European Journal of Pharmaceutics and Biopharmaceutics 137 (2019) 95–111

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Lipid gene nanocarriers for the treatment of skin diseases: Current state-of-the-art

Coralie Bellefroid[1], Anna Lechanteur[*,1], Brigitte Evrard, Géraldine Piel

Laboratory of Pharmaceutical Technology and Biopharmacy, CIRM, University of Liege, Belgium

ARTICLE INFO

Keywords:
Skin delivery
Stratum corneum
Nucleic acid
Gene
Lipidic nanoparticle
Deformable liposomes

ABSTRACT

Nucleic acids carried by non-viral nanovectors have demonstrated high potential as a therapeutic strategy for gene-related diseases. The dermal or transdermal gene delivery allow to target local skin diseases or to reach the blood stream. However, the skin is the first defense barrier of the body and must be overcome to distribute nucleic acids. Many intracellular barriers as cellular uptake, endosomal escape or cytosolic gene trafficking have to be crossed for the gene to achieve its therapeutic action. All hurdles to skin nucleic acid therapy are precisely described. Physical, active or passive methods have been proposed to improve the penetration through the stratum corneum. Lipidic-nanocarriers represent one of the most attractive methods because any skin disruption technique is requested. We give an overview of deformable lipidic-nanocarriers that have been developed to promote the skin penetration of nucleic acids. Moreover, this review describes the potential of deformable liposomes for cutaneous disorders.

1. Introduction

The use of nucleic acids as treatment options has been widely studied owing to advantages offered by gene therapy in restoring or replacing a specific or missing gene [1,2]. While the development of gene therapy is booming, some limitations still remains which make its clinical applicability uncommon nowadays [3]. These limitations include (1) the immune response activation which leads to a neutralization and destruction of the gene, (2) general low membrane permeability of nucleic acids (due to their negative charge, hydrophilic nature and high molecular weight) [3–6] resulting in low transfection efficiency and (3) the short lived nature due to problems of gene integration into the genome [2,5,7]. The whole scientific community joins forces to face these limitations. Three different classes of genetic materials are mainly studied for disease treatment by gene therapy, which allow to knock-in or knock-down a specific gene depending on the genetic cargo transfected: plasmid DNA (pDNA), RNA interference (RNAi) molecules and antisense oligonucleotides (AON) [8].

Gene therapy is considered for many administration routes (such as oral, transdermal as well as mucosal routes (i.e. vaginal, buccal, ocular, etc.)). However, multiple biological barriers are encountered like the first-pass metabolism, high systemic side effects or mucus hindrance

Abbreviations: Ago-2, Argnonaute-2; AON, Antisense Oligonucleotides; AuPT, Gold Platinum; DC-Chol, 3ß-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol; DDS, Drug Delivery System; DEFB4, Beta-Defensin-4; DMRIE, Dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium; DMSO, Dimethyl Sulfoxide; DOGS, 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl]; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOPG, 1,2-dioleoyl-sn-glycero-3-phospho-(1′-*rac*-glycerol); DOSPA, 2,3-dioleyloxy-N-[2-(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOTMA, 1,2-di-O-octadecenyl-3-trimethylammonium-propane; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DSPE-PEG_{2000}, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]; DTS, DNA Targeting Sequence; EGFR, Epidermal Growth Factor Receptor; Exp-5, Exportin-5; hBD-2, Human B Defensin-2; HBsAg, Hepatitis B virus surface Antigen; HPV, Human Papillomavirus; ICAM, Intercellular Adhesion Molecule; IL-12, Interleukin-12; IL-14, Interleukin-14; IL-4, Interleukin-4; IL-6, Interleukin-6; LUV, Large Unilamellar Vesicle; Mi221, MicroRNA-221; miRNA, Micro RNA; MLV, Multilamellar Vesicle; MN, Microneedles; NaChol, Sodium Cholate; NaDChol, Sodium Deoxycholate; NHA, Hydrogen-Bond Acceptors; NHD, Hydrogen-Bond Donors; NLS, Nuclear Localization Sequence; NPC, Nuclear Pore Complex; PC, Phosphatidylcholine; pDNA, Plasmid DNA; PEG, Polyethylene Glycol; PEI, Polyethylenimine; RISC, RNA-Induced Silencing Complex; RNAi, RNA interference; RSV, Respiratory Syncytial Virus; SC, Stratum Corneum; SECosome, Surfactant-Ethanol-Cholesterol-ome; shRNA, Short hairpin RNA; siRNA, Small interfering RNA; SNA, Spherical Nucleic Acid; SNA-NCs, Spherical Nucleic Acid Nanoparticle Congugates; SUV, Small Unilamellar Vesicle; TNF-α, Tumor Necrosis Factor-α; VCAM, Vascular Cell Adhesion Molecule; VEGF, Vascular Endothelial Growth Factor; VEGF-R2, Vascular Endothelial Growth Factor Receptor 2

* Corresponding author at: ULiège Laboratoire de Technologie Pharmaceutique et Biopharmacie, CHU Bat B36 Tour 4, 15 avenue Hippocrate, 4000 Liège, Belgium.
E-mail address: anna.lechanteur@uliege.be (A. Lechanteur).

[1] Co-first authors.

https://doi.org/10.1016/j.ejpb.2019.02.012
Received 28 September 2018; Received in revised form 21 January 2019; Accepted 15 February 2019
Available online 19 February 2019
0939-6411/ © 2019 Elsevier B.V. All rights reserved.

which consequently decrease patient's compliance [9,10]. Hence, the cutaneous gene delivery gained much interest recently since this route allows an easy topical application for dermatological pathologies but also for the transdermal delivery. Indeed, the skin is the largest organ of the human body, representing an alternative route of administration by providing an easy and pain-free drug delivery option. Cutaneous drug delivery is not associated with a significant drug loss compared to systemic administration due to low enzymatic degradation and a negligible clearance. Moreover, systemic toxicity resulting from cutaneous application is limited, side effects are easy to observe and to handle [3,9–11].

The first duty of the skin is to protect the body from external attacks. In this regard, the outer skin layer, the stratum corneum (SC), is impermeable to gene penetration and represents the major extracellular barrier to effective topical gene application [12,13]. To overcome this barrier, different active penetration enhancement techniques for nucleic acids delivery, such as microporation or electroporation, have been proposed to improve the penetration of genes in underlying skin layers containing the viable cells implicated in dermatological pathologies [6,14–17]. However, passive methods and more specifically lipidic non-viral nanocarriers are now favored since these methods are painless, inexpensive and easily applicable [13,18–20]. Moreover, they can be combined to physical enhancement methods like microneedles (MN) and offer great benefits for topic or systemic targeting [21–24].

The present review summarizes the potentialities of different genetic cargos and the corresponding extracellular and intracellular barriers that need to be overcome to deliver the gene to target cells following dermal application. An exhaustive section is dedicated to enhancement penetration methods with a thorough attention dedicated to improved lipidic-vesicles. Finally, the various therapeutic applications of non-viral nanocarriers complexed to nucleic acids are explained. Among others, the topical application of pDNA for vaccination, the treatment of psoriasis or skin cancers and the usefulness in wound healing are emphasized. Within this paper a critical and exhaustive review of the feasibility of applying genes on the skin using lipidic non-viral nanocarriers is provided.

2. Classification of gene therapies

Two different approaches are considered for therapeutic gene delivery through the skin. The first is the *ex vivo* delivery which consists of isolating human cells, treating them by gene delivery and re-grafting those treated cells to the patient. The second approach is the *in vivo* technique during which the genetic cargo is directly transferred to the skin. Although the direct cutaneous application of genes still remains challenging, the *ex vivo* method seems impracticable because of the related pain, costs and the time required for routine applications [25].

Considering the *in vivo* alternative of skin gene therapy, we will discuss in this review the different types of genetic cargos used, which are classified in three sections: pDNA, Antisense Oligonucleotides (AON) or RNAi (small interfering RNA (siRNA), short hairpin RNA (shRNA) and micro RNA (miRNA)) (Table 1). The characteristics of each gene types are related to their mechanisms of action and their intracellular activities.

2.1. Plasmid DNA

Plasmid DNA (pDNA) is a double stranded DNA with a size ranging from hundreds to thousands bases assembled in a circular shape [26]. pDNA vectors carry a transgene which is replicated independently from host chromosomal DNA and segregated to each daughter cell resulting from cell division. In their simplest form, in addition to the transgene, pDNA vectors are composed of a promoter, an enhancer as well as splicing and polyadenylation sites, transcription terminal signals and antibiotic resistance genes [27]. As already stated by Williams et al., we also emphasize the importance of plasmid composition and size since all plasmid elements affect its effectiveness in eukaryotic cells or influence host immune responses after *in vivo* administration in patients. New vectors have been proposed to enhance transgene expression or to reduce adverse effects mainly by reducing bacterial elements which compose the vector [28]. Hence, an optimal pDNA vector design is mandatory to obtain an optimal gene expression [29].

pDNA vectors are widely used since there represent the only way to knock-in a gene permanently. Indeed, once into the cytoplasm, vectors are imported into the cell nucleus and the transcription of the specific encoding mRNA occurs thanks to the promoter sequence. Following post-transcriptional modifications, mRNA is exported into the cytoplasm where it is translated into proteins. Regarding these information, pDNA vectors are classically used to re-express an endogenous gene or to correct a non-functional gene in order to treat a genetic disorder [30]. For instance, the team of Heller has intensely investigated the delivery of pDNA encoding interleukin-12 (IL-12) to treat melanoma [31,32]. The utilization of pDNA in vaccination is also widespread. Applications of plasmids in skin disorders are discussed in Section 5.

2.2. Antisense oligonucleotides

An antisense oligonucleotide (AON) is a single-stranded deoxyribonucleotide composed of 18–21 nucleotides which are complementary to a specific mRNA. By binding to this mRNA the ANO represses the synthesis of the correspondent protein. AON are not classified as a RNAi due to their mechanisms and their sites of action [33]: once in the cytoplasm, the complexation of an AON and its complementary mRNA induces (i) the activation of RNase leading to selective mRNA degradation or (ii) the steric interference of ribosomal assembly. Moreover, when AON enter into the nucleus, they can regulate the maturation of mRNA by (iii) inhibiting the 5′ cap formation, (iv) inhibiting mRNA splicing or (v) activating the RNase H protein.

One major drawback of single-stranded AON is their rapid degradation by nucleases in biological fluids [34,35]. Another inconvenient of AON is the induction of toxicity through the activation of the complement cascade [36,37]. Multiple chemical modifications like the alkylation of the ribose (2′-O-Methyl or 2′-O-Methoxyethyl) were developed to increase their resistance to nucleases or their binding affinity with a specific mRNA [38].

2.3. RNA interference

The RNAi mechanism was first discovered in 1998 in the *Caenorhabditis elegans* nematode. Indeed, Fire et al. reported that a double-stranded fragment of nucleic acids specific to a certain mRNA sequence is able to induce its degradation. In addition, they were the first to already highlight the greater therapeutic potential of a double-stranded fragment compared to a single-stranded fragment [39]. Based on these promising results, RNAi were first used *in vivo* in an experimental mouse model in 2002 and in 2006, the first trial based on a systemic siRNA delivery in primates was achieved [40,41].

The action of RNA interference is mediated through two different types of molecules: siRNA and shRNA. Although both molecules have similar functions, they are different molecules in terms of chemical structure, mechanisms of action, RNAi pathway and off-target effects [42]. Indeed, the double-stranded siRNA (composed of 36–44 bases) crosses the plasma membrane and once into the cytoplasm, is integrated into the RNA-induced silencing complex (RISC) [43,44]. The endonuclease Argonaute 2 (Ago-2) of this complex separates the two siRNA strands and only the antisense strand remains attached to the RISC while the sense strand is released into the cytoplasm. The RISC complex then orientates the antisense strand to its complementary mRNA which is cleaved by RNAse and accordingly, the translation of this mRNA into protein is repressed.

The initiation of shRNA activity is different. The vector bearing the shRNA sequence is transported into the cell nucleus in order to allow its

Table 1
Characteristics of genetic cargo.

Characteristics	pDNA	AON	RNA interference		
			siRNA	shRNA	miRNA
Schematic representation					
Final activity	Expression of protein	Repression of protein expression			
Number of bases (b)	500–8000b	18–21b	36–44b	Stem 50–58b Loop 4–23b	21–24b
Single (S) *or* double (D) strand	D	S	D	Stem D Loop S	S
Translocation	Nucleus	Nucleus and cytoplasm	Cytoplasm	Nucleus	Cytoplasm
Mechanism of action	Synthetize the encoded protein using DNA transcription machinery	-Activation of RNase -Steric hindrance with ribosomes -Interfering with synthesis of mRNA or pre-mRNA	RISC with antisense strand specifically cleaves target mRNA		
Duration of action *in vivo*	Continuous	Transient	Transient	Continuous	Transient
Ease of engineering	No	Yes	Yes	No	Yes
Direct off-target effects	/	/	+ +	+	/
Indirect off-target effects	+ +	+	+	+ +	+
Ease of chemical modification	No	Yes	Yes	No	No

transcription by polymerases and then processed by Drosha to produce a stem-loop structure. The primary transcript (pre-shRNA) is exported by Exportin 5 (Exp 5) into the cytoplasm where it interacts with DICER to be cleaved in short fragments, equivalent to siRNA. Once shRNA and siRNA are loaded to the RISC, they are supposed to act similarly [45].

Practically, siRNA and shRNA have both advantages and disadvantages. Whereas shRNA can be continuously transcripted by host cells, the action of siRNA is transient [46]. However, chemical synthesis of siRNA is easier than the development of plasmid or vector delivering shRNA, able to cross the cellular and the nuclear membranes. Finally, it is well known that siRNA and shRNA induce a lot of specific and non-specific off-target effects. Specific off-target effects are due to the presence of the sense strand in the cytoplasm which can induce off-target gene suppression mainly through their “seed region” [47–49]. Non-specific off-target effects are concerned by cellular toxicities induced by si/shRNA via immune response (Toll-like receptor activation) [50] or because of the cationic drug delivery system [51]. Off-target effects represent a major concern and consequently delay the use of RNAi molecules in therapeutic strategies. To prevent these effects, some chemical modifications have been proposed although there are less easily achievable on shRNA compared to siRNA [52]. In the context of skin diseases and according to the literature (Source Science Direct citations), it seems that siRNA are privileged to shRNA probably because of the ease of synthesis and the transient action leading to less side effects.

Finally, miRNA are also classified as endogenous RNAi molecules. Mature miRNA are small non-coding RNA molecules, synthetized within the cells, which also regulate endogenous genes via the RNAi pathway [53]. Compared to siRNA which bind perfectly to the corresponding mRNA target, miRNA only pair imperfectly with the mRNA, allowing them to mediate the repression of multiple mRNA. Dysregulation of miRNA has been recently correlated to different pathologies (i.e cancer) [54]. Hence, administering synthetic miRNA could now be used to restore its endogenous amounts to overcome its deficiency. On the other hand, the over-expression of a miRNA represents an attractive therapeutic target or biomarker for diagnosis [55,56]. The discovery of this therapeutic potential is quite novel and nowadays, only one miRNA-based therapeutic is in Phase-II clinical trials for the treatment of hepatitis C disease [57]. In terms of skin diseases, it has been found that some miRNA are implicated in psoriasis [58] or melanoma [59] and therefore could represent interesting targets (see Section 5).

3. Hurdles to skin nucleic acid therapy

3.1. Extracellular barriers

Administration of genetic materials remains a significant challenge because of their sensitive physico-chemical properties. Hence, many advantages of cutaneous drug delivery make the skin an attractive route to deliver drugs. The skin acts as an envelope protecting the body from the environment (such as chemical and physical attacks, UV lights, exogenous molecules and microorganisms). The skin also plays an important role to maintain homeostasis by regulating loss of water or the temperature [9,10,60]. The skin has three main layers, namely the epidermis, the dermis and the hypodermis which differ from each other by their properties and structures (Fig. 1).

The outermost layer of the skin is the epidermis which is divided into two distinct parts: the *stratum corneum* (SC) and the viable epidermis. The SC is the major hurdle to transdermal gene delivery because of its structure. Indeed, the SC is a 10–20 µm thick layer composed of dead and anucleated cells (corneocytes connected by corneodesmosomes [13]), which are embedded in a lipid-matrix composed of cholesterol, ceramides, free fatty acids and triglycerides [12]. The structure of the horny layer has been described by Elias et al., in 1983 [61] as the ‘bricks and mortar’ model where corneocytes represent bricks and the lipid matrix is referred to as mortar. This particular architecture makes the SC very hydrophobic and represents the more important barrier to cross for drugs and foreign compounds. Underneath the SC lies the viable epidermis. This layer is 50–100 µm thick and bears several living cells such as keratinocytes, melanocytes, Langerhans cells and Merkel cells [62]. These cells are linked together by tight junctions and are actively involved in the skin barrier, regulation of epidermal homeostasis and in the establishment of epithelial polarity [60]. The diffusion of genes in the viable epidermis is described to be similar to the diffusion through an aqueous medium. However, the polarity of this layer as well as the presence of proteins and cellular junctions can hinder gene diffusion [11]. Keratinocytes are the main component of the viable epidermis and are persistently produced by the deeper epidermis. To renew corneocytes from the SC, every 14 days keratinocytes enter in a maturation step which consists of moving upward to the surface layer, where they undergo keratinization [12]. This process in which the corneocytes are constantly renewed is called desquamation.

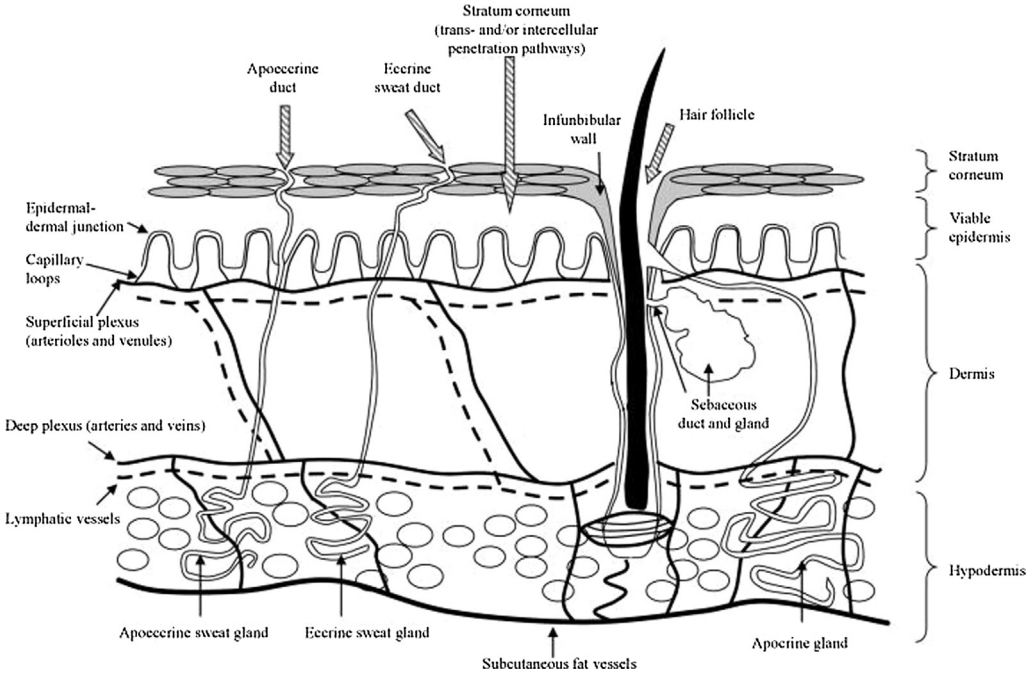

Fig. 1. Schematic overview of the skin layers, appendages, blood and lymphatic vessels. Adapted with permission from [11].

The epidermis is bound to the dermis by the dermal-epidermal junction known as the basement membrane, which provides a resistance to external strengths [12]. The 1–4 mm thick dermis is the most living part of the skin, composed of various cell types (such as fibroblasts, dermal dendritic cells, macrophages, mast cells, inflammatory cells) and of an extracellular matrix composed of collagen and elastin fibers which are responsible for skin elasticity and skin structural support. The dermis bears many neurovascular plexuses, which provide cutaneous sensation, circulation and nutrition [11,62,63]. Accordingly, the dermis is the targeted layer if nanocarriers or drugs have to reach the systemic circulation. The dermis can be altered in case of cutaneous pathologies [64–67].

The deepest layer of the skin is the hypodermis mainly composed of a fat cell network which serves to cushion and protect internal organs from external shocks. Adipose tissue acts also as a store of energy and insulates the body from external temperatures [12,62].

Regarding the skin structure, the epidermis is the most challenging layer to overcome because of its amphiphilic nature. Indeed, a hydrophobic drug could cross the SC but could not easily penetrate the hydrophilic viable epidermis. Moreover, during the diffusion through the SC, when the drug partition coefficient is high, a phenomenon called ‘reservoir effect’ occurs, meaning that the drug remains in the lipophilic part of the epidermis (SC) [11,68]. Contrariwise, while a hydrophilic drug will not penetrate the hydrophobic SC, it could easily diffuse into the viable epidermis. The penetration of a drug through the epidermis is therefore dramatically influenced by its hydro- or lipo-philicity.

Drug delivery through the epidermis is rendered possible by two main passive pathways: intracellular or intercellular. A third pathway through cutaneous appendages is the transfollicular pathway, less attended (Fig. 2). Which pathway is used by drugs to pass cutaneous barriers depends on their affinity with the lipid matrix or with the internal environment of corneocytes and on their ability to cross cell membranes [3,9,11]. There are four parameters inherent to each drug that can influence its passage through skin layers: (1) molar mass (M_w), (2) the number of hydrogen-bond donors (NHD), (3) the number of hydrogen-bond acceptors (NHA) that control interactions with the corneocyte membrane and (4) the octanol-water partition coefficient (logP) that represents the SC-water partition [9]. Similarly, Lipinski proposed “the rule of five” which states that penetration will be weak when $M_w > 500$, NHD > 5, NHA > 10 and LogP > 5 [69]. “The rule of five” thus predicts a poor passage through lipid matrix for large and hydrophilic molecules such as nucleic acids ($M_w \gg 10{,}000$ Da, LogP < 0) [3].

The intercellular route involves a pathway only through the lipid matrix that allows the diffusion of lipophilic or non-polar compounds. The intracellular route requires a drug diffusion through corneocytes which is more favorable to the diffusion of hydrophilic and polar solutes due to hydrogen bonds between polar molecules and corneocytes (NHA/NHD) [70].

Other important characteristics of the skin involved in cutaneous drug administration are the appendages including nails, hair, apocrine glands, eccrine sweat glands and sebaceous glands. In the past, transdermal absorption by skin appendages has been considered insignificant since they represent only 0.1% of the surface of the skin area. However, many studies have shown that follicular absorption represents a significant transdermal penetration pathway [71] and acts as a major reservoir for hydrophilic drugs [72–74]. Moreover, even if the intercellular route is the most commonly used way for lipophilic molecules, penetration through skin appendages related to the presence of sebum in the follicular canal could be demonstrated for hydrophobic drugs [72,75–77].

To sum up, the SC and epidermis layers are first obstacles encountered by a topically applied genetic cargo, which must thus first overcome these barriers in order to diffuse into underlying targeted cells and induce its therapeutic activity.

3.2. Intracellular barriers

When genes have faced and overcome extracellular barriers, their final outcome is to be internalized into target cells in order to induce their genetic modifications. However, many hurdles have been described as preventing the final intracellular activity of nucleic acids. Many methods have been investigated to improve the intracellular delivery of nucleic acids and as explained in Section 4, one of the most promising strategies is the complexation of the genetic materials with a drug delivery system (DDS). In this section, all steps which must be overcome by DDS containing nucleic acids are described and shown in Fig. 3.

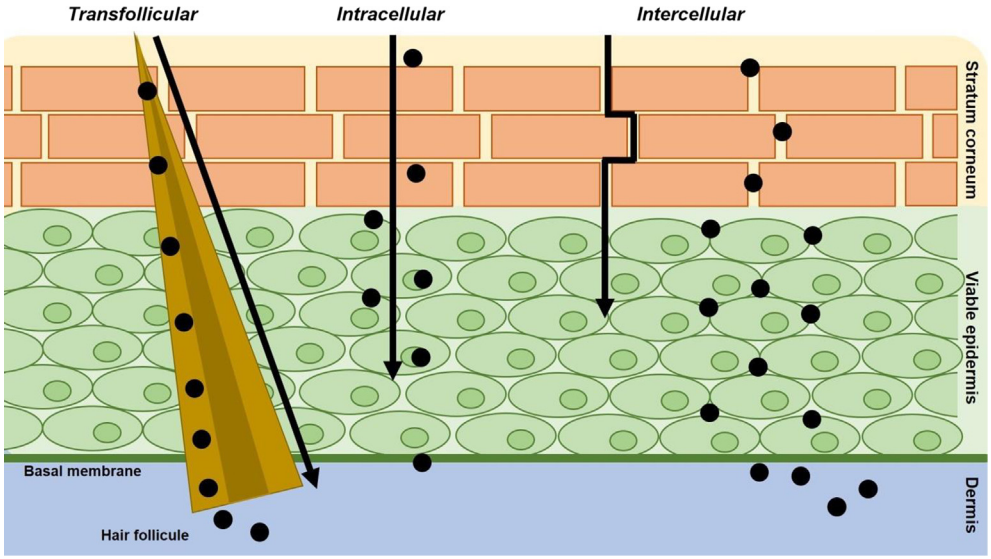

Fig. 2. Schematic pathways of skin penetration.

The following fourth first steps necessary for an intracellular delivery are common for each type of genetic cargo (pDNA, AON and RNAi). The first step involves a contact of the DDS with the cellular membrane *(Step 1)*. Nanocarriers are too large to diffuse passively into cells and are thus internalized by the endocytic pathway [78]. The cell surface is covered by a high density of negative charges due to the presence of diverse anionic proteoglycans that are considered as the major site of interaction with cationic nanoparticles [79–82]. After attachment to the cell membrane, DDS have been found to be internalized into cells via several endocytic mechanisms like the clathrin-dependent or caveola-dependent pathways *(Step 2)*, depending on the nature and the physicochemical properties of the nanovectors. Hence, their surface charge, size, polydispersity and composition are essential factors conditioning their transfectability [83]. In addition, the internalization capacity may vary depending on the cell type. In this context, Douglas et al., showed that the internalization of polymeric particles made of alginate and chitosan was different in three different cell lines [84]. In the context of skin delivery, it is clearly assume by the scientific community that the transfection efficiency is completely different between keratinocytes, macrophages, dendritic cells or fibroblasts. To the best of our knowledge, there is no study comparing transfection capacity in different skin cell types. Moreover, any studies have analyzed the

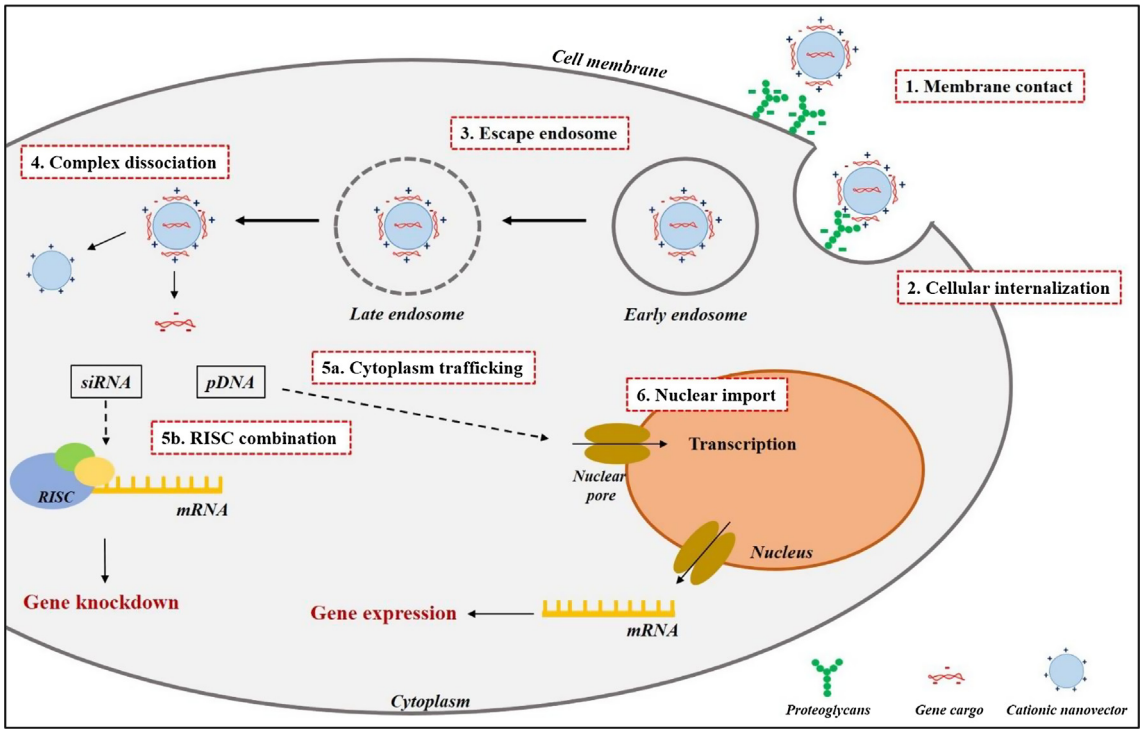

Fig. 3. Steps involved in intercellular delivery of nanoparticle-gene complexes. Following their contact with anionic proteoglycans present in the outer face of the cell membrane (1), nanovectors are internalized by endocytosis (2). The nanoparticle-gene complex must then escape from the endosome (3) and dissociate (4) to release the gene cargo into the cytoplasm. For example, siRNA can further associate to the RISC (5b) in order to induce the knockdown of complementary mRNA. Contrarily, pDNA has first to circulate through the cytoplasm (5a) before being imported into the nucleus (6). After transcription and post-transcription modifications, mRNA will be translated into proteins in the cytoplasm.

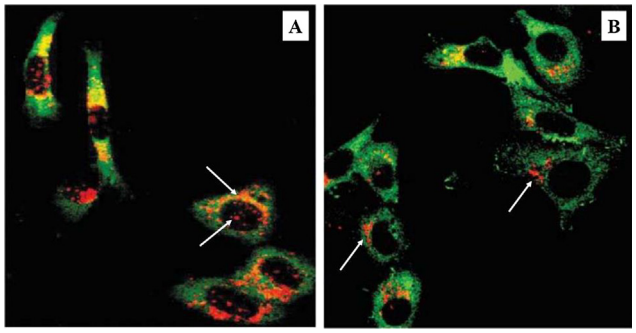

Fig. 4. Intracellular distribution of pDNA visualized by confocal microscopy. A mixture of FITC-labeled 70-kDa dextran (green) and Cy3-labeled κB-DNA (red) was microinjected into the cytoplasm of HeLa cells in the presence of p50 (A) or alone (B). Cells were fixed and the DNA was visualized by confocal microscopy after 8 h. Adapted with permission from [105]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

endocytosis pathway in function of cell type. Vercauteren et al., described all tools necessary to study endocytic mechanisms like exclusion studies using chemical inhibitors as well as fluorescence colocalization studies [85]. Although these experiments are valuable, they still remain tricky since multiple pathways are generally used to cross the cell membrane. Moreover, our group has recently pointed out the importance of performing transfection assays in the proper biological environment. Indeed, Frere et al., developed PEGylated polymeric nanoparticles and showed that their transfection rates into cells were dramatically reduced in the presence of serum [86,87].

The most critical step for nucleic acid activity is the "*endosomal escape*" (*Step 3*) [78,88]. Following cellular uptake, endocytosis vesicles fuse with early endosomes, which have an internal pH of about 6, and then mature in more acidic late endosomes. To prevent acidic and enzymatic degradations, the gene cargo has to escape before the merge with lysosomes [89]. Different strategies have been investigated to promote intracellular delivery: for cationic liposomes, the incorporation of a lipid helper such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) is frequent and involves the reorganization of lipids between the membranes of liposomes and endosomes [90–95]. As described for the first time by Koltover et al., the DOPE lipid promotes the transition of the lamellar L_α phase to the columnar inverted hexagonal H_{II} in acidic pH which facilitates the release of nucleic acids [96]. For polymer-based nanoparticles, the most recurrent approach is the "*proton sponge effect*". Here the buffer capacity of the polymers determines how protons (H^+) enter into the endosomes followed by chlorine ions (Cl^-) and water, important to keep the neutral charge and the osmotic balance. This water content of the endosome will induce the burst release of the nanocarriers into the cytoplasm [97].

Once well located in the cytoplasm, the complex formed between the DDS and the nucleic acid needs to disassemble in order to release the gene of interest (*Step 4*). This step is still controversial when liposomes are used to carry nucleic acids. The pDNA (or siRNA) can be directly released into the cytoplasm. Another hypothesis raised by Vercauteren et al. is that the entire complex is directly released from the endosome and has to be further dissociated into the cytoplasm as it has been described for polymer-based nanoparticles. This process has also been highlighted by our group when using PEGylated lipoplexes composed of DOTAP, DOPE, cholesterol, polyethylene glycol (PEG) and siRNA and developed for mucosal applications in the context of cervical cancer [98]. In this work, we demonstrated using flow cytometry assays and fluorescence inhibitors that the amounts of siRNA blocked in the endosomal compartment were equal between all tested formulations. However, gene knockdown efficiency was different when the lowest amount of PEG was used, which provides evidence that complex dissociation is the major barrier for siRNA or pDNA release [99]. The use of biodegradable lipids or bioreducible polymers may solve these limiting hurdles [85,100].

The complexation of siRNA with the RISC will induce mRNA degradation and consequently, gene knockdown (*Step 5.a*). On the other hand, shRNA and pDNA must get through the cytosol to enter the nucleus prior to transcription, regardless the method of transfection (*Step 5.b*). Two basic methods allow plasmids to reach the nucleus: diffusion or active transport [101]. Lykacs et al. stated that the diffusion coefficient of pDNA is inversely proportional to the number of base-pairs (bp) composing the pDNA [102]. In this manner, while the progression of pDNA of more than 250 bp was slowed by > 17 folds after microinjection in cells compared to its diffusion in water, pDNA with > 2000 bp were unable to diffuse. Moreover, the diffusion of free pDNA may be blocked by cytosolic nucleases [103]. Yet, by using experimental settings which disrupt the microtubule network, Vaughan et al. could demonstrate that pDNA make use of the microtubule networks to reach the nucleus [104]. Briefly, after pDNA (pCMV-GFP-DTS) microinjection into the cytoplasm of TC7 cells pretreated with DMSO or nocodazole, only cells treated with DMSO showed a precise localization of the pDNA in the nucleus or directly around it. Interestingly, pDNA injected in cells treated with nocodazole did not demonstrate any specific pattern of localization. The addition of nuclear localization sequence (NLS) like p50 into the plasmid improved its transfection efficiency. Mesika et al., showed by confocal microscopy that the injection of Cy3-labeled pDNA in the presence of p50 induced its localization around the nucleus or inside (Fig. 4.A) whereas without p50, the pDNA remained mostly at the site of injection (Fig. 4.B) [105].

Finally, the last and a substantial intracellular barrier for an efficient pDNA transfection is the crossing of the nuclear envelope (*Step 6*). Indeed, the amount of pDNA that reaches the nuclear compartment is very low when cells are not in mitosis since the membrane is not broken [106,107]. The only way to enter into the nucleus in non-dividing cells is through the nuclear pore complex (NPC) which is an aqueous channel. The transport of pDNA through NPC is promoted when the pDNA contains a sequence recognized by transcription factors [108]. These sequences are named DNA targeting sequence (DTS) and the most known is the SV40 enhancer which can bind different transcription factors [109]. Other techniques have been described to enhance the nuclear entrance and are reviewed by Dean et al. [108].

The last step concerning the nuclear entry of pDNA is not clearly explained in the literature. It is still questionable whether the pDNA-nanocarrier complex is transported and dissociated prior or after the nuclear entry. Once again, this behavior depends on the type of nanocarrier used. Indeed, with liposomes, if the hypothesis of the reorganization of lipids is verified, the pDNA is free before the nuclear entrance and thus has to circulate into the cytoplasm. However, when the complex has to be further dissociated after the endosomal escape, the release of pDNA could happen into the cytoplasm or into the nucleus.

4. Three main strategies to improve skin gene delivery

During last decades, different strategies have been elaborated to increase gene penetration through the skin and dispense nucleic acid to targeted cells. Penetration enhancement methods are classified into three categories namely active, physical and passive methods [3,5,10,15,16,110–114]. Assets and limitations of these three approaches are explained in Table 2.

Physical and active methods involve the disruption of the SC to increase the penetration in underlying layers. The transfer of genetic material carried out by means of either viral or non-viral vectors to improve penetration is considered as a passive method. Due to the infectious nature and therefore safety issues related to viral carriers, non-viral carriers will be preferred. Despite their lower transfection rates, non-viral vectors offer many advantages, being low immunogenicity, low toxicity, low antigenicity, low production costs, adaptable

Table 2
Assets and limitations of penetration enhancement techniques for nucleic acid delivery (adapted with permission from Zakrewsky et al. [3]).

Classification	Approaches	Assets	Limitations
Physical	• Microneedle • Microporation • Microdermabrasion	• High enhancement • Universal methods with a wide variety of genes • Reproducible (not depending to state skin disease) • Possibility of reaching the desired skin layer	• Costs • Limited application area • Irritation and risks of infection • No specific targeting • Relatively invasive/painful
Active	• Electroporation • Iontophoresis • Sonophoresis	• Very effective skin penetration and cell internalization • Universal with a wide variety of genes • Controlled delivery • Repeated application at the same site • Transient perturbation of skin	• Costs • Complexity of equipment • Limited application area • Localized penetration zone • Relatively invasive • Patient compliance
Passive	• Polymeric nanoparticles • Liposomes • Peptides • Dendrimers	• Low costs • Application to large skin surface • Non invasive • Protect drugs from degradation • Skin perturbation • Targeting of specific cell-types	• Can be limited by size of complexes • Lower enhancement • Delivery depending on state of skin

composition and better stability [115–118]. A large number of non-viral strategies developed to increase dermal penetration of nucleic acids have been devised.

4.1. Physicals and actives approaches to cross the skin barrier

The purpose of physical methods is to increase the penetration of nucleic acids by creating a disruption of the SC prior or after drug application [3,7,10,16]. Microporation technique is a physical method that creates ablation of corneocytes by applying a local electric current allowing penetration of large molecules into the skin [119].

One of the most used physical methods is the use of microneedles (MN) owing to their many advantages (Table 3) [21–24]. This technique consists in creating pores in the skin using micron-sized needles (100–700 μm in length [3]) favoring macromolecule and nucleic acid penetration into the viable epidermis. Other than these advantages, MN techniques also face limits such as local inflammation, a need for repeated applications due to small delivered volumes and breakage of fine MN. Nevertheless, owing to their minimally invasive technology and their impressive efficacy, MN seem to be appropriate candidates as penetration enhancer approach.

MN [21–24] can be solid, hollow, coated, biodegradable/dissolving or hydrogel-forming [16]. More details about skin drug delivery methods by MN are stated in Van Der Maaden's review [23].

MN are used to improve gene material (siRNA and pDNA) delivery. The improved delivery of genetic material using MN has been widely published. For instance, Chabri et al. [120] observed the skin penetration of 100 nm diameter-cationic lipoplexes (DOTAP:pDNA) by using silicon MN. Coulman et al. [121] also demonstrated that silicon-based MN are able to drill SC to enhance penetration of macromolecules like β-galactosidase and pDNA in the viable epidermis. A proof-of-concept looking at the increased gene expression in the human viable epidermis using steel MN on which a pDNA had been impregnated, was recently launched [122]. Using a soluble protrusion array device, Gonzalez-Gonzalez et al. [123] aimed at an anti-luciferase siRNA delivery linked to an effective gene silencing in luciferase expression in transgenic mice.

Table 3
Advantages of MN for skin gene delivery.

Advantages of MN
- Can deliver large molecules such as nucleic acids
- Are minimally invasive and painless
- Are not associated with a fear of needles
- Are easy to use
- Are not dependent on the state of the skin
- Are simple devices and relatively cheap
- Can be associated with other penetration enhancement techniques
- Can increase cellular uptake
- Can be adaptable : can target gene expression at different area on the skin depending on needle length
- Can provide a controlled drug delivery

The mechanism by which MN enhance gene penetration is based on the dissolution and diffusion principle [23]. Hence, either the MN themselves dissolve in the skin to allow the delivery of the drug enclosed in MN (dissolving MN) or it is the drug impregnated (coated MN) or contained in MN (hollow MN) which dissolves in the skin and reaches the targeted layer to induce a immune response for vaccination. This expertise has been proven effective in several vaccination studies performed to target influenza [124], hepatitis B and C [125,126], measles [127], malaria [128,129] and more recently the Ebola virus [130].

Active methods aim to increase skin diffusion and transfection of macromolecules such as nucleic acids using an electric field (electroporation and iontophoresis) [6,113,114] or ultrasound (sonoporation) [6,7,110]. These methods are very effective in terms of skin penetration and gene expression. Many reviews describe the performance of such processes [3,5–7,15–17]. Nevertheless, despite their efficiency, these methods have to deal with major disadvantages like expensive and extensive equipment, painful and invasive methods. Conjointly, their translation to clinical trials is not easy. To achieve more suitable outcomes, the above cited techniques should be combined [130–137].

4.2. Passive approaches to increase gene penetration through the skin: non-viral nanocarriers

Non-viral nanocarriers are developed for two purposes. Their first benefit is to increase macromolecule penetration through SC barrier and the second is to promote the intracellular delivery of nucleic acids. Polymers, dendrimers, cell penetrating peptides, gold nanoparticles, lipid-based carriers have thus been studied for topical gene delivery [3,15,138–141]. In this section, we focus more specifically on classical and modified liposomes to illustrate the potential of the lipid-based carriers.

Liposomes are vesicles composed of phospholipids which bear hydrophobic chains and a hydrophilic head. When they encounter an aqueous medium, liposomes have the ability of spontaneous self-assembly to form a bilayer where the hydrophobic lipid chains face each other and the hydrophilic lipid heads are in contact with the aqueous medium. The amphiphilic feature of liposomes explains why they are widely used to increase the penetration of hydrophilic molecules (in the aqueous core) and/or lipophilic molecules (within the membrane

bilayer) [142]. The liposomes classification is based on their size and their lamellarity [15]. Hence, small unilamellar vesicles (SUVs) are composed of one lipid bilayer with a size ranging from 20 nm to 100 nm, large unilamellar vesicles (LUVs) have a size > 100 nm and multilamellar vesicles (MLVs) are composed of several lipid bilayers with a size > 0.5 μm. Besides the fact that liposomes can encapsulate a wide range of molecules, the lipid composition of liposomes is close to the lipid composition of the skin which is an important factor to enhance diffusion through SC and to avoid side effects. The most used lipids are biodegradable, biocompatible and non-toxic [143,144]. We have to notice that the stability in suspension is still a drawback when lipid-based nanocarriers are considered. However concerning gene complexation, most of lipid-carriers are positively charged to promote the electrostatic interactions with anionic nucleic acid which prevents liposomes agglomeration. Moreover, different strategies are developed to increase the stability of lipid-carrier and to prevent the burst released of drugs. Nowadays, the most used is the lyophilization [145,146].

4.2.1. Conventional liposomes

Liposomes refer to a simple lipid composition mainly natural phospholipids such as phosphatidylcholine (PC) or lecithins. The term “conventional liposomes” is used to differentiate these vesicles from modified vesicles engineered to improve skin penetration.

The importance of using lipid nanocarriers for skin drug delivery and mechanisms of actions were very recently reviewed [141]. Within this paper, we thus specifically focus on skin delivery of genes.

In 1995, Li et al. [147] demonstrated that classical based-phosphatidylcholine liposomes could be used to improve DNA penetration into hair follicles of histocultured mouse skin. Indeed, only a specific accumulation of DNA entrapped in liposomes was found in hair follicles while the “naked” control DNA did not penetrate. However, due to the low density of hair follicles (0,1% of the skin surface), DNA penetration by the transappendageal follicular pathway tends to be limited. To date, this method is still controversial since nanoparticles could be identified in hair follicles only when the skin had been massaged during the experimentation and no nanoparticle transferred in the epidermis [19]. Other researches [148] already identified the massage procedure as being an important factor since it can increase (5-fold higher) drug penetration through hair follicle.

It is now generally well established in the literature that, as topical delivery system, classical liposomes are relatively ineffective. Indeed, classical liposomes are retained in the upper layer of the epidermis (SC), resulting in a liposome ‘reservoir’, where intact liposomes are unable to provide an effect in deeper skin layers [14,149–151]. This phenomenon could be explained by the fact that the classical liposomes break and disrupt the lipids of the stratum corneum, allowing a free diffusion of the molecule without any carrier (Fig. 5).

Various strategies have been designed to obtain more suitable outcomes.

One of them is the use of cationic lipids, such as DOTMA-containing liposomes, to enhance gene delivery into cells. The group of Felgner et al. described that a DOTMA-containing liposomes could facilitate the delivery of DNA into cell [152]. They suggest that, cationic liposomes spontaneously associate with the plasmid through electrostatical interactions to form a complex with a positively charged surface which can associate with the negatively charged cell surface. Later, this hypothesis was validated by Ruponen et al. [153]. In general, a slight excess of positive charges leads to higher transfection rates, however, if cationic liposomes have too much affinity for DNA, the transfection will be limited [154–156]. Several types of cationic lipids could be used to form lipoplexes: monovalent lipids (DOTMA, DOTAP…), multivalent lipids (DOGS, DOSPA), cholesterol-derivatives (DC-Cholesterol) or cationic switchable lipids [18,20,83,157–161]. As an example, the cationic head of DOTAP is an amphiphile quaternary ammonium and is widely used to effectively deliver nucleic acids despite its toxicity [18,20,162,163]. Indeed, Birchall et al. [164] demonstrated that DOTAP-composing lipoplexes were able to increase uptake and expression of plasmid DNA (33-fold higher compared with control pDNA) into a viable epidermis layer. To increase the stability of liposomes or to enhance nucleic acid delivery, other lipid helpers can be added to cationic lipids [95]. Hence, cholesterol is used to improve the stability and entrapment efficiency of liposomes [165]. As explained in the Section 3.2, another improvement is to add a fusogenic lipid such as DOPE, which can significantly increase cellular uptake, thus allowing better transfection rates. Indeed, in a recent study Kim et al. [166] showed that this transfection efficiency did not depend on the size or zeta potential of liposomes but mainly on the optimal DOTAP/DOPE ratio, highlighting the key role of DOPE to improve cellular uptake and transfection in a particular cell type [166]. In the same study, the authors emphasize the fact that the optimal DOTAP/DOPE ratio depends on the targeted cell type.

To date, the use of cationic lipids is one of the most used strategies in combination with the following modifications (surfactant/ethanol) to enhance gene delivery.

Nevertheless, while the use of cationic lipid makes sense for nucleic acid complexation, few studies have described the use of anionic lipids, such as DOPG, to complex macromolecules, oligonucleotides and plasmid DNA as well as to enhance gene transfer [153,167-171] As an example, Srinivasan and co-workers [170] used DOPG, an anionic lipid, to enhance gene transfer. Divalent cations are essential to condense nucleic acids as reviewed by Kulkarni [172]. Based on previous studies [172,173], calcium divalent was chosen to achieve complexation between DNA and anionic lipids which are strongly repulsed by their negative charge. While lower transfection efficiency was observed at low Ca^{2+} concentrations probably due to decreased DNA - anionic lipid interactions, [170] higher Ca^{2+} concentrations (> 25 mM) also correlated with low transfection rates, probably related to the large particle size (500 nm) with increased agglomeration of vesicles [174]. The optimal ratio, associated with effective transfection and no toxic effect, is thus composed of 15 mM Ca^{2+} and displaying an allowed size of about 200 nm. However, to our knowledge, such anionic complexes are not yet widely used to increase gene penetration through the skin.

4.2.2. Transfersomes

In the 1990 s, Cevc and Blume [175] introduced a new class of ultradeformable liposomes called Transfersomes®. These vesicles have the feature to be deformable owing to surfactant addition in the lipid bilayer. Transfersomes® are five to eight times more elastic than classical liposomes and are able to penetrate intact skin when applied under non occlusive conditions due to the transdermal water gradient [150,176]. Surfactants added to phospholipids, also called ‘edge activators’, act by destabilizing the membrane of the bilayer and consequently increase the flexibility of the vesicles. Surfactants thus allow vesicles to compress and distort to squeeze through the pores (Fig. 5).

Based on works of Cevc et al., a number of authors were interested in Transfersomes® as a promising delivery system for gene therapy. Surfactants bearing different characteristics are used to compose deformable liposomes, including sorbitane monoleate, polysorbate 80 (Tween®80), sodium cholate (NaChol), sodium deoxycholate (NaDChol), octadecylamine or potassium glycyrrhizinate [165,177–179].

Lee et al. [180] studied the effect of edge activator on formation and transfection efficiency of deformable liposomes encapsulating pDNA. They have tested three edge activators NaChol, NaDChol and Tween®80. While NaChol seems to be more suitable for pDNA delivery through the skin, Tween®80 turns out to be more toxic and seems to be less suitable as edge activator. These results support the study of Kim et al. [181], showing that a 6:1 ratio (DOTAP: NaChol) complexed to pDNA with a ratio 1:14 (pDNA:liposome) provides smallest, non-toxic and effective particles with maximal transfection efficiency demonstrated in four different cell lines (OVCAR-3, HepG2, H-1299 and T98G). Conflictingly, a study showed that Tween®20 can be used as an

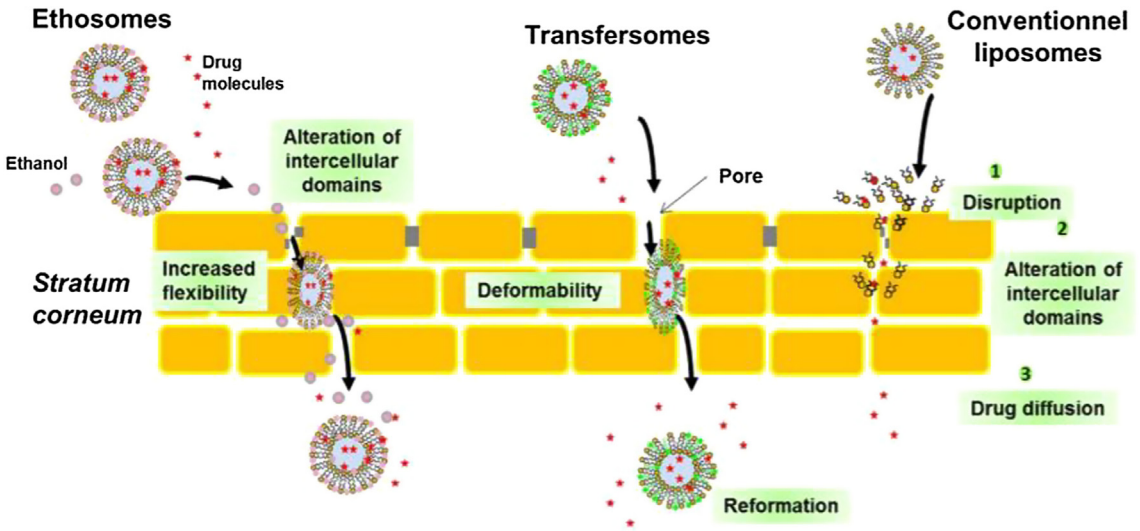

Fig. 5. Schematic overview of skin penetration of ethosomes, transfersomes and conventional liposomes. Reprint with permission from Sala et al. [141].

effective edge activator without inducing any cytotoxicity [182].

The percentage of edge activator in the final liposome complex is an essential factor determining penetration efficacy into the deep epidermis layers [183]. Indeed, authors studied the ability of formulated lipoplexes to internalize into melanoma cells, knockdown the expression of BRAF and induce cell death in melanoma cells. Several concentrations of NaChol as edge activator were tested within a DOTAP-containing formulation (DOTAP: NaChol ratio: 4:1, 6:1, 8:1, 10:1 with different amounts of siRNA). They found that liposomes at 8:1 ratio and complexed with siRNA at 16:1 ratio had the highest rate of permeation through the skin with significant deposition at upper epidermis (Fig. 6). They suggested that liposomes containing less than 16,6% of edge activator (ratio 8:1) failed to reach deeper layers of the epidermis because of the low surfactant percentages [183].

4.2.3. Ethosomes

Ethosomes are nanostructures similar to liposomes including a variable proportion of ethanol (up to 45% w/w [165]), identified as being a penetration enhancer [184]. Ethosomes were first described by Touitou et al. [185] as an approach for improving skin penetration offering a better diffusion of the drug in the deep skin layers by destructuring and solubilizing the lipids of the SC, thus making it more

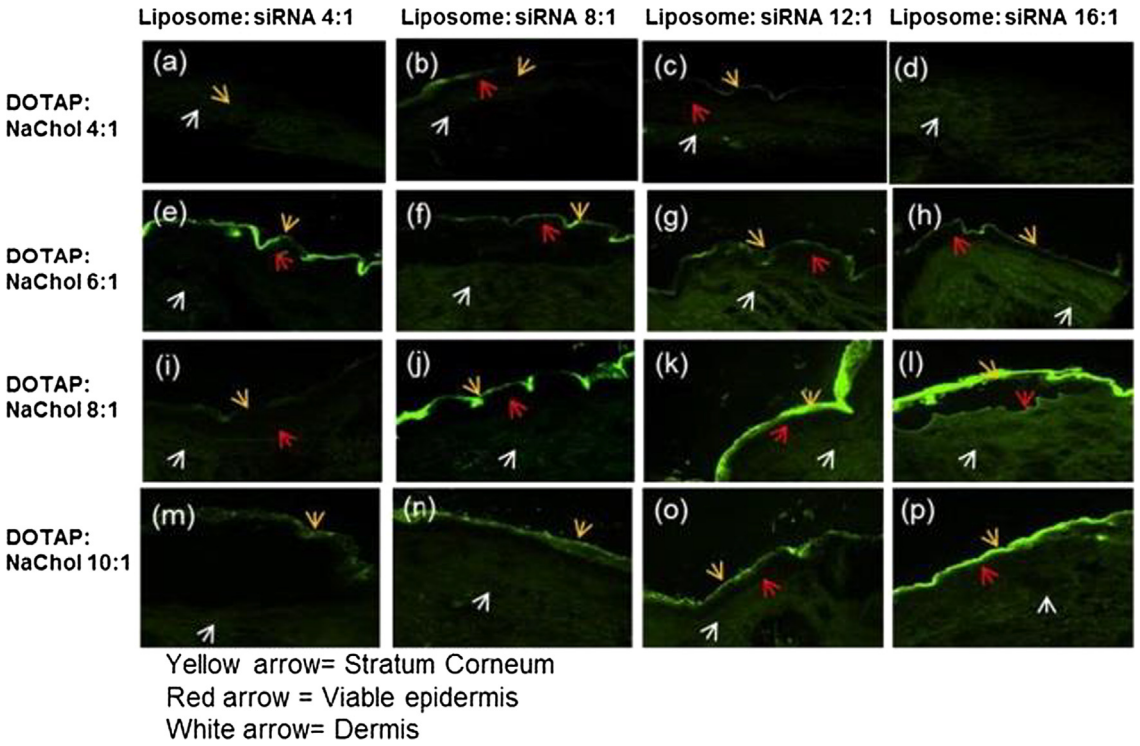

Fig. 6. Fluorescent microscopy images of liposomes (green) complexed with siRNA that permeated through skin layers. (a–d) Liposome:siRNA (Liposome:NaChol 4:1 ratio) complexes at w:w ratios of 4:1, 8:1, 12:1, 16:1, respectively. (e–h) Liposome:siRNA (Liposome:NaChol 6:1 ratio) complexes at w:w ratios of 4:1, 8:1, 12:1, 16:1, respectively. (i–l) Liposome:siRNA (Liposome:NaChol 8:1 ratio) complexes at w:w ratios of 4:1, 8:1, 12:1, 16:1, respectively. (m–p) Liposome:siRNA (Liposome:NaChol 10:1 ratio) complexes at w:w ratios of 4:1, 8:1, 12:1, 16:1, respectively. Reprinted with permission from Dorrani et al. [183]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

permeable (Fig. 5). In contrast to Transfersomes®, ethosomes appear to improve drug delivery under both occlusive and nonocclusive conditions [186].

Despite the fact that the mechanisms of action of ethosomes are not clear, authors agree on a synergy between several mechanisms. Hence, on the one hand, an interaction of ethanol with the polar group of lipids would cause an increase in the fluidity and a decrease in the density of the multilamellar bilayer by a reduction of the transition temperature of the lipids resulting in a destructuration of SC. On the other hand, ethosomes would fuse with skin lipids due to their malleability and fluidity resulting in a drug release into deeper skin layers by crossing the disturbed SC [13,151,185,187]. Also, ethanol could give a negative charge to ethosomes allowing an electrostatic repulsion avoiding the aggregation of vesicles and thus leading to a steric stabilization [188]. In addition, Manosroi et al. suggest that ethanol acts as a hydroxyl-radical scavenger protecting the drug and leading to an improved stability [189].

It is important to pinpoint that ethanol contents influence the permeation flux. Indeed, large amounts of ethanol enhance the permeation flux [190]. Additionally, ethanol affects the physicochemical properties of ethosomes. When ethanol is added to the formulation, the net surface charge of the vesicles decreases which can lead to smaller size and a favored passage through the skin [189]. Finally, ethanol could improve insertion of macromolecules such as siRNA within the bilayer membranes due to its destabilization effect on bilayers [20]. As the concentration of surfactant in ultradeformable liposomes, ethanol concentration in ethosomes is crucial. The common concentration of ethanol used in ethosome formulations is between 10% and 50% w/w [165,191].

Considering the advantages of cationic lipids, surfactants and ethanol, it is interesting to combine these techniques to obtain more valuable results. Geusens and co-workers [14,149] have developed a system named SECosomes (surfactant-ethanol-cholesterol-omes) composed of DOTAP to bind siRNA, cholesterol as stabilizer, NaChol as surfactant and 30% ethanol. Interestingly, it has been demonstrated very recently that SECosomes bearing siRNA targeting human beta-defensin 2 expression, [192] could deliver the siRNA to a skin-humanized mouse model of psoriasis [149]. Therefore, the concomitant use of several enhancement strategies seems to be useful for transport of nucleic acids through the skin and could be promising therapeutic tools to treat skin pathologies such as psoriasis.

5. Biomedical applications

The topical application of non-viral nanocarriers carrying different gene cargos (pDNA, siRNA, shRNA or miRNA) has been considered as a therapeutic strategy for several diseases.

Genetic skin diseases are a group of disease induced by genetic mutations, such as epidermolysis bullosa, xeroderma pigmentosum, pachyonychia congenita [193] or epidermolytic hyperkeratosis, dermatitis atopic [194–196], scleroderma [197], alopecia areata [198] or vitiligo [199] and are ideal candidates for gene therapy treatment. In this section, we focus on cutaneous applications of non-viral nanocarriers complexed to nucleic acids for topical pDNA vaccination, psoriasis, wound healing and skin cancers. The Table 4 summarizes the type of nanocarriers and genetic cargo involved in studies discussed according to these different diseases.

5.1. Plasmid DNA-vaccination

One of the fields in which topical gene delivery is greatly attractive is the needle-free skin vaccination. Instead of an intramuscular injection, needle-free vaccination offers two major advantages. First, cutaneous vaccination has a high potential from an immunologic facet since many Langerhans and dendritic cells are located in the epidermis and in the dermis. Secondly, skin vaccination is interesting in virtue of its ease of application and the avoidance of pain which will increase patient's compliance [200,201].

Despite these above-cited advantages, the impermeable SC is the major hurdle to skin vaccination. Physical methods to cross this barrier have been tested for skin vaccination such as MN, skin patches, tape stripping as well as active techniques like gene gun, liquid jet injection or electroporation [202,203]. They have demonstrated some attractive results but are associated to disadvantages as explained in Section 4.1. Moreover, these methods are often associated to passive strategies which consist of using drug delivery system (DDS) to increase the skin penetration. For instance, PEGylated liposomes combined to a multiple-needle tattoo device were proposed for the vaccination against influenza A [204] and poly(methyl methacrylate)-particles were associated to gene gun to target HPV infections [205]. All MN techniques developed for vaccination have been very elegantly summarized in a recent review [206].

The utilization of nanocarriers alone and mainly lipidic nanocarriers are also considered as the only way to cross the SC. Wang et al. developed deformable liposomes (made of phosphatidylcholine from soybean, DPPC and octadecylamine) complexed to pDNA encoding for the surface antigen (HBsAg) and their transfection efficacy was tested *versus* conventional liposomes in an *in vivo* mouse model. The deformable formulation can induce HBsAg translation in the same manner as pure recombinant HBsAg or naked pDNA applied by intramuscular injection whereas normal liposomes were not able to induce HBsAg expression [207]. The group of Vyas have also investigated the use of deformable liposomes and of pDNA-HBsAg for topical vaccination against hepatitis B and showed promising results [208,209]. Xu et al., have developed flexible liposomes with DOTMA and NaDChol in the context of respiratory syncytial virus (RSV) vaccination. They showed a significant anti-RSV IgG antibody response after topical application compared to intramuscular injection. Moreover, topical skin vaccination to treat pneumonia induced in mice following RSV challenge was associated with fewer histopathologic anomalies when compared to the intramuscular vaccination alternative. One has however to be careful in interpreting those results, since the flexible formulation was not compared to classic one, which makes it tricky to highlight the benefit of it [210].

Nowadays, it is difficult to discern whether physical methods combined to nanocarriers offer a real benefit for vaccination strategies. Although the use of nanoparticles without any other techniques is sufficient to cross the SC and target immune cells in the epidermis and dermis in order to induce immune response, it is obvious that lipid nanoparticles as liposomes are preferred for cutaneous vaccination since polymer-based carriers are very rarely used for this purpose. This observation is probably due to their lipid composition similar to the epidermis which enables them to penetrate the impermeable SC. For a complete review of nanocarriers-based DNA vaccines, we refer to Hansen et al., [201] and Geusens et al. [14,211].

5.2. Psoriasis

Psoriasis is considered as an inherited skin disease since this inflammatory dermatologic pathology is the result of genetic and environmental disorders [212]. This pathology is characterized by a dramatic hyperplasia of the epidermis, hyperkeratosis and parakeratosis. Different gene targets have been exploited for therapy but only those involving their delivery by nanoparticles are identified hereunder.

pDNA encoding for interleukin-4 (IL-4) was considered as a treatment option since subcutaneous injection of IL-4 induced the T helper 2 differentiation in CD4 + T cells which consequently showed a positive impact against psoriasis plaques [213]. While Zhang et al. associated pDNA IL-4 with DMSO (concentration of 10%) [214], Li et al. developed ultradeformable liposomes composed of DOTAP/DOPE/NaChol (8/8/4.16 mass ratio) complexed to pDNA in different molar ratios

Table 4
Type of nanocarriers and genetic cargo according to skin diseases.

Disease	Type of nanocarriers	Genetic cargo	References
Vaccines			
Hepatitis B	SPC/DPPC/octadecylamine/cholesterol	pDNA (HBsAg)	[207]
Hepatitis B	SPC/Span85/cholesterol	pDNA (HBsAg)	[208]
Hepatitis B	DOTMA/sodium deoxycholate	pDNA (HBsAg)	[209]
RSV (Pneumonia)	DOTMA/sodium cholate	pDNA (RSV-F)	[210]
Psoriasis			
	DOTAP/DOPE/sodium deoxycholate	pDNA (IL-4)	[215]
	Monoolein/oleic acid/PEI	siRNA (IL-6)	[216]
	DOTAP/Cholesterol/sodium cholate + 30% ethanol	siRNA (DEFB4)	[218,150,219,220]
	FuGENE®-6 (Promega Corporation)	pDNA (shRNA TNF-α)	[221]
	DOPyCl/DOPE/PC/DSPE-PEG_{2000}	siRNA (TNF-α + STAT3)	[222]
Wound healing			
	Lipofectamine®	pDNA (FGF1)	[227]
	DMRIE/cholesterol	pDNA (IGF1)	[228]
	DMRIE/cholesterol	pDNA (KGF)	[229]
	SNA-Gold nanoparticle	siRNA (ganglioside GM3S)	[230]
	DOTAP/sodium cholate/CSP protein	siRNA (Kelch ECH)	[231]
Skin cancer			
	/	siRNA (BRAF)	[233]
	Cationized gelatin microsphere	siRNA (VEGF)	[234]
	DOSPER® Liposomal Transfection Reagent (Roche Life Science)	pDNA (angiostatin)	[235]
	DPPC/DOPE/Stearylamine	pDNA (IL-12)	[236]
	DOTAP/DOPE/DSPE-PEG_{2000}	siRNA (B-Raf-Akt3)	[238]
	SNA-NCs	siRNA (EGFR)	[239]
	Cationic AuPT nanoparticle	pDNA (miRNA 221 inhibitor)	[240]

ranging from 1:1 to 8:1 [215]. Both of these studies used *in vitro* and *in vivo* assays to prove the ability of their formulations to express IL-4 following cutaneous application. For this, their pDNA-interleukin-4-containing preparations were applied in the dorsal ear skin of transgenic K14-VEGF mice, which spontaneously display phenotypic characteristics of psoriasis. One application of the ultradeformable liposomes per day during 31 consequent days already allowed repressing psoriasis markers such as ICAM, VCAM or VEGF-R2. Depieri et al. recently developed liquid crystalline nanodispersion with monoolein (MO), oleic acid (OA) and polyethylenimine (PEI) in an aqueous phase (AP: Tris-HCl buffer (pH 6.5, 0.1 M) with 1.5% of Poloxamer 407 in nuclease free water) and siRNA against the interleukin-6 (IL-6) to treat psoriasis-related inflammation [216]. Making use of reconstructed human epidermis (EpiDerm™), this article first emphasized cytotoxicity of used nanodispersions. Hence, since all gene cargos are highly negatively charged, nanovectors involved in their complexation are often positively charged and therefore could induce toxicity [217]. The induction of toxicity due to the positive charge of nanocarriers has already been highlighted by our group and thus has a huge importance in gene delivery [99]. Depieri et al. showed in their attractive work that the cytotoxicity increased with PEI concentrations as this polymer confers positive charges to the complex. Furthermore, confocal microscopy analyses illustrated that a safe formulation composed of MO:OA:PEI:AP (8:2:1:89) was able to cross the porcine ear SC compared to non-deformable carriers PEI:AP (1:99) (Fig. 7A). The effectiveness of anti-IL-6 siRNA was evaluated by ELISA in the supernatant of cells which composed the *in vitro* psoriasis skin model (MatTek Corporation). Therefore, they evaluated the action of siRNA but not the real impact of the formulation against psoriasis lesions *in vivo*. Finally, treatment of psoriasis by skin application of elastic liposomes carrying siRNA anti-DEFB4 has largely investigated. DEFB4 encodes for the human b defensin-2 (hBD-2) which is highly up-regulated in psoriatic lesions [218]. Previously, SECosomes made of DOTAP/cholesterol/NaChol (6:1:1 M ratio) and 30% of ethanol demonstrated their deformability behavior and their potential to cross the SC [149,219]. This formulation combined to siRNA anti-DEFB4, revealed a positive therapeutic effect in the skin-humanized mouse model for psoriasis [220]. Very recently, the increased effectiveness of the nanocarrier combined to different edge activators (NaChol, Tween® 20 or Tween® 80), DOPE and cholesterol [4] was proven. However, based on siRNA transfection effectiveness *in vitro* and penetration capacity in excised human skin, the best formulation selected (DDC642) is composed of DOTAP/DOPE/cholesterol (molar ratio 6/4.2/1.8) and 30% of ethanol. Surprisingly, this formulation does not contain any edge activator but only ethanol. Moreover, they show contradictory results compared to their previous study since the SECosome formulation cannot cross the excised skin contrarily to DDC642 formulation, both of them were able to cross psoriatic skin biopsies (Fig. 7.B). Overall, this study highlights the importance of ethanol in deformable formulation and questions about the real usefulness of edge-activators.

Ultimately, another target to treat psoriasis is the tumor necrosis factor-α (TNF-α) which is upregulated in psoriatic skin. Some authors have already tested the utilization of shRNA [221] or siRNA anti-TNF-α [222] and proved the potentiality of this target. The first clinical trial with TNF-α siRNA complexed in Spherical Nucleic Acid (SNA™) called AST-005 is now in progress. The Exicure company announced in 2016, the positive phase 1 data for topical psoriasis treatment [223,224].

5.3. Wound healing

Another purpose in which cutaneous gene therapy is suitable is regenerative medicine. Following tissue injury, wound repair is a dynamic process which involves cell-cell and cell-matrix interactions as well as proinflammatory cytokines, growth factors and chemokines secretions. When a disturbance happens during the repair process, the healing is not properly completed [225,226]. Clinically, these situations often arise among type 2 diabetes patients as non-healing ulcers and chronic scars appear on the plantar surface. Chronic nonhealing wounds can lead to further bacterial infections.

In this context, due to the impaired properties of the SC, we can hypothesize that the intervention of physical methods or of new drug delivery systems is not needed. However, as mentioned by Geusens et al., many studies involve subcutaneous injection in order to improve the efficacy of the gene cargo [14]. pDNA encoding for growth factors such as fibroblast growth factor (FGF)-1 [227], insulin-like growth factor (IGF)-1 [228] or keratinocyte growth factor (KGF) [229] can be

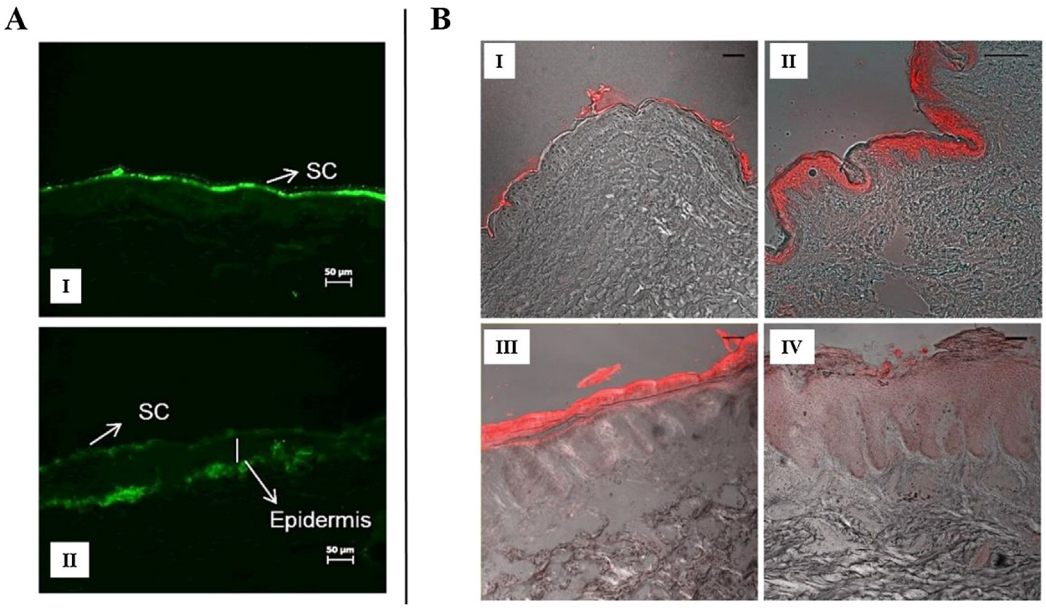

Fig. 7. (A) *Ex vivo* skin penetration of siRNA across porcine ear skin. Fluorescence microscopy of porcine ear skin sections after treatment for 12 h with a non-deformable carrier made of PEI (I) and deformable carrier made of PEI, monoolein and oleic acid (II), both complexed to FAM-GAPDH siRNA 10 µM. (B) Skin penetration capacity of siRNA in normal and lesioned psoriatic skin. Representative confocal images from skin biopsy cross sections, both of normal (I-II) and lesional psoriatic skin (III-IV) 6 h after incubation with Cy5-siRNA complexes of SECosomes (I,III) and DDC642 (II,IV). Reprinted with permission from [216] for picture A and [4] for picture B.

used. On the other hand, siRNA strategy has also been employed to increase wound repair in diabetic patients. In an experimental mouse model of type 2 diabetes, siRNA directed against the ganglioside-monosialic acid 3 synthase complexed to spherical nucleic acid particles led to accelerate wound closure [230]. After complexation with lipopolyplex, siRNA against Kelch like ECH-associated protein 1 penetrated deep into target tissues and accelerated the wound healing in a murine diabetic wound healing model [231].

5.4. Skin cancer

Cutaneous melanoma is one of the deadliest cancers and its incidence is rising steadily. Melanocytes are present in the junction between the epidermis and the dermis [232]. As for other type of cancers, several numbers of targets have been proposed for the treatment of melanoma using gene therapy [14]. Recently, miRNA-153-3p has been proposed as a potential biomarker for the diagnosis and the treatment of melanoma [59]. In melanoma mouse model, siRNA directed against the proto-oncogene BRAF [233] or against the promoter of angiogenesis VEGF [234] were effective in reducing melanoma size after subcutaneous or intratumoral injections, respectively. Liposomes complexed to pDNA coding for angiostatin [235] or IL-12 [236] have also been proposed as new targets but not yet tested through a cutaneous application neither. The administration of pDNA encoding for IL-12 was also considered against melanoma using physical methods such as gene gun [237] or electroporation [31]. However, examples about the use of non-viral carriers alone carrying gene therapy to treat skin cancers are few.

Tran et al., developed conventional liposomes made of DOTAP, DOPE and DSPE-PEG_{2000} (molar ratio 4.75:4.75:0.5) combined to siRNA (targeting ^{V600E}B-Raf and Akt3) at a weight ratio of 10:1. They showed that the delivery of siRNA-liposomal complexes decreased melanocytic lesions in mouse skin but only following ultrasound treatment to permeabilize the skin [238]. Spherical nucleic acid nanoparticle conjugates (SNA-NCs), which are gold cores coated with highly oriented oligonucleotides, were produced and complexed to anti-EGFR siRNA [239]. The topical delivery of this nanovector was recommended without any combination to barrier disruption methods for the treatment of cutaneous tumors and skin inflammation. After successful assessment of nanoparticle preparation, *in vitro* gene expression, *ex vivo* penetration in skin of nude mice, *in vivo* skin penetration, cationic nanocomplexes combined with pDNA encoding miRNA-221 inhibitor were validated to successfully cross all extracellular and intracellular barriers in the context of melanoma and to display long-awaited *in vivo* anti-tumor effects [240].

6. Conclusions and remaining challenges

The field of topical gene delivery is broad and could offer many therapeutic possibilities. Different genetic cargos have opposite actions since pDNA allows gene transcription whereas siRNA or shRNA knockdown a specific protein. Numbers of dermatologic diseases concerned by topical gene delivery can be very large. Although this novel gene delivery technique is contemplated for systemic administration, it gains the highest interest for topical diseases.

The development of lipid-based nanovectors and mainly liposomes is actually one of the best strategies for topical administration. Surprisingly, we found out that the most cationic lipid used is the DOTAP lipid which is quite old and known to present some toxicity. We can wonder why multiple lipids like pH-sensitive lipids, lipid-polyethylene glycol or cholesterol-derivatives which have been developed for gene delivery since many years, have never been considered for topical application. The combination of new lipids with edge activators, ethanol or cell-penetrating peptides has also never been envisioned. Moreover, the real benefit of edge activators or ethanol in the lipidic formulation remains to be proven. Advanced studies on penetration and intracellular behaviors of nanocarriers must be done and future efforts must focus on skin toxicity. To conclude, despite that the topical application of gene can substantially influence new therapies, many efforts are still needed to reach this expectative.

Author contributions

C. Bellefroid and A. Lechanteur conceived and wrote this review in

an equal manner. All authors read and approved the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

Authors would like to acknowledge the Fonds Leon Fredericq (Liege, Belgium), FRS-FNRS Télévie (Belgium) and SPW-DGO6-Compl FEDER (n°1510606).

References

[1] N. Nayerossadat, T. Maedeh, P.A. Ali, Viral and nonviral delivery systems for gene delivery, Adv. Biomed. Res. 1 (2012) 27, https://doi.org/10.4103/2277-9175.98152.
[2] X.X. Zhang, T.J. McIntosh, M.W. Grinstaff, Functional lipids and lipoplexes for improved gene delivery, Biochimie 94 (2012) 42–58, https://doi.org/10.1016/j.biochi.2011.05.005.
[3] 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, https://doi.org/10.1016/j.jconrel.2015.09.017.
[4] E. Desmet, S. Bracke, K. Forier, L. Taevernier, M.C. Stuart, B. De Spiegeleer, et al., 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, https://doi.org/10.1016/j.ijpharm.2016.01.042.
[5] K. Ita, Dermal/transdermal delivery of small interfering RNA and antisense oligonucleotides- advances and hurdles, Biomed. Pharmacother. 87 (2017) 311–320, https://doi.org/10.1016/j.biopha.2016.12.118.
[6] J.M. Meacham, K. Durvasula, F.L. Degertekin, A.G. Fedorov, Physical methods for intracellular delivery: practical aspects from laboratory use to industrial-scale processing, J. Lab. Autom. 19 (2014) 1–18, https://doi.org/10.1177/2211068213494388.
[7] O. Germershaus, K. Nultsch, Localized, non-viral delivery of nucleic acids: opportunities, challenges and current strategies, Asian J. Pharmaceut. Sci. 10 (2015) 159–175, https://doi.org/10.1016/j.ajps.2014.10.001.
[8] M. Elsabahy, M. Foldvari, Needle-free gene delivery through the skin: an overview of recent strategies, Curr. Pharm. Design 19 (2013) 7301–7315, https://doi.org/10.2174/13816128113199990369.
[9] M.-A. Bolzinger, S. Briançon, J. Pelletier, Y. Chevalier, Penetration of drugs through skin, a complex rate-controlling membrane, Curr. Opin. Colloid In. 17 (2012) 156–165, https://doi.org/10.1016/j.cocis.2012.02.001.
[10] Y. Shahzad, R. Louw, M. Gerber, J. du Plessis, Breaching the skin barrier through temperature modulations, J Control Release 202 (2015) 1–13, https://doi.org/10.1016/j.jconrel.2015.01.019.
[11] O.G. Jepps, Y. Dancik, Y.G. Anissimov, M.S. Roberts, Modeling the human skin barrier–towards a better understanding of dermal absorption, Adv. Drug. Deliv. Rev. 65 (2013) 152–168, https://doi.org/10.1016/j.addr.2012.04.003.
[12] G. Honari, H. Maibach, Skin Structure and Function, Applied Dermatotoxicology, Academic Press, 2014, pp. 1–10, , https://doi.org/10.1016/B978-0-12-420130-9.00001-3.
[13] N. Bibi, N. Ahmed, G. Khan, Nanostructures in transdermal drug delivery systems, in: A.M. Grumezescu (Ed.), Nanostructures in Transdermal Drug Delivery System, Elsevier, 2017, pp. 639–668.
[14] B. Geusens, T. Strobbe, S. Bracke, P. Dynoodt, N. Sanders, M. Van Gele, et al., Lipid-mediated gene delivery to the skin, Eur. J. Pharm. Sci. 43 (2011) 199–211, https://doi.org/10.1016/j.ejps.2011.04.003.
[15] X. Chen, Current and future technological advances in transdermal gene delivery, Adv. Drug Deliver. Rev. 127 (2018) 85–105, https://doi.org/10.1016/j.addr.2017.12.014.
[16] S. Munch, J. Wohlrab, R.H.H. Neubert, Dermal and transdermal delivery of pharmaceutically relevant macromolecules, Eur. J. Pharm. Biopharm. 119 (2017) 235–242, https://doi.org/10.1016/j.ejpb.2017.06.019.
[17] B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur. J. Pharm. Sci. 14 (2001) 101–114, https://doi.org/10.1016/S0928-0987(01)00167-1.
[18] S. Rietwyk, D. Peer, Next-generation lipids in RNA interference therapeutics, ACS Nano. 11 (2017) 7572–7586, https://doi.org/10.1021/acsnano.7b04734.
[19] A. Patzelt, W.C. Mak, S. Jung, F. Knorr, M.C. Meinke, H. Richter, et al., Do nanoparticles have a future in dermal drug delivery? J Control Release 246 (2017) 174–182, https://doi.org/10.1016/j.jconrel.2016.09.015.
[20] Y. Singh, S. Tomar, S. Khan, J.G. Meher, V.K. Pawar, K. Raval, et al., Bridging small interfering RNA with giant therapeutic outcomes using nanometric liposomes, J. Control Release 220 (2015) 368–387, https://doi.org/10.1016/j.jconrel.2015.10.050.
[21] J. McCaffrey, R.F. Donnelly, H.O. McCarthy, Microneedles: an innovative platform for gene delivery, Drug Deliv. Transl. Res. 5 (2015) 424–437, https://doi.org/10.1007/s13346-015-0243-1.
[22] M. Ogundele, H. Okafor, Microneedles: a potent modern tool in enhancing transdermal drug delivery, Curr. Trends Biomed. Eng. & Biosci. 10 (2017) 1–9, https://doi.org/10.19080/CTBEB.2017.10.555776.
[23] K. Van Der Maaden, W. Jiskoot, J. Bouwstra, Microneedle technologies for (trans) dermal drug and vaccine delivery, J. Control Release 161 (2012) 645–655, https://doi.org/10.1016/j.jconrel.2012.01.042.
[24] S.H. Bariya, M.C. Gohel, T.A. Mehta, O.P. Sharma, Microneedles: an emerging transdermal drug delivery system, J. Pharm. Pharmacol. 64 (2012) 11–29, https://doi.org/10.1111/j.2042-7158.2011.01369.x.
[25] M.N. Primo, R.O. Bak, J.G. Mikkelsen, Lentiviral vectors for cutaneous RNA managing, Exp. Dermatol. 21 (2012) 162–170, https://doi.org/10.1111/j.1600-0625.2011.01436.x.
[26] W. Yin, P. Xiang, Q. Li, Investigations of the effect of DNA size in transient transfection assay using dual luciferase system, Anal. Biochem. 346 (2005) 289–294, https://doi.org/10.1016/j.ab.2005.08.029.
[27] J.A. Williams, Vector design for improved DNA vaccine efficacy, safety and production, Vaccines 1 (2013) 225–249, https://doi.org/10.3390/vaccines1030225.
[28] J. Mairhofer, R. Grabherr, Rational vector design for efficient non-viral gene delivery: challenges facing the use of plasmid DNA, Mol. Biotechnol. 39 (2008) 97–104, https://doi.org/10.1007/s12033-008-9046-7.
[29] J.A. Williams, A.E. Carnes, C.P. Hodgson, Plasmid DNA vaccine vector design: impact on efficacy, safety and upstream production, Biotechnol. Adv. 27 (2009) 353–370, https://doi.org/10.1016/j.biotechadv.2009.02.003.
[30] M. Foldvari, D. Weng Chen, N. Nafissi, D. Calderon, L. Narsineni, A. Rafiee, Non-viral gene therapy: gains and challenges of non-invasive administration methods, J. Control Release 240 (2016) 165–190, https://doi.org/10.1016/j.jconrel.2015.12.012.
[31] M.L. Lucas, L. Heller, D. Coppola, R. Heller, IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma, Mol. Ther. 5 (2002) 668–675, https://doi.org/10.1006/mthe.2002.0601.
[32] A.I. Daud, R.C. DeConti, S. Andrews, P. Urbas, A.I. Riker, V.K. Sondak, et al., Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma, J. Clin. Oncol. 26 (2008) 5896–5903, https://doi.org/10.1200/JCO.2007.15.6794.
[33] J. Chan, S. Lim, W. Wong, Antisense oligonucleotides: from design to therapeutic application, Clin. Exp. Pharmacol. Physiol. 33 (5-6) (2006) 533–540, https://doi.org/10.1111/j.1440-1681.2006.04403.x.
[34] N. Dias, C.A. Stein, Antisense oligonucleotides: basic concepts and mechanisms, Mol. Cancer Ther. 1 (2002) 347–355.
[35] R.S. Geary, D. Norris, R. Yu, C.F. Bennett, Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides, Adv. Drug Deliv. Rev. 87 (2015) 46–51, https://doi.org/10.1016/j.addr.2015.01.008.
[36] T.L. Jason, J. Koropatnick, R.W. Berg, Toxicology of antisense therapeutics, Toxicol. Appl. Pharm. 201 (2004) 66–83, https://doi.org/10.1016/j.taap.2004.04.017.
[37] X. Chi, P. Gatti, T. Papoian, Safety of antisense oligonucleotide and siRNA-based therapeutics, Drug Discov. Today 22 (2017) 823–833, https://doi.org/10.1016/j.drudis.2017.01.013.
[38] S.T. Crooke, Progress in antisense technology, Annu. Rev. Med. 55 (2004) 61–95, https://doi.org/10.1146/annurev.med.55.091902.104408.
[39] A. Fire, S. Xu, M. Montgomery, S. Kostas, S. Driver, C. Mello, Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans, Nature 391 (1998) 806–811, https://doi.org/10.1038/35888.
[40] J. Conde, A. Ambrosone, Y. Hernandez, F. Tian, M. McCully, C.C. Berry, et al., 15 years on siRNA delivery: beyond the State-of-the-Art on inorganic nanoparticles for RNAi therapeutics, Nano Today 10 (2015) 411–532, https://doi.org/10.1016/j.nantod.2015.06.008.
[41] M. Gujrati, Z.-R. Lu, Gene Therapy of Cancer. Chapter 4: Targeted Systemic Delivery of Therapeutic siRNA, in: E. Lattime, S. Gerson (Eds.), 2014. pp. 47–65. DOI: 10.1016/b978-0-12-394295-1.00004-4.
[42] D.D. Rao, J.S. Vorhies, N. Senzer, J. Nemunaitis, siRNA vs. shRNA: similarities and differences, Adv. Drug Deliv. Rev. 61 (2009) 746–759, https://doi.org/10.1016/j.addr.2009.04.004.
[43] D. Castanotto, J.J. Rossi, The promises and pitfalls of RNA-interference-based therapeutics, Nature 457 (2009) 426–433, https://doi.org/10.1038/nature07758.
[44] M. Videira, A. Arranja, D. Rafael, R. Gaspar, Preclinical development of siRNA therapeutics: towards the match between fundamental science and engineered systems, Nanomedicine 10 (2013) 689–702, https://doi.org/10.1016/j.nano.2013.11.018.
[45] C.B. Moore, E.H. Guthrie, M.T. Huang, D.J. Taxman, Short hairpin RNA (shRNA): design, delivery, and assessment of gene knockdown, Methods Mol. Biol. 629 (2010) 141–158, https://doi.org/10.1007/978-1-60761-657-3_10.
[46] D.H. Kim, M.A. Behlke, S.D. Rose, M.S. Chang, S. Choi, J.J. Rossi, Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy, Nat. Biotechnol. 23 (2005) 222–226, https://doi.org/10.1038/nbt1051.
[47] A.L. Jackson, J. Burchard, J. Schelter, B.N. Chau, M. Cleary, L. Lim, et al., Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity, RNA 12 (2006) 1179–1187, https://doi.org/10.1261/rna.25706.
[48] A. Jackson, S. Bartz, J. Schelter, S. Kobayashi, J. Burchard, M. Mao, et al., Expression profiling reveals off-target gene regulation by RNAi, Nat. Biotechnol. 21 (2003) 635, https://doi.org/10.1038/nbt831.
[49] A. Birmingham, E.M. Anderson, A. Reynolds, D. Ilsley-Tyree, D. Leake, Y. Fedorov, et al., 3' UTR seed matches, but not overall identity, are associated with RNAi off-targets, Nat. Methods 3 (2006) 199–204, https://doi.org/10.1038/nmeth854.
[50] C. Sledz, M. Holko, M. de Veer, R. Silverma, B. Williams, Activation of the

interferon system by short-interfering RNAs, Nat. Cell Biol. 5 (2003) 834–839, https://doi.org/10.1038/ncb1038.
[51] R. Petrilli, J.O. Eloy, M.C. de Souza, J.P. Abriata Barcellos, J.M. Marchetti, B. Yung, et al., Nanobiomaterials in Drug Delivery: Applications of Nanobiomaterials. Chapter 3: Lipid nanoparticles as non-viral vectors for siRNA delivery, A. Grumezescu (Ed.), 2016. 75–109 DOI: 10.1016/b978-0-323-42866-8.00003-4.
[52] A.L. Jackson, J. Burchard, D. Leake, A. Reynolds, J. Schelter, J. Guo, et al., Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing, RNA 12 (2006) 1197–1205, https://doi.org/10.1261/rna.30706.
[53] C. Chakraborty, A. Sharma, G. Sharma, C.G. Priya Doss, S.-S. Lee, Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine, Mol. Ther. 8 (2017) 132–143, https://doi.org/10.1016/j.omtn.2017.06.005.
[54] G.A. Calin, C.D. Dumitru, M. Shimizu, R. Bichi, S. Zupo, E. Noch, et al., Frequent deletions and down-regulation of microRNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia, Proc. Natl. Acad. Sci. USA 99 (2002) 15524–15529, https://doi.org/10.1073/pnas.242606799.
[55] M. Ishida, F.M. Selaru, miRNA-based therapeutic strategies, Curr. Anesthesiol. Rep. 1 (2013) 63–70, https://doi.org/10.1007/s40139-012-0004-5.
[56] Y. Chen, D.Y. Gao, L. Huang, In vivo delivery of miRNAs for cancer therapy: challenges and strategies, Adv. Drug Deliv. Rev. 81 (2015) 128–141, https://doi.org/10.1016/j.addr.2014.05.009.
[57] M. Lindow, S. Kauppinen, Discovering the first microRNA-targeted drug, J. Cell Biol. 199 (2012) 407–412, https://doi.org/10.1083/jcb.201208082.
[58] H. Hermann, T. Runnel, A. Aab, H. Baurecht, E. Rodriguez, N. Magilnick, et al., miR-146b Probably Assists miRNA-146a in the suppression of keratinocyte proliferation and inflammatory responses in psoriasis, J. Invest Dermatol. 137 (2017) 1945–1954, https://doi.org/10.1016/j.jid.2017.05.012.
[59] H.F. Zeng, S. Yan, S.F. Wu, MicroRNA-153-3p suppress cell proliferation and invasion by targeting SNAI1 in melanoma, Biochem. Biophys. Res. Co. 487 (2017) 140–145, https://doi.org/10.1016/j.bbrc.2017.04.032.
[60] M. Svoboda, Z. Bilkova, T. Muthny, Could tight junctions regulate the barrier function of the aged skin? J. Dermatol. Sci. 81 (2016) 147–152, https://doi.org/10.1016/j.jdermsci.2015.11.009.
[61] Elias, Epidermal lipids, barrier function and desquamation, J. Invest. Dermatol. 80 (1983) 44–49, https://doi.org/10.1038/jid.1983.12.
[62] J.S. Barbieri, K. Wanat, J. Seykora, Skin: basic structure and function, Pathobiol. Human Disease (2014) 1134–1144, https://doi.org/10.1016/b978-0-12-386456-7.03501-2.
[63] J.E. Lai-Cheong, J.A. McGrath, Structure and function of skin, hair and nails, Medicine 45 (2017) 347–351, https://doi.org/10.1016/j.mpmed.2017.03.004.
[64] M.A. Fung, Inflammatory Diseases of the Dermis and Epidermis, in Dermatopathology, in: K.J. Busam (Ed.), W.B. Saunders, Philadelphia, 2010. pp. 11–81. DOI: 10.1016/B978-0-443-06654-2.00001-9.
[65] D.Y.M. Leung, M. Boguniewicz, Atopic Dermatitis and Allergic Contact Dermatitis, in: S.T. Holgate, A. Sheikh (Eds.), Middleton's Allergy Essentials, Elsevier, 2017, pp. 265–300 DOI: 10.1016/b978-0-323-37579-5.00011-8.
[66] M.C. Simoes, J.J. Sousa, A.A. Pais, Skin cancer and new treatment perspectives: a review, Cancer Lett. 357 (2015) 8–42, https://doi.org/10.1016/j.canlet.2014.11.001.
[67] K. Taghipour, G.K. Perera, Autoimmune blistering skin diseases, Medicine 45 (2017) 405–412, https://doi.org/10.1016/j.mpmed.2017.04.007.
[68] M.S. Roberts, S.E. Cross, Y.G. Anissimov, The skin reservoir for topically applied solutes, in: R.L. Bronaugh, H.I. Maibach (Eds.), Percutaneous Absorption, Marcel Dekker, 2005, pp. 213–234.
[69] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug. Deliver Rev. 64 (2012) 4–17, https://doi.org/10.1016/j.addr.2012.09.019.
[70] G.M. El Maghraby, B.W. Barry, A.C. Williams, Liposomes and skin: from drug delivery to model membranes, Eur. J. Pharm. Sci. 34 (2008) 203–222, https://doi.org/10.1016/j.ejps.2008.05.002.
[71] J. Lademann, N. Otberg, U. Jacobi, R.M. Hoffman, U. Blume-Peytavi, Follicular penetration and targeting, J. Investig. Dermatol. Symp. Proc. 10 (2005) 301–303, https://doi.org/10.1111/j.1087-0024.2005.10121.x.
[72] J. Lademann, F. Knorr, H. Richter, U. Blume-Peytavi, A. Vogt, C. Antoniou, et al., Hair follicles-an efficient storage and penetration pathway for topically applied substances. Summary of recent results obtained at the Center of Experimental and Applied Cutaneous Physiology, Charite -Universitatsmedizin Berlin, Germany, Skin Pharmacol. Physiol. 21 (2008) 150–155, https://doi.org/10.1159/000131079.
[73] J. Lademann, H. Richter, A. Teichmann, N. Otberg, U. Blume-Peytavi, J. Luengo, et al., Nanoparticles-an efficient carrier for drug delivery into the hair follicles, Eur. J. Pharm. Biopharm. 66 (2007) 159–164, https://doi.org/10.1016/j.ejpb.2006.10.019.
[74] A. Vogt, N. Mandt, J. Lademann, H. Schaefer, U. Blume-Peytavi, Follicular targeting–a promising tool in selective dermatotherapy, J. Investig. Dermatol. Symp. Proc. 10 (2005) 252–255, https://doi.org/10.1111/j.1087-0024.2005.10124.x.
[75] T. Ogiso, T. Shiraki, K. Okajima, T. Tanino, M. Iwaki, T. Wada, Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of penetration between scalp and abdominal skins in vitro, J. Drug Target. 10 (2002) 369–378, https://doi.org/10.1080/1061186021000001814.
[76] R.H. Neubert, Potentials of new nanocarriers for dermal and transdermal drug delivery, Eur. J. Pharm. Biopharm. 77 (2011) 1–2, https://doi.org/10.1016/j.ejpb.2010.11.003.
[77] R. Alvarez-Roman, A. Naik, Y.N. Kalia, R.H. Guy, H. Fessi, Skin penetration and distribution of polymeric nanoparticles, J. Control Release 99 (2004) 53–62, https://doi.org/10.1016/j.jconrel.2004.06.015.
[78] Y. Wang, L. Miao, A. Satterlee, L. Huang, Delivery of oligonucleotides with lipid nanoparticles, Adv. Drug Deliv. Rev. 87 (2015) 68–80, https://doi.org/10.1016/j.addr.2015.02.007.
[79] A. Elouahabi, J. Ruysschaert, Formation and intracellular trafficking of lipoplexes and polyplexes, Mol. Ther. 11 (2005) 336–347, https://doi.org/10.1016/j.ymthe.2004.12.006.
[80] Z. Rehman, I.S. Zuhorn, D. Hoekstra, How cationic lipids transfer nucleic acids into cells and across cellular membranes: recent advances, J. Control Release. 166 (2013) 46–56, https://doi.org/10.1016/j.jconrel.2012.12.014.
[81] L.M. Bareford, P.W. Swaan, Endocytic mechanisms for targeted drug delivery, Adv. Drug. Deliv. Rev. 59 (2007) 748–758, https://doi.org/10.1016/j.addr.2007.06.008.
[82] Z. Rehman, D. Hoekstra, I.S. Zuhorn, Protein kinase A inhibition modulates the intracellular routing of gene delivery vehicles in HeLa cells, leading to productive transfection, J. Control Release 156 (2011) 76–84, https://doi.org/10.1016/j.jconrel.2011.07.015.
[83] L. Wasungu, D. Hoekstra, Cationic lipids, lipoplexes and intracellular delivery of genes, J Control Release 116 (2006) 255–264, https://doi.org/10.1016/j.jconrel.2006.06.024.
[84] K.L. Douglas, C.A. Piccirillo, M. Tabrizian, Cell line-dependent internalization pathways and intracellular trafficking determine transfection efficiency of nanoparticle vectors, Eur. J. Pharm. Biopharm. 68 (2008) 676–687, https://doi.org/10.1016/j.ejpb.2007.09.002.
[85] D. Vercauteren, J. Rejman, T.F. Martens, J. Demeester, S.C. De Smedt, K. Braeckmans, On the cellular processing of non-viral nanomedicines for nucleic acid delivery: mechanisms and methods, J. Control Release 161 (2012) 566–581, https://doi.org/10.1016/j.jconrel.2012.05.020.
[86] A. Frere, M. Kawalec, S. Tempelaar, P. Peixoto, E. Hendrick, O. Peulen, et al., Impact of the structure of biocompatible aliphatic polycarbonates on siRNA transfection ability, Biomacromolecules 16 (2015) 769–779, https://doi.org/10.1021/bm501676p.
[87] A. Frere, A. Baroni, E. Hendrick, A.S. Delvigne, F. Orange, O. Peulen, et al., PEGylated and functionalized aliphatic polycarbonate polyplex nanoparticles for intravenous administration of HDAC5 siRNA in cancer therapy, ACS Appl. Mater. Inter. 9 (2017) 2181–2195, https://doi.org/10.1021/acsami.6b15064.
[88] P. Resnier, T. Montier, V. Mathieu, J.P. Benoit, C. Passirani, A review of the current status of siRNA nanomedicines in the treatment of cancer, Biomaterials 34 (2013) 6429–6443, https://doi.org/10.1016/j.biomaterials.2013.04.060.
[89] B. Yameen, W.I. Choi, C. Vilos, A. Swami, J. Shi, O.C. Farokhzad, Insight into nanoparticle cellular uptake and intracellular targeting, J Control Release 190 (2014) 485–499, https://doi.org/10.1016/j.jconrel.2014.06.038.
[90] D. Harries, S. May, W.M. Gelbart, A. Ben-Shaul, Structure, stability, and thermodynamics of lamellar DNA-lipid complexes, Biophys. J. 75 (1998) 159–173, https://doi.org/10.1016/S0006-3495(98)77503-4.
[91] S. Mochizuki, N. Kanegae, K. Nishina, Y. Kamikawa, K. Koiwai, H. Masunaga, et al., The role of the helper lipid dioleoylphosphatidylethanolamine (DOPE) for DNA transfection cooperating with a cationic lipid bearing ethylenediamine, Biochim. Biophys. Acta 1828 (2013) 412–418, https://doi.org/10.1016/j.bbamem.2012.10.017.
[92] Z. Du, M.M. Munye, A.D. Tagalakis, M.D. Manunta, S.L. Hart, The role of the helper lipid on the DNA transfection efficiency of lipopolyplex formulations, Sci. Rep. 4 (2014) 7107, https://doi.org/10.1038/srep07107.
[93] R. Kanasty, J.R. Dorkin, A. Vegas, D. Anderson, Delivery materials for siRNA therapeutics, Nat. Mater. 12 (2013) 967–977, https://doi.org/10.1038/nmat3765.
[94] J. Smisterova, A. Wagenaar, M.C. Stuart, E. Polushkin, G. ten Brinke, R. Hulst, et al., Molecular shape of the cationic lipid controls the structure of cationic lipid/dioleylphosphatidylethanolamine-DNA complexes and the efficiency of gene delivery, J. Biol. Chem. 276 (2001) 47615–47622, https://doi.org/10.1074/jbc.M106199200.
[95] X. Cheng, R.J. Lee, The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery, Adv. Drug Deliv. Rev. 99 (2016) 129–137, https://doi.org/10.1016/j.addr.2016.01.022.
[96] I. Koltover, T. Salditt, J.O. Rädler, C.R. Safinya, An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery, Science 281 (1998) 78–81, https://doi.org/10.1126/science.281.5373.78.
[97] A. Frère, B. Evrard, D. Mottet, G. Piel, Polymeric Nanoparticles as siRNA Drug Delivery System for Cancer Therapy: The Long Road to Therapeutic Efficiency, in: Nanoarchitectonics for Smart Delivery and Drug Targeting, 2016, pp. 503–540, https://doi.org/10.1016/b978-0-323-47347-7.00018-5.
[98] A. Lechanteur, T. Furst, B. Evrard, P. Delvenne, P. Hubert, G. Piel, Development of anti-E6 pegylated lipoplexes for mucosal application in the context of cervical preneoplastic lesions, Int. J. Pharm. 483 (2015) 268–277, https://doi.org/10.1016/j.ijpharm.2015.02.041.
[99] A. Lechanteur, T. Furst, B. Evrard, P. Delvenne, P. Hubert, G. Piel, PEGylation of lipoplexes: the right balance between cytotoxicity and siRNA effectiveness, Eur. J. Pharm. Sci. 93 (2016) 493–503, https://doi.org/10.1016/j.ejps.2016.08.058.
[100] Y.S. Lee, S.W. Kim, Bioreducible polymers for therapeutic gene delivery, J. Control Release 190 (2014) 424–439, https://doi.org/10.1016/j.jconrel.2014.04.012.
[101] E. Vaughan, J.V. DeGiulio, D.A. Dean, Intracellular trafficking of plasmids for gene therapy: mechanisms of cytoplasmic movement and nuclear import, Curr. Gene Ther. 6 (2006) 671–681, https://doi.org/10.2174/156652306779010688.
[102] G. Lukacs, P. Haggie, O. Seksek, D. Lechardeur, N. Freedman, A.S. Verkman, Size-dependent DNA Mobility in Cytoplasm and Nucleus, J. Biol. Chem. 275 (2000) 1625–1629, https://doi.org/10.1074/jbc.275.3.1625.

[103] H. Pollard, G. Toumaniantz, J. Amos, D. Ascande, Ca2+ - Sensitive cytosolic nucleases prevent efficient delivery to the nucleus of injected plasmids, J. Gene Med. 3 (2001) 153–164, https://doi.org/10.1002/jgm.160.
[104] E.E. Vaughan, D.A. Dean, Intracellular trafficking of plasmids during transfection is mediated by microtubules, Mol. Ther. 13 (2006) 422–428, https://doi.org/10.1016/j.ymthe.2005.10.004.
[105] A. Mesika, V. Kiss, V. Brumfled, G. Ghosh, Z. Reich, Enhanced intracellular mobility and nuclear accumulation of DNA plasmids associated with a karyophilic protein, Hum. Gene Ther. 16 (2005) 200–208, https://doi.org/10.1089/hum.2005.16.200.
[106] A. Coonrod, F. Li, M. Horwitz, On the mechanism of DNA transfection: efficient gene transfer without viruses, Gene Ther. 4 (1997) 1313–1321, https://doi.org/10.1038/sj.gt.3300536.
[107] A. Fasbender, J. Zabner, B. Zeiher, M. Welsh, A low rate of cell proliferation and reduced DNA uptake limit cationon lipid-mediated gene transfer to primary cultures of ciliated human airway epithelia, Gene Ther. 4 (1997) 1173–1180, https://doi.org/10.1038/sj.gt.3300524.
[108] D.A. Dean, D.D. Strong, W.E. Zimmer, Nuclear entry of nonviral vectors, Gene Ther. 12 (2005) 881–890, https://doi.org/10.1038/sj.gt.3302534.
[109] D. Dean, Import of plasmid DNA into the nucleus is sequence specific, Exp. Cell Res. 230 (1997) 293–302, https://doi.org/10.1006/excr.1996.3427.
[110] A. Tsuchiya, J.H. Kang, T. Mori, Y. Naritomi, S. Kushio, T. Niidome, et al., Efficient delivery of signal-responsive gene carriers for disease-specific gene expression via bubble liposomes and sonoporation, Colloids Surf B Biointerfaces 160 (2017) 60–64, https://doi.org/10.1016/j.colsurfb.2017.09.021.
[111] B. Combadiere, B. Mahe, Particle-based vaccines for transcutaneous vaccination, Comp. Immunol. Microbiol. 31 (2008) 293–315, https://doi.org/10.1016/j.cimid.2007.07.015.
[112] C.M. Newman, T. Bettinger, Gene therapy progress and prospects: ultrasound for gene transfer, Gene Ther. 14 (2007) 465–475, https://doi.org/10.1038/sj.gt.3302925.
[113] F. Lin, X. Shen, J.R. McCoy, J.M. Mendoza, J. Yan, S.V. Kemmerrer, et al., A novel prototype device for electroporation-enhanced DNA vaccine delivery simultaneously to both skin and muscle, Vaccine 29 (2011) 6771–6780, https://doi.org/10.1016/j.vaccine.2010.12.057.
[114] J.J. Drabick, J. Glasspool-Malone, A. King, R.W. Malone, Cutaneous transfection and immune responses to intradermal nucleic acid vaccination are significantly enhanced by in vivo electropermeabilization, Mol. Ther. 3 (2001) 249–255, https://doi.org/10.1006/mthe.2000.0257.
[115] M. Foldvari, D.W. Chen, N. Nafissi, D. Calderon, L. Narsineni, A. Rafiee, Non-viral gene therapy: Gains and challenges of non-invasive administration methods, J. Control Release 240 (2016) 165–190, https://doi.org/10.1016/j.jconrel.2015.12.012.
[116] X. Guo, L. Huang, Recent advances in nonviral vectors for gene delivery, Acc. Chem. Res. 45 (2012) 971–979, https://doi.org/10.1021/ar200151m.
[117] M.A. Mintzer, Nonviral vectors for gene delivery, Chem. Rev. 109 (2009) 259–302, https://doi.org/10.1021/cr800409e.
[118] B. Thapa, R. Narain, Mechanism, current challenges and new approaches for non viral gene delivery, Polymers and Nanomaterials for Gene Therapy, Woodhead Publishing, 2016, pp. 1–27, , https://doi.org/10.1016/B978-0-08-100520-0.00001-1.
[119] B. Singh, R.K. Khurana, A. Jain, R. Kaur, R. Kumar, Microporation and nanoporation for effective delivery of drugs and genes, in: M. Vijay (Ed.), Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes, Elsevier, 2017, pp. 485–514 https://doi.org/10.1016/b978-0-12-809717-5.00004-x.
[120] F. Chabri, K. Bouris, T. Jones, D. Barrow, A. Hann, C. Allender, et al., Microfabricated silicon microneedles for nonviral cutaneous gene delivery, Brit. J. Dermatol. 150 (2004) 869–877, https://doi.org/10.1111/j.1365-2133.2004.05921.x.
[121] S. Coulman, D. Barrow, A. Anstey, C. Gateley, A. Morrissey, N. Wilke, et al., Minimally invasive cutaneous delivery of macromolecules and plasmid DNA via microneedles, Curr. Drug Deliv. 3 (2006) 65–75, https://doi.org/10.2174/156720106775197510.
[122] M. Pearton, V. Saller, S.A. Coulman, C. Gateley, A.V. Anstey, V. Zarnitsyn, et al., Microneedle delivery of plasmid DNA to living human skin: Formulation coating, skin insertion and gene expression, J Control Release. 160 (2012) 561–569, https://doi.org/10.1016/j.jconrel.2012.04.005.
[123] E. Gonzalez-Gonzalez, T.J. Speaker, R.P. Hickerson, R. Spitler, M.A. Flores, D. Leake, et al., Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD), Mol. Ther. 18 (2010) 1667–1674, https://doi.org/10.1038/mt.2010.126.
[124] Y.C. Kim, F.S. Quan, R.W. Compans, S.M. Kang, M.R. Prausnitz, Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity, J. Control Release 142 (2010) 187–195, https://doi.org/10.1016/j.jconrel.2009.10.013.
[125] L. Guo, Y. Qiu, J. Chen, S. Zhang, B. Xu, Y. Gao, Effective transcutaneous immunization against hepatitis B virus by a combined approach of hydrogel patch formulation and microneedle arrays, Biomed. Microdevices 15 (2013) 1077–1085, https://doi.org/10.1007/s10544-013-9799-z.
[126] Dongfeng Yin, W. Liang, Shuxing Xing, Zhixiang Gao, Wei Zhang, Zhili Guo, Shen Gao, Hepatitis B DNA vaccine-polycation nano-complexes enhancing immune response by percutaneous administration with microneedle, Biol. Pharm. Bull. 36 (2013) 1283–1291, https://doi.org/10.1248/bpb.b13-00050.
[127] C. Edens, M.L. Collins, J.L. Goodson, P.A. Rota, M.R. Prausnitz, A microneedle patch containing measles vaccine is immunogenic in non-human primates, Vaccine 33 (2015) 4712–4718, https://doi.org/10.1016/j.vaccine.2015.02.074.
[128] K. Matsuo, S. Hirobe, Y. Yokota, Y. Ayabe, M. Seto, Y.S. Quan, et al., Transcutaneous immunization using a dissolving microneedle array protects against tetanus, diphtheria, malaria, and influenza, J. Control Release 160 (2012) 495–501, https://doi.org/10.1016/j.jconrel.2012.04.001.
[129] J.B. Carey, A. Vrdoljak, C. O'Mahony, A.V. Hill, S.J. Draper, A.C. Moore, Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route, Sci. Rep. 4 (2014) 6154, https://doi.org/10.1038/srep06154.
[130] H.W. Yang, L. Ye, X.D. Guo, C. Yang, R.W. Compans, M.R. Prausnitz, Ebola vaccination using a DNA vaccine coated on PLGA-PLL/gammaPGA nanoparticles administered using a microneedle patch, Adv. Healthc. Mater. 6 (2017), https://doi.org/10.1002/adhm.201600750.
[131] 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, https://doi.org/10.1016/j.biomaterials.2011.06.058.
[132] A. Jose, S. Labala, V.V. Venuganti, Co-delivery of curcumin and STAT3 siRNA using deformable cationic liposomes to treat skin cancer, J. Drug Target 25 (2017) 330–341, https://doi.org/10.1080/1061186X.2016.1258567.
[133] S. Labala, A. Jose, S.R. Chawla, M.S. Khan, S. Bhatnagar, O.P. Kulkarni, et al., Effective melanoma cancer suppression by iontophoretic co-delivery of STAT3 siRNA and imatinib using gold nanoparticles, Int. J. Pharm. 525 (2017) 407–417, https://doi.org/10.1016/j.ijpharm.2017.03.087.
[134] V.V. 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, https://doi.org/10.1039/c4nr05241b.
[135] 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, https://doi.org/10.7150/thno.19894.
[136] 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 265 (2017) 66–74, https://doi.org/10.1016/j.jconrel.2017.04.027.
[137] Y. Hu, B. Xu, J. Xu, D. Shou, E. Liu, J. Gao, et al., Microneedle-assisted dendritic cell-targeted nanoparticles for transcutaneous DNA immunization, Polym. Chem. 6 (2015) 373–379, https://doi.org/10.1039/c4py01394h.
[138] M.S. Roberts, Y. Mohammed, M.N. Pastore, S. Namjoshi, S. Yousef, A. Alinaghi, et al., Topical and cutaneous delivery using nanosystems, J. Control Release 247 (2017) 86–105, https://doi.org/10.1016/j.jconrel.2016.12.022.
[139] T. Lehto, K. Ezzat, M.J.A. Wood, S. El Andaloussi, Peptides for nucleic acid delivery, Adv. Drug Deliv. Rev. 106 (2016) 172–182, https://doi.org/10.1016/j.addr.2016.06.008.
[140] C. Carrillo, N. Sánchez-Hernández, E. García-Montoya, P. Pérez-Lozano, J.M. Suñé-Negre, J.R. Ticó, et al., DNA delivery via cationic solid lipid nanoparticles (SLNs), Eur. J. Pharm. Sci. 49 (2013) 157–165, https://doi.org/10.1016/j.ejps.2013.02.011.
[141] M. Sala, R. Diab, A. Elaissari, H. Fessi, Lipid nanocarriers as skin drug delivery systems: properties, mechanisms of skin interactions and medical applications, Int. J. Pharm. 535 (2018) 1–17, https://doi.org/10.1016/j.ijpharm.2017.10.046.
[142] G. Bozzuto, A. Molinari, Liposomes as nanomedical devices, Int. J. Nanomed. 10 (2015) 975–999, https://doi.org/10.2147/IJN.S68861.
[143] K. Egbaria, W. Weiner, Liposomes as a topical drug delivery system, Adv. Drug Deliv. Rev. 5 (1990) 287–300, https://doi.org/10.1016/0169-409X(90)90021-J.
[144] A. Gillet, B. Evrard, G. Piel, Liposomes and parameters affecting their skin penetration behaviour, J. Drug Deliv. Sci. Tec. 21 (2011) 35–42, https://doi.org/10.1016/s1773-2247(11)50004-8.
[145] C. Chen, D. Han, C. Cai, X. Tang, An overview of liposome lyophilization and its future potential, J. Control Release 142 (2010) 299–311, https://doi.org/10.1016/j.jconrel.2009.10.024.
[146] S. Ghanbarzadeh, H. Valizadeh, P. Zakeri-Milani, The effects of lyophilization on the physico-chemical stability of sirolimus liposomes, Adv. Pharm. Bull. 3 (2013) 25–29, https://doi.org/10.5681/apb.2013.005.
[147] L. Li, R.M. Hoffman, Model of selective gene therapy of hair growth: liposome targeting of the active lac-z gene to hair follicles of histocultured skin, Vitro Cell Dev-An. 31 (1995) 11–13, https://doi.org/10.1007/BF02631332.
[148] S. Trauer, H. Richter, J. Kuntsche, R. Buttemeyer, M. Liebsch, M. Linscheid, et al., Influence of massage and occlusion on the ex vivo skin penetration of rigid liposomes and invasomes, Eur. J. Pharm. Biopharm. 86 (2014) 301–306, https://doi.org/10.1016/j.ejpb.2013.11.004.
[149] B. Geusens, M. Van Gele, S. Braat, S. De Smedt, M. Stuart, T. Prow, et al., 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, https://doi.org/10.1002/adfm.201000484.
[150] P.L. Honeywell-Nguyen, G.S. Gooris, J.A. Bouwstra, Quantitative assessment of the transport of elastic and rigid vesicle components and a model drug from these vesicle formulations into human skin in vivo, J. Investig. Dermatol. Symp. Proc. 123 (2004) 902–910, https://doi.org/10.1111/j.0022-202X.2004.23441.x.
[151] M.M. Elsayed, O.Y. Abdallah, V.F. Naggar, N.M. Khalafallah, Lipid vesicles for skin delivery of drugs: reviewing three decades of research, Int. J. Pharm. 332 (2007) 1–16, https://doi.org/10.1016/j.ijpharm.2006.12.005.
[152] P.L. Felgner, T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, et al., Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure, Proc. Natl. Acad. Sci. USA 84 (1987) 7413–7417, https://doi.org/10.1073/pnas.84.21.

7413.
[153] M. Ruponen, S. Ronkko, P. Honkakoski, J. Pelkonen, M. Tammi, A. Urtti, Extracellular glycosaminoglycans modify cellular trafficking of lipoplexes and polyplexes, J. Biol. Chem. 276 (2001) 33875–33880, https://doi.org/10.1074/jbc.M011553200.
[154] N. Düzgüneş, P.L. Felgner, Intracellular delivery of nucleic acids and transcription factors by cationic liposomes, Methods in Enzymology, Academic Press, 1993, pp. 303–306 https://doi.org/10.1016/0076-6879(93)21025-4.
[155] P. Pires, S. Simões, S. Nir, R. Gaspar, N. Düzgünes, M.C. Pedroso de Lima, Interaction of cationic liposomes and their DNA complexes with monocytic leukemia cells, BBA-Biomembranes 1418 (1999) 71–84, https://doi.org/10.1016/S0005-2736(99)00023-1.
[156] C. Tros de Ilarduya, Y. Sun, N. Duzgunes, Gene delivery by lipoplexes and polyplexes, Eur. J. Pharm. Sci. 40 (2010) 159–170, https://doi.org/10.1016/j.ejps.2010.03.019.
[157] D.A. Balazs, W. Godbey, Liposomes for use in gene delivery, J. Drug Deliv. 2011 (2011) 326497, https://doi.org/10.1155/2011/326497.
[158] N. Belmadi, P. Midoux, P. Loyer, C. Passirani, C. Pichon, T. Le Gall, et al., Synthetic vectors for gene delivery: an overview of their evolution depending on routes of administration, Biotechnol. J. 10 (2015) 1370–1389, https://doi.org/10.1002/biot.201400841.
[159] W. Viricel, S. Poirier, A. Mbarek, R.M. Derbali, G. Mayer, J. Leblond, Cationic switchable lipids: pH-triggered molecular switch for siRNA delivery, Nanoscale 9 (2017) 31–36, https://doi.org/10.1039/c6nr06701h.
[160] W. Viricel, A. Mbarek, J. Leblond, Switchable lipids: conformational change for fast ph-triggered cytoplasmic delivery, Angew. Chem. Int. Ed. Engl. 54 (2015) 12743–12747, https://doi.org/10.1002/anie.201504661.
[161] Y. Obata, S. Saito, N. Takeda, S. Takeoka, Plasmid DNA-encapsulating liposomes: effect of a spacer between the cationic head group and hydrophobic moieties of the lipids on gene expression efficiency, BBA-Biomembranes 1788 (2009) 1148–1158, https://doi.org/10.1016/j.bbamem.2009.02.014.
[162] H. Lv, S. Zhang, B. Wang, S. Cui, J. Yan, Toxicity of cationic lipids and cationic polymers in gene delivery, J. Control Release 114 (2006) 100–109, https://doi.org/10.1016/j.jconrel.2006.04.014.
[163] M.C. Pedroso de Lima, S. Simões, P. Pires, H. Faneca, N. Düzgüneş, Cationic lipid–DNA complexes in gene delivery: from biophysics to biological applications, Adv. Drug Deliv. Rev. 47 (2001) 277–294, https://doi.org/10.1016/S0169-409X(01)00110-7.
[164] J.C. Birchall, C. Marichal, L. Campbell, A. Alwan, J. Hadgraft, M. Gumbleton, Gene expression in an intact ex-vivo skin tissue model following percutaneous delivery of cationic liposome–plasmid DNA complexes, Int. J. Pharm. 197 (2000) 233–238, https://doi.org/10.1016/S0378-5173(00)00336-7.
[165] I.M. Abdulbaqi, Y. Darwis, N.A.K. Khan, R.A. Assi, A.A. Khan, Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials, Int. J. Nanomed. 11 (2016) 2279–2304, https://doi.org/10.2147/IJN.S105016.
[166] B.K. Kim, G.B. Hwang, Y.B. Seu, J.S. Choi, K.S. Jin, K.O. Doh, DOTAP/DOPE ratio and cell type determine transfection efficiency with DOTAP-liposomes, Biochim. Biophys. Acta 1848 (2015) 1996–2001, https://doi.org/10.1016/j.bbamem.2015.06.020.
[167] M. Kapoor, D.J. Burgess, S.D. Patil, Physicochemical characterization techniques for lipid based delivery systems for siRNA, Int. J. Pharm. 427 (2012) 35–57, https://doi.org/10.1016/j.ijpharm.2011.09.032.
[168] S.D. Patil, D.G. Rhodes, D.J. Burgess, Anionic liposomal delivery system for DNA transfection, AAPS J. 6 (2004) 13–22, https://doi.org/10.1208/aapsj060429.
[169] M. Somiya, K. Yamaguchi, Q. Liu, T. Niimi, A.D. Maturana, M. Iijima, et al., One-step scalable preparation method for non-cationic liposomes with high siRNA content, Int. J. Pharm. 490 (2015) 316–323, https://doi.org/10.1016/j.ijpharm.2015.05.072.
[170] C. Srinivasan, D.J. Burgess, Optimization and characterization of anionic lipoplexes for gene delivery, J. Control Release 136 (2009) 62–70, https://doi.org/10.1016/j.jconrel.2009.01.022.
[171] A. Gillet, P. Compere, F. Lecomte, P. Hubert, E. Ducat, B. Evrard, et al., Liposome surface charge influence on skin penetration behaviour, Int. J. Pharm. 411 (2011) 223–231, https://doi.org/10.1016/j.ijpharm.2011.03.049.
[172] V.I. Kulkarni, V.S. Shenoy, S.S. Dodiya, T.H. Rajyaguru, R.R. Murthy, Role of calcium in gene delivery, Expert Opin. Drug Del. 3 (2006) 235–245, https://doi.org/10.1517/17425247.3.2.235.
[173] D.D. Lasic, N.S. Templeton, Liposomes in gene therapy, Adv Drug Deliv. Rev. 20 (1996) 221–266, https://doi.org/10.1016/0169-409X(96)00002-6.
[174] S.D. Patil, D.G. Rhodes, D.J. Burgess, Biophysical characterization of anionic lipoplexes, BBA-Biomembranes 1711 (2005) 1–11, https://doi.org/10.1016/j.bbamem.2005.03.004.
[175] G. Cevc, G. Blume, Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force, BBA-Biomembranes 1104 (1992) 226–232, https://doi.org/10.1016/0005-2736(92)90154-E.
[176] G. Cevc, D. Gebauer, Hydration-driven transport of deformable lipid vesicles through fine pores and the skin barrier, Biophys. J. 84 (2003) 1010–1024, https://doi.org/10.1016/S0006-3495(03)74917-0.
[177] M.H. Abdallah, Transfersomes as a transdermal drug delivery system for enhancement the antifungal activity of nystatin, J. Pharm. Pharm. Sci. 5 (2013) 560–567.
[178] A. Gillet, A. Grammenos, P. Compere, B. Evrard, G. Piel, Development of a new topical system: drug-in-cyclodextrin-in-deformable liposome, Int. J. Pharm. 380 (2009) 174–180, https://doi.org/10.1016/j.ijpharm.2009.06.027.
[179] A. Gillet, F. Lecomte, P. Hubert, E. Ducat, B. Evrard, G. Piel, Skin penetration behaviour of liposomes as a function of their composition, Eur. J. Pharm. Biopharm. 79 (2011) 43–53, https://doi.org/10.1016/j.ejpb.2011.01.011.
[180] E.H. Lee, A. Kim, Y.-K. Oh, C.-K. Kim, Effect of edge activators on the formation and transfection efficiency of ultradeformable liposomes, Biomaterials 26 (2005) 205–210, https://doi.org/10.1016/j.biomaterials.2004.02.020.
[181] A. Kim, E.H. Lee, S.-H. Choi, C.-K. Kim, In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome, Biomaterials 25 (2004) 305–313, https://doi.org/10.1016/s0142-9612(03)00534-9.
[182] S.-E. Jin, C.-K. Kim, Charge-mediated topical delivery of plasmid DNA with cationic lipid nanoparticles to the skin, Colloids Surf. B Biointerfaces 116 (2014) 582–590, https://doi.org/10.1016/j.colsurfb.2014.01.053.
[183] 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, https://doi.org/10.1016/j.jconrel.2016.03.010.
[184] Nina Dragicevic, Jelena Predic Atkinson, Howard I. Maibach, Chemical penetration enhancers: classification and mode of action, in: Nina Dragicevic, Howard I. Maibach (Eds.), Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, pp. 11–27 https://doi.org/10.1007/978-3-662-47039-8_2 http://link.springer.com/10.1007/978-3-662-47039-8_2.
[185] E. Touitou, N. Dayan, L. Bergelson, B. Godin, M. Eliaz, Ethosomes- novel vesicular carriers for enhanced delivery: characterization and skin penetration properties, J. Control Release 65 (2000) 403–418, https://doi.org/10.1016/S0168-3659(99)00222-9.
[186] B. Godin, E. Touitou, Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier, J. Control Release 94 (2004) 365–379, https://doi.org/10.1016/j.jconrel.2003.10.014.
[187] N. Dayan, E. Touitou, Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes, Biomaterials 21 (18) (2000) 1879–1885, https://doi.org/10.1016/S0142-9612(00)00063-6.
[188] P. Verma, K. Pathak, Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation, Nanomedicine 8 (2012) 489–496, https://doi.org/10.1016/j.nano.2011.07.004.
[189] J. Manosroi, N. Khositsuntiwong, W. Manosroi, F. Götz, R.G. Werner, A. Manosroi, Enhancement of transdermal absorption, gene expression and stability of tyrosinase plasmid (pMEL34)-loaded elastic cationic niosomes: potential application in vitiligo treatment, J. Pharm. Sci. 99 (2010) 3533–3541, https://doi.org/10.1002/jps.22104.
[190] D.D. Verma, S. Verma, G. Blume, A. Fahr, Particle size of liposomes influences dermal delivery of substances into skin, Int. J. Pharm. 258 (2003) 141–151, https://doi.org/10.1016/s0378-5173(03)00183-2.
[191] M.J. Choi, H.I. Maibach, Elastic vesicles as topical/transdermal drug delivery systems, Int. J. Cosmetic Sci. 27 (2005) 211–221, https://doi.org/10.1111/j.1467-2494.2005.00264.x.
[192] K. Ross, Towards topical microRNA-directed therapy for epidermal disorders, J. Control Release 269 (2018) 136–147, https://doi.org/10.1016/j.jconrel.2017.11.013.
[193] S.A. Leachman, R.P. Hickerson, P.R. Hull, F.J. Smith, M.L. Milstone, E.B. Lane, et al., Therapeutic siRNAs for dominant genetic skin disorders including pachyonychia congenita, J. Dermatol. Sci. 51 (2008) 151–157, https://doi.org/10.1016/j.jdermsci.2008.04.003.
[194] I. Ishimoto, Y. Takei, Y. Yuzawa, K. Hanai, S. Nagahara, Y. Tarumi, et al., Downregulation of monocyte chemoattractant protein-1 involving short interfering RNA attenuates hapten-induced contact hypersensitivity, Mol. Ther. 16 (2008) 387–395, https://doi.org/10.1038/sj.mt.6300360.
[195] H. Ibaraki, T. Kanazawa, Y. Takashima, H. Okada, Y. Seta, Development of an innovative intradermal siRNA delivery system using a combination of a functional stearylated cytoplasm-responsive peptide and a tight junction-opening peptide, Molecules 21 (2016), https://doi.org/10.3390/molecules21101279.
[196] H. Ibaraki, T. Kanazawa, Y. Takashima, H. Okada, Y. Seta, Transdermal anti-nuclear kappaB siRNA therapy for atopic dermatitis using a combination of two kinds of functional oligopeptide, Int. J. Pharm. 542 (2018) 213–220, https://doi.org/10.1016/j.ijpharm.2018.03.026.
[197] X. Zhou, F.K. Tan, X. Guo, F.C. Arnett, Attenuation of collagen production with small interfering RNA of SPARC in cultured fibroblasts from the skin of patients with scleroderma, Arthritis Rheum. 54 (2006) 2626–2631, https://doi.org/10.1002/art.21973.
[198] M. Nakamura, J.-I. Jo, Y. Tabata, O. Ishikawa, Controlled delivery of T-box21 small interfering RNA ameliorates autoimmune alopecia (Alopecia Areata) in a C3H/HeJ mouse model, Am. J. Pathol. 172 (2008) 650–658, https://doi.org/10.2353/ajpath.2008.061249.
[199] A. Manosroi, N. Khositsuntiwong, F. Gotz, R.G. Werner, J. Manosroi, Transdermal enhancement through rat skin of luciferase plasmid DNA loaded in elastic nanovesicles, J. Liposome Res. 19 (2009) 335–348, https://doi.org/10.1080/08982100902731523.
[200] K. Peachman, M. Rao, C.R. Alving, Immunization with DNA through the skin, Methods 31 (2003) 232–242, https://doi.org/10.1016/s1046-2023(03)00137-3.
[201] S. Hansen, C.M. Lehr, Nanoparticles for transcutaneous vaccination, Microb. Biotechnol. 5 (2012) 156–167, https://doi.org/10.1111/j.1751-7915.2011.00284.x.
[202] Y.C. Kim, C. Jarrahian, D. Zehrung, S. Mitragotri, M.R. Prausnitz, Enabling skin vaccination using new delivery technologies, Drug Deliv. Transl. Res. 1 (2011) 7–12, https://doi.org/10.1007/s13346-010-0005-z.
[203] J.H. Van den Berg, B. Nuijen, T.N. Schumacher, J.B. Haanen, G. Storm, J.H. Beijnen, et al., Synthetic vehicles for DNA vaccination, J. Drug Target. 18

(2010) 1–14, https://doi.org/10.3109/10611860903278023.
[204] J.H. Van den Berg, B. Nuijen, J.H. Beijnen, A. Vincent, H. van Tinteren, J. Kluge, et al., Optimization of Intradermal Vaccination by DNA Tattooing in Human Skin, Hum. Gene Ther. 20 (2009) 181–189, https://doi.org/10.1089/hgt.2008.073.
[205] P.J. Lou, W.F. Cheng, Y.C. Chung, C.Y. Cheng, L.H. Chiu, T.H. Young, PMMA particle-mediated DNA vaccine for cervical cancer, J. Biomed. Mater. Res. A 88 (2009) 849–857, https://doi.org/10.1002/jbm.a.31919.
[206] J. Arya, M.R. Prausnitz, Microneedle patches for vaccination in developing countries, J. Control Release 240 (2016) 135–141, https://doi.org/10.1016/j.jconrel.2015.11.019.
[207] J. Wang, J.H. Hu, F.Q. Li, G.Z. Liu, Q.G. Zhu, J.Y. Liu, et al., Strong cellular and humoral immune responses induced by transcutaneous immunization with HBsAg DNA-cationic deformable liposome complex, Exp. Dermatol. 16 (2007) 724–729, https://doi.org/10.1111/j.1600-0625.2007.00584.x.
[208] S.P. Vyas, R.P. Singh, S. Jain, V. Mishra, S. Mahor, P. Singh, et al., Non-ionic surfactant based vesicles (niosomes) for non-invasive topical genetic immunization against hepatitis B, Int. J. Pharm. 296 (2005) 80–86, https://doi.org/10.1016/j.ijpharm.2005.02.016.
[209] S. Mahor, A. Rawat, P.K. Dubey, P.N. Gupta, K. Khatri, A.K. Goyal, et al., Cationic transfersomes based topical genetic vaccine against hepatitis B, Int. J. Pharm. 340 (2007) 13–19, https://doi.org/10.1016/j.ijpharm.2007.03.006.
[210] J. Xu, Y. Ding, Y. Yang, Enhancement of mucosal and cellular immune response in mice by vaccination with respiratory syncytial virus DNA encapsulated with transfersome, Viral Immunol. 21 (2008) 483–489, https://doi.org/10.1089/vim.2008.0044.
[211] B. Geusens, N. Sanders, T. Prow, M. Van Gele, J. Lambert, Cutaneous short-interfering RNA therapy, Expert Opin. Drug Deliv. 6 (2009) 1333–1349, https://doi.org/10.1517/17425240903304032.
[212] E. Higgins, Psoriasis, Medicine 45 (2017) 368–378, https://doi.org/10.1016/j.mpmed.2017.03.010.
[213] K. Ghoreschi, P. Thomas, S. Breit, M. Dugas, R. Mailhammer, W. van Eden, et al., Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease, Nat. Med. 9 (2003) 40–46, https://doi.org/10.1038/nm804.
[214] Y. Zhang, J. Li, C.Y. Liu, X.K. Zhou, J. Qiu, Y.B. Zhang, et al., A novel transdermal plasmid-dimethylsulfoxide delivery technique for treatment of psoriasis, Dermatology 221 (2010) 84–92, https://doi.org/10.1159/000314154.
[215] J. Li, X. Li, Y. Zhang, X.K. Zhou, H.S. Yang, X.C. Chen, et al., Gene therapy for psoriasis in the K14-VEGF transgenic mouse model by topical transdermal delivery of interleukin-4 using ultradeformable cationic liposome, J. Gene Med. 12 (2010) 481–490, https://doi.org/10.1002/jgm.1459.
[216] L.V. Depieri, L.N. Borgheti-Cardoso, P.M. Campos, K.K. Otaguiri, F.T. Vicentini, L.B. Lopes, et al., RNAi mediated IL-6 in vitro knockdown in psoriasis skin model with topical siRNA delivery system based on liquid crystalline phase, Eur. J. Pharm. Biopharm. 105 (2016) 50–58, https://doi.org/10.1016/j.ejpb.2016.05.012.
[217] H.Y. Xue, S. Liu, H.L. Wong, Nanotoxicity: a key obstacle to clinical translation of siRNA-based nanomedicine, Nanomedicine 9 (2014) 295–312, https://doi.org/10.2217/nnm.13.204.
[218] S. Bracke, E. Desmet, S. Guerrero-Aspizua, S.G. Tjabringa, J. Schalkwijk, M. Van Gele, et al., Identifying targets for topical RNAi therapeutics in psoriasis: assessment of a new in vitro psoriasis model, Arch. Dermatol. Res. 305 (2013) 501–512, https://doi.org/10.1007/s00403-013-1379-9.
[219] B. Geusens, J. Lambert, S.C. De Smedt, K. Buyens, N.N. Sanders, M. Van Gele, Ultradeformable cationic liposomes for delivery of small interfering RNA (siRNA) into human primary melanocytes, J. Control Release 133 (2009) 214–220, https://doi.org/10.1016/j.jconrel.2008.10.003.
[220] S. Bracke, M. Carretero, S. Guerrero-Aspizua, E. Desmet, N. Illera, M. Navarro, et al., Targeted silencing of DEFB4 in a bioengineered skin-humanized mouse model for psoriasis: development of siRNA SECosome-based novel therapies, Exp. Dermatol. 23 (2014) 199–201, https://doi.org/10.1111/exd.12321.
[221] M. Jakobsen, K. Stenderup, C. Rosada, B. Moldt, S. Kamp, T.N. Dam, et al., Amelioration of psoriasis by anti-TNF-alpha RNAi in the xenograft transplantation model, Mol. Ther. 17 (2009) 1743–1753, https://doi.org/10.1038/mt.2009.141.
[222] S. Marepally, C.H. Boakye, A.R. Patel, C. Godugu, R. Doddapaneni, P.R. Desai, et al., Topical administration of dual siRNAs using fusogenic lipid nanoparticles for treating psoriatic-like plaques, Nanomedicine 9 (2014) 2157–2174, https://doi.org/10.2217/nnm.13.202.
[223] http://globenewswire.com/news-release/2016/10/11/878535/0/en/Exicure-Announces-Positive-Phase-1-Data-for-Topical-Anti-TNF-Compound-AST-005-in-Patients-with-Mild-to-Moderate-Psoriasis.html.
[224] http://www.sciencemag.org/news/2016/08/spherical-rna-therapy-shows-promise-against-psoriasis-first-human-trial.
[225] S.A. Eming, T. Krieg, J.M. Davidson, Gene therapy and wound healing, Clin. Dermatol. 25 (2007) 79–92, https://doi.org/10.1016/j.clindermatol.2006.09.011.
[226] O. Bleiziffer, E. Eriksson, F. Yao, R.E. Horch, U. Kneser, Gene transfer strategies in tissue engineering, J. Cell Mol. Med. 11 (2007) 206–223, https://doi.org/10.1111/j.1582-4934.2007.00027.x.
[227] L. Sun, L. Xu, H.I. Chang, F.A. Henry, R.M. Miller, J.M. Harmon, et al., Transfection with aFGF cDNA improves wound healing, J. Invest. Dermatol. 108 (1997) 415–426, https://doi.org/10.1111/1523-1747.ep12286471.
[228] M.G. Jeschke, R.E. Barrow, H.K. Hawkins, M.T. Chrysopoulo, J.R. Perez-Polo, D.N. Herndon, Effect of multiple gene transfers of insulin like growth factor I complementary DNA gene constructs in rats after thermal injury, Arch. Surg. 134 (1999) 12–19, https://doi.org/10.1001/archsurg.134.10.1137.
[229] C.T. Pereira, D.N. Herndon, R. Rocker, M.G. Jeschke, Liposomal gene transfer of keratinocyte growth factor improves wound healing by altering growth factor and collagen expression, J. Surg. Res. 139 (2007) 222–228, https://doi.org/10.1016/j.jss.2006.09.005.
[230] P.S. Randeria, M.A. Seeger, X.Q. Wang, H. Wilson, D. Shipp, C.A. Mirkin, et al., siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown, Proc. Natl. Acad. Sci. USA 112 (2015) 5573–5578, https://doi.org/10.1073/pnas.1505951112.
[231] P.S. Rabbani, A. Zhou, Z.M. Borab, J.A. Frezzo, N. Srivastava, H.T. More, et al., Novel lipoproteoplex delivers Keap1 siRNA based gene therapy to accelerate diabetic wound healing, Biomaterials 132 (2017) 1–15, https://doi.org/10.1016/j.biomaterials.2017.04.001.
[232] K. Satyamoorthy, M. Herlyn, Cellular and molecular biology of human melanoma, Cancer Biol. Ther. 1 (2002) 14–17, https://doi.org/10.4161/cbt.1.1.32.
[233] K. Hoeflich, D. Gray, M. Eby, J. Toien, L. Wong, J. Bower, et al., Oncogenic BRAF is required for tumor growth and maintenance in melanoma models? Cancer Res. 66 (2006) 999–1006, https://doi.org/10.1158/0008-5472.CAN-05-2720.
[234] G. Matsumoto, T. Kushibiki, Y. Kinoshita, U. Lee, Y. Omui, E. Kubota, et al., Cationized gelatin delivery of a plasmid DNA expressing small interference RNA for VEGF inhibits murine squamous cell carcinoma, Cancer Sci. 97 (2006) 313–321, https://doi.org/10.1111/j.1349-7006.2006.00174.x.
[235] M. Rodolfo, E.M. Cato, S. Soldati, R. Ceruti, M. Asioli, E. Scanziani, et al., Growth of human melanoma xenographs is suppressed by systemic angiostatin gene therapy, Cancer Gene Ther. 8 (2001) 491–496, https://doi.org/10.1038/sj.cgt.7700331.
[236] L. Speroni, J. Gasparri, A.B.V. de los, N.S. Chiaramoni, A. Smagur, S. Szala, et al., Antitumoral effect of IL-12 gene transfected via liposomes into B16F0 cells, Acta Biochim. Pol. 56 (2009) 249–253.
[237] A. Rakhmilevich, J. Timmins, K. Janssen, E. Pohlmann, M. Sheehy, N. Yang, Gene gun-mediated IL-12 gene therapy induces antitumor effects in the absence of toxicity: a direct comparison with systemic IL-12 protein therapy, J. Immunother. 22 (1999) 135–144.
[238] M.A. Tran, R. Gowda, A. Sharma, E.J. Park, J. Adair, M. Kester, et al., Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development, Cancer Res. 68 (2008) 7638–7649, https://doi.org/10.1158/0008-5472.CAN-07-6614.
[239] D. Zheng, D.A. Giljohann, D.L. Chen, M.T. Massich, X.Q. Wang, H. Iordanov, et al., Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation, Proc. Natl. Acad. Sci. USA 109 (2012) 11975–11980, https://doi.org/10.1073/pnas.1118425109/-/DCSupplemental.
[240] J. Niu, Y. Chu, Y.F. Huang, Y.S. Chong, Z.H. Jiang, Z.W. Mao, et al., Transdermal gene delivery by functional peptide-conjugated cationic gold nanoparticle reverses the progression and metastasis of cutaneous melanoma, ACS Appl. Mater. Inter. 9 (2017) 9388–9401, https://doi.org/10.1021/acsami.6b16378.