Expert Opinion

Cutaneous short-interfering RNA therapy

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Since the 1990s, RNA interference (RNAi) has become a major subject of interest, not only as a tool for biological research, but also, more importantly, as a therapeutic approach for gene-related diseases. The use of short-interfering RNAs (siRNAs) for the sequence-specific knockdown of disease-causing genes has led to numerous preclinical and even a few clinical studies. Applications for cutaneous delivery of therapeutic siRNA are now emerging owing to a strong demand for effective treatments of various cutaneous disorders. Although successful studies have been performed using several different delivery techniques, most of these techniques encounter limitations for translation to the clinic with regards to patient compliance. This review describes the principal findings and applications in cutaneous RNAi therapy and focuses on the promises and pitfalls of the delivery systems.

Keywords: cutaneous disorders, delivery techniques, siRNA, skin

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1. Introduction

The quest for the complete sequence of the human genome was completed in 2004 and resulted in ~ 25,000 genes [1]. The expression of each of these individual genes needs to be controlled appropriately to suit the functions and environment of each cell, and must change to respond to new conditions or signals [2]. Inappropriate expression therefore often leads to gene-related diseases. With the availability of DNA sequence information, the development of gene-based technologies for the therapeutic treatment of these diseases is accelerating.

One of these technologies focuses on the RNA interference (RNAi) mechanism. RNAi or RNA interference is a Nobel prize-winning discovery of the late 1990s that demonstrated how gene expression can be controlled at the mRNA level by means of double-stranded RNA [3]. Briefly, introduction of synthetic double-stranded RNA (dsRNA), short-interfering RNAs (siRNAs), or short-hairpin RNA (shRNA) expressing plasmid DNA into the cell leads to sequence-specific silencing of the complementary target gene. The dsRNA unwinds and hybridises with the messenger RNA, which leads to mRNA cleavage and inhibition of protein translation. Of all antisense technologies [4-6], RNA interference has the major advantage that this mechanism recruits the same proteins as the miRNAs and thus benefits from the biological innate system [6]. It also allows endogenous expression of siRNA molecules that can be recycled after being used [4]. Although it has been demonstrated that 'later generation' antisense molecules show similar *in vitro* properties [7], others claim that RNAi provides a stronger method of gene silencing than either antisense molecules or ribozymes [8-11]. In addition, RNAi has a broader therapeutic potential than small-molecule drugs owing to the possibility of sequence-specific suppression of any disease-associated genes. With the potential power of RNA-based medicine as an excellent mechanism to block disease-causing genes, the pharmaceutical world is ready for a new area of drug development.

The types of study using siRNAs or shRNAs are highly diverse and are being performed on an increasingly routine basis. Most reports describe the knockdown of

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one or a limited number of genes directly relevant to the biological question under investigation. Other studies have used RNAi to identify causal genes within disease-associated genomic regions or biomarkers associated with compound activity. Several clinical trials are focusing on the specific reduction of a disease-causing gene by means of RNAi as a therapeutic approach (for an overview, see [12]). One of the main concerns with siRNA therapeutics is their safety. Off-target effects, stimulation of immune responses and competition with endogenous RNAs, that is, miRNAs, need to be monitored while testing RNAi therapies. These concerns have been expertly summarised previously [13-15], and fall outside the scope of this review.

Resistance to exonuclease digestion, the maintenance of duplex stability, good pharmacokinetics and the minimisation of nonspecific immunological responses are critical considerations when applying siRNAs or shRNAs *in vivo* [16]. Chemically modified siRNAs and carrier molecules have been used to mitigate premature RNAi digestion. Localised siRNA delivery avoids many problems associated with systemic delivery and offers higher bioavailability. However, feasibility is dependent on access to the target. The skin is the most accessible tissue for topical or localised RNAi therapy. This review focuses on delivery strategies of siRNA molecules to skin cells, *in vitro* and *in vivo*, and addresses the most important studies with relevant outcome for a variety of skin disorders.

2. Mechanism of RNA interference

RNA interference is a regulatory mechanism within eukaryotic cells. This process uses small dsRNA molecules to direct homology-dependent control of gene activity through RNA degradation. Exogenously introduced (long) dsRNA into cells is processed by the RNase III-like protein Dicer, yielding 21- to 23-bp RNA fragments with 2-nt 3′ overhangs (**Figure 1**). Known as small interfering RNAs, these fragments are bound to the dsRNA binding protein (transactivation response binding protein [TRBP]) of the RNA-induced silencing complex (RISC) that targets complementary mRNA. A multifunctional protein Argonaute-2 (Ago-2) is contained within RISC. Ago-2 unwinds the siRNA, after which the sense strand of the siRNA is cleaved. The now activated RISC contains the antisense strand or guide strand and allows the single-stranded siRNA selectively to recognise and cleave complementary RNA. Selectivity of strand incorporation into the RISC is based on differential thermodynamic stabilities of the siRNA ends. The least thermodynamically stable end unwinds the 5′ end of the guide strand. The guide strand binds to the PIWI domain of Ago-2. Cleavage of mRNA occurs at a position between nucleotides 10 and 11 on the complementary antisense strand, relative to the 5′ end. The products are then released and the RISC with the antisense strand can interact with another target mRNA molecule [17,18].

RNA interference can be triggered by two different pathways. The first uses synthetic 21 – 23 base duplexes (siRNAs), whereas the second option makes use of DNA expression vector with Pol II or Pol III promoters that are transcribed into RNA hairpins. Nuclear synthesis of these shRNAs is followed by their transport into the cytoplasm by means of the miRNA machinery where they are processed into siRNAs by Dicer. The advantage of the latter siRNA expression system is that the RNAi effects last much longer because of continued shRNA expression [16,19].

The direct introduction of synthetic siRNAs is an effective strategy for RNAi therapeutics. Chemical modifications can be introduced into siRNA to increase stability, promote efficacy, block binding to unintended targets that contain sequence mismatches (specific off-target effects) and reduce potential immune stimulatory effects (general off-target effects) [13]. The effects of these synthetic siRNA molecules are transient, whereas the promoter-expressed shRNAs can potentially mediate long-term silencing with a single application due to constitutive expression.

3. siRNA delivery into the skin

3.1 Structure of the skin

The structure of the skin comprises a series of layers, ranging from the fatty subcutaneous layer (hypodermis), the dermis of connective tissue to the stratified avascular, cellular epidermis. All layers are penetrated by hair shafts and gland ducts. Its structure and barrier functions have been described extensively in literature [20-23]. A schematic drawing of the structure of human skin is given in **Figure 2**.

The dermis is composed of fibrous proteins (collagen and elastin) and an interfibrillar gel of glycosaminoglycans, salts and water. Blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicles and sebaceous glands) and sweat glands are embedded within the dermis. The hair follicles and sweat ducts open directly into the environment at the skin surface and provide the so-called appendageal route of skin permeation [22,24].

The epidermis does not contain any blood vessels. As a consequence, nutrients and waste products must diffuse across the dermal–epidermal junction to maintain its vitality. In most areas of the body, the epidermis consists of four layers, which from inside to outside are the stratum basale (basal layer), stratum spinosum (spinous layer), stratum granulosum (granular layer) and stratum corneum (SC) [22,24].

The very efficient barrier properties of the skin are exerted by the stratum corneum and impede penetration of topically administered therapeutics in the skin [22]. The SC is composed of terminally differentiated anucleated keratinocytes, so-called corneocytes, tightly packed in columns that run perpendicular to the skin surface. The matrix between corneocytes is composed of hydrophobic non-polar lipids, which are assembled into lamellar stacks and form a very densely packed structure [25]. The structure of the SC is often depicted in the so-called bricks and mortar arrangement [26], where the keratin-rich corneocytes (bricks) are embedded in the intercellular lipid-rich matrix (mortar).

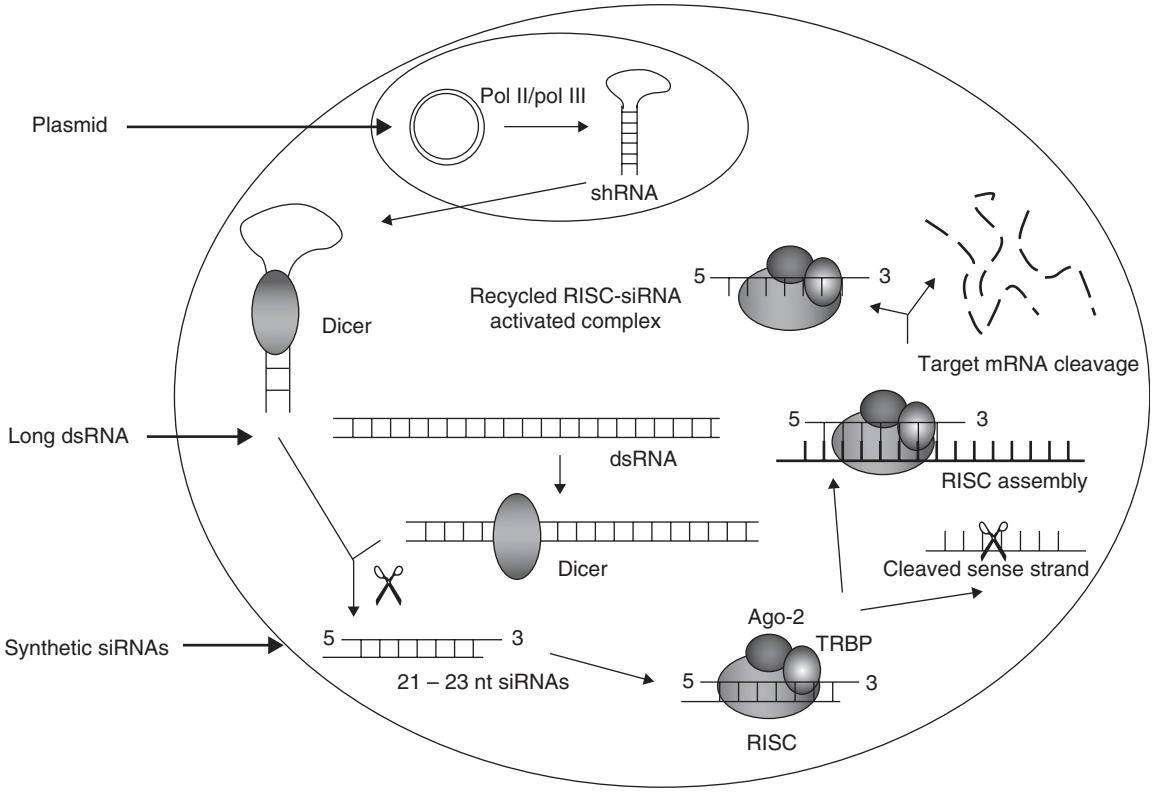

Figure 1. The mechanism of RNA interference. The right-hand side shows a schematic diagram of the mammalian RNAi pathway and formation of small interfering RNAs (siRNA) that mediate homology-dependent target mRNA degradation in the cytoplasm through the RNA-induced silencing complex (RISC). The left-hand side shows different entry points for artificial DNA-based (plasmid) and RNA-based (long dsRNA and synthetic siRNAs) siRNA drugs that enter and activate RISC for gene silencing.

3.2 Applications of RNAi in therapy and research

Attraction and interest in RNAi-based therapeutics for skin diseases is emerging. This approach applies particularly to monogenic dominant negative skin diseases where the silencing of a specific disease-causing gene is expected to have a clinical effect. Owing to their extreme specificity and potency, siRNA agents show promise as new therapeutic tools for individualised medicine, in which siRNAs theoretically can be designed to target any given gene mutation. A wide spectrum of heritable skin diseases has been found, with > 80 distinct genes involved in > 100 disease phenotypes [27], a high proportion of which is keratin diseases without effective treatment options. An overview of diseases that can be treated by means of RNAi is given later in this paper.

In addition to therapeutic applications, siRNA may also be useful for discovering disease-controlling genes or deciphering the gene function. A prototypical approach is to test gene function by disabling and examining resulting phenotypes. Knockout animal models are a time-consuming and expensive alternative. RNAi can be used to perform surrogate gene-deficiency experiments in cell culture or appropriate skin models in a matter of days [2]. In accordance with DNA, mRNA or protein-arrayed libraries for large-scale analysis, the use of RNAi-based methods is enabling corresponding functional analysis to be conducted on a similar scale. Genome-wide RNAi analysis was first developed for the model organisms *Caenorhabditis elegans* and *Drosophilia* [16]. A year later Ganesan *et al.* reported a study that used a genome-wide synthetic library of chemically synthesised siRNAs to identify new biological pathways that govern melanin biogenesis in human melanocytes. This effort identified 92 new genes that support pigment production by using RNAi [28].

On a smaller scale, RNAi has helped clarify the role of p53 in premature senescence [29] and hyperpigmentation [30]. Borlon *et al.* concluded that p53 was not involved in the differential expression of senescence-associated genes in UVB-induced premature senescence of skin fibroblasts [29]. Later, RNAi experimental data in UVB-exposed mouse skin showed that intradermal injection of p53-specific siRNA significantly suppressed the expression of mRNAs and proteins encoding paracrine cytokines as well as melanogenic factors. This knockdown resulted in the inhibition of hyperpigmentation [30].

Multiple other *in vitro* RNAi studies were performed and identified potential markers and regulators of skin ageing (e.g., growth-regulated protein alpha (GRO-α) [31], FOXO3a [32]

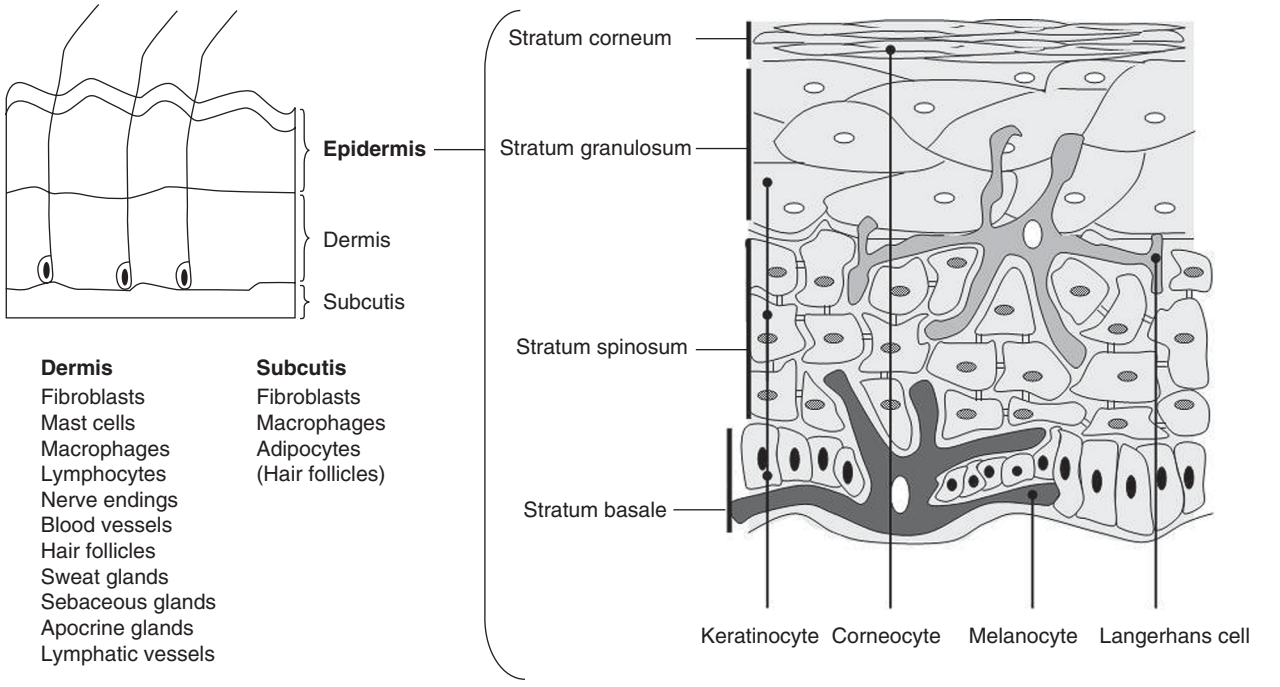

Figure 2. Structure of human skin tissue.

and K6PC-5 [33]), melanoma (e.g., FABP7 [34], ASK/Dbf4 [35], angiopoietin-2 [36], SphK1 [37]) or skin inflammation (e.g., G2A [38], phospholipase C epsilon [39], S100/A11 [40,41]). However, detailed description of these and other functional studies using RNAi fall out of the scope of this review and will not be discussed further.

3.3 Accessibility

The skin is an easy target organ for topical siRNA delivery as it allows direct access to target cells in the skin [42]. Application of topical or local siRNA therapy is easily confined to the affected skin area and systemic absorption of topically applied siRNA molecules is significantly less than for intravenous administration. This is due to very little penetration of siRNA through the barriers of the skin. Generally, only small lipophilic molecules (< 500 Da) have higher levels of penetration [43]. As a consequence, systemic toxicity is minimal [44]. Further advantages of cutaneous siRNA therapy include the ability to monitor visually the genetically modified region and the possibility of surgical removal of aberrant tissue if unwanted side effects occur. Also, treated skin can be biopsied more conveniently than for any other organ.

3.4 Barriers to cutaneous siRNA delivery

In vivo delivery of 'naked' siRNAs is challenging. siRNA alone is not taken up efficiently, and has low biodistribution and low stability [45]. Naked siRNA is susceptible to degradation by endogenous enzymes. siRNA also has a negative charge and is relatively large (~ 13 kDa); both of which contribute to poor penetration through cell membranes. Three studies have described the uptake of naked, unmodified siRNAs by keratinocytes after intradermal injection in mouse skin [46-48]. This is in accordance to Hengge, who observed gene expression from intradermally injected plasmid DNA [49,50]. The observation that keratinocytes are able to take up unmodified oligonucleotides and plasmid DNA holds promise for cutaneous gene therapy. If widely applicable, the use of extra chemical and/or physical uptake-enhancing techniques could become redundant. One challenge for this approach is patient compliance with repeated intradermal injections. Another challenge is that intradermal injection can be applied only to small areas of skin, making treatment of full-body skin diseases unlikely.

Topical siRNA application, on the other hand, requires penetration-enhancing techniques to reach therapeutic concentrations in the skin. The outermost layer of the epidermis, the stratum corneum, acts as the main barrier to penetration of molecules (**Figure 2**). The densely packed corneocytes containing hydrophobic non-polar intracellular lipids form a permeation resistance unit to topically applied RNAi. Penetration of RNAi through the extracellular matrix into deeper skin layers is also inhibited by the dense network of polysaccharides and fibrous proteins.

Although intradermal injection overcomes the stratum corneum barrier, injected oligonucleotides face further barriers before reaching target mRNA. The electron-dense adhesion structure of the basal membrane forms an obstruction for transport within the epidermis to the target cell membrane [51]. The precise mechanism of siRNA transfection has not been characterised. One possibility is endosomal uptake, as described for DNA [51-53]. Subsequent endosomal degradation and difficulties with endosomal escape can result in decreased

functional siRNA, the final consequence being lower silencing efficiency. All the above-described obstacles also apply for siRNA-expressing plasmid vectors, including migration through the cytosol and penetration through the nuclear envelope.

3.5 Methods to overcome the barriers in cutaneous siRNA delivery

The transappendageal and transepidermal pathways are the two main routes of skin permeation [54]. The transappendageal route is the shunt route and includes migration through follicular openings and sweat gland pores [55]. The sizes of the sweat pores and the follicular openings of the skin are 30 – 100 nm, so it is reasonable to expect skin penetration of DNA or small oligonucleotides such as siRNAs [56]. Cevc, on the other hand claims that the most important and most abundant permeation route is the transepidermal pathway [57]. The transepidermal pathway – with an average width of 10 nm – can be defined as the pathway where compounds permeate through intact stratum corneum. Original pore sizes of 0.4 nm have even been detected and colloid-induced opening of these narrow funnels is a prerequisite of transport through the skin. The only colloids capable of achieving this funnel opening are specialised lipid-based ultra-deformable vector systems that have enabled targeted drug delivery deep below the application site, into the systemic circulation [57].

Five hundred daltons is generally accepted as the largest molecular mass for lipophilic molecules that can passively diffuse through skin [43]. Applying this rule to high-molecular-mass molecules that are hydrophilic – siRNA or plasmids expressing shRNA – translates to no expectations of skin penetration. As a consequence, penetration-enhancing techniques to improve dermal or transdermal delivery of nucleic acids in the skin are an active area of research. **Table 1** lists, but is not limited to, the major non-viral gene delivery techniques for cutaneous therapy. It gives an overview of the different chemical and physical delivery methods and describes their advantages and disadvantages. Among the physical delivery techniques, procedures such as tape-stripping, gene gun, ultrasound, intradermal injection (in combination with) electroporation and microneedles are commonly applied. Chemical methods described here are chemical penetration enhancers, liposomal vesicle systems and chemical depilation. All methods have their advantages and limitations, and the choice of one over the others depends essentially on the proposed therapeutic application, cost-effectiveness and, very importantly, patient compliance.

Although these methods have been described mainly for cutaneous DNA delivery only, they could easily be extrapolated for siRNA molecules. Thus far, publications describing cutaneous siRNA delivery using electroporation are scarce and have been limited to a combination with intradermal injection [58,59]. Despite the very high efficacy of this combined delivery method, concerns may rise about the invasiveness and associated pain of the procedure as well as the difficulty of self-administration. Also, elastic liposomal formulations have been described often for their efficient (*trans*)dermal delivery of pDNA *in vivo* [60-68]; only a few studies describe the use of liposomal or lipid-based formulations for topical cutaneous application of siRNA *in vivo* [69,70]. Nevertheless, their use in combination with an ultrasound permeation-enhancing technique [71] or an agarose-based matrix has been demonstrated [72]. Tran *et al.* described the use of a nanoliposomal formulation in combination with ultrasound for the treatment of cutaneous melanoma with siRNA therapy [71]. Low-frequency ultrasound offers a non-invasive, non-painful permeability-enhancing technique causing transient cavitational effects and subsequent perturbation in the stratum corneum lipid layers and cell membranes, thereby enhancing transdermal transport. Thanik *et al.* used an agarose-based matrix system to deliver siRNA–Lipofectamine complexes in a murine wound model to improve wound healing [72].

A very recent report by Lee *et al.* describes the use of an erbium:yttrium-alumunium-garnet (Er:YAG) laser to ablate precisely the superficial SC. This is accomplished with minimal residual thermal damage and enhances delivery of topical applied siRNA molecules into mouse skin [73]. This method achieves the desired therapeutic effects, but concerns may arise about the induced skin damage, patient tolerability and the cost of this technique.

4. The skin as a tool

Before clinical implementation, it is important to use appropriate *ex vivo* or *in vivo* models, such as reconstructed or excised human or animal skin, as a tool to evaluate siRNA penetration capacity, gene silencing efficacy and biological/therapeutic effects. Unfortunately, no standards exist for animal models, skin-equivalent cell culture models, and organ culture models that use human skin. Validation of skin models would be beneficial in this field as it allows direct testing, evaluation and comparison of the various delivery strategies.

One challenge in the field of siRNA delivery is to screen the efficacy of newly developed patient-friendly methodologies that can be rapidly translated into the clinic. Gonzalez-Gonzalez *et al.* engineered a transgenic reporter mouse (transgenic click beetle luciferase/humanised green fluorescent protein (Tg CBL/hMGFP)), in which hMGFP from *Montastrea cavernosa* and CBL expression is confined to the epidermis [48]. They demonstrated that intradermal injection of the most potent CBL siRNA reproducibly inhibited hMGFP expression. This effect was quantified by multiple independent assays, including intravital imaging, RT-qPCR and *ex vivo* microscopy. This mouse model can be used as a test model for future experiments in which other delivery methodologies can be evaluated and improved using intradermal injection of CBL3 siRNA as a positive control. According to the authors, two such patient-friendly methodologies, hollow dissolvable microneedle arrays – loaded with siRNA – and cream

Table 1. Overview of the different physical and chemical skin delivery techniques.

Method	Description	Advantages	Disadvantages	Remarks	Ref.
Physical					
Tape-stripping	Disruption/removal of SC by means of repeated tape trips	Simple Inexpensive Minimally invasive	No universal standards Reproducibility Patient variability (age, gender, ethnicity, anotomical site) Irritation	Induces enhancement of the T-cell-mediated immune response: more benefit to topical immunisation	[111,112]
Ballistic methods/ gene gun	Delivery of naked nucleid acids in target cells using accelerated inert particles as physical carriers. Acceleration is made by gas discharge in a gun	Effective at low amounts Delivery directly into cytoplasm: avoids endocytotic degradation plus immunostimulatory effects No DNA size limit Stable	Small target area Precisely controlled operating conditions Mild skin damage High equipment cost Disposition of metal particles	Induces strong Th2 response: therefore, mainly used for gene immunisation	[101,113,114]
Microneedles	Increase skin permeability by creating micrometre-scale pathways into skin plus drive drugs actively into skin	No pain or bleeding Well tolerated Inexpensive when large-scale production	Small target area Fragile microneedle tips can break and cause skin irritation Design is complicated, special application needed; self-administration difficult	No siRNA application described so far, only DNA vaccination	[62,115]
Intradermal injection		Efficient delivery Inexpensive equipment that is readily available to clinicians	Variability (operator skills plus skin properties) Painful Needlestick injuries No self-administration		[116]
Cavitational ultrasound/ sonophoresis	The accoustic pressure of ultrasound waves induces cavitation bubbles in the coupling medium causing structure-disordering effects in the SC	Non-invasive Convenient Painless Well tolerated Patient compliant	Thermal heating (only with high-intensity continuous wave ultrasound) Expensive equipment	Enhanced skin permeation in conjunction with other enhancement techniques	[117]
Electroporation	Short high-voltage pulses disrupt cell membranes and lipid bilayer structures in the skin. Electrodes can be invasive or topical	Transfection efficiency higher than naked DNA injection or liposome-mediated delivery [118] High transfection efficiency of a variety of cells Ability of this technique to spread the nucleic acids from an i.d. injection to a wider area	Complexity of device design No self-administration Acute tissue damage Pain	Closely spaced microelectrodes can constrain the electric field within the SC and avoid pain [119] Best combination – highest efficiency – with i.d. injection	[10,120,121]
Jet injection	Local injection of molecules diluted in liquid by means of a device that uses high pressure to	No use of needles or particles Minimal patient discomfort	Efficiency depends on various parameters Standard procedures needed Only small target areas	Not described for siRNA	[122]

i.d.: Intradermal; SC: Stratum corneum.

Table 1. Overview of the different physical and chemical skin delivery techniques (continued).

Method	Description	Advantages	Disadvantages	Remarks	Ref.
	force microdroplets of liquid into the skin				
Iontophoresis	The electrical driving of charged molecules into tissue by passing a small direct current through a drug-containing electrode in contact with skin	Non-invasive Effective Self-applicable	Skin irritation Not applicable for patients carrying electrically sensitive implantable devices Not advisable for patients with broken or damaged skin surfaces	Only described for antisense oligonucleotides [123,124]	[125]
Chemical					
Chemical enhancers/ combinations of chemical enhancers	Disruption of the highly ordered bilayer structures in SC by means of sulphoxides, azones, glycols, alkanols, terpenes, etc	Increased skin permeability Cheap Easy to use	Skin irritation Degradation of the siRNAs	Specific combination of chemical enhancers results in increased enhancement and low skin irritation [126]	[127-129]
Lipid-based systems	Elastic vesicles (Transfersomes®, niosomes, ethosomes) penetrate intact skin using different mechanisms [55]	Easy to use Applicable to large areas Painless Cheap	Low efficacy Skin irritation Immune response Unstable		[130]
Chemical depilation	Facilitates removal of cornified epithelium	Gentler than tape-stripping	Degradation of the siRNAs Immune response/skin irritation		[131]

i.d.: Intradermal; SC: Stratum corneum.

formulations that enable siRNA penetration through the stratum corneum, are being developed.

Takahashi *et al.* used a luciferase/GFP-expressing model system in which the efficiency of the different gene transfer methods was evaluated by their gene silencing effect *in vivo*. The goal was to develop an effective RNAi-based tumour therapy [74]. A similar but more generalised approach was reported in 2007 by Wang *et al.*, where siRNAs against green fluorescent protein (eGFP) with a bicistronic firefly luciferase (fLuc)/eGFP were intradermally injected in mouse skin. The authors observed that the siRNA efficiently blocked expression of both fLuc and eGFP in keratinocytes. These results indicate that siRNA can inhibit ectopically expressed genes. This supports the hypothesis that targeting endogenous genes is also feasible [47].

In another approach, the skin can be used as a 'tool' or transport medium for the transdermal delivery of siRNA in order to treat non-skin related diseases, such as rheumatoid arthritis and airway inflammation [75,76]. Administration of siRNAs to the skin for the treatment of non-skin diseases requires efficient transport of the siRNA – through the epidermis - into the dermal vasculature. Two studies have been reported using a topically applied siRNA containing cream-based formulation. Takanashi *et al.* used the skin as a delivery route for the topical application of siRNA against osteopontin [75]. Osteopontin is an extracellular matrix cytokine and is a validated target in rheumatoid arthritis and other inflammatory diseases. The anti-osteopontin siRNA was applied in a lipid/alcohol-based 'GeneCream' formulation onto the skin of an arthritis mouse model. The authors reported penetration of the siRNA cream into dermal fat tissue and an amelioration of arthritic symptoms. This technology appears to support the hypothesis that topical siRNA can be used effectively to treat certain diseases.

One year earlier, Wang *et al.* also described the use of a cream-based formulation that contained imiquimod and chitosan nanoparticles for the transdermal delivery of siRNA targeted against the natriuretic peptide receptor. Topical delivery of siRNA resulted in decreased lung inflammation and airway hyper-responsiveness in a mouse model of allergic asthma. Moreover, transdermally applied siRNA chitosan particles have proved to be safe and effective in mice and may provide an innovative new treatment approach for preventing airway inflammation and asthma in humans [76].

5. Skin diseases

Diseases of the skin are amenable to RNAi-based therapies [42,77]. There is a variety of potential applications for RNAi therapy in the skin. The availability of disease lesions to topical treatments has been a motivating force for RNAi researchers. This abrogates the need for complex targeting strategies common with other disease models. The skin also has a high turnover rate and can be removed without complicated surgeries if necessary. The skin represents a good model for treatment and monitoring.

5.1 *In vitro* studies

To understand the function and dysfunction of the skin, it is necessary to study partial aspects in models of manageable size and to reintegrate the results back into context. Selective cultivation of cell components of the skin has added considerably to our understanding of their biology in health and disease. In addition, the use of cell cultures has enormously stimulated dermatopharmacological research and the development of new cutaneous therapies, including RNAi delivery. Tissue culture models range from simple monolayer cultures of one cell type to co-cultures of epithelial and mesenchymal cells and finally to three-dimensional skin equivalents – organotypic cultures. The latter probably represent the best *in vitro* alternative for intact skin with respect to animal welfare considerations. However, it is unlikely that a single *in vitro* model will incorporate all the complex series of reactions, such as a local skin inflammatory reaction occurring at different regions of the skin [78].

Table 2 gives an overview of the publications describing *in vitro* studies as a very first phase in the development of new RNAi skin therapeutics. Although cell cultures provide a good indication of the cytotoxicity and effectiveness of the treatment, it remains difficult to predict how the culture-induced changes in cell properties and/or the greater complexity of the *in vivo* environment in higher models will ultimately affect the phenotypical outcome aimed for.

5.1.1 Viral skin diseases

The conventional therapeutic approaches for viral infection of the skin are complicated because the viral disease processes are entangled with normal cell function. RNAi therapy offers a solution to this problem; it can specifically target viral gene products leaving normal cell function intact. Viral diseases of the skin are particularly important targets because of their carcinogenic potential.

Herpes simplex virus type 1 is a double-stranded DNA virus that infects up to 80% of individuals by adulthood worldwide and causes significant morbidity among immune-compromised hosts. To inhibit the cell-to-cell spread of the Herpes simplex virus type 1 in cultured human keratinocytes, Bhuyan *et al.* used a siRNA targeted against viral glycoprotein E. This glycoprotein is a transmembrane protein that is essential for immune evasion and replication. Keratinocytes were transfected with glycoprotein E-specific siRNA using Lipofectin and then infected with Herpes simplex virus type 1. This resulted in the formation of smaller plaques *in vitro* and reproduced the phenotype of the glycoprotein E null virus, a glycoprotein E deletion mutant strain. This study demonstrates that exogenous siRNA can suppress Herpes simplex virus type 1 glycoprotein E expression and function during active infection *in vitro* through RNAi [79].

5.1.2 Wound healing

Jazag *et al.* conducted a study where simultaneous knockdown of transforming growth factor-β (TGF-β) pathway-related Smads – Smad2, Smad3 and Smad4 – was performed in a

Table 2. Overview of the siRNA *in vitro* studies as a primary step in the development for possible future cutaneous siRNA treatments.

Authors	siRNA	Target cell	Model	(Possible) treatment	Delivery system
Bhuyan *et al.* [79]	sigE	HaCaT	Monolayer	HSV-1	Lipofectin
Jazag *et al.* [80]	siSmad2, 3, 4	HaCaT	Monolayer	Wound healing	Effectene
Sumimoto *et al.* [93]	siBRAF + siSkp-2	Melanoma cell lines	Monolayer	Melanoma	HIV-lentiviral vectors
Mildner *et al.* [84]	siVEGF/siMatriptase-1	Keratinocytes	Threee-dimensional organotypic skin model	Wound healing plus tumours/ichtyosis	Lipofectamine 2000
Van Gele *et al.* [81]	siMyosin Va exon F	Human melanocytes	Monolayer	Hyperpigmentation	HiPerfect plus HIV-lentiviral vector
Geusens *et al.* [82]	siMyosin Va exon F	Human melanocytes	Monolayer	Hyperpigmentation	Ultradeformable cationic liposomes
An *et al.* [83]	siTyrosinase	Human melanocytes	Monolayer	Pigmentation	Lipofectamine RNAiMAX

keratinocyte cell line HaCaT in a search for wound healing therapy. They described that loss of Smad3 alone or together with Smad2 or Smad4 accelerated wound closure in wounded cell monolayers. However, Smad4 knockdown alone blocked wound closure induced by TGF-β. TGF-β is known to promote wound closure by activating cell migration. These results showed that each Smad seems to contribute differently to cell migration, even though all Smads are downstream mediators of the same TGF-β pathway. The phenotypic changes that resulted from knockdown of different Smads are very complex and future investigation is needed to dissect the biological responses at the molecular level [80].

5.1.3 *Pigmentation*

Pigmentation of the skin is a complex process that involves synthesis and transfer of melanin from the melanocytes to the keratinocytes. Van Gele *et al.* developed a siRNA against Myosin Va exon F, a motor protein that is part of the tripartite complex with Slac2-a and Rab27a, which is involved in intracellular melanosome transport in the melanocytes [81]. Silencing this protein in cultured human primary melanocytes resulted in perinuclear aggregation of the melanosomes. In addition, a lentiviral vector system expressing shRNAs against Myosin Va exon F was developed and led to the inhibition of the formation of the Rab27a-Slac2-a-MyoVa tripartite complex as observed by redistribution of the melanosomes from the peripheral to the perinuclear region. This approach could lead to a treatment for hyperpigmentation disorders and is consistent with the phenotypic effect seen in melanocytes of Griscelli patients. In a next step, Geusens *et al.* developed ultradeformable liposomes as a delivery agent for this Myosin Va exon F siRNA [82]. The first *in vitro* results demonstrated efficient knockdown of the target gene in human primary melanocytes, and the favourable characteristics of the liposomes suggest a promising outcome for topical application to the skin.

An alternative method to control skin pigmentation is to inhibit melanogenesis in melanocytes by targeting tyrosinase by means of siRNA [83]. Melanin synthesis was reported to be efficiently inhibited. Consequently, cell viability after UV exposure was significantly decreased. *In vitro* studies also revealed a minor cytotoxic effect of antityrosinase siRNA in the absence of UV radiation. This implies that melanin plays a role in cell survival beyond UV absorption. Targets other than melanin as suggested by Van Gele *et al.* may be safer.

5.1.4 *Silencing in a human organotypic skin model*

The above-described data on RNAi applications *in vitro* use conventional monolayer culture systems. To improve on such models, Mildner and colleagues investigated whether RNAi technology could also be used in more complex *in vitro* cell culture systems. siRNAs were targeted against two genes strongly expressed by KC, VEGF and matriptase-1. KC were transfected using Lipofectamine 2000 before being seeded onto a fibroblast collagen gel. Although VEGF knockdown did not alter the epidermal phenotype, in the case of matriptase-1 knockdown terminal differentiation of KC was affected, manifesting as thickening of the stratum corneum and incomplete removal of the nuclei from end-differentiated KC. This phenotype recapitulates the phenotype of the matriptase-1 knockout animal. These results show that the use of RNAi in an organotypic model represents an important intermediate step in the evaluation of the feasibility, phenotypical outcome and safety of using siRNA for clinical applications [84].

5.2 *In vivo* studies

5.2.1 *Allergic skin disease*

Dermatitis, or inflammation of the skin, is a common cause of rashes, which can appear in several different forms. The distinct types of dermatitis are delineated by a causative agent or a cellular mechanism responsible for the rash. Contact

dermatitis is a delayed hypersensitivity reaction involving allergens and antibodies, whereas atopic dermatitis is an allergic-type reaction that is accompanied by hay fever, asthma and very dry skin. Ishimoto *et al.* developed an efficient siRNA against monocyte chemoattractant protein-1 (MCP-1) in order to treat contact hypersensitivity [85]. MCP-1 is secreted by activated skin cells, especially macrophages, during the elicitation phase after hapten re-exposure and is a trigger of contact hypersensitivity. In a first step, skin fibroblasts as well as macrophages were cultured and transfected with anti-MCP-1 siRNA using Lipofectamine. The *in vitro* data showed a more efficient transfection and gene silencing in the fibroblasts than in macrophages. Next, sensitised mice were exposed to 2,4-dinitrofluorobenzene and the resulting ear swelling was used as a model for contact hypersensitivity. When they compared local treatment versus systemic intravenous injection of anti-MCP-1 siRNA mixed with atelocollagen, a significant suppression of the ear swelling after two injections (at 30 min and 8 h) was reported. The local treatment showed no therapeutic effects.

Where Ishimoto *et al.* used anti-MCP-1 siRNA to treat contact hypersensitivity, Ritprajak *et al.* developed a cream-emulsion containing anti-CD86 siRNA to target cutaneous dendritic cells in atopic dermatitis-prone NC/Nga mouse models [70]. Systemic administration in this case may suppress protective immunity against infectious agents. So, topical application of a siRNA-based ointment was chosen to treat murine contact hypersensitivity and atopic dermatitis-like disease. Gene gun delivery was used to target skin dendritic cells *in vivo* [86]. This method was convenient and caused less irritation of the already inflamed skin. Moreover, repeated administration was reported to cause less irritation, achieved higher compliance and was more reproducible than intradermal injection.

Another study focusing on atopic dermatitis used the same NC/Nga mouse model. This study focused on the role of cyclooxygenase-1 gene in the scratching behaviour of mice. Targeted siRNA silencing of the cyclooxygenase-1 gene was accomplished by using intradermal injection. Electroporation reportedly accelerated the scratching behaviour of NC/Nga mice and revealed that cyclooxygenase-1-derived prostaglandins might act as endogenous inhibitors of itching. These data may be relevant to patients with atopic dermatitis [87].

5.2.2 Wound healing

Open wounds represent a substantial biomedical burden and offer a target-rich environment for candidate gene silencing. Thanik *et al.* developed an agarose matrix-based method to deliver siRNA effectively to cutaneous areas in a murine wound model [72]. The agarose matrix is a 12 kDa natural colloid seaweed extract and is used as a delivery system for MAPK1 and lamin A/C siRNA complexed with Lipofectamine 2000. Expression of both target genes was effectively reduced in the wound bed and lasted until a week after complete wound healing. There was no difference in treated groups compared to control in either MAPK1 or lamin A/C. This study reports effective gene silencing in open wounds and suggests the enormous potential for other targets involved in diabetic wounds, ulceration and scaring.

5.2.3 Alopecia Areata

Alopecia areata is an autoimmune disease affecting hair follicles, resulting in hair loss. This is thought to be triggered by a collapse of immune privilege. Nakamura *et al.* suggest a dominant role for Th1 cells in alopecia lesions. Therefore, they established a gene therapy experiment in an alopecia areata C3H/HeJ mouse model focused on the *Tbx21* gene. *Tbx21* plays a key role in Th1 cell development. The hypothesis was that silencing this gene with siRNA would restore hair growth. The authors reported that intralesional injections of anti-Tbx21 siRNA conjugated with cationised gelatin microspheres resulted in controlled release of the siRNA, which restored the hair shaft elongation [88].

5.2.4 Psoriasis

Psoriasis is a chronic inflammatory skin disorder affecting 1 – 3% of the world population characterised by demarcated erythematous scaly plaques in which keratinocytes show hyperproliferation and abnormal differentiation leading to epidermal hyperplasia. Within psoriatic lesions expression of tumour necrosis factor-α (TNF-α), a pro-inflammatory cytokine, is upregulated. Therefore, TNF-α mRNA is a siRNA therapy target. Jakobsen *et al.* engineered a xenograft transplantation model by transplanting lesional human psoriatic skin onto immunodeficient mice. A single intradermal injection of shRNA-encoding lentiviral vectors against TNF-α resulted in reduced levels of TNF-α. The authors reported reduced epidermal thickness, and resolution of the psoriasis phenotype was observed. These improved clinical scores showed that TNF-α mRNA is a useful target for treatment of psoriasis [89].

5.2.5 Skin cancer

Melanocytes are perhaps the most obvious non-keratinocyte target cell type in skin. Malignant melanoma is one of the most significant skin diseases in terms of morbidity, mortality and treatment costs. Not surprisingly, a considerable number of RNAi strategies have been reported for treatment of this condition. The proto-oncogene BRAF is one of the most frequently altered genes in cutaneous melanomas and is a downstream effector of another key oncogene, RAS. A single point mutation of BRAF, T1799A (or V599E amino acid substitution), has been demonstrated to be strongly tumorigenic in malignant melanoma cell models. This mutation is an important target for RNAi therapy in malignant melanoma tumours [90,91]. Experimental models of a siRNA selectively targeted to the mutated BRAF (V599E) in a melanoma cell line have shown that the cell line growth is strongly inhibited. Also, *in vitro* invasiveness and ability to form tumours in xenograft models are also decreased by means of siRNA therapy [92-94].

Sumimoto *et al.* found that simultaneous inhibition of mutated BRAF and Skp-2 in melanoma cells resulted in an effective inhibition of *in vitro* cell growth and invasive ability. Skp-2 is a substrate recognition subunit of a SCF ubiquitin–protein ligase complex, which is involved in the degradation of several proteins, including a cdk inhibitor, $p27^{Kip1}$ whose expression is often lost in many human cancers [19,93]. In these cancers, overexpression of Skp-2 is related to the decrease of $p27^{Kip1}$ through the enhanced degradation. Specific inhibition of the overexpressed Skp-2 therefore leads to reversion of the malignant phenotype owing to the increase of $p27^{Kip1}$.

A similar synergistic effect of two siRNAs was observed by Tran *et al.*, where the cooperative acting of siRNAs against ^{V600E}BRAF and Akt3 led to inhibition of tumour development *in vivo*. *In vitro* reconstructed melanoma skin models and xenograft melanoma mouse models were permeabilised with ultrasound before topical application of siRNA–nanoliposomal complexes. This non-surgical approach has the potential to decrease early melanocytic lesion development in skin or prevent the spread of cutaneous melanoma metastases [71].

Microphthalmia-associated transcription factor (Mitf) is critically involved in melanin synthesis and may promote cell progression as well as increase the survival of melanocytes and melanomas. There is controversy over whether Mitf functions as a positive or a negative regulator for melanoma proliferation and survival. Nakai *et al.* investigated the silencing effect of Mitf in B16 melanoma cell cultures and in preestablished B16 tumour mice. Their findings indicate that RNAi-mediated silencing of Mitf suppressed viability of B16 *in vitro*. The RNAi was also capable of inhibiting melanoma growth after intratumoral injection, followed by repetitive electroporation of RNAi in melanoma mouse models [59].

VEGF is a key promoter of angiogenesis in tumour growth. Matsumoto *et al.* introduced a siRNA expressing plasmid DNA conjugated with cationised gelatine microspheres by intratumoral injection [95]. The siRNA was targeted to VEGF isoform 5 in a mouse squamous cell carcinoma model. They successfully demonstrated sustained release of siVEGF-5 and expression in the tumours for at least 10 days. Similar results were obtained for malignant melanoma cell lines and mouse models by Tao *et al.* In this case, a melanoma cell line was stably transfected with a pU-VEGF-siRNA plasmid and subsequently injected subcutaneously in mice. The tumorigenicity in the pU-VEGF-siRNA-treated mice was significantly less than in those treated with the control plasmid [96]. Numerous studies demonstrated the involvement of proteins such as SOX10 [97], CDK2, FABP7 [34], ASK/Dbf4 [35], angiopoietin-2 [36] and SphK1 [37] in the development of skin cancers, making them potential targets for RNAi therapy. **Table 3** summarises a series of *in vivo* studies using RNAi therapy for skin cancers.

5.2.6 *Pachyonychia congenita*

Pachyonychia congenita is a well-characterised genetic disorder predominantly affecting nails and skin caused by mutations in keratins *KRT6A* or *KRT16* (PC-1) and *KRT6B* or *KRT17* (PC-2). So far, 55 causative mutations responsible for pachyonychia congenita have been published [98]. In the search for the optimal siRNA, effective and efficient screening revealed KGa.513a.12 as a siRNA target [46,99,100]. The siRNA targeted N171K mutant *KRT6A* mRNA and had no effect on wild-type K6a expression in tissue culture or animal studies [46]. The first clinical trial was initiated in early 2008. The study investigated the safety and tolerability of intralesional injections of siRNA into pachyonychia congenita patient calluses. Early findings in this first-in-man clinical trial with siRNA for a skin disorder indicated improvement at the site of siRNA treatment. For an overview of the treatment of pachyonychia congenita, see Leachman *et al.* [98].

6. Conclusion

Since the discovery of the RNAi mechanism, the number of published review papers on siRNA-based topics has exceeded a thousand. Interest in RNAi for cutaneous therapy is increasing, as reflected by the number of publications that have appeared in the last 3 years. This review describes the latest finding in cutaneous RNAi therapy. It gives an overview of the different delivery techniques and lists the *in vitro* and *in vivo* studies that have demonstrated effective gene silencing in target skin cells with RNAi. So far, only one siRNA treatment has entered the clinical phase.

7. Expert opinion

RNAi is a powerful technology with potential for therapeutics in dermatology. RNAi has not just been applied to understanding the functions of genes or signalling pathways inside skin cells, it has also been used as a therapeutic for skin disorders. The effectiveness and usefulness of RNAi therapy have been demonstrated in a clinical trial for pachyonychia congenita patients. The RNAi approach gave promising therapeutic results in this clinical trial. The pain from intralesional injections was a major obstacle that necessitated regional nerve blocks and oral pain medication to make the treatment bearable.

Clinical and laboratory results suggest that siRNAs do not necessarily need to be complexed with or encapsulated in a carrier system to be effective. Uptake of naked siRNA by keratinocytes has been shown repeatedly [46-48] and is probably not the rate-limiting factor for siRNA therapeutics. Overcoming the stratum corneum, on the other hand, is an important barrier. As a consequence, the best strategy for effective siRNA therapeutics is physically targeting the siRNA to the treatment area, as they do not readily penetrate the SC. So far, this can be achieved only by means of invasive delivery techniques, that is, dermabrasion, microneedles and intradermal injection. Unfortunately, naked siRNA therapy implicates nonspecific cell targeting and is prone to enzymatic and endosomal degradation. Therefore, it is often better to increase siRNA concentrations in order to obtain therapeutic effects. This is inevitably associated with a higher economical cost.

Table 3. Summary of RNAi studies targeting different genes in skin cancer.

Authors	siRNA	Type of skin cancer	RNAi delivery strategies	Established mouse model
Hoeflich *et al.* [92]	siBRAF	Melanoma	Stable A375 melanoma cell line transduced with retroviral expression plasmid for shRNA	Subcutaneous injection of shRNA-containing cell line in mice
Sharma *et al.* [94]	si^{V599E}B-Raf	Melanoma	siRNA nucleofected melanoma cell lines	Subcutaneous injection of siRNA-containing melanoma cell line in mice
Tran *et al.* [71]	si^{V600E}B-Raf + siAkt3 synergystic targeting	Melanoma	Topical application of siRNA–nanoliposomal complexes plus ultrasound	Melanoma skin reconstructs and xenograft melanoma mouse models
Nakai *et al.* [59]	siMitf	Melanoma	Intratumoral injection plus electroporation	Pre-established xenograft melanoma mouse model
Matsumoto *et al.* [95]	shVEGF-5	Squamous cell carcinoma	VEGF siRNA expressing plasmid conjugated with cationised gelatine microspheres	Intratumoral injection in xenograft squamous cell carcinoma mouse model
Tao *et al.* [96]	siVEGF	Melanoma	Stable melanoma cell line with U-VEGF-siRNA plasmid	Subcutaneous injection of siRNA-cell line in melanoma mouse models

An understanding of the underlying mechanism of the disease and how the pathology affects the barrier properties of the skin is necessary before developing the optimal delivery vehicle/method for RNAi treatment of a specific cutaneous disorder. Several key issues need to be addressed during this process:

- efficacy of the delivery method in the tissue to be treated
- associated pain and safety
- treatment area – what is the percentage of body surface area that requires treatment?
- translation into a home-based setting
- the cost of development.

Rating of importance of these issues can be a guideline for the development of a siRNA therapeutic strategy, including the search for the most suitable delivery system. This rating can be very subjective and vary from one application to another. That is why a variety of methodologies for cutaneous therapy exist (**Table 1**) and why the search for new techniques is still continuing. Current delivery methods have advantages and limitations, highlighting that no ideal technique exists. Quantifying patient compliance and tolerability is challenging because of patient variability and the differences in degree of pathology. Lack of standardised skin models and validated evaluation procedures may lead to misinterpretations of the efficacy, difficulty reproducing results and differences in the final cost of therapeutics. There needs to be a non-invasive method to assess siRNA delivery using clinically relevant imaging technologies in order to optimise experimental treatment strategies. Developing RNAi into therapeutic modalities would benefit from a combination of imaging techniques for RNAi delivery (biodistribution) with the quantitative assessment of RNAi effects *in vivo*.

Many modalities (e.g., gene gun, jet injection, microneedles, iontophoresis) for cutaneous siRNA delivery are available but more data are needed to identify the best technology for different applications. Different delivery techniques have been compared by Peachman *et al.* for DNA vaccine delivery and the gene gun has been identified as the most effective modality [101]. Although the gene gun is particularly suited for cutaneous vaccination, it does not imply that the same technology would be an efficient siRNA delivery device. Unfortunately, there may be an inverse relationship between the effectiveness of the RNAi delivery systems described in this review and factors such as patient compliance and economical feasibility for home-based settings. The available data indicate that intradermal or intralesional injection is the most efficient siRNA delivery method and can even be enhanced in combination with electroporation. The limitations of this method are that it is unsuitable for self-application, it is limited to restricted areas, painful, and accompanied with risk for infections, and needle stick injuries. Electroporation equipment is also relatively costly. Topical application of liposomes containing siRNA, on the other hand, scores very well with regard to patient compliance, production costs and treatment of whole body parts. By contrast, topical administration on skin leads to uptake confined to the upper epidermal layers without complete skin penetration [102].

Topical formulations could potentially end up being the most expensive alternative if most of the costly siRNA does not penetrate through the stratum corneum barrier. Controversy exists about the efficacy of a special type of liposome, ultradeformable transfersomes®. They have been reported to penetrate intact skin deep enough to reach the systemic circulation after topical administration, and deliver high-molecular-mass molecules with an efficacy similar to subcutaneous injection [103-105].

Liposomes can have safety issues that are primarily the result of preparation techniques associated with first-generation

'conventional' liposomes involving volatile organic solvents or detergents. These solvents exert toxicity towards cell membranes [106]. Liposomes have been described in combination with auxiliary compounds, such as ethanol or surfactants, for efficient skin penetration. The incorporation of glycols has enhanced liposomal stability [107]. Toxic effects from volatiles, surfactants and glycols can be minimised or avoided by optimising the formulation and/or preparation method. In addition, toxic effects are often not attributed to the liposome itself but are the result of the encapsulated drug [108]. In general, side effects from liposomes are not to be expected because liposomes are similar to epidermal lipids, biodegradable and non-toxic [109].

The principal advantages of liposomes are the ability to protect siRNAs from degradation and the possibility of receptor-mediated targeting [11]. siRNAs can be encapsulated in the inner core of the liposomes and/or complexed by means of electrostatic interactions. This protects the siRNA from shear forces, enzymes and the low endosomal pH. Cell-specific targeting reduces off-target effects and can be accomplished by integrating a ligand into the liposomal membrane.

The search for an improved cream-based siRNA delivery system is demanding a scientific marriage between pharmacists, biotechnologists and dermatologists. The question remains whether an effective formulation will be developed or whether the future lies in combining the use of liposomal formulations with other non-invasive techniques such as ultrasound or iontophoresis. Low-frequency ultrasound offers a non-invasive, non-painful permeability-enhancing technique that is suited for large body area applications. Combining the protective qualities of nanoliposomes with the permeabilising activities of low-frequency ultrasound has shown its efficacy for the treatment of cutaneous melanoma [71]. Iontophoresis has not been described for cutaneous siRNA delivery, neither has it been used in combination with liposomal siRNA complexes. Its applicability is more limited because it is not ideally suited for chronic wounds or diseases where the skin is broken. Manipulation or treatment of larger areas is also time-consuming and labour-intensive.

In conclusion, siRNA therapy has numerous applications in dermatology, particularly with monogenic dominant negative skin diseases where the silencing of a specific disease-causing gene is expected to have a clinical effect. siRNA agents show promise as new therapeutic tools for individualised medicine, in which siRNAs theoretically can be designed to target gene mutations. The application of siRNAs to therapeutics has raised some concerns about their safety [13]. siRNA degradation problems can be overcome by the use of appropriate backbone modifications. Delivery systems that mask RNAs from the receptors of the innate immune system will also increase functional siRNA at the targeted site [110]. The development of efficient siRNA delivery techniques for cutaneous therapy remains a major bottleneck. Successful (*trans*) dermal siRNA delivery is based on achieving a suitable balance between effective delivery and safety. Clinical impact does not just rely on safe and effective siRNAs or gene administration. Low-cost and easy to use siRNA delivery tools that allow self-administration at home are long-range goals for this technology. Research is now focusing on patient-friendly and efficient siRNA delivery methods for all types of skin disorders. Synergistic effects of non-invasive technologies could increase skin permeability. Translation of siRNA as a therapeutic will require collaboration between medical doctors, researchers and engineers to develop the first successful clinical siRNA therapy.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Stein LD. Human genome: end of the beginning. Nature 2004;431(7011):915-6
2. Sun BK, Tsao H. Small RNAs in development and disease. J Am Acad Dermatol 2008;59(5):725-37; quiz 738-740
3. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391(6669):806-11
4. Yao Y, Wang C, Varshney RR, Wang DA. Antisense makes sense in engineered regenerative medicine. Pharm Res 2009;26(2):263-75
5. Tachikawa K, Briggs SP. Targeting the human genome. Curr Opin Biotechnol 2006;17(6):659-65
6. Eckstein F. The versatility of oligonucleotides as potential therapeutics. Expert Opin Biol Ther 2007;7(7):1021-34

•• **Overview of the different oligonucleotides and their applications in clinical trials.**

7. Vickers TA, Koo S, Bennett CF, et al. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. J Biol Chem 2003;278(9):7108-18
8. Wadhwa R, Kaul SC, Miyagishi M, Taira K. Know-how of RNA interference and its applications in research and therapy. Mutat Res 2004;567(1):71-84
9. Chen X, Dudgeon N, Shen L, Wang JH. Chemical modification of gene silencing oligonucleotides for drug discovery and development. Drug Discov Today 2005;10(8):587-93
10. Prud'homme GJ, Glinka Y, Khan AS, Draghia-Akli R. Electroporation-enhanced nonviral gene transfer for the prevention or treatment of immunological, endocrine and neoplastic diseases. Curr Gene Ther 2006;6(2):243-73
11. Yu B, Zhao X, Lee LJ, Lee RJ. Targeted delivery systems for oligonucleotide therapeutics. Aaps J 2009;11(1):195-203
12. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009;8(2):129-38
13. Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. Nature 2009;457(7228):426-33

• **Great summary on the RNAi mechanism.**

14. Schlee M, Hornung V, Hartmann G. siRNA and isRNA: two edges of one sword. Mol Ther 2006;14(4):463-70
15. Aagaard L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. Adv Drug Deliv Rev 2007;59(2-3):75-86

• **Complete overview of the potential of RNAi therapeutics.**

16. Martin SE, Caplen NJ. Applications of RNA interference in mammalian systems. Annu Rev Genomics Hum Genet 2007;8:81-108
17. Dykxhoorn DM, Lieberman J. Knocking down disease with siRNAs. Cell 2006;126(2):231-5
18. Dykxhoorn DM, Palliser D, Lieberman J. The silent treatment: siRNAs as small molecule drugs. Gene Ther 2006;13(6):541-52
19. Raoul C, Abbas-Terki T, Bensadoun JC, et al. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. Nat Med 2005;11(4):423-8
20. Scheuplein RJ, Blank IH. Permeability of the skin. Physiol Rev 1971;51(4):702-47
21. Elias PM. Epidermal lipids, membranes, and keratinization. Int J Dermatol 1981;20(1):1-19
22. Barry BW. Dermatological Formulations: Percutaneous Absorption. New York/ Basel: Marcel Dekker; 1983
23. Williams AC. Transdermal and Topical Drug Delivery; from Theory to Clinical Practice. London: Pharmaceutical Press; 2003
24. Bissett DL. Anatomy and biochemistry of the skin. In: Kydonieus AF, Berner B, editors, Transdermal Delivery of Drugs. Boca Raton: CRC Press, Inc, 1987. p. 29-42
25. Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Ponec M. Structure of the skin barrier and its modulation by vesicular formulations. Prog Lipid Res 2003;42(1):1-36

•• **Excellent review on vesicle–skin interactions.**

26. Barbero AM, Frasch HF. Modeling of diffusion with partitioning in stratum corneum using a finite element model. Ann Biomed Eng 2005;33(9):1281-92
27. Uitto J, Pulkkinen L. The genodermatoses: candidate diseases for gene therapy. Hum Gene Ther 2000;11(16):2267-75
28. Ganesan AK, Ho H, Bodemann B, et al. Genome-wide siRNA-based functional genomics of pigmentation identifies novel genes and pathways that impact melanogenesis in human cells. PLoS Genet 2008;4(12):e1000298

• **High-throughput approach that identified 92 new genes involved in pigmentation.**

29. Borlon C, Vankoningsloo S, Godard P, et al. Identification of p53-dependent genes potentially involved in UVB-mediated premature senescence of human skin fibroblasts using siRNA technology. Mech Ageing Dev 2008;129(3):109-19
30. Murase D, Hachiya A, Amano Y, et al. The essential role of p53 in hyperpigmentation of the skin via regulation of paracrine melanogenic cytokine receptor signaling. J Biol Chem 2009;284(7):4343-53
31. Fimmel S, Devermann L, Herrmann A, Zouboulis C. GRO-alpha: a potential marker for cancer and aging silenced by RNA interference. Ann N Y Acad Sci 2007;1119:176-89
32. Kyoung Kim H, Kyoung Kim Y, Song IH, et al. Down-regulation of a forkhead transcription factor, FOXO3a, accelerates cellular senescence in human dermal fibroblasts. J Gerontol A Biol Sci Med Sci 2005;60(1):4-9
33. Youm JK, Jo H, Hong JH, et al. K6PC-5, a sphingosine kinase activator, induces anti-aging effects in intrinsically aged skin through intracellular Ca2+ signaling. J Dermatol Sci 2008;51(2):89-102
34. Slipicevic A, Jorgensen K, Skrede M, et al. The fatty acid binding protein 7 (FABP7) is involved in proliferation and invasion of melanoma cells. BMC Cancer 2008;8:276-88
35. Nambiar S, Mirmohammadsadegh A, Hassan M, et al. Identification and functional characterization of ASK/Dbf4, a novel cell survival gene in cutaneous melanoma with prognostic relevance. Carcinogenesis 2007;28(12):2501-10
36. Helfrich I, Edler L, Sucker A, et al. Angiopoietin-2 levels are associated with

disease progression in metastatic malignant melanoma. Clin Cancer Res 2009;15(4):1384-92

37. Bektas M, Jolly PS, Muller C, et al. Sphingosine kinase activity counteracts ceramide-mediated cell death in human melanoma cells: role of Bcl-2 expression. Oncogene 2005;24(1):178-87

38. Hattori T, Obinata H, Ogawa A, et al. G2A plays proinflammatory roles in human keratinocytes under oxidative stress as a receptor for 9-hydroxyoctadecadienoic acid. J Invest Dermatol 2008;128(5):1123-33

39. Ikuta S, Edamatsu H, Li M, et al. Crucial role of phospholipase C epsilon in skin inflammation induced by tumor-promoting phorbol ester. Cancer Res 2008;68(1):64-72

40. Howell MD, Fairchild HR, Kim BE, et al. Th2 cytokines act on S100/A11 to downregulate keratinocyte differentiation. J Invest Dermatol 2008;128(9):2248-58

41. Bin L, Howell MD, Kim BE, et al. Inhibition of S100A11 gene expression impairs keratinocyte response against vaccinia virus through downregulation of the IL-10 receptor 2 chain. J Allergy Clin Immunol 2009;124(2):270-77

42. Khavari PA, Rollman O, Vahlquist A. Cutaneous gene transfer for skin and systemic diseases. J Intern Med 2002;252(1):1-10

43. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 2000;9(3):165-9

• **The limitations of skin penetration.**

44. Fattal E, Bochot A. State of the art and perspectives for the delivery of antisense oligonucleotides and siRNA by polymeric nanocarriers. Int J Pharm 2008;364(2):237-48

45. Paroo Z, Corey DR. Challenges for RNAi in vivo. Trends Biotechnol 2004;22(8):390-4

46. Hickerson RP, Smith FJ, Reeves RE, et al. Single-nucleotide-specific siRNA targeting in a dominant-negative skin model. J Invest Dermatol 2008;128(3):594-605

47. Wang Q, Ilves H, Chu P, et al. Delivery and inhibition of reporter genes by small interfering RNAs in a mouse skin model. J Invest Dermatol 2007;127(11):2577-84

48. Gonzalez-Gonzalez E, Ra H, Hickerson RP, et al. siRNA silencing of keratinocyte-specific GFP expression in a transgenic mouse skin model. Gene Ther 2009;16(8):963-72

• **Interesting *in vivo* model for evaluating different patient-friendly delivery techniques.**

49. Hengge UR, Chan EF, Foster RA, et al. Cytokine gene expression in epidermis with biological effects following injection of naked DNA. Nat Genet 1995;10(2):161-6

•• **First paper describing the property of keratinocytes to take up naked DNA.**

50. Hengge UR, Walker PS, Vogel JC. Expression of naked DNA in human, pig, and mouse skin. J Clin Invest 1996;97(12):2911-6

51. Basner-Tschakarjan E, Mirmohammadsadegh A, Baer A, Hengge UR. Uptake and trafficking of DNA in keratinocytes: evidence for DNA-binding proteins. Gene Ther 2004;11(9):765-74

52. Wattiaux R, Laurent N, Wattiaux-De Coninck S, Jadot M. Endosomes, lysosomes: their implication in gene transfer. Adv Drug Deliv Rev 2000;41(2):201-8

53. Budker V, Budker T, Zhang G, et al. Hypothesis: naked plasmid DNA is taken up by cells in vivo by a receptor-mediated process. J Gene Med 2000;2(2):76-88

54. Scheuplein RJ. Mechanism of percutaneous adsorption. I. Routes of penetration and the influence of solubility. J Invest Dermatol 1965;45(5):334-46

55. El Maghraby GM, Williams AC. Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin. Expert Opin Drug Deliv 2009;6(2):149-63

56. Vogt A, Combadiere B, Hadam S, et al. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. J Invest Dermatol 2006;126(6):1316-22

57. Cevc G. Lipid vesicles and other colloids as drug carriers on the skin. Adv Drug Deliv Rev 2004;56(5):675-711

58. Draghia-Akli R, Khan AS. In vivo electroporation of gene sequences for therapeutic and vaccination applications. Recent Pat DNA Gene Seq 2007;1(3):207-13

59. Nakai N, Kishida T, Shin-Ya M, et al. Therapeutic RNA interference of malignant melanoma by electrotransfer of small interfering RNA targeting Mitf. Gene Ther 2007;14(4):357-65

60. Alexander MY, Akhurst RJ. Liposome-medicated gene transfer and expression via the skin. Hum Mol Genet 1995;4(12):2279-85

61. Babiuk S, Baca-Estrada ME, Pontarollo R, Foldvari M. Topical delivery of plasmid DNA using biphasic lipid vesicles (Biphasix). J Pharm Pharmacol 2002;54(12):1609-14

62. Coulman S, Allender C, Birchall J. Microneedles and other physical methods for overcoming the stratum corneum barrier for cutaneous gene therapy. Crit Rev Ther Drug Carrier Syst 2006;23(3):205-58

63. Cui Z, Mumper RJ. Topical immunization using nanoengineered genetic vaccines. J Control Release 2002;81(1-2):173-84

64. Kim A, Lee EH, Choi SH, Kim CK. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials 2004;25(2):305-13

65. Lee EH, Kim A, Oh YK, Kim CK. Effect of edge activators on the formation and transfection efficiency of ultradeformable liposomes. Biomaterials 2005;26(2):205-10

66. Niemiec SM, Latta JM, Ramachandran C, et al. Perifollicular transgenic expression of human interleukin-1 receptor antagonist protein following topical application of novel liposome-plasmid DNA formulations in vivo. J Pharm Sci 1997;86(6):701-8

67. Mori M, Kobayashi H, Sugiyama C, et al. Induction of unscheduled DNA synthesis in hairless mouse epidermis by skin carcinogens. J Toxicol Sci 1999;24(3):217-26

68. Watabe S, Xin KQ, Ihata A, et al. Protection against influenza virus challenge by topical application of influenza DNA vaccine. Vaccine 2001;19(31):4434-44

69. Dugour A, Hagelin K, Smus C, et al. Silencing the androgen receptor: new skills for antiandrogen oligonucleotide skin and hair therapy. J Dermatol Sci 2009;54(2):123-5

70. Ritprajak P, Hashiguchi M, Azuma M. Topical application of cream-emulsified CD86 siRNA ameliorates allergic skin disease by targeting cutaneous dendritic cells. Mol Ther 2008;16(7):1323-30

71. Tran MA, Gowda R, Sharma A, et al. Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. Cancer Res 2008;68(18):7638-49

• **Example of combined non-invasive cutaneous delivery techniques.**

72. Thanik VD, Greives MR, Lerman OZ, et al. Topical matrix-based siRNA silences local gene expression in a murine wound model. Gene Ther 2007;14(17):1305-8

73. Lee WR, Shen SC, Zhuo RZ, et al. Enhancement of topical small interfering RNA delivery and expression by low-fluence erbium: YAG laser pretreatment of skin. Hum Gene Ther 2009;20(6):580-8

74. Takahashi Y, Nishikawa M, Kobayashi N, Takakura Y. Gene silencing in primary and metastatic tumors by small interfering RNA delivery in mice: quantitative analysis using melanoma cells expressing firefly and sea pansy luciferases. J Control Release 2005;105(3):332-43

75. Takanashi M, Oikawa K, Sudo K, et al. Therapeutic silencing of an endogenous gene by siRNA cream in an arthritis model mouse. Gene Ther 2009;16(8):982-89

76. Wang X, Xu W, Mohapatra S, et al. Prevention of airway inflammation with topical cream containing imiquimod and small interfering RNA for natriuretic peptide receptor. Genet Vaccines Ther 2008;6:7-15

77. Pfutzner W, Vogel JC. Advances in skin gene therapy. Expert Opin Investig Drugs 2000;9(9):2069-83

78. Suskind RR. Environment and the skin. Med Clin North Am 1990;74(2):307-24

79. Bhuyan PK, Kariko K, Capodici J, et al. Short interfering RNA-mediated inhibition of herpes simplex virus type 1 gene expression and function during infection of human keratinocytes. J Virol 2004;78(19):10276-81

80. Jazag A, Kanai F, Ijichi H, et al. Single small-interfering RNA expression vector for silencing multiple transforming growth factor-beta pathway components. Nucleic Acids Res 2005;33(15):131-140

81. Van Gele M, Geusens B, Schmitt AM, et al. Knockdown of myosin Va isoforms by RNAi as a tool to block melanosome transport in primary human melanocytes. J Invest Dermatol 2008;128(10):2474-84

82. Geusens B, Lambert J, De Smedt SC, et al. Ultradeformable cationic liposomes for delivery of small interfering RNA (siRNA) into human primary melanocytes. J Control Release 2009;133(3):214-20

83. An SM, Koh JS, Boo YC. Inhibition of melanogenesis by tyrosinase siRNA in human melanocytes. BMB Rep 2009;42(3):178-83

84. Mildner M, Ballaun C, Stichenwirth M, et al. Gene silencing in a human organotypic skin model. Biochem Biophys Res Commun 2006;348(1):76-82

85. Ishimoto T, Takei Y, Yuzawa Y, et al. Downregulation of monocyte chemoattractant protein-1 involving short interfering RNA attenuates hapten-induced contact hypersensitivity. Mol Ther 2008;16(2):387-95

86. Kim TW, Lee JH, He L, et al. Modification of professional antigen-presenting cells with small interfering RNA in vivo to enhance cancer vaccine potency. Cancer Res 2005;65(1):309-16

87. Inoue T, Sugimoto M, Sakurai T, et al. Modulation of scratching behavior by silencing an endogenous cyclooxygenase-1 gene in the skin through the administration of siRNA. J Gene Med 2007;9(11):994-1001

88. Nakamura M, Jo J, Tabata Y, Ishikawa O. Controlled delivery of T-box21 small interfering RNA ameliorates autoimmune alopecia (Alopecia Areata) in a C3H/HeJ mouse model. Am J Pathol 2008;172(3):650-8

89. Jakobsen M, Stenderup K, Rosada C, et al. Amelioration of Psoriasis by Anti-TNF-alpha RNAi in the Xenograft Transplantation Model. Mol Ther 2009;17(10):1743-53

90. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417(6892):949-54

91. Karasarides M, Chiloeches A, Hayward R, et al. B-RAF is a therapeutic target in melanoma. Oncogene 2004;23(37):6292-8

92. Hoeflich KP, Gray DC, Eby MT, et al. Oncogenic BRAF is required for tumor growth and maintenance in melanoma models. Cancer Res 2006;66(2):999-1006

93. Sumimoto H, Hirata K, Yamagata S, et al. Effective inhibition of cell growth and invasion of melanoma by combined suppression of BRAF (V599E) and Skp2 with lentiviral RNAi. Int J Cancer 2006;118(2):472-6

94. Sharma A, Trivedi NR, Zimmerman MA, et al. Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors. Cancer Res 2005;65(6):2412-21

95. Matsumoto G, Kushibiki T, Kinoshita Y, et al. Cationized gelatin delivery of a plasmid DNA expressing small interference RNA for VEGF inhibits murine squamous cell carcinoma. Cancer Sci 2006;97(4):313-21

96. Tao J, Tu YT, Huang CZ, et al. Inhibiting the growth of malignant melanoma by blocking the expression of vascular endothelial growth factor using an RNA interference approach. Br J Dermatol 2005;153(4):715-24

97. Cook AL, Smith AG, Smit DJ, et al. Co-expression of SOX9 and SOX10 during melanocytic differentiation in vitro. Exp Cell Res 2005;308(1):222-35

98. Leachman SA, Hickerson RP, Hull PR, et al. Therapeutic siRNAs for dominant genetic skin disorders including pachyonychia congenita. J Dermatol Sci 2008;51(3):151-7

•• **Overview of the first clinical trial using siRNA for cutaneous purposes.**

99. Schwarz DS, Ding H, Kennington L, et al. Designing siRNA that distinguish between genes that differ by a single nucleotide. PLoS Genet 2006;2(9):e140

100. Smith FJ, Hickerson RP, Sayers JM, et al. Development of therapeutic siRNAs for pachyonychia congenita. J Invest Dermatol 2008;128(1):50-8

101. Peachman KK, Rao M, Alving CR. Immunization with DNA through the skin. Methods 2003;31(3):232-42

102. El Maghraby GM, Williams AC, Barry BW. Can drug-bearing liposomes penetrate intact skin? J Pharm Pharmacol 2006;58(4):415-29

103. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers,

Transfersomes. Biochim Biophys Acta 2001;1514(2):191-205

•• **First description of transfersomes as the first ultradeformable liposomal carriers for (trans)dermal drug delivery.**

104. Cevc G, Blume G. Biological activity and characteristics of triamcinolone-acetonide formulated with the self-regulating drug carriers, Transfersomes. Biochim Biophys Acta 2003;1614(2):156-64

105. Cevc G, Blume G. Hydrocortisone and dexamethasone in very deformable drug carriers have increased biological potency, prolonged effect, and reduced therapeutic dosage. Biochim Biophys Acta 2004;1663(1-2):61-73

106. de Leeuw J, de Vijlder HC, Bjerring P, Neumann HA. Liposomes in dermatology today. J Eur Acad Dermatol Venereol 2009;23(5):505-16

107. Lautenschläger H. Liposomes. In: Barel AO, Paye M, Maibach HI, editors, Cosmetic science and technology. Boca Raton: CRC Press Taylor & Francin Group, 2006. p. 155-63

108. Fiume Z. Final report on the safety assessment of lecithin and hydrogenated Lecithin. Int J Toxicol 2001;20(Suppl 1):21-45

109. Dreher F, Walde P, Luisi PL, Elsner P. Human skin irritation studies of a lecithin microemulsion gel and of lecithin liposomes. Skin Pharmacol 1996;9(2):124-9

110. Sioud M. Does the understanding of immune activation by RNA predict the design of safe siRNAs? Front Biosci 2008;13:4379-92

111. Choi MJ, Zhai H, Löffler H, et al. Effect of tape stripping on percutaneous penetration and topical vaccination. Exogenous Dermatology 2003;2:262-9

112. Surber C, Schwarb FP, Smith EW. Tape-stripping technique. In: Broncugh RL, Maibach HI, editors, Percutaneous absorption: drug - cosmetics - mechanism - methodology. New York: Dekker, 1999. p. 395-409

113. Liu Y, Truong NK, Kendall MA, Bellhouse BJ. Characteristics of a micro-biolistic system for murine immunological studies. Biomed Microdevices 2007;9(4):465-74

114. Dileo J, Miller TE Jr, Chesnoy S, Huang J. Gene transfer to subdermal tissues via a new gene gun design. Hum Gene Ther 2003;14(1):79-87

115. McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles for gene and drug delivery. Annu Rev Biomed Eng 2000;2:289-313

116. Walters KA, Roberts MS. Dermatologic, cosmeceutic, and cosmetic development: therapeutic and novel approaches. New York: Informa Healthcare; 2008

117. Rao R, Nanda S. Sonophoresis: recent advancements and future trends. J Pharm Pharmacol 2009;61(6):689-705

• **This review describes the potential application of ultrasound in cutaneous delivery.**

118. Heller LC, Jaroszeski MJ, Coppola D, Heller R. Comparison of electrically mediated and liposome-complexed plasmid DNA delivery to the skin. Genet Vaccines Ther 2008;6:16-22

119. Pliquett U, Weaver JC. Feasibility of an electrode-reservoir device for transdermal drug delivery by noninvasive skin electroporation. IEEE Trans Biomed Eng 2007;54(3):536-8

120. Bodles-Brakhop AM, Heller R, Draghia-Akli R. Electroporation for the delivery of DNA-based vaccines and immunotherapeutics: current clinical developments. Mol Ther 2009;17(4):585-92

121. Wong TW, Ko SF, Hui SW. Enhancing transdermal drug delivery with electroporation. Recent Pat Drug Deliv Formul 2008;2(1):51-7

122. Villemejane J, Mir LM. Physical methods of nucleic acid transfer: general concepts and applications. Br J Pharmacol 2009;157(2):207-19

123. Sakamoto T, Miyazaki E, Aramaki Y, et al. Improvement of dermatitis by iontophoretically delivered antisense oligonucleotides for interleukin-10 in NC/Nga mice. Gene Ther 2004;11(3):317-24

124. Aramaki Y, Arima H, Takahashi M, et al. Intradermal delivery of antisense oligonucleotides by the pulse depolarization iontophoretic system. Biol Pharm Bull 2003;26(10):1461-6

125. Kasha PC, Banga AK. A review of patent literature for iontophoretic delivery and devices. Recent Pat Drug Deliv Formul 2008;2(1):41-50

126. Karande P, Jain A, Mitragotri S. Discovery of transdermal penetration enhancers by high-throughput screening. Nat Biotechnol 2004;22(2):192-7

127. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev 2004;56(5):603-18

128. Ahad A, Aqil M, Kohli K, et al. Chemical penetration enhancers: a patent review. Expert Opin Ther Pat 2009;19(7):969-88

129. Thong HY, Zhai H, Maibach HI. Percutaneous penetration enhancers: an overview. Skin Pharmacol Physiol 2007;20(6):272-82

130. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci 2008;34(4-5):203-22

•• **Very extended and useful review on skin structure and its interaction with different liposomal formulations.**

131. Kanikkannan N, Singh J, Ramarao P. Transdermal iontophoretic delivery of bovine insulin and monomeric human insulin analogue. J Control Release 1999;59(1):99-105

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