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Cutaneous gene transfer for skin and systemic diseases

P. A. KHAVARI1, O. ROLLMAN2 & A. VAHLQUIST2
From the 1VA Palo Alto Healthcare System and the Program in Epithelial Biology, Stanford University School of Medicine, Stanford, CA, USA and 2Department of Medical Sciences, Dermatology, Uppsala University, Uppsala, Sweden

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Recent progress in molecular genetics has illuminated the basis for a wide variety of inherited and acquired diseases. Gene therapy offers an attractive therapeutic approach capitalizing upon these new mechanistic insights. The skin is a uniquely attractive tissue site for development of new genetic therapeutic approaches both for its accessibility as well as for the large number of diseases that are amenable in principle to cutaneous gene transfer. Amongst these opportunities are primary monogenic skin diseases, chronic wounds and systemic disorders characterized by low or absent levels of circulating polypeptides. For cutaneous gene therapy to be effective, however, significant progress is required in a number of domains. Recent advances in vector design, administration, immune modulation, and regulation of gene expression have brought the field much nearer to clinical utility.

Keywords: epidermolysis bullosa, gene therapy, genodermatosis, ichthyosis, wound healing.

Introduction

The skin is a uniquely attractive tissue site for development of new genetic therapeutic approaches both for its accessibility as well as for the large number of diseases that are amenable, in principle, to cutaneous gene transfer. Amongst these opportunities are primary monogenic skin diseases, chronic wounds and systemic disorders characterized by low or absent levels of circulating polypeptides. Recent advances in vector design, administration, immune modulation, and regulation of gene expression have brought the concept of gene therapy much nearer to clinical utility. This, coupled to the recent progress in molecular genetics of the skin has opened up the possibility for the future use of gene therapy in a variety of inherited and acquired diseases. In this review, we will focus on the latest developments as they have been applied to gene transfer to the skin, beginning with a short recapitulation of the biology of the integument.

Structure and function of the skin

Human skin consists of three layers: (i) epidermis, which is an ectodermally derived keratinized stratified squamous epithelium; (ii) dermis, which is a collagen/elastin-rich connective tissue containing
blood and lymphatic vessels, nervous elements and scattered cells (fibroblasts, mast cells, macrophages, lymphocytes, etc.); and (iii) subcutis, which contains fat and blood vessels (Fig. 1). Of ectodermal origin, but extending deep into dermis (and occasionally subcutis) are the adnexal structures, including sweat glands, hair follicles and sebaceous glands. The latter structures are lacking in palmo-plantar skin which has an disproportionately thick epidermis mainly composed of stratum corneum. In non-palmo-plantar skin the thickness of epidermis is only in the order of 0.2 mm and the barrier against dehydration resides in a 10–20 μm thick layer [1]. Although, this barrier is poorly permeable to water, transepidermal water loss (TEWL) in an adult person is still in the order of 0.5 L day⁻¹. The cornified or horny layer may allow some transport of substances from the surface of the skin inwards, although water-soluble compounds do so with greatest difficulty. Small, lipid-soluble compounds may, however, readily diffuse through the intercellular layers in the stratum corneum. Detailed information about these processes is of course crucial when nucleic acids are therapeutically applied to the skin.

The cornified layer is produced by keratinocytes undergoing a specialized type of apoptosis [2]. This process is completed in the granular layer where most of the cellular organelles are degraded. Prior to that, the keratinocytes posses all the elements of a normal cell, i.e. a lipid membrane, a cytoskeleton composed of keratins, a nucleus surrounded by a cytosol containing numerous organelles. The differentiated keratinocytes arise from a pool of transient amplifying cells located in the basal layer of epidermis, which in its turn is derived from epidermal stem cells located at the tips of the dermal papillae [3]. These interfollicular stem cells originate from the multipotent stem cells located in the bulge area of the hair follicle. Stem cells can be isolated from epidermis in vitro and typically express certain cell surface markers, but are presently impossible to specifically target in vivo.

The water-containing compartment of the stratum corneum consists of cornocytes that are packed with keratins surrounded by a cornified envelope composed of cross-linked proteins and covalently attached lipid molecules [1]. Water-soluble compounds are believed to pass the horny

Fig. 1 Structure of human skin tissue.
layer mainly via the corneocytes that have a variable water content (from 5 to 60%) depending on the state of humidification. The horny layer has many characteristic structural elements, including corneosomes that are specialized desmosomes attaching the cells to one another until they are proteolytically degraded during desquamation of the horny cells from the skin surface. Although the architecture of the stratum corneum is an obstacle to transdermal therapy, removal of the stratum corneum, i.e. by stripping or dermabrasion, greatly enhances the penetrability of the skin; so does occlusion. A more physiological route of entrance to the skin is perhaps via the hair follicles and sweat glands, which both penetrate the stratum corneum. However, the surface area of these openings is very limited and located quite far form the structures to be targeted by gene therapy.

The epidermis is characterized by a preponderance of keratinocytes connected to one another by desmosomes and to the underlying basal membrane by hemidesmosomes. However, there are also other cell types present, for example, Langerhans cells. The former cell type is an antigen presenting cell that forms a part of the immunological barrier of the skin. The epidermis thus accomplishes protection against both mechanical, immunological, bacterial and actinic (UV) insults. In addition, it is a water barrier and a dynamic tissue which is continuously renewing itself and engaged in continual cross-talk with the surrounding tissues via release of cytokines and growth factors [2]. The basement membrane, which separates epidermis from dermis, is a fairly loose layer of proteins and glycosaminoglycans allowing free diffusion of intermediate size proteins and other macromolecules across the membrane. All these features combine to make the epidermis a challenging tissue for various gene delivery and targeting approaches, especially because the underlying dermis is rich in capillaries that efficiently clear any injected or released compounds.

These approaches include gene restoration, gene augmentation, gene correction and gene inhibition. In the case of recessive loss-of-function mutations such as those evident in many genodermatoses, simple re-introduction of the wild-type gene by viral or nonviral insertion may be sufficient for correction. In this setting, integrating viral vectors have been of central interest for their ability to stably integrate therapeutic genes into host cell genome and support durable correction [4]. Similar approaches have been used to augment expression of a gene whose overexpression is necessarily therapeutically, as in erythropoietin-responsive anaemia [5].

In the case of recessive or dominant disorders because of small genetic lesions such as point mutations, in situ genome targeting to correct the altered sequences has been undertaken using small DNA/RNA chimeric molecules. Site-specific correction of mutations in mammalian cells have been achieved at frequencies of 5–10% of cells [6, 7]. Similarly, using recombinant adeno-associated viral (rAAV) vectors containing corrected nucleic acid sequences homologous to the DNA surrounding known genetic lesions, gene defects at multiple loci may be corrected in murine embryonic stem cells [8]. However, all in situ repair strategies currently suffer from low efficiency of the process. In the case of pathogenic trans-dominant mutations, gene inhibition approaches have been used. Delivery of antisense nucleic acids [9] and ribozymes [10] represent two strategies for gene inhibition that act at the level of mRNA inhibition via recognition of specific sequences in the targeted gene. In a recent study, phosphorothioate oligonucleotides were used in a cream formulation that appeared to enhance their penetration through stratum corneum and achieve therapeutic concentrations in epidermis and dermis after topical application [11]. These and other results show the ability of the topical formulation to deliver oligonucleotides rapidly to the intended targets within the skin and cause inhibition of specific proteins [12]. Finally, introduction of ‘suicide genes’, such as those encoding the herpes virus thymidine kinase gene, has been explored as a means of killing cancers cells and potentially inducing a clearing immune response to eliminate them [13]. An underlying theme regarding selection of any of these mechanistic strategies for cutaneous gene transfer concerns the need to tailor the strategy...
selected to the underlying genetic mechanism of the disease being addressed.

**Approaches to cutaneous gene delivery**

As the skin provides a primary barrier to microbial invasion and desiccation, cutaneous tissue poses substantial obstacles to effective insertion of foreign DNA. The two basic approaches to therapeutic gene delivery to the skin involve *ex vivo* and *in vivo* gene delivery (Fig. 2). *Ex vivo* delivery involves a skin biopsy to harvest cells for growth and gene insertion in culture followed by re-grafting to the patient. Whilst cumbersome, painful, costly and potentially scarring, the *ex vivo* approach has several major advantages. First, it utilizes well-established autologous skin grafting techniques used in the treatment of burns and wounds. Second, it allows confirmation of the efficiency of gene transfer and the cell-type targeted (i.e. keratinocytes, fibroblasts) prior to delivery to the patient. Third, gene transfer in culture is useful in engineering growing cells with the most common studied viral vector, murine Type C retroviral vectors, which only infect actively dividing cells. Finally, the *ex vivo* approach avoids administering vectors directly to the patient with the concomitant risk of systemic spread and thus permits safety studies such as screens for replication competent retrovirus (RCR) prior to patient contact. For these reasons, *ex vivo* cutaneous gene transfer has been a major focus of early efforts in the field and has produced all the published examples of corrective cutaneous gene delivery to date [14–17].

In contrast to *ex vivo* cutaneous gene delivery, *in vivo* gene transfer delivers genetic material directly to intact patient skin tissue and is thus generally more simple and direct. Direct administration has been undertaken using a variety of approaches with both nonviral and viral vectors, including topical application, direct injection, application to wounded skin surfaces, electroporation and bioplastic particle insertion [18].

Topical application has generally focused on nonviral plasmid vectors that exhibit a follicular predominance in uptake and expression [19–21]. Because of the low efficiency and transient nature of this process, to date topical application of plasmids has only shown potential utility as a means of vaccination [20], a process also reported with application of topical adenovirus [22]. Direct injection of

![Gene delivery diagram](image)

*Fig. 2 Strategies for cutaneous gene transfer.*
genetic material into intact skin has achieved epidermal, dermal and subcutaneous gene transfer using both viral and non-viral vectors [5, 23-25]. Durable gene delivery via direct injection to intact skin, however, has only been observed with viral vectors. These include lentiviruses primarily engineered dermal fibroblasts and endothelial cells [5] and AAV directed to the panniculus carnosus [25]; the fact that the latter muscular structure is not present in humans limits the clinical relevance of the latter findings. Application of pseudotyped retroviral vectors to skin wounded via dermabrasion offers another means of stable cutaneous gene transfer. This approach involves placement of virus under wound eschar and is uniquely effective amongst in vivo gene transfer approaches in achieving prolonged gene transfer to cells of the epidermis and appendages [26]. Cutaneous electroporation following direct injection offers another means of targeting multiple cell types in skin [27] and has been used to boost efficiency in vaccination efforts [28]. Finally, bioplastic particle acceleration (the ‘gene gun’)-based bombardment of vector-coated microparticles has been used for immune modulation in vaccination and cancer therapy models [29] as well as in wound healing [30]. Whilst promising in terms of ease of application in many cases, current approaches to direct in vivo gene transfer still fall short of the ideal safe, efficient and regulated attributes desirable for many cutaneous gene transfer applications.

Gene therapy for genodermatoses

The rapid increase in knowledge about genetic diseases over the last 10 years is unprecedented in the history of medicine and has raised hopes amongst doctors and their patients that some of the severe genetic diseases will soon be possible to cure once and for all. The identification of genes responsible for almost 100 diseases affecting the skin has raised hopes in this regard [31]. A number of these genetic disorders, especially those involving mutations in structural proteins, are likely to be resistant to conventional pharmacotherapy and current efforts centre on returning normal protein expression. Furthermore, the genomic explosion has revolutionized our understanding of the molecular biology of human tissues, including the skin. This too has raised hopes about future possibility of efficient modulation of many pathophysiological events such as carcinogenesis, wound healing, fibrosis and inflammatory reactions of the skin. However, the therapeutic advances because of the genomic revolution have so far been meagre.

The most obvious candidate diseases for cutaneous gene therapy are the severe forms of certain genodermatoses (monogenetic skin disorders), for example, mechno-bullous disorders because of defects in anchoring molecules in epidermis (e.g., epidermolysis hyperkeratosis because of keratin mutations and various forms of epidermolysis bullosa) and disorders of the skin barrier because of defects in the keratinization process (e.g., lamellar ichthyosis because of transglutaminase-1-deficiency and Netherton’s syndrome because of SPINK5 mutations). In all these disorders the genetic aetiology has been pin-pointed and can be fairly easily identified in affected individuals.

Epidermolysis bullosa (EB) comprises a large group of congenital blistering diseases extending from mild and rather common forms with blisters appearing on friction-exposed skin areas (e.g., EB simplex) to more rare severe forms with widespread epidermolysis and chronic ulcerations with mutilating scarring (e.g., EB junctionalis and recessive dystrophic EB) (Fig. 3). Some of these diseases of the basal membrane also extends to the oral and intestinal mucosa and in rare cases EB may even be associated with muscular involvement [32]. A restoration of adequate levels of the missing structural protein in all these tissues is an obvious aim of therapy. To date, two subtypes of human EB tissue have been corrected in vivo in a preclinical model of gene therapy. Using ex vivo gene transfer in the human skin graft/immune deficient mouse xenograft model [33], both BP180-deficient and laminin 5-deficient junctional EB have been corrected at all levels studied [14, 15]. These studies provide the framework for the first genodermatoses trials planned in humans.

Ichthyosis is also a large family of diseases with different aetiologies but having in common a massive cutaneous hyperkeratosis [34]. This results in intense scaling, skin dryness (xerosis), hypohidrosis, and superficial constrictions of joints and eyelids (ectropion) (Fig. 4). The most severe types of ichthyosis are lamellar ichthyosis, congenital ichthyosisiform erythroderma and bullous ichthyosis (syn. epidermolysis hyperkeratosis). These rare and
always congenital diseases are usually associated with a life-long functional and psychological handicap. In a similar approach to that used for junctional EB subtypes noted above, both X-linked ichthyosis and lamellar ichthyosis patient skin tissue has been normalized in vivo in preclinical xenograft studies [16, 17, 35]. There is also reason to believe that an antisense probe targeted against a mutated allele that harbours a mismatch compared with the wild type allele could specifically knockout the expression of the dominant negative protein. Examples of such monogenetic skin diseases are congenital bullous ichthyosiform erythroderma of the Brocq and Siemens types, caused by mutations in keratins 1, 2e or 10. However, the chance of finding a good antisense probe is limited, because the choice of the target region on the mRNA is dictated by the location of the mutation that varies from one family to the other. Another potentially promising approach is to use ribozyme technology to specifically silence mutated keratin alleles in epidermolysis bullosa simplex cells [36]. Taken together, preclinical advances in the correction of the genodermatoses prototype diseases of EB and recessive ichthyosis provide a platform for future efforts to extend cutaneous gene therapy efforts to human clinical application.

**Gene therapy for healing of cutaneous wounds**

The concept of gene transfer in clinical dermatology is not restricted to genodermatoses but may embrace nonheritable diseases such as severe burns and refractory skin wounds of decubital, vascular or
diabetic origin. The overall goal would be to enhance wound-healing rate or tensile strength, or to inhibit postulcer complications, e.g. scarring and keloid formation by transiently modulating the panel of gene expression.

Mechanistic strategies in gene therapy protocols for chronic wounds have aimed at boosting a mitogenic factor known to participate in acute wound healing rather than to reduce inhibitory components involved in chronic ulceration. Experimental models have revealed that acute wound repair comprises a network of finely tuned cascades involving both resident and immigrating cells within the area of injury. The immediate haemostatic and subsequent inflammatory stages seem to orchestrate healing mainly via cytokines and peptide growth factor signalling that co-ordinates migration, proliferation and synthetic activities of, e.g. dermal fibroblasts and endothelial cells. A provisional, capillary-rich granulation tissue then forms within the dermal defect and provides necessary support for regrowth of epidermis from the wound edges or underlying adnexal structures. As wound closure is completed, the final remodeling phase of dermis will continue for months or years.

Which precise repair mechanisms go wrong in nonhealing conditions probably differ from one type of ulcer to the other. Taken together, a number of abnormalities have been reported to be linked to inadequate wound healing, e.g. reduced growth factor level or growth factor receptor density, abnormal proteolytic activity [37], impaired nitric oxide synthetase activity [38] and defective dermal fibroblast function [39]. Specifically, reduced expression of platelet-derived growth factor (PDGF-BB) and its receptor has been reported in delayed skin repair in animals [40]. Thus, attempts to stimulate healing in rabbit ischaemic ulcers have been made by introducing a collagen-embedded PDGF-B DNA plasmid [41] or the PDGF-B gene [42, 43] using a viral vector. Similarly, grafting of PDGF-A-transduced human skin to athymic mice was reported to cause local overexpression of PDGF-AA with concomitant reduction in wound contraction [44], increase in blood vessel formation and dermal cell density [45]. With the purpose of transforming epidermis to a PDGF-sensitive tissue, the PDGF receptor gene was recently transduced into a human skin equivalent [46]. By exposing the skin model to exogenous ligand, the keratinocytes transduced with the foreign gene responded by increased proliferation similarly to nonepidermal cells expressing the natural PDGF receptor. Other preclinical examples of experimental gene therapy studies are summarized in Table 1.

Delivery modes to wounds are somewhat different from those in genodermatoses with preserved epidermis. Not only is the epidermal barrier lacking which facilitates gene transfer to the wound bed; the ulcer area is usually limited and the time period required for transgene expression need not be very long. These advantages makes it feasible to use nonintegrative viral vectors or nonviral transfection options such as gene-gun delivery, direct application of naked DNA, liposomal or electroporative transfer, intraventricular injection by syringe, microvascular transfection, or wound bed implantation of genetically modified cells. Also, antisense oligonucleotides have been used, e.g. to regulate the activity of collagen genes thus controlling fibrosis [47].

### Table 1 Preclinical examples of wound gene transfer

<table>
<thead>
<tr>
<th>Target component</th>
<th>Gene of interest</th>
<th>Transfer principle</th>
<th>Vehicle</th>
<th>Recipient</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulation</td>
<td>PDGF-A</td>
<td>Ex vivo</td>
<td>Retrovir</td>
<td>Mouse</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>PDGF-B</td>
<td>In vivo</td>
<td>Adenoviral</td>
<td>Rabbit</td>
<td>[42]</td>
</tr>
<tr>
<td>Vascularization</td>
<td>KGF, IGF-1</td>
<td>In vivo</td>
<td>Liposomal</td>
<td>Rat</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>In vivo</td>
<td>Adenoviral</td>
<td>Mouse</td>
<td>[62]</td>
</tr>
<tr>
<td>Repithelialization</td>
<td>EGR-1</td>
<td>In vivo</td>
<td>Liposomal</td>
<td>Rat</td>
<td>[63]</td>
</tr>
<tr>
<td>Scar quality</td>
<td>KGF</td>
<td>Ex vivo</td>
<td>Gene gun</td>
<td>Mouse/rat</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>In vivo</td>
<td>Plasmid</td>
<td>Mouse</td>
<td>[65]</td>
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<tr>
<td></td>
<td>TGFbeta</td>
<td>In vivo</td>
<td>Retrovir</td>
<td>Pig</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>PFG</td>
<td>In vivo</td>
<td>Antisense</td>
<td>Mouse</td>
<td>[67]</td>
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EGR-1: early growth response factor 1 (transcription factor).

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tissue-specific promoters or inducible elements into recombinant vectors, transgene expression can be targeted to specific cells or sites, e.g. wound edge keratinocytes [48].

Potential delivery obstacles with gene application to chronic wounds – at least with genes directed towards resident cells in the wound bed – include physical hindrance because of necrotic tissue, fibrin and inflammatory exudate. Pharmacokinetic problems because of nonspecific binding or degradation of the genetic material by wound exudate components may occur. Moreover, dermal cells are sparsely distributed and more difficult to reach than epidermal keratinocytes; thus, specific cell targeting and gene dosing to, e.g. wound fibroblasts or endothelial cells may be difficult to achieve. As with gene therapy for other indications, the potential of immunological or toxic side-effects, and tumour development – in particular with growth factor gene therapies – has to be considered. Using ex vivo gene therapy the 'take rate' of genetically engineered cells or epidermal sheets on chronic wounds will probably be lower than in acute wounds – as is the case with native cells. Biotechnological refinements such as the wound chamber technique [49] and gene-delivering gel/matrix products [50, 51] or special dressings incorporating the DNA may, however, help to improve the efficacy of gene delivery to ulcers.

The prospect of wound-directed gene therapy will depend on future progress in chronic wound biology at the molecular level. Considering the complexity of tissue repair it is likely that genetic manipulation of healing processes has to include multiple and repeated applications of therapeutic genes.

The skin as bioreactor for systemic therapy
Because of its accessibility, rich vascularization and ability to deliver skin-produced polypeptides to the bloodstream, the skin is an attractive tissue site for gene-based systemic protein delivery. Accordingly, the skin has been used via both ex vivo and in vivo approaches to deliver polypeptides to blood. Amongst the gene products delivered are growth hormone, transferrin, erythropoietin, apolipoprotein E and Factor IX [5, 52–55]. In the case of ex vivo gene transfer, gene delivery has relied on a variety of grafting approaches, including epidermal sheets [56], fibroblast-rich composites [57] as well as full thickness transgenic skin [58]. Interestingly, all of these approaches are capable of successfully delivering specific gene products to the circulation. The versatility of the skin 'bioreactor' approach has been further enhanced by introduction of the capacity for topical regulation of gene products including growth hormone to the bloodstream via such approaches as modified progesterone receptor driven transcription [59]. Whilst it is not surprising that dermal gene transfer can support this process, the fact that epidermis-produced proteins can traverse the dermal epidermal junction and the dermal matrix to cross into the vasculature speaks to the remarkable potential of the skin as a bioreactor.

As in the treatment of primary of primary skin diseases, direct in vivo gene delivery represents the most straightforward strategy to harness the skin as a bioreactor. Unlike the case in the genodermatoses, however, systemic delivery currently appears very amenable to direct gene transfer by a number of approaches. One promising avenue includes intradermal injection of lentiviral vectors. Because of their capacity for genomic integration into cells within tissue upon direct administration and resulting durable gene expression, these vectors have been widely studied for direct gene delivery [60]. In support of their promise in skin, a single direct injection of HIV1-based lentivectors encoding the erythropoietin gene into full thickness human skin xenografts on immune deficient mice sustained serum erythropoietin levels and boosted haematocrit for nearly 1 year [5]. Given the tractability of skin to topical regulation combined with the ready ability to remove engineered tissue in the event of unwanted hypersensitivity reactions to introduced gene products, the use of skin as a primary site for systemic gene product delivery demonstrates great future potential.

Conclusions
The genetic basis for a number of heritable skin disorders is now well understood and corrective models of human genetic skin disease have been established. Future success in widespread application of these initial advances to humans will depend on development of new capabilities allowing effective gene transfer to the skin. Fundamental to such capabilities will be improved gene delivery vectors and strategies for their administration.

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Correspondence: Dr Paul A. Khavari, Room 2153, CCSB Building, Stanford University School of Medicine, Stanford, CA 94305-5168, USA (fax: +1 650 723 8672; e-mail: khavari@crgm Stanford.edu).