosa, D.G. Anderson, Polymer-lipid nanoparticles for systemic delivery of mRNA to the lungs, Angew. Chem. Int. Ed., 55 (2016) 13808-13812.

[164] Q.F. Zhang, C.R. Luan, D.X. Yin, J. Zhang, Y.H. Liu, Q. Peng, Y. Xu, X.Q. Yu, Amino acid-modified polyethylenimines with enhanced gene delivery efficiency and biocompatibility, Polymers, 7 (2015) 2316-2331.

[165] X.Q. Zhang, M. Chen, R. Lam, X.Y. Xu, E. Osawa, D. Ho, Polymer-functionalized nanodiamond platforms as vehicles for gene delivery, ACS Nano, 3 (2009) 2609-2616.

[166] C. Yang, J. Uertz, D. Yohan, B.D. Chithrani, Peptide modified gold nanoparticles for improved cellular uptake, nuclear transport, and intracellular retention, Nanoscale, 6 (2014) 12026-12033.

[167] Y.Q. Xia, J. Tian, X.Y. Chen, Effect of surface properties on liposomal siRNA delivery, Biomaterials, 79 (2016) 56-68.

[168] R.V. Iozzo, Matrix proteoglycans: From molecular design to cellular function, Annu. Rev. Biochem., 67 (1998) 609-652.

[169] J.H. van den Berg, K. Oosterhuis, W.E. Hennink, G. Storm, L.J. van der Aa, J.F.J. Engbersen, J.B.A.G. Haanen, J.H. Beijnen, T.N. Schumacher, B. Nuijen, Shielding the cationic charge of nanoparticle-formulated dermal DNA vaccines is essential for antigen expression and immunogenicity, J. Control. Release, 141 (2010) 234-240.

[170] E. Wagner, K. Zatloukal, M. Cotten, H. Kirlappos, K. Mechtler, D.T. Curiel, M.L. Birnstiel, Coupling of adenovirus to transferrin polylysine DNA complexes enhances receptor-mediated gene delivery and expression of transfected genes, Proc. Natl. Acad. Sci. U. S. A., 89 (1992) 6099-6103.

[171] R.J. Lee, L. Huang, Lipidic vector systems for gene transfer, Crit. Rev. Ther. Drug Carr. Syst., 14 (1997) 173-206.

[172] S. Katayose, K. Kataoka, Water-soluble polyion complex associates of DNA and poly(ethylene glycol)-poly(L-lysine) block copolymer, Bioconjugate Chem., 8 (1997) 702-707.

[173] A.R. Holmes, A.F. Dohrman, A.R. Ellison, K.K. Goncz, D.C. Gruenert, Intracellular compartmentalization of DNA fragments in cultured airway epithelial cells mediated by cationic lipids, Pharm. Res., 16 (1999) 1020-1025.

[174] D. Luo, W.M. Saltzman, Synthetic DNA delivery systems, Nat. Biotechnol., 18 (2000) 33-37.

[175] Y.S. Cho, G.Y. Lee, H.K. Sajja, W.P. Qian, Z.H. Cao, W.L. He, P. Karna, X.Y. Chen, H. Mao, Y.A. Wang, L. Yang, Targeted delivery of siRNA-generating DNA nanocassettes using multifunctional nanoparticles, Small, 9 (2013) 1964-1973.

[176] K. Lee, J.D. Kim, C.Y. Lee, S. Her, H. Jung, A high-capacity, hybrid electro-microneedle for in-situ cutaneous gene transfer, Biomaterials, 32 (2011) 7705-7710.

[177] S.O. Choi, Y.C. Kim, J.H. Park, J. Hutcheson, H.S. Gill, Y.K. Yoon, M.R. Prausnitz, M.G. Allen, An electrically active microneedle array for electroporation, Biomed. Microdevices, 12 (2010) 263-273.

[178] T.W. Prow, X.F. Chen, N.A. Prow, G.J.P. Fernando, C.S.E. Tan, A.P. Raphael, D. Chang, M.P. Ruutu, D.W.K. Jenkins, A. Pyke, M.L. Crichton, K. Raphaelli, L.Y.H. Goh, I.H. Frazer, M.S. Roberts, J. Gardner, A.A. Khromykh, A. Suhrbier, R.A. Hall, M.A.F. Kendall, Nanopatch-targeted skin vaccination against West Nile virus and chikungunya virus in mice, Small, 6 (2010) 1776-1784.

[179] J. McCaffrey, C.M. McCrudden, A.A. Ali, A.S. Massey, J.W. McBride, M.T.C. McCrudden, E.M. Vicente-Perez, J.A. Coulter, T. Robson, R.F. Donnelly, H.O. McCarthy, Transcending epithelial and intracellular biological barriers; a prototype DNA delivery device, J. Control. Release, 226 (2016) 238-247.

[180] Y. Hu, B. Xu, Q. Ji, D. Shou, X. Sun, J. Xu, J. Gao, W. Liang, A mannosylated cell-penetrating peptide-graft-polyethylenimine as a gene delivery vector, Biomaterials, 35 (2014) 4236-4246.

[181] S. Labala, A. Jose, V.V.K. Venuganti, Transcutaneous iontophoretic delivery of STAT3 siRNA using layer-by-layer chitosan coated gold nanoparticles to treat melanoma, Colloid Surf. B-Biointerfaces, 146 (2016) 188-197.

[182] H.W. Yang, L. Ye, X.D. Guo, C.L. Yang, R.W. Compans, M.R. Prausnitz, Ebola vaccination using a DNA vaccine coated on PLGA-PLL/gamma PGA nanoparticles administered using a microneedle patch, Adv. Healthc. Mater., 6 (2017) 1600750.

[183] A.A. Ali, C.M. McCrudden, J. McCaffrey, J.W. McBride, G. Cole, N.J. Dunne, T. Robson, A. Kissenpfennig, R.F. Donnelly, H.O. McCarthy, DNA vaccination for cervical cancer; a novel technology platform of RALA mediated gene delivery via polymeric microneedles, Nanomed.-Nanotechnol. Biol. Med., 13 (2017) 921-932.

[184] J.F. Liao, J.C. Lee, C.K. Lin, K.C. Wei, P.Y. Chen, H.W. Yang, Self-assembly DNA polyplex vaccine inside dissolving microneedles for high-potency intradermal vaccination, Theranostics, 7 (2017) 2593-2605.

[185] H. Seok, J.Y. Noh, D.Y. Lee, S.J. Kim, C.S. Song, Y.C. Kim, Effective humoral immune response from a H1N1 DNA vaccine delivered to the skin by microneedles coated with PLGA-based cationic nanoparticles, J. Control. Release, (2017) doi: 10.1016/j.jconrel.2017.1004.1027.

[186] Y.Q. Qiu, L. Guo, S.H. Zhang, B. Xu, Y.H. Gao, Y. Hu, J. Hou, B.K. Bai, H.H. Shen, P.Y. Mao, DNA-based vaccination against hepatitis B virus using dissolving microneedle arrays adjuvanted by cationic liposomes and CpG ODN, Drug Deliv., 23 (2016) 2391-2398.

[187] Y. Hu, B.H. Xu, J.J. Xu, D. Shou, E.G. Liu, J.Q. Gao, W.Q. Liang, Y.Z. Huang, Microneedle-assisted dendritic cell-targeted nanoparticles for transcutaneous DNA immunization, Polym. Chem., 6 (2015) 373-379.

[188] N.W. Kim, M.S. Lee, K.R. Kim, J.E. Lee, K. Lee, J.S. Park, Y. Matsumoto, D.G. Jo, H. Lee, D.S. Lee, J.H. Jeong, Polyplex-releasing microneedles for enhanced cutaneous delivery of DNA vaccine, J. Control. Release, 179 (2014) 11-17.

[189] A. Kumar, P. Wonganan, M.A. Sandoval, X.R. Li, S.J. Zhu, Z.R. Cui, Microneedle-mediated transcutaneous immunization with plasmid DNA coated on cationic PLGA nanoparticles, J. Control. Release, 163 (2012) 230-239.

[190] A. Jose, S. Labala, V.V.K. Venuganti, Co-delivery of curcumin and STAT3 siRNA using deformable cationic liposomes to treat skin cancer, J. Drug Target., 25 (2017) 330-341.

[191] A. Jose, S. Labala, K.M. Ninave, S.K. Gade, V.V.K. Venuganti, Effective skin cancer treatment by topical co-delivery of curcumin and STAT3 siRNA using cationic liposomes, AAPS PharmSciTech, (2017) doi: 10.1208/s12249-12017-10833-y.

[192] S. Labala, A. Jose, S.R. Chawla, M.S. Khan, S. Bhatnagar, O.P. Kulkarni, V.V.K. Venuganti, Effective melanoma cancer suppression by iontophoretic co-delivery of STAT3 siRNA and imatinib using gold nanoparticles, Int. J. Pharm., 525 (2017) 407-417.

[193] V.V.K. Venuganti, M. Saraswathy, C. Dwivedi, R.S. Kaushik, O.P. Perumal, Topical gene silencing by iontophoretic delivery of an antisense oligonucleotide-dendrimer nanocomplex: the proof of concept in a skin cancer mouse model, Nanoscale, 7 (2015) 3903-3914.

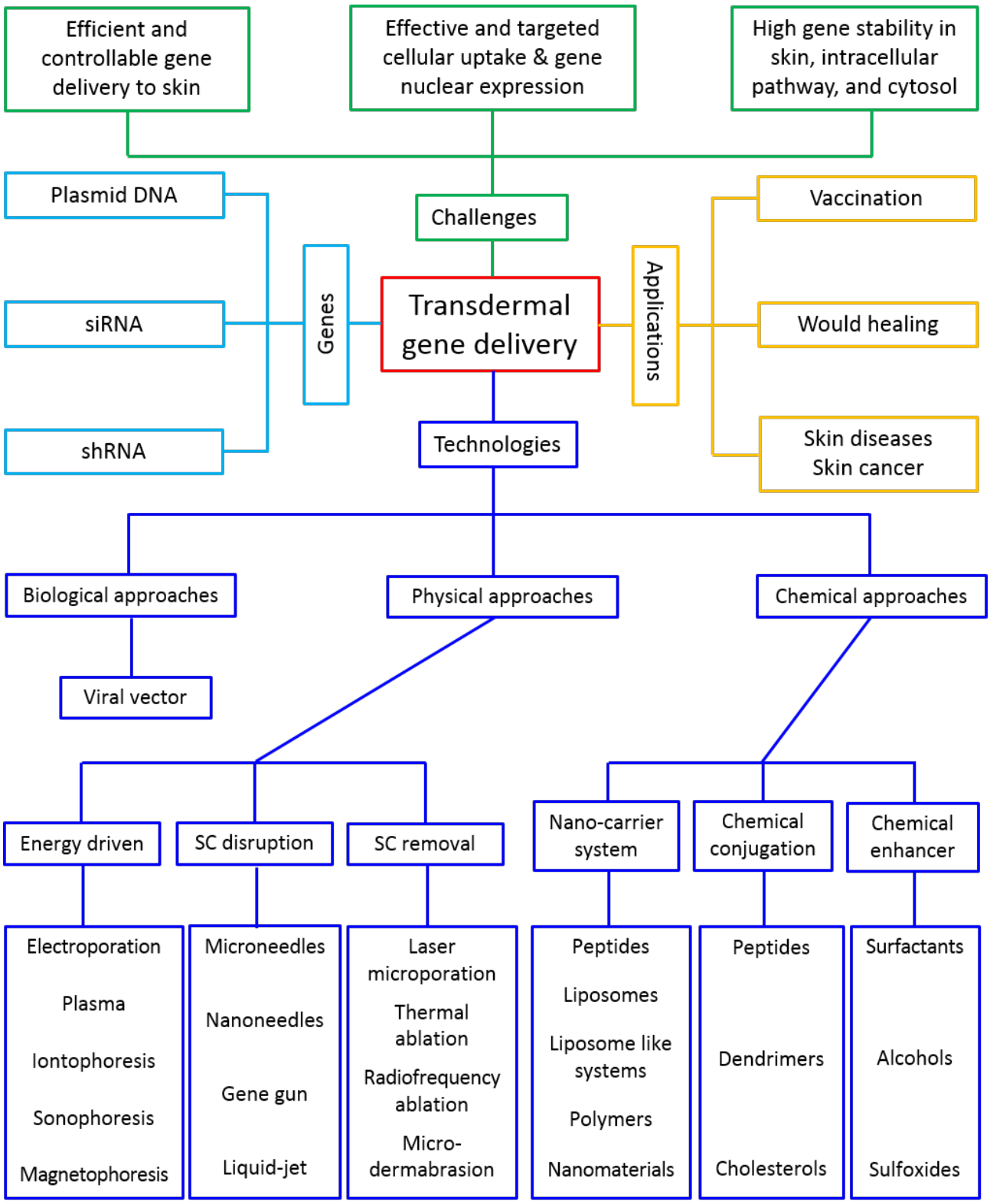

Fig. 1. The applications, challenges and technologies of gene delivery via skin.

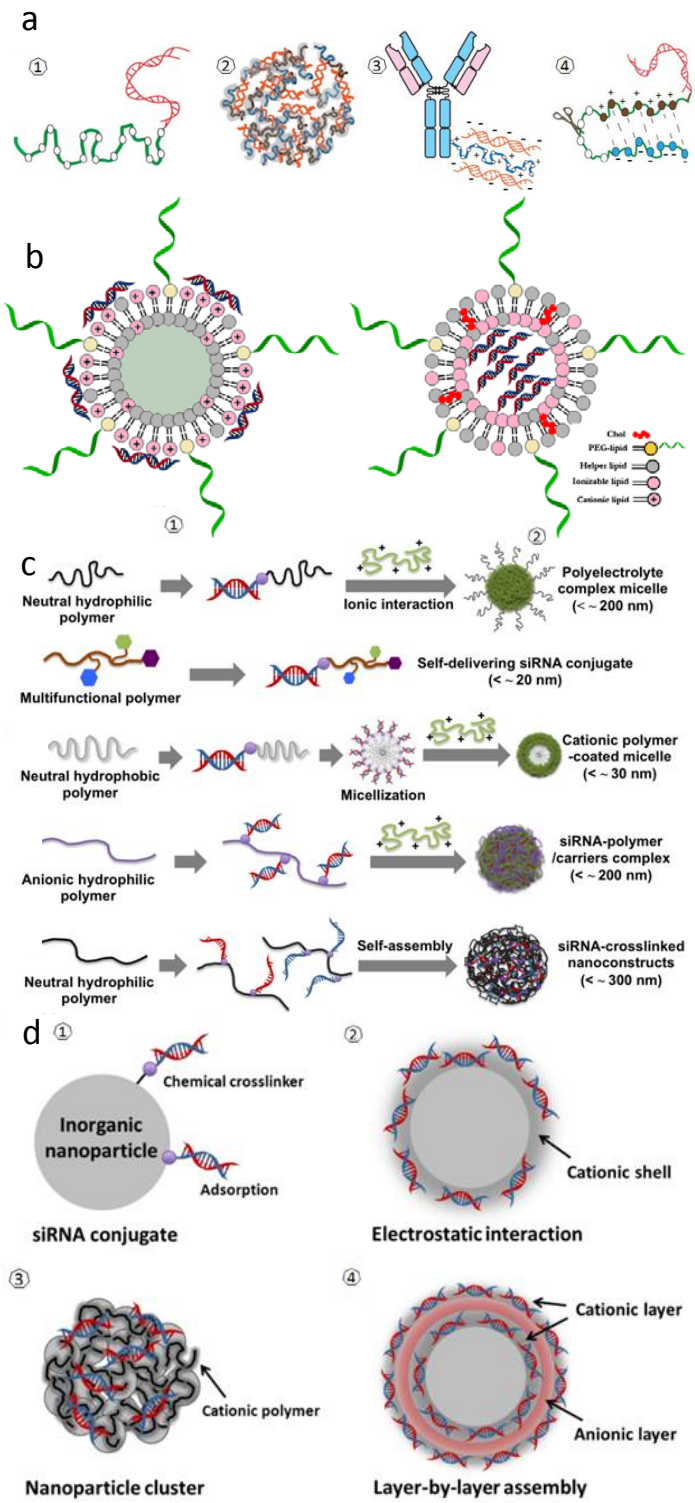

Fig. 2. Representative biochemical approaches for gene delivery [4]. a): CPP-mediated siRNA intracellular delivery. (1) CPP–siRNA conjugate; (2) CPP–siRNA nanocomplex; (3) siRNA anchored on an antibody-CPP conjugate; (4) hairpin-structured activatable CPP conjugated with siRNA. b): Two types of interactions between siRNA and liposomes [5]: cationic liposomes with electrostatically complex siRNA on their surface (1) and siRNA entrapped in liposomes made of ionizable lipids (2). c): the preparation and delivery strategies of different siRNA-polymer conjugates [6]. d): Strategy to load siRNA on inorganic nanoparticles [6]: (1) Chemical conjugation and adsorption of siRNA on a single nanoparticle surface; (2) electrostatic interaction of siRNA and cationic shell on (2) a single nanoparticle and (3) cationic polymer-coated nanoparticle clusters; (4) layer-by-layer assembly of siRNA and cationic polymers on a single nanoparticle surface. Reprinted with permission from ref. 4. Copyright 2017, Elsevier. Reprinted with permission from ref. 5. Copyright 2015, Springer. Reprinted with permission of Creative Commons Attribution (CC BY-NC) license from ref. 6.

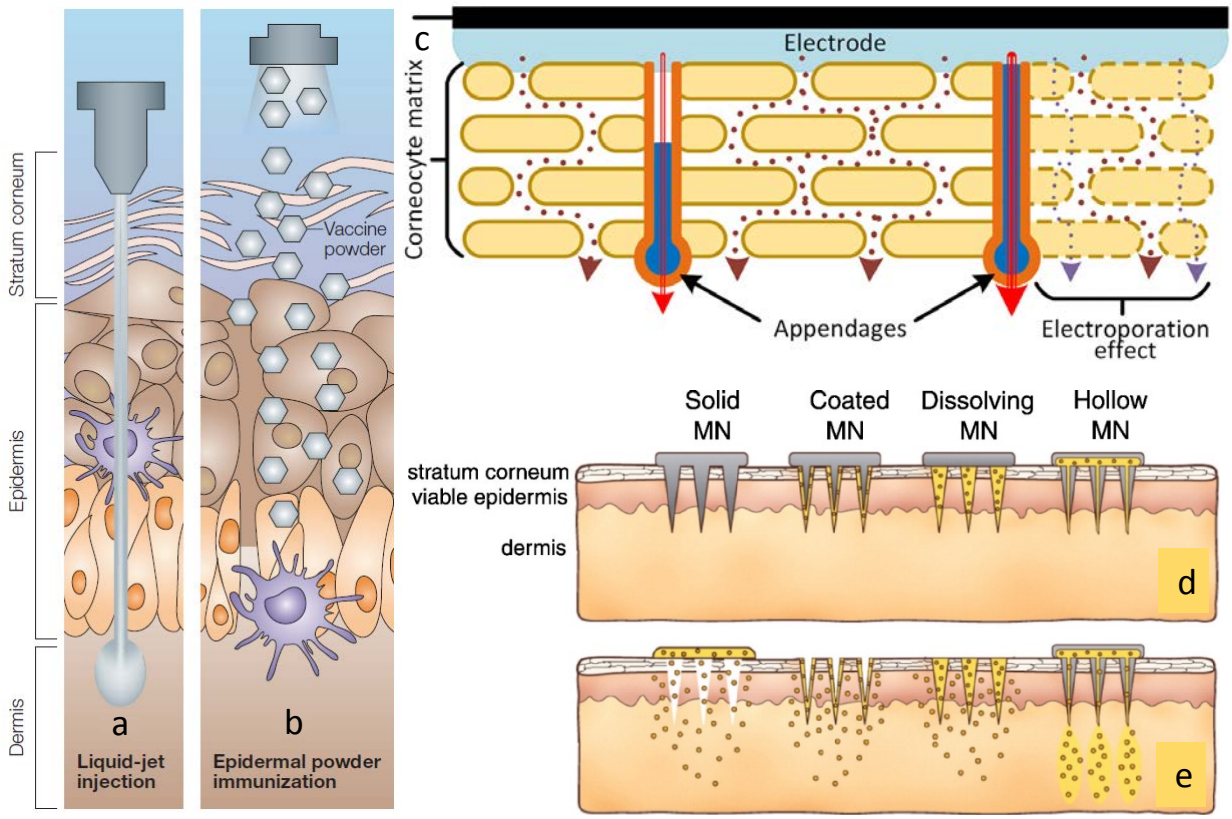

Fig. 3. Representative physical approaches for drug delivery via skin. It is worth noting that the subcutaneous layer is not included in the schematics of the skin anatomy. a): Liquid-jet injection delivers vaccine to muscular, subcutaneous or dermal regions, depending on the parameters of the injection [7]. b): Epidermal powder immunization delivers vaccine powders to the superficial layers of the skin (that is, the epidermis and the superficial layers of the dermis), where they are recognized by Langerhans cells [7]. c): Simplify model of the skin-electrode interface, the stratum Corneum and its ionic pathways. Pathways in the appendages are shown in hard lines, and pathways going between the stratification of the stratum corneum matrix are shown in dotted lines. The electroporation effect on the corneocyte matrix is shown at the right, while the current flow increase due to the pore filling is shown in the appendages [68]. d) and e): Methods of drug delivery to the skin using microneedles (MN) [10]. Microneedles are first applied to the skin (d) and then used for drug delivery (e). Solid microneedles are used as a pretreatment, after which drug can diffuse through residual holes in skin from a topical formulation (solid MN). After insertion of drug-coated microneedles into the skin, the drug coating dissolves off the microneedles in the aqueous environment of the skin (coated MN). Drug-loaded microneedles are made of water-soluble or biodegradable materials encapsulating drug that is released in the skin upon microneedle dissolution (dissolving MN). Hollow microneedles are used to inject liquid formulations into the skin (hollow MN). Reprinted with permission from ref. 7. Copyright 2005, Nature Publishing Group. Reprinted with permission of Creative Commons Attribution (CC BY-NC) license from ref. 68. Reprinted with permission from ref. 10. Copyright 2012, Elsevier.

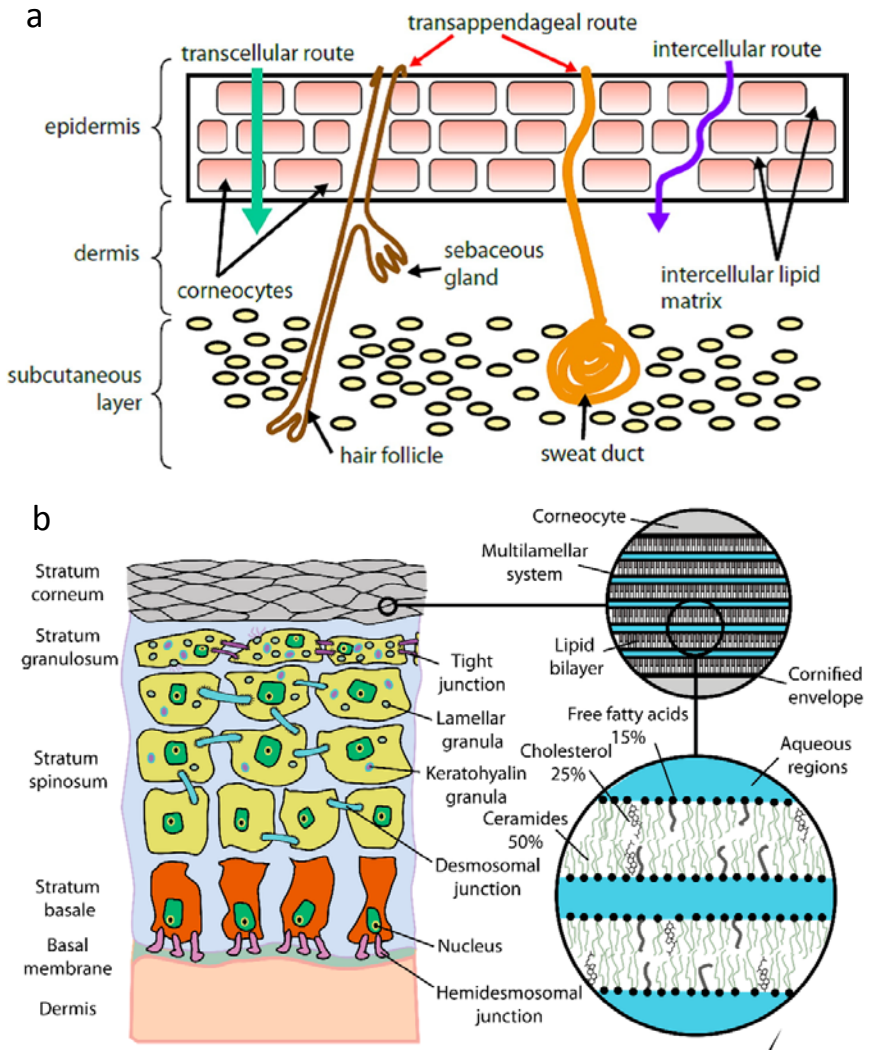

Fig. 4. (a) Schematic of skin and routes of percutaneous absorption [15]; (b) Schematic of the detailed structure of the SC and epidermis [11]. Reprinted with permission of Creative Commons Attribution license from ref. 11. Reprinted with permission from ref. 15. Copyright 2016, Elsevier.

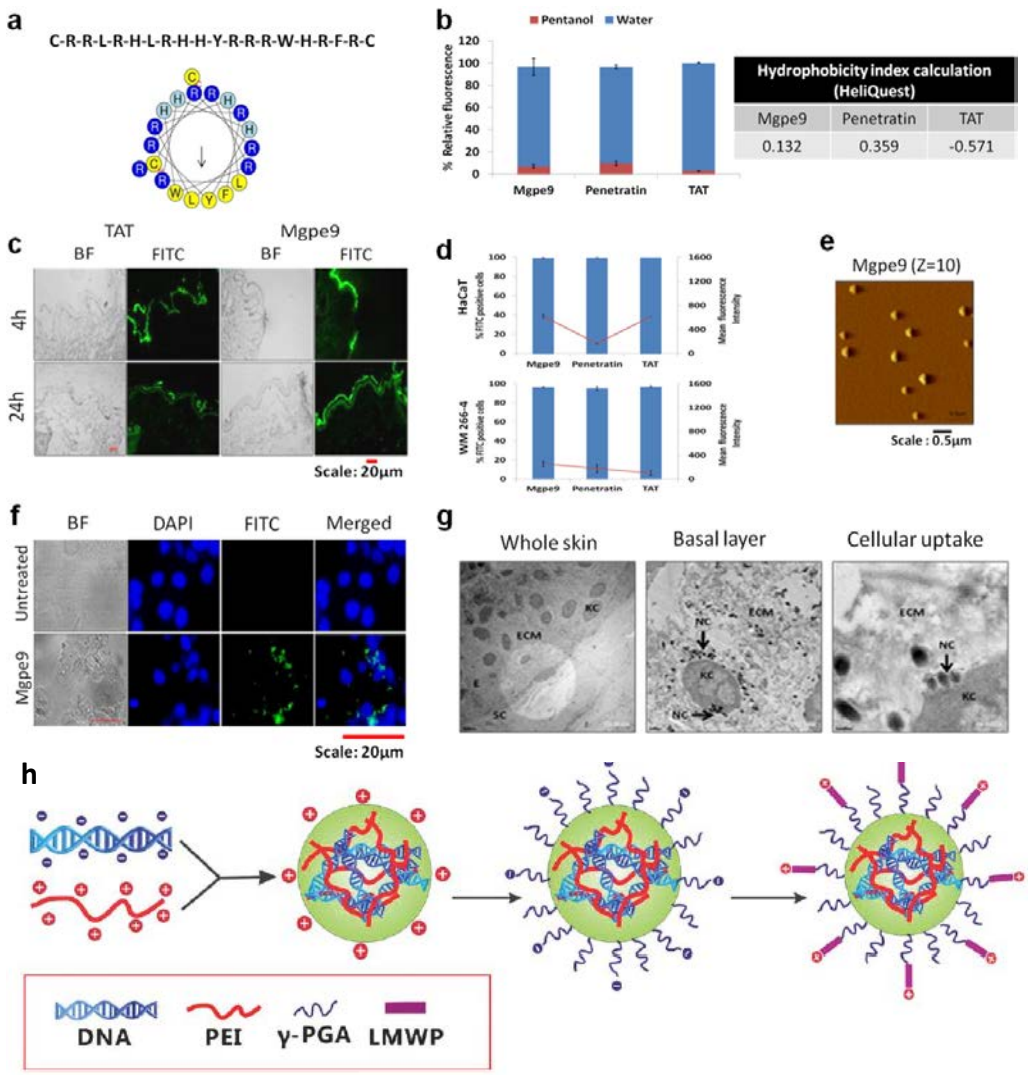

Fig. 5. Entry ofMgpe9 peptide and nanocomplexes in the skin [34]. a): Sequence of Mgpe9 peptide is shown. The helical wheel diagram represents the top-down view of the peptide along the helical axis when it forms a secondary structure. b): Pentanol–water partitioning of Mgpe9 and control peptides (penetratin and TAT) was studied using the fluorescence estimation method. The percentage relative fluorescence (bars) in each phase (red bars indicate hydrophilic phase, blue bars indicate hydrophobic phase) was recorded after 24 h of peptide treatment. Hydrophobicity index calculation for all the peptides was carried out using HELIQUEST software. c): Human skin penetration ability of FITC labeled peptides (Mgpe9 and TAT) 4 h and 24 h after application was studied using the peptide skin penetration test in independent experiments. Direct visualization of FITC fluorescence was performed after single topical application of peptides using fluorescence microscopy at 10×magnification. Scale bar: 20 µm. d): Cellular uptake of peptides (labeled with FITC) was investigated using flow cytometry in skin cell lines. The percentage of FITC positive cells (bars) and the mean fluorescence intensity (line) were recorded after 4 h. The data are shown as mean±SD. e): Atomic force microscopy was carried out to show the formation of nanocomplexes (<100 nm) between Mgpe9 and plasmid DNA at a charge ratio of 10. Scale bar: 0.5 µm. f): Cellular uptake of labeled Mgpe9 nanocomplexes (formed using FITC labeled plasmid DNA and unlabeled peptide) in HaCaT cells was studied using fluorescence microscopy. Cells were imaged at 100× magnification and uptake of nanocomplexes was visualized as green fluorescence inside the cells. DAPI (blue) has been used to stain the cellular nuclei. Scale bar: 20 µm. g): Transmission Electron Microscopy was carried out to demonstrate the entry of nanocomplexes across stratum corneum and into the viable epidermal cells of the skin. Imaging was performed at magnifications of 170×, 800× and 5000× respectively to locate the nanocomplexes in the skin. Scale bar: 5 µm, 1 µm, 0.2 µm respectively. SC denotes stratum corneum; E denotes epidermis; KC denotes keratinocytes; ECM denotes extracellular matrix, NC denotes Mgpe9 nanocomplexes. h): Schematic illustration of the self-assembling LMWP/PGA/PEI/DNA quaternary NPs [35]. Reprinted with permission from ref. 34. Copyright 2016, Elsevier. Reprinted with permission from ref. 35. Copyright 2012, Royal Society of Chemistry.

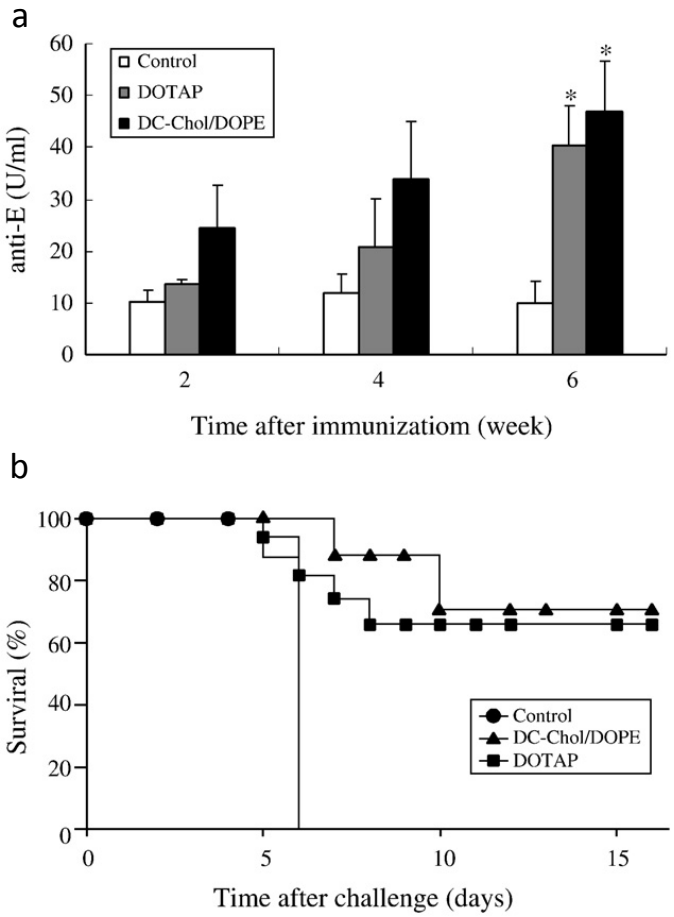

Fig. 6. Protectiveness of the developed JEV lipoplex-patches [42]. a): C3H/HeN mice were transcutaneously immunized three times with a given JEV lipoplex-patch in a 3-week time interval. The anti-JEV E antibodies were measured by ELISA in due course. Mice were immunized with pCJ-3 as a negative control. The asterisk (*) indicates significant difference ($P < 0.05$) at week 6 when the antibody level of a given test sample was compared with that of control. b): Survival rates were plotted for the immunized mice challenged with $50 \times LD_{50}$ of Beijing-1 JEV at week 6 after the first immunization; they were recorded for 15 days. Reprinted with permission from ref. 42. Copyright 2009, Elsevier.

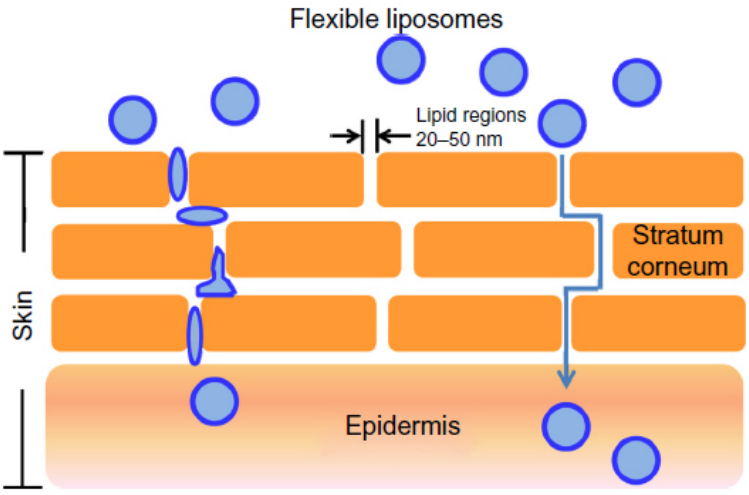

Fig. 7. Flexible liposomes and their mechanism of action [48]. The liposomes are believed to travel through lipidic regions (pores) in the stratum corneum until they reach the epidermis. Reprinted with permission of Creative Commons Attribution (CC BY-NC) license from ref. 48.

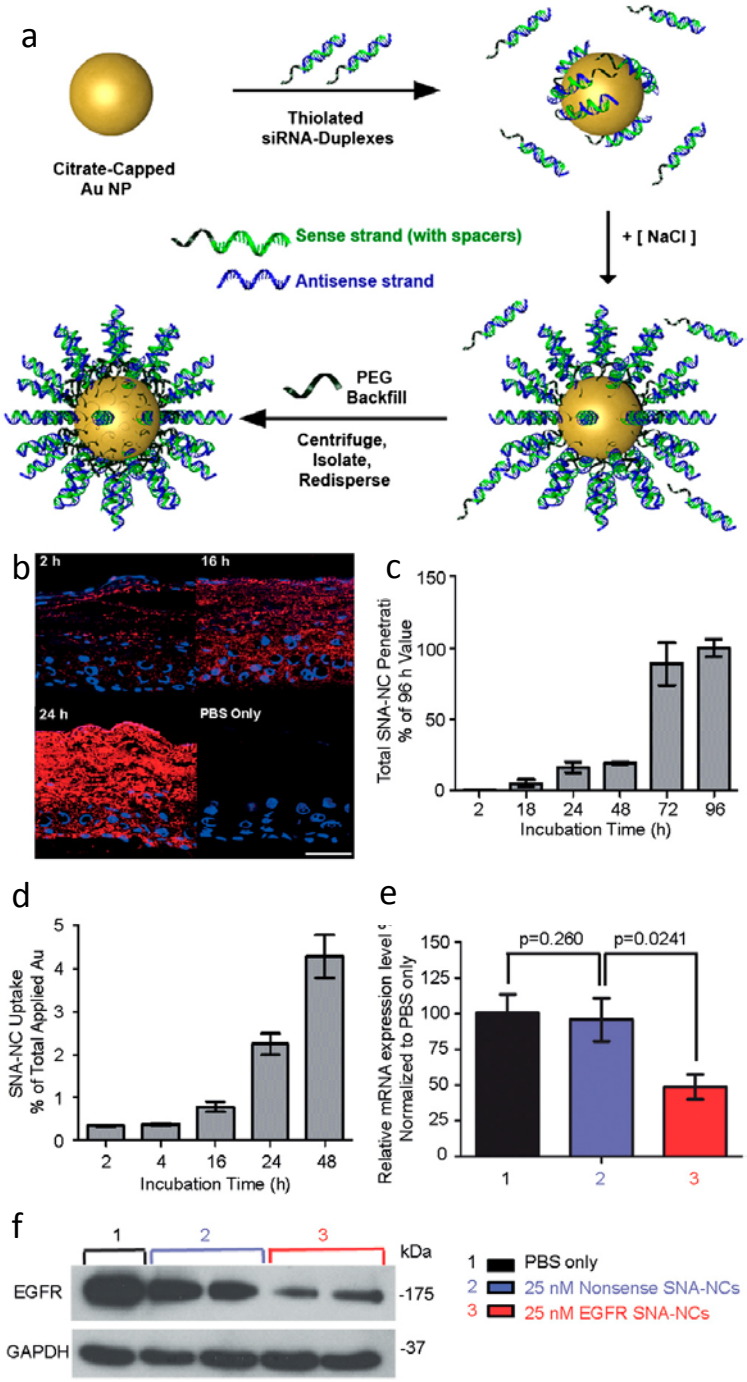

Fig. 8. a): Synthesis of siRNA-based SNA-NCs. Hybridized siRNA duplexes are added to solutions of citrate-stabilized gold colloid and attached through thiolgold chemistry. Addition of salt screens repulsive charges, resulting in densely functionalized nanoconjugates. The number of siRNAs/nanoparticle can be tightly controlled; tested SNA-NCs have approximately 30 siRNAs densely arrayed around a 13 nM core. b-f): Penetration and gene knockdown in human skin equivalents. b): Skin equivalents (EpiDerm; MatTek) treated with a single application of 25 nM Cy5-labeled (red) SNA-NCs or PBS for up to 48 h. Blue, Hoechst 33343-stained nuclei. Note the presence of SNA-NCs throughout the stratum corneum and nucleated epidermis. Scale bar, 50 µm. c): ICP-MS measurements of gold show time-dependent uptake of the SNA-NCs in the epidermis. d): The amount of gold in the cell culture medium, representing the number of particles passing through skin equivalents as measured by ICP-MS, increases with time. EGFR mRNA expression measured by RT-qPCR. e): and EGFR protein expression measured by immunoblotting. f): in skin equivalents treated with a single application of 25 nM EGFR SNA-NCs for 60 h demonstrate effective gene knockdown in human skin. Each study was performed at least three times in triplicate. Data are expressed as mean±SD. [66] Reprinted with permission from ref. 66. Copyright 2012, American Chemical Society.

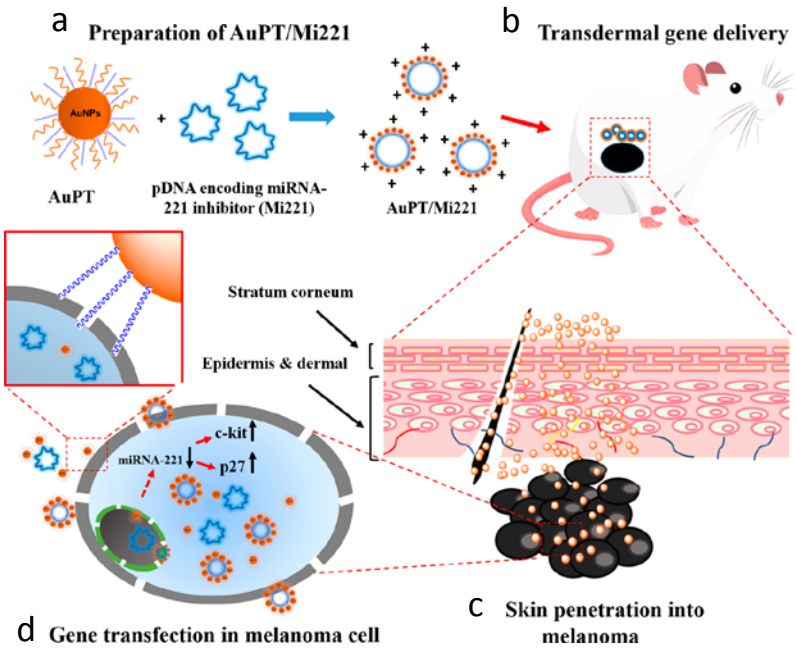

Fig. 9. Schematic illustration of the transdermal delivery of pDNAs encoding microRNA-221 inhibitor gene (Mi221) by AuPT nanoparticles for skin cutaneous melanoma treatment [64]. The therapy consists of four major steps, including a): preparation of AuPT/Mi221 nanocomplexes; b): topical application of AuPT/Mi221 and the skin penetration of AuPT/Mi221; c): skin penetration into melanoma, and d): gene transfection of AuPT/Mi221 in melanoma cells for tumor therapy. Reprinted with permission from ref. 64. Copyright 2017, American Chemical Society.

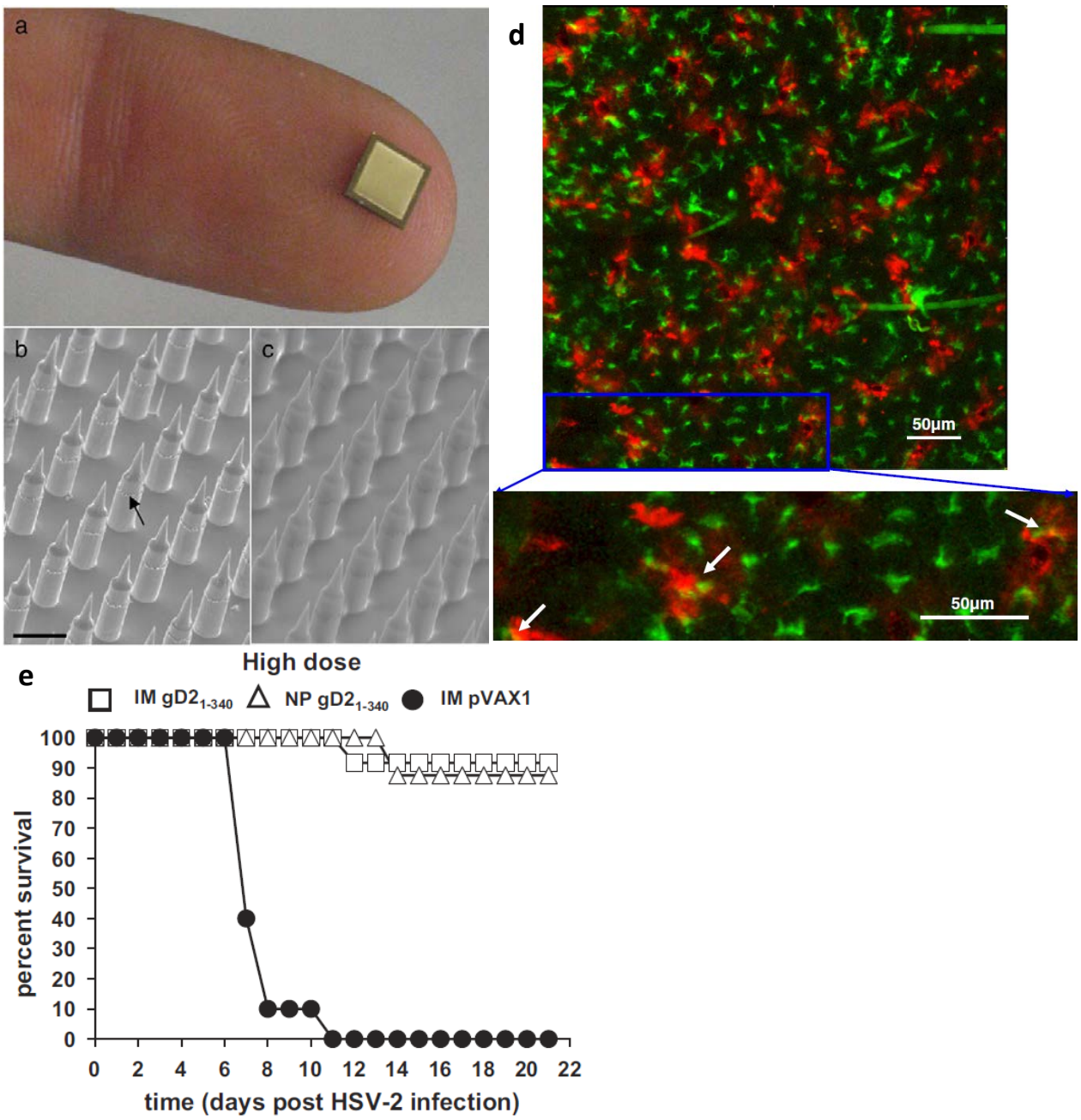

Fig. 10. a): Prototype of Nanopatch™, b): uncoated Nanopatch™ and c): HSV-2-gD2 vaccine coated Nanopatch™. The scale bar indicates 50 µm for (b) and (c). d): Delivery of fluorescent labeled HSV-2-gD2 DNA in skin. Green and red indicate antigen presenting cells and delivered HSV-2-gD2 DNA, respectively. a-d) in [122] e): gD2$_{1-340}$ DNA vaccine delivered by Nanopatch™ protects against lethal HSV-2 challenge [123]. Animals were challenged with 50×LD$_{50}$ HSV-2 intravaginally and mortality was recorded twice daily for 21 days. Pooled data from 2 independent experiments are represented. Reprinted with permission from ref. 122 and 123. Copyright 2010, Elsevier.

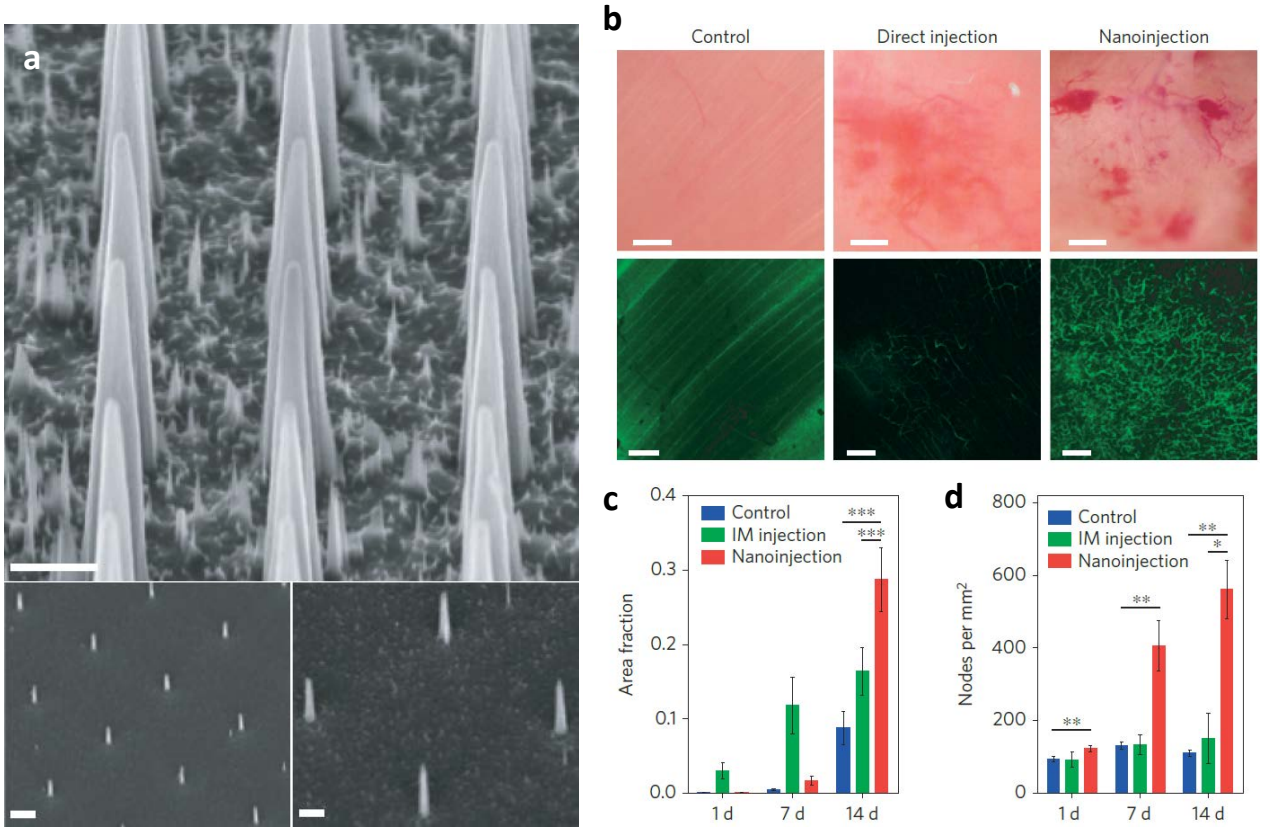

Fig. 11. a): SEM micrographs showing the morphology of porous silicon nanoneedle arrays with pitches of 2 µm, 10 µm and 20 µm, respectively. Scale bars, 2 µm. **b)-d):** Nanoneedles mediate neovascularization [124]. **b):** Intravital bright-field (top) and confocal (bottom) microscopy images of the vasculature of untreated (left) and hVEGF-165-treated muscles with either direct injection (centre) or nanoinjection (right). The fluorescence signal originates from systemically injected FITC–dextran. Scale bars, bright-field 100 µm; confocal 50 µm. **c) and d):** Quantification of the fraction of fluorescent signal (dextran) **c**): and the number of nodes in the vasculature per mm^2 **d**): within each field of view acquired for untreated control, intramuscular injection (IM) and nanoinjection. $*p = 0.05$, $**p < 0.01$, $***p < 0.001$. Error bars represent the s.d. of the averages of 5 areas taken from 3 animals. Reprinted with permission from ref. 124. Copyright 2015, Nature Publishing Group.

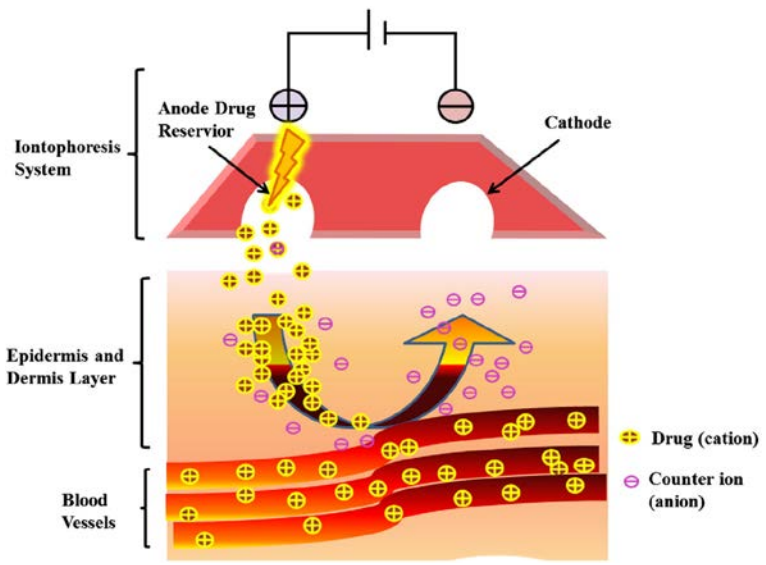

Fig. 12. Iontophoresis method [146]. The iontophoresis unit carries an anode drug reservoir that holds and releases the drug and a cathode that collects the opposite ions when a potential is applied across the two polarities. Reprinted with permission from ref. 146. Copyright 2014, Elsevier.

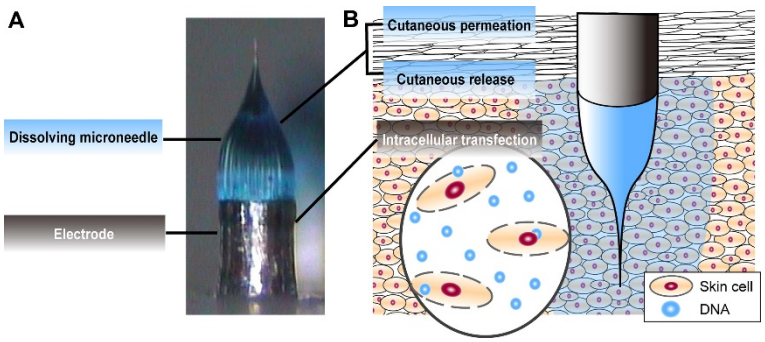

Fig. 13. Schematic representation of in-situ cutaneous gene transfer by the hybrid electro-microneedle (HEM) [176]. (A) Monolithic hybrid assembly of a dissolving microneedle and an electrode to produce a HEM. The dissolving microneedle of the HEM induces cutaneous permeation by bypassing the skin, and followed by cutaneous release from the encapsulated reservoir. The electrode of the HEM facilitates intracellular transfection by generating electric field pulses. (B) Stepwise-aligned cutaneous permeation, cutaneous release, and intracellular transfection using the HEM. Reprinted with permission from ref. 176. Copyright 2011, Elsevier.

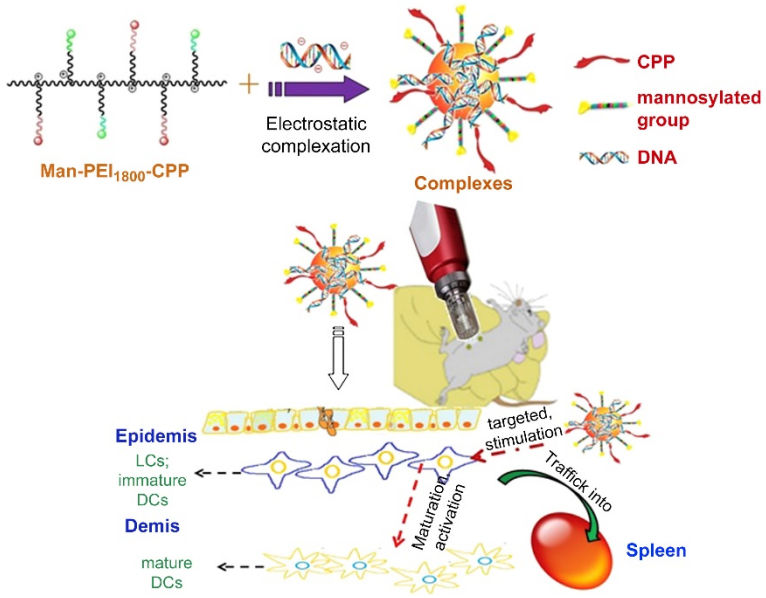

Fig. 14. A schematic diagram showing the formation of Man-PEI$_{1800}$-CPP/DNA complexes and the cell targeting of the complexes for microneedle-mediated transcutaneous delivery [180]. Reprinted with permission from ref. 180. Copyright 2014, Elsevier.

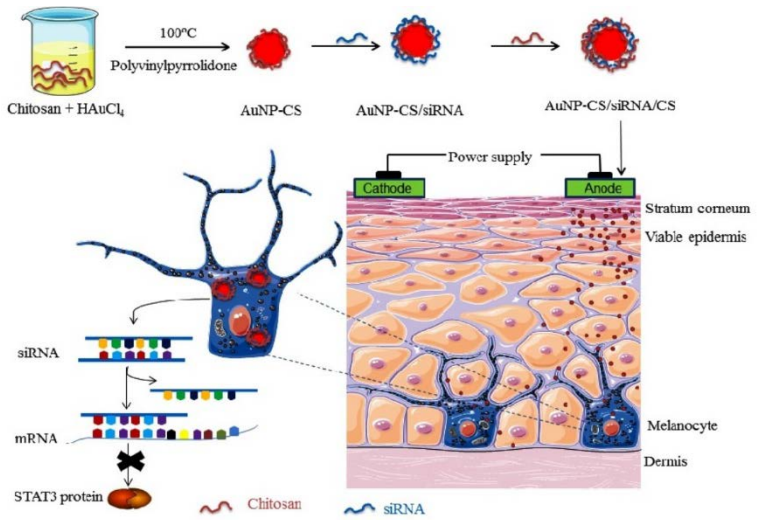

Fig. 15. Iontophoresis aid transcutaneous delivery of chitosan and siRNA coated gold nanoparticles for treating melanoma [181]. Reprinted with permission from ref. 181. Copyright 2016, Elsevier.