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CRISPR-Mediated Gene Editing: A Revolutionary Strategy for Treatment of Cutaneous Diseases

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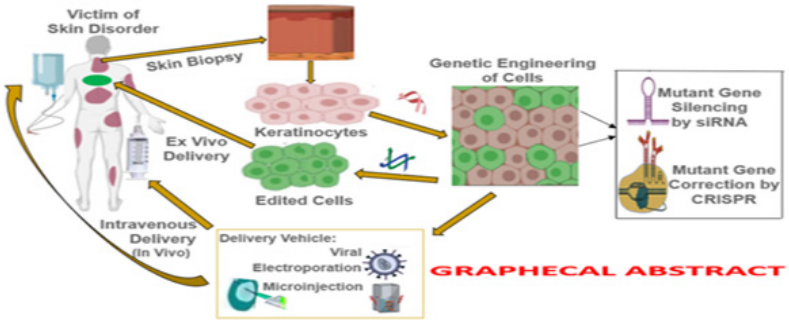

Abstract

There is a wide range of chemotherapeutic options for many cutaneous diseases. Despite their usefulness in many skin conditions, some patients either do not respond to traditional therapies or are concerned about the serious side effects. Gene editing (GE) treatments, particularly those Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-mediated ones, are good alternatives. This review aims to integrate all classes of CRISPR nucleases—DNA and RNA targeting—into the collection of clinical tools available to the practicing dermatologist. For this purpose, the PubMed and Google Scholar search engines were explored. The CRISPR systems beyond the commonly used Cas9, such as the advancement of Cas12 and Cas13 variants, as well as base editing (BE), and the prime editing (PE) innovations, have taken center stage. This confirms remarkable precision and efficiency in targeted ex vivo and in vivo gene modifications, allowing more intricate reformation and therapeutic interventions. Various delivery modalities for CRISPR therapeutics, including microneedles and transdermal areas, are uniquely right for dermatological diseases. Several preclinical clinical trials for the treatment of monogenic cutaneous disorders are in the initial phases on their way. However, there are, so far, no CRISPR-mediated treatments for complex or polygenic cutaneous conditions that exist today. Such therapies may become a real fact shortly. Certain limitations and safety concerns can be resolved. Integrating microarray analysis, Artificial Intelligence (AI), machine learning (ML), and molecular docking will be important for identifying core target genes and exploring the toxic mechanisms associated with drug-induced diseases.

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Introduction

Skin Anatomy

The human skin is the largest organ of the body, accounting for 15 % of the total body weight. It forms the first line of defense against chemical, physical, and microbial threats. The homeostasis of the skin is vital for reducing water loss and contributing to thermoregulation of the body. Its integrity is achieved by a complicated superstructural system of proteins that join the outermost epidermal layer to the underlying dermis (Figure 1). The skin is made of two main layers—the epidermis and the dermis [1]. The epidermis represents the outermost layer of the skin and has self-renewing features. The epidermis is predominantly made of keratinocytes that are distinguished by their expression of cytokeratins and the formation of desmosomes and tight junctions that are important for cell-cell adhesion [1]. The dermis is a connective tissue found beneath the epidermal layer and superficial to the subcutaneous fat layer. The major resident cells of the dermis are the fibroblasts, which produce collagen and elastic fibers. The dermis shelters key skin structures, including hair follicles, nerves, sebaceous glands, and sweat glands. Fibroblasts and immune cells are the most abundant cell types, and they play an integrative role in providing efficient host defense and maintaining appropriate skin function. The epidermis and dermis are tightly connected by several anchoring structures found within the basement membrane (BM) zone [1].

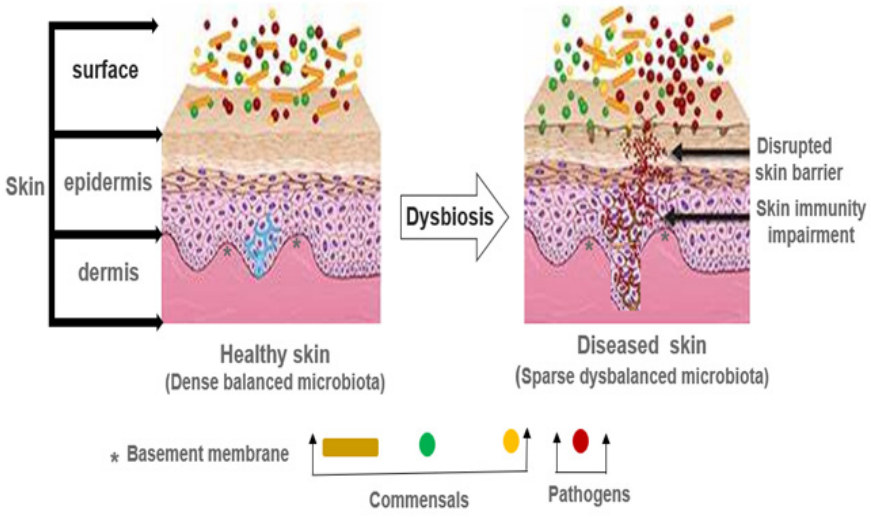

Figure 1: Schematic diagrams in healthy and diseased skin show how defects in the skin structural barrier permit penetration of the epidermis by pathogens. Adapted from: [2,3].

Microbial Origin of Skin Diseases

Previous studies in the microbiome field revealed complex microbial populations inhabiting the skin. The constitution of the skin microbiome differs across different body spots and across individuals [4]. These variations are influenced by various host habits, including, for instance, diet, age, sex, and lifestyle. There is an established balance relationship (Figure 1) between the host cells and local and/or transient bacterial populations that is always influenced by intrinsic (host) and extrinsic (environmental) factors [2,3]. This balance plays a pivotal role in the preservation of skin health and the orchestration of skin homeostasis. Preservatives are applied to stabilize the microbial population and protect the user from the effects of pathogenic microbes [5]. However, this is believed to result in antiseptic effects and may affect the diversity of the cutaneous microbiota.

The well-coordinated but delicate balance can be disturbed by changes in the skin microbial communities, affecting the skin barrier function. Defects in the skin's structural barrier enable penetration of the epidermis by chemical, allergic, and/or infectious agents. This may lead to chronic inflammation and a loss of microbial multiplicity with a concomitant increase in Staphylococci, including *S. aureus,* among others. This is often owing to commensals defeated by pathogens battling for space and nutrients, which results in the occurrence of multiple cutaneous disorders [3]. This induced dysbalanced microbial state or dysbiosis may be evidenced in chronic skin inflammatory disorders, such as Atopic Dermatitis (AD, eczema), psoriasis, rosacea, or acne [2,3,5].

Genetic Origin of Skin Diseases

There is an increasing interest in identifying the molecular basis underlying skin diseases. It is well known that alterations in genes vital for proper skin function can result in a broad spectrum of heritable disorders. The next-generation sequencing has modernized the detection of disease-causing genes and has a great impact on deciphering gene and protein signatures in rare and frequent skin diseases. Some diseases have a clear-cut genetic background, but some others have genetic causes as an important etiological agent.

Xeroderma pigmentosum (XP) is a hereditary monogenic skin disease, meaning caused by a mutation in a known single gene. This disease is recognized by abnormal pigmentation and a very hypersensitive phenotype to sunlight. The most frequently affected genetic variation in XP genetic skin diseases (also known as genodermatoses) is the group XP- C disease group, which results primarily from nonsense mutations in the XPC gene. These mutations induce the nucleotide excision repair (NER) track. The NER can be classified into two subpathways: the first is the global-genome nucleotide excision repair (GG-NER) and the second is the transcription-coupled nucleotide excision repair (TC-NER). The two subpathways differ in their approaches to detecting DNA damage [6]. Deficiencies in NER are associated with different disorders, including XP and others. The GG-NER track functions in various cellular activities and has a key role in base excision repair (BER). For example, it is dramatically involved in the first step of BER, namely in the removal of oxidative DNA damage [7]. Furthermore, it was found that the loss of XPC tends to disrupt skin differentiation [8].

Genodermatoses comprise a large group of cutaneous diseases. Though these diseases are rare, with a prevalence incidence of less than 1 in 50,000 – 200,000, they often appear at birth or early in life and are usually chronic, severe, and could be life-threatening [9]. About 3,000 different conditions influence the human skin, with nearly one-third of the world's population being affected by one [10].

Genodermatoses include clinically heterogeneous conditions that are demonstrated in the skin and other organs [11]. Genodermatoses clinical manifestations vary from mild forms, mainly influencing the skin, nails, and hair, to severe forms, with effects extended to other organ systems. The severe complications accompanying clinical manifestations and secondary problems can adversely impact patients' quality of life, such as psychological and co-morbidities.

Two Epidermolysis Bullosa (EB) subtypes- Dominant Dystrophic EB (DDEB) and EB Simplex (EBS)—are produced by dominant negative mutations in the collagen 7 A1 *(COL7A1* or *C7A1)* gene, which codes for collagen 7 (C7). The Recessive dystrophic EB (RDEB) is a monogenic disease that is caused by

the loss of or attenuated function of anchoring fibrils (AFs), a vital architectural constituent of the skin. RDEB and impaired AF formation and function are the consequence of pathogenic *COL7A1* gene variants [12]. The RDEB disorders are a varied group of inherited blistering disorders that involve the skin and mucous membranes and other organs. In some subtypes, patients with RDEB present a substantial risk for squamous cell carcinoma, and their anticipated life expectancy is typically 30 years [13].

Most pathogenic variants of *COL17A1* result from missense or splicing mutations that permit the expression of a residual quantity of protein, leading to intermediate and less severe phenotypes. 5–10 % of residual protein already improves clinical phenotype [14]. Many subtypes of psoriasis harbor gene mutations in *CARD14,* which lead to the upregulation of inflammatory cytokines, and likewise, eczema carries similar causative mutations in *CARD11* [15,16]. Contrary to cases carrying gene mutations in *CARD*, one of the most common associations of genetic mutation with AD is in the Filaggrin (FLG) gene, which encodes profilaggrin, a protein that helps form and preserve the skin barrier [17]. Another genetic similarity between eczema and psoriasis is in an inflammasome called NLRP3, which, when activated, results in a greater inflammatory response and additional resistance to glucocorticoid therapy.

In contrast to monogenic skin disorders, some, like acne, are complex multifactorial diseases, meaning that many factors in addition to genes combine to cause the disease. A gene that might be faulty in people with acne is called Forkhead Box protein O1 *(FOXO1)* [18]. Normally, *FOXO1* acts like a volume regulator button, suppressing other genes that are involved in key steps in acne development: inflammation, overgrowth of skin cells, and overproduction of skin oil. Scientists suppose that in people with acne, the *FOXO1* gene might be underperforming. Another motive to suspect *FOXO1* of being defective in acne is that some acne therapies, like the topical retinoid adapalene, actually work by boosting the activity of *FOXO1* [19]. The control and function of *FOXO1* may provide a significant way for the prevention and treatment of acne. Of course, it is probable that acne is a polygenic disease, and the malfunction of some of these genes will possibly increase the probability of a person developing acne. This means that all the genes that contribute to acne must be identified before attempting to develop a genetic cure for it. Although genetic treatment (s) for acne may look like a real dream in the future, none exist today, and none are in progress now. Reduction in *FoxO1* expression appears to be a vital mechanism of action of isotretinoin in acne [19].

Methods

Pubmed and Google Scholar were explored for CRISPR-mediated strategies in the treatment of cutaneous diseases. The snowball method was also used to extract other publications. The keywords used as search terms included Acne, CRISPR-Cas, Cutaneous diseases, Gene editing, Genome editing, Genodermatoses, and Skin disorders. References were reviewed and synthesized for descriptive review. The "Blind" collection/analysis of the data was the main criterion that was used to eliminate "bias" and to ensure the quality of studies. Studies were selected based on their relevance to the topic and the quality of the evidence presented. Due to the exponential growth of papers published in the field and space limitations, refined searching, vigorous screening, and detailed evaluation were followed. Several hundred publications were selected for data extraction. After the exclusion of studies with insufficient data, duplications, and non-English publications, a total of 143 articles were selected according to their titles and abstracts and chosen for further analysis. Only 2 papers between 1994 and 2001 and another 9 papers between 2006 and 2013 were used to lay down basic principles and mechanisms. One hundred sixty-eight (132) of the examined research works appeared between January 20214 and June 2025.

Results and Discussion

Traditional Treatment of Skin Diseases: Chemotherapy

The present traditional pharmacotherapy for cutaneous diseases, like topical steroids, anti-inflammatory biologics, or antibiotics, is generally prescribed to patients with the most common chronic conditions, such as AD or psoriasis. They largely aim at curing the symptoms rather than the original cause of the disease [20]. Although these medications are typically potent, they frequently cause side effects and are often short-term solutions for long-term chronic problems. Innovative treatment options, immune-suppressive agents,

Innovative treatment options, immune-suppressive agents, and emollients that target the immune components of these diseases are efficient [21]. Interleukin-4 (IL-4) inhibitor-based treatments for AD (e.g., Dupixent) showed promising clinical benefits; however, they offer short-term relief and thus limit long-term perfection in patient satisfaction [22]. Patients must keep on the medication for life and often suffer major side effects. Therefore, they pose an economic burden on general healthcare systems [23]. For example, Dupilumab, a monoclonal antibody used for AD that blocks interleukin (IL)-4 and IL-13, has an annual cost of more than $30,000 per patient [24]. Because of the prolonged use, corticosteroids for AD can lead to darkening and thinning of the skin and stretch marks [21].

Furthermore, the chronic use of antibiotics for acne vulgaris can cause resistance and poor outcomes [21]. Antimicrobial resistance is a global problem, and acne specialists have expressed clinical worries about the use of antibiotics to treat acne [25]. Even more so with the finding of resistance plasmid (encoding for Macrolide-Clindamycin resistance gene erm and Tetracycline resistance gene *tet(W))*, in C. acnes that can be transmitted to other Cutibacterium species, thus modifying the skin microflora [26,27] (Figure 1). Additionally, these therapies can result in serious side effects such as breaking skin microbiome homeostasis-since they are not selectively killing bacteria, or photosensitivity, in the case of antibiotics, or even birth defects or severe scaling of the skin, as observed in the case of Isotretinoin (ISO) [21]. The potential for ISO to cause transcriptionally mediated drug-drug interactions (DDIs) has not been fully explored. More importantly, cases refractory to chemotherapy are at risk of disturbing the protective stratum corneum layer, and consequently, patients are at a higher risk of having secondary bacterial infections. The *C. acnes* is still acknowledged to have a key role in acne pathogenesis. Evidence indicates that an imbalance of individual *C. acnes* phylotypes and changes of the skin microbiome cause acne [25]. In addition, it is now believed that *S. epidermidis* is also an actor in acne development. Together, *C. acnes* and *S. epidermidis* maintain and control the homeostasis of the skin microbiota [25].

Modern Treatment of Skin Diseases: Genotherapy

There are arguments for limiting the use of chemotherapeutics, and it is obvious that there is an urgent need for treatment modalities to target the true cause of the disease rather than focus on symptomatic management. Previous studies have revealed that many cutaneous diseases stem from genetic changes—either in the DNA of invading viruses, pathogenic bacteria (the DNA of the host, as in Genodermatoses [28-30]. Therefore, genotherapies are promising alternatives.

Although developing a genetic therapy for polygenic cutaneous diseases (e.g., acne) presents a serious challenge, genetic treatment may help with acne, but perhaps may not cure the disease. However, finding a genetic solution for monogenic skin disorders is more reachable. Recently, the U.S. Food and Drug Administration (FDA) has approved, for the first time, a therapy known as Beremagene Geperpavec (B-VEC) for patients with RDEB (31). The B-VEC is a topical gene replacement therapy utilizing engineered herpes simplex virus 1 (HSV-1) as a gene delivery vector. Early success in the usage of gene therapy for the treatment of monogenic inherited RDEB disorders afforded particular promise for the improvement of curative therapies for genodermatoses.

CRISPR-Mediated Gene Editing for Skin Diseases

Gene editing (GE) is a type of genetic engineering (GEN). The concept of GE is to repair the faulty genes in the person's body. Genetic therapy aims to repair or replace a defective or absent gene that initiates a disease. This means that, instead of adding "good" genes as in gene therapy, we go to fix the existing "bad" ones so they will function accurately.

Gene editing involves the:

(i) Addition of a wild-type gene to patients' cells harboring recessive mutations [32].

(ii) Delivering antisense oligonucleotides (ANOs), short interfering RNA (siRNA) into patients' cells/tissues with dominant mutation to silence defective alleles, or spliceosomes [33-35).

(iii) Direct, site-specific manipulation of the genomic DNA utilizing recently advanced GE toolkits such as meganuclease, Zinc-finger nucleases (ZFNs), transcription activator-like effector nuclease (TALEN) [12,36]. Clustered regularly interspaced short palindromic repeats -associated protein 9 (CRISPR-Cas9)

and programmed base editing (BE), and prime editing (PE) [37-39]. Compared to classical gene therapy, which comprises the random introduction of one or more exogenous genes into cells to replace the function of a lacking or mutated gene, GE is still in its infancy. However, GE is a rapidly growing and promising area of research.

In the early years, GE techniques such as ZFNs and TALENs were applied to correct mutations in *COL7A1* in keratinocytes [12]. However, these tools are not simply adaptable owing to either complex design implicating protein manufacturing for each target gene or reduced editing efficacy in certain cells or tissue types. The advent of CRISPR/Cas9 technology revolutionized gene manipulation, bringing a new era in GE because it is easy, cost-effective, multipurpose, and has great precision editing machinery to accomplish gene correction. CRISPR/Cas9 is the perfect tool of the future for the therapy of diseases by permanently amending deleterious base mutations or disrupting disease-causing genes (Figure 2).

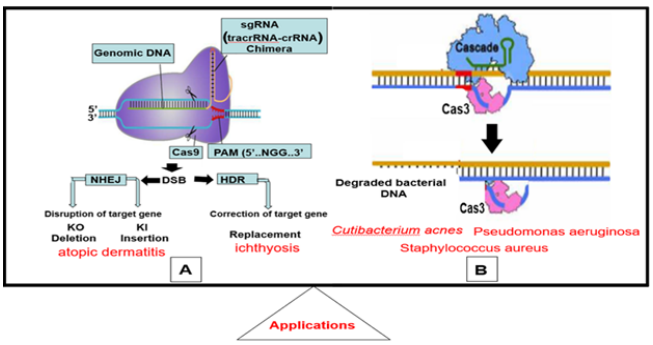

Figure 2: CRISPR-based therapies for three classes of cutaneous disorders. Cas9 loaded with an sgRNA recognizes the PAM, hybridizes at a specific genomic locus, and generates a double-stranded break DSB in DNA. The target gene disrupted by the DSBs is repaired by the cellular intrinsic mechanisms, either Nonhomologous DNA end-joining (NHEJ) or homology-directed repair (HDR) through the integration of a donor DNA template carrying the correct sequence. While Cas9 is a single protein that has both DNA-targeting and cutting activity, Cas3 contains a complex of multiple proteins called the Cascade complex and recruits a trans-nuclease helicase called Cas3 to make the initial cut in DNA. After cutting the target DNA, Cas3 initiates single-strand DNA degradation. The degradation can continue for many kilobases in one or both directions from the target region, making it useful for the cleavage of long segments of bacterial DNA.

Abbreviations: Cas, CRISPR-associated; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat; DBS: Double-strand breaks; HDR: Homology-directed repair; Nonhomologous end joining; PAM: Protospacer Adjacent Motif sequence; sgRNA: Small (single-) guided RNA.

The sgRNA guides Cas9 to cleave the DNA strand that disrupts the homologous spacer region. The recognition process of the sgRNA needs the involvement of protospacer-adjacent motifs (PAMs), a short guanine-enriched sequence. The favored PAM by Streptococcus pyogenes Cas9 (SpCas9) is NGG, which is usual in the genomes of most organisms, thus enabling the application of CRISPR technology across different fields.

The wild-type Cas9 only cleaves double-stranded DNA (dsDNA) to generate double-strand breaks (DSBs), which are repaired through cellular intrinsic mechanisms of DNA repair, specifically, homology-directed repair (HDR) and nonhomologous end joining (NHEJ) (40). The base sequence of the initial gene is damaged, leading to inactivation; however, the inactivation of a single harmful gene can't address the complex stages of all disease events. Consequently, scientists searched for possible means to modify Cas9 by clarifying the physicochemical structure of Cas9, the mechanism of action by which Cas9 shears dsDNA, and other properties. They granted Cas9 new functions by mutating the structural domain of Cas9 and inserting effectors, involving transcriptional regulatory kits such as dead Cas9 (dCas9). These Cas9 variants enrich the GE paradigm and can be adapted to extra types of diseases.

Representative variants, Cas9 variants that have been modified to widen the scope of application, include: Cas9 nickase (Cas9n), Cas12a, and Cas13a [41]. The first variant (Cas9n) cuts the single DNA strand, the second (Cas12a) cleaves double DNA strands to create sticky ends, while the third variant (Cas13a) is an RNA-specific nuclease that identifies and cleaves RNA strands. It added functionality to the CRISPR-Cas system, allowing further therapeutic options for inherited

diseases where permanent modifications of the DNA might not be tenable or dangerous due to unintended off-target effects. Taking the example specified earlier, KO the NLRP3 gene using Cas9 may be efficient, but may also produce unintended off-target effects, specifically the buildup of unexpected, unwanted, or even adverse changes in the genome that would be lasting for the length of the cell lifetime. Contrary to this, editing the mRNA product before translation would avoid the necessity to target the genome directly and instead inhibit the expression of the proinflammatory protein. This type of accuracy and targeted therapy would be valuable in cases where systemic corticosteroid treatment is untenable due to adverse side effects or resistance. In addition, two reversible modifications of CRISPR-dCas9 activation (CRISPRa) and CRISPR interference (CRISPRi) were designed to greatly reduce the unknown problems caused by off-target effects [42,43].

CRISPR-mediated therapies have huge implications for three categories of cutaneous disorders: epidermal blistering diseases, genodermatoses, inflammatory disorders, and bacterial infections (Figure 1). Many genodermatoses are monogenic skin disorders, such as congenital ichthyosis and RDEB are ideal candidates for CRISPR targeting. Moreover, the skin is an easily manageable organ that enables the extraction and in vitro cultivation of target cells as well as direct confined transfer of CRISPR-Cas therapeutics using topical, grafting, or injection techniques. Also, the visibility of skin allows for easy monitoring of the genetically edited skin tissues for both efficiency and potential harmful effects.

Other variants are the single-base substitution tools and transcriptional regulatory tools (e.g., dCas9-effector). Fusion of Cas9n with adenosine deaminase or cytidine deaminase allows for the induction of point mutations in the genome, C-G to T-A and C-G to G-C substitutions. Accomplishing base substitutions is important for single-GE efforts; nonetheless, multiple types of base mutations cause disease, and attaining random substitutions between bases was an urgent mission for applying CRISPR technology to disease treatment [44,45]. Prime editors, composed of CRISPR Cas9n and an RNA template, solve the challenges of traditional HDR-based gene correction, which provides the modified customized sequence concurrently at the target site with a nuclease [46]. It has been reported that the PE can delete up to 80 base pairs (bp) of nucleotides or integrate up to 44 bp and correct point mutations, including transversions. The platform can also execute combination edits without inducing explicit DNA DSBs. Accordingly, it has been anticipated that PE could fix up to ~89% of human genetic variants [9]. The correction of insertion or transversion edits by PE has been examined in three human cell lines, including HeLa, K562, and U2OS, with editing efficiencies in these cell types varying between 12% and 30% and reduced indels of 0.13 – 2.2%. In general, the correction efficiencies were comparable to CRISPR/Cas9-based HDR GE with much lower off-target rates [47]. The fusion of a deactivated form of Cas13 (dCas13) to adenosine deaminases acting on RNA (ADAR) enzymes can deaminate adenosine into inosine (A-to-I) that is identified as a guanine, and fusion to Cas9-based RNA BE (ceRBE) can induce A-to-I and C-to-U conversions, without RNA cut [48]. As PE is a novel developed technology, more tests in different cell types for editing efficacy and off-target impact are needed, specifically in hard-to-transduce cells such as keratinocytes. So far, no application of PE in keratinocytes has been reported.

Cutaneous Bacterial Infections

Other therapeutic targets for CRISPR include inflammatory disorders, such as AD, in which certain disease-inducing mutations are well-described [49]. These cases have been the spotlight of CRISPR/Cas9-based therapy in mouse and cellular models [49,50]. CRISPR-Cas antimicrobials have been developed as a novel therapeutic strategy against bacterial infections [51]. Particularly, CRISPR-Cas9 has been exploited to selectively delete antimicrobial resistance genes from populations of bacteria, re-sensitizing populations of bacteria to usual antimicrobials [52].

As shown in Figure 2, CRISPR/Cas complexes with sgRNAs complementary in sequence to pathogenic bacterial sequences can be repurposed to target and kill specific bacterial species.

Researchers have repurposed other Cas nucleases, such as CRISPR-Cas3, to modify or edit host DNA by making thousands of cuts in the bacterial DNA and leaving the human DNA intact. Another advantage of

the CRISPR/Cas strategy is that normal flora can be spared by programming the CRISPR machinery to target bacterial genes conserved within a strain or even a specific species. In contrast to Cas9, which makes a single cut in DNA, Cas3 is a processive nuclease and helicase that uses adenosine triphosphate (ATP) to unwind DNA and successively degrade long segments of DNA [53]. Consequently, the bacteria cannot replicate with their genetic code disabled. For example, Cas9 has been recently used to exclusively target one of two strains of E. coli in a mouse model, and Cas3 was recently used to target Clostridium difficile in vivo [54,55].

CRISPR/Cas-mediated antimicrobial treatment of skin infections would be beneficial in instances involving antibiotic resistance (for example, methicillin-resistant *S. aureus*) or *P. aeruginosa* folliculitis. The situations in which a patient cannot tolerate the traditional antibiotic owing to an allergy or is refractory to treatment due to antibiotic resistance would be indicators for successful application using CRISPR-based therapy. Whereas antibiotics (even relatively selective ones) may kill both bad bacteria and normal flora present on the skin, the benefit of the CRISPR/Cas approach is that normal flora can be secured by programming the CRISPR apparatus to target bacterial genes preserved within a strain or even a specific species.

Staphylococcus Aureus

S. aureus, an ordinary cutaneous bacterial pathogen acknowledged for its antimicrobial resistance, is responsible for about 76% of all skin and soft tissue infections and is correlated with high morbidity and mortality [56-57]. Antimicrobial resistance to *S. aureus* continues to arise as the pathogen acquires plasmids and other mobile genetic elements that grant antibiotic resistance and virulence genes [58].

An innovative approach to target virulent strains of S. Aureus utilizing CRISPR-Cas9 was developed [52]. sgRNAs were advanced to target *S. Aureus* antimicrobial resistance genes, including the methicillin resistance gene (*mecA*). When delivered with Cas9 by a phage capsid to diverse populations of bacteria in vitro, these sgRNA/Cas9 combinations were able to eliminate resistant *S. Aureus* strains and remove specific plasmids having antimicrobial resistance genes. In addition, when transported topically to a mouse model of *S. Aureus* skin colonization in vivo, the constructs of sgRNA/Cas9 were able to substantially reduce colonization by resistant bacteria. These outcomes validated the promise for the topical treatment of CRISPR antimicrobials in vivo and laid the basis for potential multiplexed CRISPR antimicrobials devised to concurrently target either some bacterial species or multiple gene sequences in the same bacterium. These results also confirmed the possibility for CRISPR antimicrobials to modify the cutaneous microbiome. The function of the cutaneous microbiome in dermatologic disorders continues to be disclosed as researchers examine metagenomic sequencing information from diverse skin samples [59]. For instance, decreased microbiome diversity and boosted *S. aureus* skin colonization has been involved in the pathogenesis of AD [60]. The potential role of CRISPR antimicrobials in AD patients has yet to be studied, but antimicrobials aiming at pathogenic *S. aureus* strains may complement tactics designed to enlarge commensal bacterial inhabitants in the cutaneous microbiota [61].

Cutibacterium Acnes

Cutibacterium acnes (formerly Propionibacterium acnes) causes acne [62]. This bacterium is a highly predominant and the most abundant skin commensal that resides deep in the hair follicle, a unique location for host access [63]. Sebum may be involved in encouraging the growth of particular subtypes of *C. acnes* [25]. The hair follicle is a physiological niche relevant to immune education and the focal route for topical absorption of therapeutics. The hair follicle makes C. acnes an appealing chassis to create therapeutics for dermal biotechnological applications [64, 65]. In the pilosebaceous units, it metabolizes sebum lipids, leading to the blockage or inflammation of the pilosebaceous follicles. Acne has a wide array of treatment selections, ranging from oral contraceptives to topical creams to antibiotics to ISO. However, some patients either do not respond to therapy or are concerned about important side effects (e.g., birth defects, liver failure, etc.). This may be attributed to the balance that maintains and regulates the homeostasis of the skin microbiota (*C. acnes* and *S. epidermidis)* [25], leading to intimate interaction with the innate immune system.

Until the past few years, *C. acnes* has been considered an intractable, unengineerable bacterium. It has been

incredibly challenging to introduce DNA and get proteins formed or secreted from an element implanted into its genome. However, bioengineers insisted on editing the genome of *C. acnes* because it seems an attractive synthetic biology framework for treating skin diseases, owing to its niche ecology deep inside hair follicles, particularly where sebum is released. Also, its importance for skin homeostasis, its close contact with relevant treatment targets, plus the fact that it has been revealed to successfully engraft when applied to human skin.

Recently, researchers have succeeded in editing the genome of *C. acnes* to produce and secrete a therapeutic molecule proper for curing acne symptoms [64]. This molecule is the Neutrophil Gelatinase-associated Lipocalin (NGAL) protein, which in humans is encoded by the Lipocalin-2 (*LCN2*) gene. NGAL is known to be a mediator of the acne drug, ISO, which has been shown to decrease sebum by inducing apoptosis of sebocytes. The engineered bacterium has been confirmed in skin cell lines, and its delivery has been proven in mice. When applied to the skin of the only animal model able to scan engineered bacteria to date, they engrafted, lived, and produced the protein. However, mice's skin is different from humans; it is less compact, has more hair, has less lipids, and has a dissimilar sweat mechanism. Hence, there is a necessity for another model, a more representative human skin, such as 3D skin models [66].

Human adults have a unique combination of *C. acnes* strains [62,67]. Preferably, the engineering of various *C. acnes* strains could allow for strain-specific engineered live biotherapeutic products (eLBPs) tailored to the patient's unique microbial profile, consequently increasing engraftment. For this reason, a group of natural and synthetic inducible systems has been designed for the environmental or exogenous stimulation of gene expression. To prove the idea of potential therapeutic applications, researchers created an antioxidant-producing strain capable of rescuing keratinocytes from UV-generated oxidative stress. To engineer the bacterium, the schematics of the *C. acnes* replicative plasmid was optimized. These researchers created an average of population median fluorescent values of three *C. acnes* autonomous replicates for nine distinctive constitutive endogenous promoters [65]. Further, a synthetic consensus promoter (BBa_J23119) from *E. coli*, and a control harboring a no-insert (empty) plasmid were grown for exponential and stationary growth phases. When compared with an empty-plasmid control in the exponential phase using flow cytometry, an increase in fluorescent signal for all reporters was observed.

Unlike in most eukaryotes, Cas9 cuts in the bacterial chromosome have been shown to kill the cell [68]. However, the mechanism of cell killing remains to be investigated. Bacteria chiefly depend on homologous recombination (HR) with sister chromosomes to repair DSBs. Owing to the low genome integration efficacy in *C. acnes*, the use of Cas9 was first tested as a means to screen for genes that would render *C. acnes* auxotrophic upon disruption [65]. However, genome targeting by Cas9 seemed to be toxic for *C. acnes,* which is usually the case for bacteria due to non-repaired DSBs, as indicated by the lack of transformants [68]. The expression of Cas9 was reduced by the use of weaker promoters, generating transformants but without any edit in the genome. It was anticipated that using the plasmid-borne CRISPRi as a non-lethal method to screen for *C. acnes* metabolic genes would yield *C. acnes* strains with a single-gene KO amino acid auxotrophy based on homology. The growth of *C. acnes* in the presence or absence of a specific amino acid (Histidine for the auxotroph) was compared with a control (dCas9) (i.e., without gRNA and its corresponding promoter) [65]. It was found that the Histidine, leucine, and tryptophan pathway repression showed growth defects. However, growth was also decreased in the dCas9-only control, implying that the wild-type expression of the targeted genes can't compensate for the lack of those amino acids in the growth medium.

Taken together, the outcome of these studies paved the way for engineering *C. acnes* for microbiome-based therapies for cutaneous disorders such as AD. The complexity of human skin's microbiome, which includes a dense range of microorganisms, such as bacteria, fungi, and viruses, should always be kept in mind. In healthy skin, commensal microbes act in symbiosis with the physical structure of the skin to create a barrier against external insults [25].

Cutaneous Viral Infections

CRISPR-Cas systems have evolved in bacteria against invading bacteriophages. Scientists have repurposed

these systems to serve a comparable function in virally influenced human cells. In human cells, CRISPR-Cas nucleases can target latent viruses that are skilled at escaping elimination by immune surveillance and regular antiviral therapies. As such, there has been massive research into the capability of CRISPR-Cas systems to target particular viral genomic sequences, allowing targeted destruction and even complete removal of constituents of the viral genome [28]. Additionally, researchers have leveraged the elevated sensitivity of some Cas enzymes for viral pathogen recognition in human tissue samples [42]. Two Cas enzymes (Cas12 and Cas13) that show unselective trans-cleavage of ssDNA when triggered by their guide-complementary target nucleotide sequence—allow ultrasensitive nucleic acid detection in viral biosensing systems [42,43]. Earlier research indicated that CRISPR-Cas technology may be efficient in detecting and modifying herpes simplex virus (HSV), human papillomavirus (HPV), and Kaposi sarcoma-associated herpesvirus (KSHV).

Herpesviruses

Herpesviruses are large dsDNA viruses that cause lifelong infection in human hosts. Classically, HSV-1 and HSV-2 infect the oral and genital mucosal epithelium, respectively, resulting in local production of ulcers. After going through partial clearance by the host immune system, HSV starts a latent infection as an episomal DNA in the sensory ganglia. Latent herpes infections escape immunological surveillance by restricting viral gene transcription and are very difficult to treat. Because of this, CRISPR-based targeting of viral DNA has appeared as an alternative strategy for HSV, KSHV, and other HSVs. HSV-1 duplication in cultured human fibroblasts was abrogated by disrupting two key viral genes using CRISPR-Cas9 [69,70]. Similar success against HSV-1 was achieved, where in vitro viral replication was inhibited in epithelial cells by Cas9/sgRNA editing complexes with no evident off-target effects [70]. Furthermore, the burden of KSHV in latently infected epithelial and endothelial cell lines by Adeno-associated Virus (AAV)-CRISPR-Cas9-based disruption of the KSHV latency-associated nuclear antigen has been demonstrated [71].

Still, obstacles remain in the application of CRISPR-Cas for HSVs: the efficacy of CRISPR-Cas used to eliminate latent HSV-1 in neurons remains to be proved, and the efficiency of anti-HSV and anti-KSHV CRISPR-Cas systems in vivo needs to be demonstrated [1,2,71].

Human Papillomavirus

Like the HSV, the HPV is a dsDNA virus that infects the basal cells of stratified epithelium. It can integrate the viral DNA into the host genome. The proteins of some high-risk strains like HPV E6 and E7 induce malignant transformation of epithelial cells through inactivation of Tumor protein (P53) and Retinoblastoma (Rb), respectively, leading to anogenital *squamous* cancers and other neoplasms. 73 CRISPR-Cas9 has been successfully used to disrupt *E6* and E7 genes in cervical cancer cells of animal models, both in vitro and in vivo [43,73]. Antiviral CRISPR-Cas9 clinical trial in humans is one of the first in vivo targeting of *E6/E7* in HPV-infected neoplastic cervical cells [74].

Fewer studies have been done with the aim of applying CRISPR-Cas to cure the dermatological symptoms of HPV. However, researchers have started to develop CRISPR-Cas composes targeting the virus in HPV-associated anal cancer. In a mouse model of HPV-16-associated anal cancer, the tumor burden was successfully reduced [75]. Using an AAV vector, researchers delivered Cas9 nuclease in combination with two sgRNAs: one specific for HPV-16 E6, while the other specific for HPV-16 E7 [76]. Three intratumoral injections for one week to mice with patient-derived xenografts of HPV-16 anal malignant cells, the CRISPR-Cas9, and dual-gRNA caused a 2-fold reduction in tumor volume [76]. This protocol proved the concept for a new in vivo GE approach for HPV-16-associated anal cancer. Aimed at HPV-associated genital warts, CRISPR-Cas9 was successfully applied to target the *E7* gene in vitro, enhancing apoptosis of HPV-6 and -11-infected keratinocyte cell lines [76]. Remarkably, however, the Cas9 and sgRNAs were transfected transiently into the keratinocyte cell line and achieved only partial *E7* inactivation. Furthermore, the efficiency of the genome editing components was not demonstrated in vivo.

CRISPR-Cas technologies hold the potential to serve not only for HPV therapeutic purposes but also for

HPV diagnosis. The nuclease Cas12a and an ssDNA fluorescent reporting system were used as a nucleic acid biosensing system, known as DETECTR, to detect specific sequences in amplified dsDNA from human samples [77]. The assay was carried out in just one hour and needed only isothermal amplification of DNA, indicating that DETECTR could act as a fast, low-cost, point-of-care identifying assay for HPV with comparable sensitivity, particularly to traditional diagnostic PCR.

Cutaneous Genetic Disorders

Genodermatoses

CRISPR technology for treating genodermatoses has been investigated in animal and cellular models. In humans, RDEB is one of the most extensively studied genodermatoses as a potential candidate for GE therapy [78]. Virtually all researchers have leveraged CRISPR-Cas constructs to advance diverse treatment strategies for RDEB, including the targeted introduction of genes to particular genomic sites, the repair of disease-producing point mutations, and the deletion of disease-causing genes or genomic sequences [38]. The RDEB disorders result from mutations in different genes that code for different proteins expressed at the cutaneous BM zone [79]. CRISPR's ability to correct both dominant and recessive disease-causing mutations has been repeatedly verified. However, so far, only in vitro and in xenografted rodent models [80-82]. Gene replacement therapies are ideal to treat recessive monogenic diseases. However, dominant mutations require corrective gene editing instead of gene editing. Such approaches enable corrective GE and the targeted KO of mutant alleles in dominant negative diseases. It is also hoped that targeted GE strategies will lower the risks associated with the random placement of exogenous transgenes.

EBS is initiated by dominant negative missense mutations in either the keratin 14 (*KRT14*) or keratin 5 (*KRT5*) genes. The genes code for intermediate filaments (IFs) expressed in the basal layer of the epidermis [83]. Both DDEB and EBS are specifically well-fit for treatment with CRISPR-Cas GE, which enables the direct modification of the dominant, disorder-causing allele. CRISPR-Cas9-caused HDR was utilized to correct the disease-causing mutated *KRT14* allele in EBS patient keratinocytes in culture [16]. Gene-engineered clones showed normal phenotypes without typical mutant cytoplasmic aggregates in vitro. In one particular pretrial, researchers have applied Cas9 to successfully return sufficient gene function (gene encoding type VII collagen, *COL7A1*) in rodent and cellular models of RDEB that are considered enough for a scarless phenotype following engraftment onto a human body [80,81]. Each of these studies returned the gene function in a substantial number of cells (≤70%), which is considered adequate for the generation of a scarless phenotype once engrafted onto a human body. CRISPR-Cas9 was used to induce site-directed mutagenesis of the mutated *COL7A1* allele in induced Pluripotent Stem Cells (iPSCs)- cells that have been genetically modified by CRISPR-Cas9 to escape inciting a host immune response- taken from DDEB patient keratinocytes. The engineered cells expressed a truncated version of C7 that was unable to form harmful trimers with wild-type C7 and would hypothetically enable normal anchoring fibril assembly at the DEJ [7].

The use of CRISPR-Cas9-based treatments for RDEB was extended to include in vivo strategies. DSBs on either side of the mutation-carrying exon 80 in the *COL7A1* gene function were induced [80]. Two sgRNAs targeting the 5' and 3' sides of exon 80 in *COL7A1* were designed and transferred as a sgRNA/Cas9RNP complex by intradermal injection into mouse tail skin, followed by direct electroporation to enable the transfection of epidermal stem cells (ESCs). Full excision of the disease-producing mutation in ESCs was achieved, restoring *C7* gene function in RDEB mouse models. Mice's dermal-epidermal connection zone improved from 30% to 60% after one treatment. The achievement of this approach proved the capacity for CRISPR-Cas9-induced gene correction of ESC in vivo with no cost and technical complications of ex vivo cell modification. Still, however, several restrictions of this method exist. Only 2% of epidermal cells were capable of being targeted with this innovative in vivo delivery route, and no long-term follow-up to evaluate the sustainability of the treatment could be carried out. Moreover, potential off-target impacts of the Cas9/sgRNA RNPs at locations other than exon 80 were not examined. Whether transdermal transfer of Cas9/sgRNA RNPs by electroporation would be safe and efficient in humans has yet to be investigated. However, a lot of pain and collateral injury may likely accompany the transdermal electroporation

procedure, particularly for RDEB patients [84]. Electroporation protocols for RDEB patients would need high voltages to penetrate the nuclei of ESC and would need to be dispensed over large surface areas of skin.

Another approach for gene silencing is AON-mediated strategies, which have been broadly explored for the therapy of EB. In 2006, a study pioneered the design of AONs for EB treatment , where AONs restored the C7 function in 6% of EB patient cells and, to a low extent, in a xenografted rat model. Unfortunately, ultimate proof for therapeutic success was not provided since no anchoring fibrils were observed, which would have offered decisive proof for therapeutic success [85]. After that, several studies have confirmed the treatment benefit of AONs in EB therapy [86]. Lipofectamine was employed for the transfection of primary skin cells ex vivo, restoring type VII collagen expression in 6%–50% of the cells. Remarkably, in vivo delivery either intradermally or intravenously led to substantially lesser restoration rates (10%–14%). Overall, preclinical CRISPR data reveal its superiority over AONs.

However, it remains to be understood how CRISPR/Cas9-based treatment for RDEB compares with existing gene treatments. Future research will determine the safety and efficiency of Cas9-based therapy for RDEB and eventually other genodermatoses like congenital ichthyosis, Netherton syndrome, and other monogenic skin diseases. It has been recently shown that co-transporting Cas9-targeting NLRP3 with dexamethasone in mouse models relieved symptoms—lessening skin edema, decreased infiltration of mast cells, and overall progress in inflammatory activity—compared with the Cas9–NLRP3 therapy alone or the dexamethasone treatment alone [49, 87].

Although HDR- and NHEJ-based GE therapies, including CRISPR-Cas9, often lead to the generation of insertion or deletions (indels) at the break site, they offer a potentially powerful approach for targeted gene editing of mutations in the *COL7A1* gene [36, 37]. NHEJ-independent GE therapies, such as BE and PE, provide much more efficient repair than HDR-mediated technology and involve site-specific modification using CBEs or ABEs. PE doesn't need DSBs or an exogenous donor template. Yet, it can only be used in base substitution mutations in *COLA71* [38, 88]. It is still rarely established for RDEB therapy [89]. PE, ABE, and CBE have been used for *COL7A1* gene correction in RDEB patient cells. Using an early version of ABE (ABE 7.10). It was shown that electroporation of mRNA into fibroblasts could achieve correction of mutations in primary fibroblasts and iPSCs from two RDEB patients [12]. Using a developed ABE (ABE8e), correction rates of >90% without the need for a donor molecule or selection have been achieved [88]. More recently, BE platforms-mediated transversions have evolved, but the mutational constellation of *COL7A1*, such as deletions, insertions, transitions, and transversions, was reported [90-92]. Using PE, two COL7A1 patient mutations have been corrected and stemmed skin equivalents that exhibited deposition of type VII collagen and (AF) formation in mice in vivo [89]. Newer repetitions of the PE offered more activity and smaller architectures, and both BE and PE allowed sequence alteration without the need for a donor template and facilitated editing events with reduced rates of DSB and thus NHEJ indels than occur with nucleases [93,94]. For rare disease symptoms and/or genes with a great and diverse group of disease-causing mutations, as observed in RDEB/COL7A1, the advance of patient-specific substances for individualized therapy represents a potential obstacle. This is due to the variability in the activity of individual reagents and the potential elevated cost and lengthy control process to get personalized clinical-grade reagents [12].

One possible solution is to merge the principles of gene therapy and gene editing [95]. Gene therapy offers the ability to generate a cDNA vector approach applicable to most or all patients. However, these vectors can either be temporary, needing re-dosing, or incorporate semi-random and present genomic insertional mutagenic concerns. Moreover, the inclusion of non-native gene-controlling elements and lasting transgene expression at supranormal levels may not be specified in every instance [21]. Gene editing allows the retention of locus-specific regulation at the expense of wide applicability. To bridge this gap, CRISPR programs that allow precision installation of huge genetic cargo, such as the ~9 kb COL7A1 cDNA, are considered exciting technologies. Progresses toward this include (1) Programmable Addition by Site-specific Targeting Elements (PASTE), (2) PE-Assisted Site-specific

Integrase GE (PASSIGE), (3) engineered [ee] PASSIGE, and CRISPR-associated transposases [47, 96-98]. All facilitate the insertion of bulky genetic elements without the need for/induction of DSBs. With this concept, a cDNA could be established, without DSBs, proximal to the promoter region and upstream of related mutations to achieve the wide applicability of gene therapy with conservation of the in-place expression controller afforded by locus-specific targeting.

Despite the immense interest in CRISPR, siRNA-mediated therapies are still the most broadly investigated approaches. Pachyonychia congenita, an autosomal dominant genodermatosis which results from mutations in keratin, was the first skin disorder to undergo clinical studies related to siRNA treatments (NCT00716014) [99]. The siRNA that targets the disease-related mutations was intradermally injected, producing promising regression of the disease [99]. Unluckily, the pain accompanying frequent intralesional administration excludes a clinical translation. Therefore, considering the promise of siRNA therapeutics for skin diseases, the interest in effective and less invasive delivery techniques is increasing.

Melanoma

Melanoma has exceptional immunogenic potential, due mainly to the great mutational burden that drives the production of immune-stimulating neoantigens [100]. Consequently, under ideal conditions, melanoma cells are specifically susceptible to destruction by the human immune system. But, clinically, in melanoma, the tumor microenvironment is greatly immunosuppressive, and highly developed disease has an exceptionally poor therapeutic response [101]. Accordingly, melanoma serves as an optimal target for immunotherapies that are planned to relieve tumor immunosuppression.

The CRISPR-Cas machinery has been used in some of the first CRISPR-Cas clinical trials for immunotherapy for cancers, including melanoma [102]. Much of this research has focused on the use of GE to inactivate essential immune checkpoint inhibitors like Cytotoxic T-lymphocyte-associated -4 (CTLA-4) protein and programmed cell death-1 (PD-1) protein. These two proteins normally inhibit the anti-tumor cytotoxic impact of endogenous and exogenous T cells [103].

The first human trial intended to test the application CRISPR-Cas for metastatic melanoma was based on the established success of earlier immunotherapies, including PD-1 inhibitors and T cells transduced with the NY-ESO-1 T-Cell Receptor (TCR). Particularly, researchers aimed to strengthen the therapeutic impact of these existing methods by employing CRISPR-Cas9 to KO *PD-1* gene loci in autologous NY-ESO-1 TCR-transduced T cells. The autologous T cells were first obtained from a patient and transduced with a Lentivirus (LV) vector that expresses the NY-ESO-1 TCR, priming them to identify an abundantly immunogenic NY-ESO-1 antigen expressed on melanoma cells [104].

They are then electroporated by RNA-guided CRISPR-Cas9 nucleases created to disrupt the expression of both PD-1 and the two (TCRα and TCRβ) endogenous subunits of TCR [102]. Disrupting PD-1 blocks immune-suppressive signaling, and preventing the endogenous TCR subunits stops aberrant immune responses that may be caused by TCR-mediated targeting of unspecified antigens. By re-introduction of these melanoma-targeted, immune-dedicated T cells, researchers expected to get a more robust tumor-precise immune response—a response that has not been easy to accomplish with preceding T-cell treatments for solid tumors [104].

Most recent melanoma studies pursue a systemic method by using, for example, genetically modified T cells to enhance their antitumor activity; however, this is beyond the scope of this review.

Delivery Strategies for the CRISPR System in Skin Applications

Cutaneous diseases are both varied and complex, and the choice of appropriate GE methods and delivery vectors for various diseases is important. The delivery mechanism(s) of the genome-modifying components are equally critical and can be achieved in a variety of ways. Delivery procedures of the GE components to treat cutaneous diseases can be classified into two large categories: (A) ex vivo, in which primary cells are cultivated outside the body and returned to patients on gene correction, and (B) in vivo, , in which the the

CRISPR cargo are directly delivered to patients (Figure 3). Almost all those GE therapies are designed as ex vivo applications of diseased primary cell lines (11, 105) due to a lack of safe and efficient techniques for in vivo delivery of the CRISPR construct.

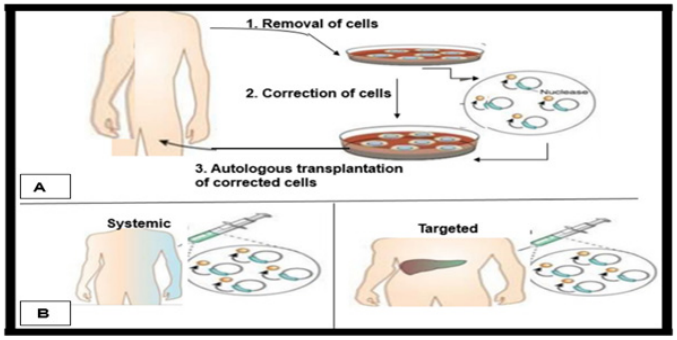

Figure 3: Outline of the ex vivo and in vivo CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-mediated gene editing procedures for clinical therapy. (A) Ex vivo delivery involves deriving patient stem cells, correcting them with CRISPR/Cas + short guide RNA against the targeted gene, and reintroducing the corrected cells into patients. (B) In vivo delivery, where the designed CRISPR system is administered using a viral or non-viral vector and directly injected locally into the affected tissue using hypodermic needles. Source: [40].

The ex vivo approaches enable the targeting and transfer of the CRISPR-Cas materials and, by allowing for the enrichment of engineered cells, decrease the requirement for highly effective and specific CRISPR-Cas editing constructs. However, cell growth in culture can result in undesired cellular differentiation. Furthermore, cell-based transplantations can be technically challenging, specifically for non-hematopoietic cells. Contrary to ex vivo gene manipulation, in vivo GE includes the direct alteration of somatic cells in situ (Figure 3). The in vivo approach is specifically challenging due to the barrier characteristics of human skin and the imperfect properties of genetic cargo, such as high molecular weight, negative charge, and biological instabilities. Further, the target cells, for example, keratinocytes and ESCs, are considered 'difficult to transfect' among primary cells. Using CRISPR-Cas components, in vivo GE is accomplished through systemic or localized delivery of packaged CRISPR-Cas constructs (protein, DNA, and/or RNA) into the body toproduce GE outcomes in particular organs or cells

A variety of effective Cas9 variants and derivatives have been developed to deal with the complex genomic alterations that occur during diseases. The availability of the skin allows for the delivery of CRISPR components via suitable topical formulations and positions cutaneous bacterial infections at the forefront of CRISPR antimicrobial investigation [106]. Moreover, although many of these studies have been within the context of in vitro and animal models, continued improvement in this area will allow for the application of these therapeutics to human patients.

Delivery technologies to move CRISPR enzymes and sgRNAs into living cells include both viral (Adeno-associated viral; AAV and Lentiviral; LV) vectors as well as non-viral vector (Chemical and physical) delivery strategies [107,108]. In comparison with *viral* vectors, non-viral vectors are more appealing to clinical application, as they offer some advantages over viral vectors:

- high cargo encapsulation capability
- low off-target effects, reduced immunogenicity, genotoxicity, and oncogenicity
- easier and more economic to manufacture [105].

Therefore, nonviral vectors with target recognition functions may be the focus of future research. Pathological and physiological changes resulting from disease onset are expected to serve as identifying factors for targeted delivery or targets for GE.

The use of dual vectors has been suggested to overcome the size caveat: the first for the Cas9 and the second for the sgRNA [109]. Still, the success of this method depends on the concurrent intracellular carriage of sgRNA and Cas9. In addition, curative protocols for recessive genodermatoses need the simultaneous transfer of donor templates, which cannot be accomplished using viral vectors. Recent developments of the CRISPR-Cas technology, like PE, allow more precise cleavage and higher editing efficiencies; however, viral vectors cannot act as delivery tools as the RT (7 kb) cannot be encapsulated [47].

To avoid the drawbacks of virus vectors, a highly branched poly (β amino ester) (HPAE, also called PTTA-DATOD) has been designed and synthesized as transporters for the delivery of plasmids encoding dual sgRNA-guided CRISPR-Cas9 machinery

to excise COL7A1 exon 80 containing the c.6527dupC mutation [87]. HPAEs are biodegradable polymers with reversible charge characteristics that support nucleic acid binding and allow modularity and customization for designed tissue/gene delivery, including the skin. The HPAEs facilitated the transfection of skin stem cells and increased skin linkage in a mouse model of RDEB. Moreover, CRISPR-Cas9 plasmids carried by PTTA-DATOD achieved effective targeted deletion and restored bulk C7 assembly in RDEB patient keratinocyte polyclones [87].

Still, the SaCas9 requires more than one vector to concurrently deliver the CRISPR components, thereby lowering the efficiency of targeting. Another viral-based delivery that involves reprogramming Cas3 to treat bacterial infections utilizing CRISPR is phage therapy [54,55]. In this procedure, the CRISPR elements are packaged within viral vectors (bacteriophage: viruses that replicate in bacteria) that selectively infect bacterial cells but not human cells. The crucial tenet in this case is that bacteriophages can replicate so much within the cell that they induce bacterial lysis exclusively [110]. They may also combine with bacterial DNA to modify the bacteria and lower pathogenicity [110]. Bacteriophages advance the potential for targeting particular bacteria with minimal disruption to the rest microbiome [110]. Phages can target a particular bacterial species' DNA when packaged with CRISPR/Cas3, as has been effectively accomplished in a recent clinical trial for the treatment of lower urinary tract infections resulting from *E. coli* (NCT04191148). Lytic bacteriophages to *C. acnes* have been established, but their clinical applications have not been well examined [110,111]. The present Cas3 clinical trial is testing the delivery of the phage therapy directly into patients' bladders (through catheterization) with an immediate next goal of intravenous or intramuscular delivery, all of which largely require treatment by medical professionals.

Nonviral delivery systems such as lipid-based and polymeric nanoparticles, as well as electroporation, ultrasound, and microneedles, have gained traction to deliver gene therapies that hold promise for CRISPR-based therapeutics. Lipid nanoparticles (LNPs) are potent, well-tolerated, with low immunogenicity, able to deliver BE and PE, scalable, and safe [112,113]. It has been shown that LNPs could deliver ABE to RDEB fibroblasts in vitro, attaining more than 80% *COL7A1* GE efficiencies that verify important proof of principle for expanded delivery-options for topical use of gene editors [114].Indeed, LNPS can deliver CRISPR GE constructs in DNA, RNA, or RNP form, thus overcoming typical delivery difficulties such as proteolytic digestion in the skin. CRISPR-Cas9 mRNA or RNP complexes represent a possible strategy to correct genes in situ, in vivo, that maintain endogenous gene control in a more durable manner, which would potentially reduce the need for repetitive delivery [49].

Both in vitro and in vivo studies have confirmed the potential of polyethyleneimine (PEI)- based NPs for the treatment of cutaneous diseases owing to their high transfection efficiency and effective endosomal escape [115]. At the same time, PEI is also identified by its pronounced toxicity. Although the branched PEI complexes genetic cargo 15-fold more effectively than linear PEI, the linear PEI is more appropriate for in vivo applications because of its better biocompatibility [116]. For instance, linear PEI was found to be a complex viral RNA that triggers innate immune receptors in melanoma highly efficiently. Intratumoral injection of these polyplexes induced prominent T-cell infiltration, leading to potent antitumor activity with no major side effects [117]. These findings formed the foundations for an ongoing Phase I/II clinical trial for melanoma immunotherapy.

Limitations of CRISPR-Based Cutaneous Disease Therapy

Nowadays, the targeted transfer of CRISPR/Cas gene drugs to the body can treat many patients with adverse dermatologic cases that have not responded to traditional chemotherapy. The advantages of simplicity, effectiveness, and high specificity make it one of the best sought-after approaches of the future. However, researchers have uncovered some unexpected conditions when applying CRISPR technology to edit genes. Genetic manipulation of stem cells in vivo would target both undifferentiated and differentiated cells. In the skin, this is likely not a limitation because stem cells will persist, while the differentiated cells will ultimately die and slough off through the normal course of skin cell maturation. However, researchers

have to work on several challenges to the expanded application of CRISPR-mediated therapeutics. For in vivo genome editing by the CRISPR-Cas strategy to be clinically adaptable in dermatology, three main challenges have to be well addressed. CRISPR-Cas sgRNAs and nucleases have to (a) be optimized for vigorous and precise on-target effects with minimal off-target effects, b) be delivered effectively to particular human cells, and c) have minimal antigenic features so that they are not rejected by human immune systems.

Delivery Obstacles

After gene modification, the delivery of genetically edited cells to patients is the next critical step for the achievement of therapeutic success. Systemic delivery of CRISPR-mediated antimicrobials remains a challenge. Therefore, advances in delivering CRISPR-based therapies to the skin require specific attention before bringing CRISPR into the clinic; the adopted strategy for transdermal drug delivery must overcome the resilient barrier of the epidermis [118]. The success of any therapy depends on the efficacy of uptake and downstream bioavailability of the drug. The absence of vasculature in the viable epidermis and the tight epidermal-dermal junction region prevent the transfer or penetration of biomacromolecules to the viable epidermis, which is the target for most cutaneous diseases [1]. Skin tight junctions are considered a challenge for the delivery of therapeutic agents. Systemic delivery of CRISPR-based approaches for combating antimicrobial resistance remains a challenge [118].

Laser-assisted drug delivery generates microscopic ablation regions, with vertical channels penetrating through the stratum corneum and reaching deep into the dermal layer [119]. Although this strategy may offer a practical solution to cell delivery difficulties, it remains to be shown whether laser-assisted drug vehicles can be repurposed for transferring CRISPR/Cas machinery. Correction of epidermal-dermal adhesion was attained by electroporation-based ex vivo and in vivo CRISPR-Cas9 RNP [36,80]. However, the high electrical voltage needed in those protocols is difficult to apply directly to patients' skin for in vivo topical treatment. Intradermal injections using hypodermic needles followed by electroporation of CRISPR/Cas9 complexes [80]. Hypodermic needles or intradermal injections improved bioavailability [81]. Still, the physical delivery routes, such as electroporation and microinjection difficult to directly apply in vivo due to the painful penetration into the dermis [120,121]. This is particularly important for RDEB patients, who have fragile skin. Thereby, these methods may increase patient reluctance to the therapeutic procedure. Relatively new and innovative strategies use microneedles to create small pores in the epidermis and positively deliver drugs into the dermal layer [49].

There are novel delivery procedures, but these techniques are still under investigation for assessment of their safety and effectiveness. Gene delivery approaches with viral vectors can cause integrational mutagenesis and permanent Cas9 expression in cells. Accordingly, delivery of Cas9 nuclease through a transiently expressed Cas9/sgRNA RNP may be favored. Researchers have advanced LINPs that can transport CRISPR-Cas nucleotide sequences or RNP constructs that are directed to particular organs.

Off-Target Effects

In vivo, editing needs the advancement of efficient targeting strategies to produce cell-specific alterations with minimal off-target effects and to exclude the comprehensive characterization of all engineered cells. Safe in vivo GE procedures could have utility for a broad range of systemic and local diseases, but many obstacles and concerns remain to be dealt with. The main risk of CRISPR-based therapeutics is "off-target" effects, namely the deposition of unexpected, unwanted, or even adverse alterations to the genome [122]. The base mismatches between sgRNA and nontarget sequences may lead introduction of one or even multiple new mutations. When sgRNA combines with the DNA strand, the proximal end of the seed sequence at the PAM binds the target strand precisely according to base complementary pairing. However, sometimes the distal three to five bases do not separate as anticipated when mismatching occurs but form an uncommon duplex configuration under a strong force [123]. There are concerns about these effects, even though there have been claims that these effects are insignificant or can be minimized to undetectable levels. Still, others have clear certifications of large insertions or deletions that may occur as a result of unintended events as recorded in animal models [124].

As stated before, the potential off-target impacts of the Cas9/sgRNA RNPs at locations of the COL7A1 Gene in the ESC from RDEB patients have yet to be explored. Whether transdermal transfer of Cas9/sgRNA RNPs by electroporation would be safe and efficient in humans has yet to be investigated. The permanent expression of Cas9 in large numbers of cells increases the possibility of off-target effects, and controlling Cas9 activation may decrease their occurrence [41].

Improving the accuracy of sgRNAs and detaching them from the DNA strand when mismatches arise is the key to overcoming the limitations of off-target impacts in therapeutic GE [36]. To improve the efficiency of CRISPR-Cas9, researchers have developed sensitive methods to scan the whole genome for accidental off-target genome editing effects. Such methods would enable screening of off-target effects of a specific Cas/sgRNA construct before therapeutic application. More precise algorithms can be improved to design more specific sgRNAs and pegRNAs. New modifications in Cas proteins to change their detection properties can enhance their fidelity. A portion of the hairpin structure at the 5′ end of sgRNA, which decreases the energy during mismatch and inhibits the formation of an R-loop when a mismatch happens, has been designed [125]. The R-loop is needed for Cas9 activation, and thus it also prevents DNA duplex cuts in the presence of mismatches [41]. Furthermore, developing targeted delivery routes that bring the GE constituents, particularly to the cells or tissues of interest, can decrease the risk of off-target influences in other parts of the body. Strategies for regulating the timing of GE can reduce off-target impacts by limiting the time of enzyme activity [36].

DNA-Damage Toxicity

The cleavage of dsDNA by Cas9 generally triggers NHEJ repair, and as expected, these mended DNA strands usually have a few fewer or a few extra base pairs. Large deletions crossing kb and complex rearrangements as accidental consequences of on-target activity have been described in several cases [126]. This highlights a main safety concern for clinical applications of DSB-producing CRISPR therapy. Other variations of Cas9, such as dCas9, where the nuclease domains are dead or deactivated, may confertherapeutic value while mitigating the risks of DSBs [127]. By fusion of transcriptional activating or repressing domains or proteins to the DNA-binding effector, dCas9 can briefly manipulate the expression of particular genes without causing DSBs. Other variants, such as Cas9n, can also be taken into consideration, where SSBs rather than DSBs are induced. Further manipulations of these Cas9 variants have led to the improvement of BEs and PEs, a key invention for the safe therapeutic use of the CRISPR technique. Furthermore, CRISPR-caused DSBs often trigger apoptosis instead of the intended gene edit. More safety concerns were disclosed when using this tool in hPSCs, which confirmed that p53 activation in response to the toxic DSBs established by CRISPR frequently triggers subsequent apoptosis [128]. Consequently, successful CRISPR edits are more probably to occur in p53-repressed cells, leading to a bias toward selection for oncogenic cell survival [129].

Immunogenicity

In addition to the technological limitations, CRISPR techniques, like conventional GE and therapy, still raise concerns about immunogenic toxicity. The usually used Cas9 proteins developed from S. pyogenes and S. aureus have been reported to trigger an immune reaction in humans [41]. The advance of CRISPR inhibitors gave a solution to immunogenicity by inactivating the GE enzymes after DNA cleavage [130]. As a method to address this challenge, a modified Cas9 lacking response-causing exons was delivered via AAV to successfully avoid humoral and cellular immune responses in young and adult mice [131].

Further, even the modified Cas9 has to be delivered in a vector designed to evade sparking the host immune response. In vivo, exosomes, lipids, and viruses are effective at avoiding immune clearance, while synthetic chemical nanoparticles need a protective coating on the surface, such as modified PEG, which also stabilizes the polymer in the blood environment, or the inclusion of modified CD47 protein [132]. In this regard, plant exosomes are more likely to escape recognition by the immune system because of their natural origin. The use of plant exosomes for carrying CRISPR/dCas9 systems is also more welcomed for safety reasons, owing to the great differences between plant and mammalian pathogens. However, verification on the transfer of gene drugs by plant exosomes is stillespecially as many plants manufacture

exosomes with different characteristics [133]. To limit the potential immunologic response to Cas9 by predesigned antibodies in human serum, researchers have found new Cas enzymes such as the structurally discrete Cas12e and Cas12d from soil bacteria [132,134]. CRISPR therapeutics using nucleases from bacteria to which humans are not exposed may not be exposed to pre-existing immunity, enabling a more robust genome editing influence. In this regard, plant exosomes are more likely to escape recognition by the immune system because of their natural origin. The use of plant exosomes for carrying CRISPR/dCas9 systems is also more welcomed for safety reasons, owing to the great differences between plant and mammalian pathogens. However, verification of the transfer of gene drugs by plant exosomes is still immature, especially as many plants manufacture exosomes with different characteristics [133].

Safety and Ethical Considerations in CRISPR Technology for Skin Therapies

As with any new technology, CRISPR technology for skin therapies brings up important safety and ethical considerations. The safety of CRISPR-mediated GE technology is a crucial topic of concern for researchers. While the current technologies represent the safest approach to GE in humans, safety concerns about such technologies limit their applications for routine clinical practice. In vivo, treatment choices would be ideal, but many fewer studies have investigated these possibilities. Studies on the in vivo treatment of non-dermatologic diseases have revealed great promise in animal models [135]. But of the studies in dermatology dedicated to in vivo delivery of CRISPR-therapeutics, all have been constrained to localized impacts in mouse models, and none have proved high efficacy or success in the long-term to go through [75, 80]. Also, further studies in the field of dermatology must be carried out to resolve certain concerns existing for the implementation of CRISPR for GE to assure safety (concerning the balanced microbiota) and efficacy (*C. acnes* specific treatment) in humans.

When confirming editing efficiency, scientists realized that immense base deletions and chromosomal structural translocations occasionally occurred. These faults may cause positional diseases such as malignant tumors and are not acceptable in clinical uses, although the likelihood of their occurrence is low [136]. Therefore, one of the main challenges in GE today is how to target only the cells that are involved in a disease. In other words, for acne, targeting only skin cells and not, say, heart muscle or brain cells. Altering the genes in the wrongcells could cause serious side effects. This means researchers must find a way to zero in on just skin cells for gene treatment to be a safe selection for acne. An exonuclease structural domain was joined with Cas9 to decrease the occurrence of these mutations [136].

When it comes to modifying the genes in a person's somatic cells, such as the skin, brain, or kidneys, most scientists agree that GE is ethical. Simply because changes in the somatic cells only affect that individual, but are not passed on to his or her children. In other words, we are not changing the DNA of future generations of humans. On the other hand, altering the genes in a person's germline cells (like a sperm or an egg) is a subject of deep debate around the world. The issue is that alterations in germline cells are passed on to the children of the person who undergoes the treatment. The idea of modifying the DNA of future generations is not acceptable. Changing a person's genes can result in unpredictable health risks. This is why any novel genetic treatment must undergo meticulous testing and receive approval from regulatory agencies before physicians can prescribe it. This process can take over a decade.

Still, GE is risky: accidentally editing the mistaken genes could spell disaster, which is why wide testing is necessary before GE becomes widely available. Additionally, future work will be required to confirm the safety and efficacy of the delivery method in patients, and also the possibility of extending this approach to whole-body administration.

Conclusions and Perspectives in CRISPR/Cas9-Based Skin Disorders Research

The studies reported in this review largely confirm the ability of CRISPR-Cas systems to treat human skin diseases both in in vitro models and through ex vivo modification of basic patient cell lines. Furthermore, with the introduction of hypoimmunogenic common donor iPSCs, ex vivo gene modification approaches for genodermatoses could be used in patients more broadly [137]. Despite the fear of the tumorigenic potential of iPSCs, CRISPR-engineered iPSCs were not correlated with tumorigenesis

in a mouse model of RDEB32 [137]. By optimizing the re-differentiation development of iPSCs and without cells havingoncogenic potential, iPSCs may be safe for clinical application [138]. Preclinical trials in animal models and cell lines of cutaneous diseases have been extremely promising and pose a hopeful opportunity to translate these outcomes into humans.

So far, while GEN treatments, where bioengineers have to repair or replace a single faulty gene, have been fruitful and have become a certainty for a small number of life-threatening disorders, no such treatments are yet on the horizon for multifactorial conditions such as the rare cases of EB. These diseases are caused by multiple co-occurring mutations and could be treated with unique CRISPR-Cas composed targeting several genetic loci [102]. Concerning CRISPR-guided therapies for other multifactorial diseases involving many genes, like skin cancer, intensive research of the literature shows that many clinical trials are on their way. For a range of reasons, dermatology is expected to continue to be at the heart of the improvement and clinical implementation of CRISPR-Cas therapeutics. For instance, one of the first human trials involving CRISPR-Cas9 is directed toward treating refractory melanoma, among other neoplasms [102].

Still, many other genetic skin diseases and cutaneous infections can be targeted with CRISPR-Cas therapeutics. Xeroderma pigmentosum and Pachyonychia congenita have been targeted with designer nucleases and RNAi-based therapies, respectively, and could also be targeted with CRISPR-based editing [32].

Reasonable success in GE treatment of cutaneous diseases will require addressing proper delivery techniques to diseased cells in which the entire body is influenced (including multiorgan involvement). Additionally, GE proficiency has never been 100%, thereby producing a heterogeneous mixture of cells that contain corrected DNA and the original, defective DNA. Restoration of only 10% of the normal gene function is considered satisfactory to improve skin conditions significantly [139]. This seems reachable even in the light of present gene delivery challenges [140].

Confirming the safety and efficiency of these treatments, tackling ethical considerations, and boosting responsible use is vital for realizing the full potential of GE in medicine. It is expected that upcoming advances in improving editing efficacy and reducing undesirable off-target effects will bring us closer and closer to integrating CRISPR-mediated therapies in the dermatological setting. The process of satisfactory application of GE is a long one, but one day it may be a real possibility, and dermatologists may recommend CRISPR-based therapy for those patients who experience key psychosocial burdens or scarring, who will also be considered candidates for GE therapy. Many research laboratories and clinics have already started exploring and testing genetic treatments for skin disorders, and an acne treatment may be available soon. In the case of acne, it is unlikely to completely eliminate it, since acne is likely a polygenic disease. However, this shouldn't be discouraging since there is reason to be optimistic. But it means that technical advances are necessary before we can attempt to treat acne genetically.

In the future, skin therapeutic approaches may involve bacteriophages, pre- and postbiotic solutions, and other management of specific bacteria within the microbiome. Research is ongoing to determine which of these may have clinical usefulness. Ongoing research aimed at refining GE efficiency, developing novel delivery systems, and increasing therapeutic applications. Future directions may include exploring CRISPR/Cas GE technology for treating acquired skin disorders, such as cancer, and investigating the possible application of CRISPR-based diagnostics for early detection and treatment of infectious diseases.

Recently, the first-ever CRISPR-Cas9 therapy has been approved by the UK Medicines and Healthcare Products Agency to treat sickle cell disease, strengthening the hope that GE treatments for EBS are close on the horizon [141]. Genome-scale screening using the CRISPR-Cas system has been of enormous interest and is anticipated to accomplish unprecedented advances. The global GE market size was assessed at USD 0.49 billion in 2024 and is predicted to touch USD 2.95 billion by 2034 (Figure 4), with a compound annual growth rate (CAGR) of 12.30% from 2025 to 2034.

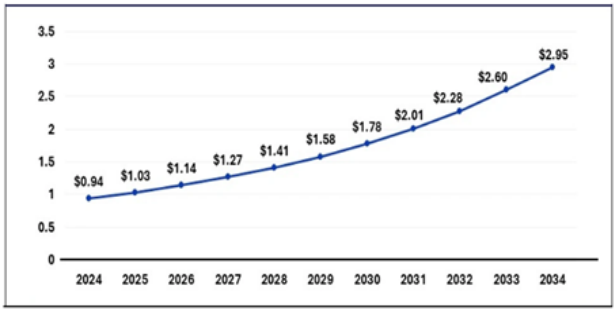

Figure 4: U.S. CRISPR-based gene editing market size between and 2034 (USD Billion).

* Source: https://precedence research.com/crispr-based-gene-editing-market.

The addition of artificial intelligence (AI) in therapeutic GE represents a topic of growing interest and investigation and brings new possibilities for refining component design and outcome expectations. AI can analyze genomic data from patients to detect mutations and biomarkers correlated with particular diseases. It can estimate optimal gRNA sequences in view of the genomic context, the edit to create, off-target sites, and possible effects on gene function and cell phenotype, allowing personalized approaches based on genetic profiles [142]. More precisely, deep-learning-based computational models, such as DeepBE and DeepCas9 variants, have been advanced to predict the efficacy and editing outcomes of different Cas9 and BE variants [143-153].

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