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Advances in Treatments for Epidermolysis Bullosa (EB): Emphasis on Stem Cell-Based Therapy

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Abstract

Epidermolysis bullosa (EB) is a rare genetic dermatosis characterized by skin fragility and blister formation. With a wide phenotypic spectrum and potential extracutaneous manifestations, EB poses significant morbidity and mortality risks. Currently classified into four main subtypes based on the level of skin cleavage, EB is caused by genetic mutations affecting proteins crucial for maintaining skin integrity. The management of EB primarily focuses on preventing complications and treating symptoms through wound care, pain management, and other supportive measures. However, recent advancements in the fields of stem cell therapy, tissue engineering, and gene therapy have shown promise as potential treatments for EB. Stem cells capable of differentiating into skin cells, have demonstrated positive outcomes in preclinical and early clinical trials by promoting wound healing and reducing inflammation. Gene therapy, on the other hand, aims to correct the underlying genetic defects responsible for EB by introducing functional copies of mutated genes or modifying existing genes to restore protein function. Particularly for severe subtypes like Recessive Dystrophic Epidermolysis Bullosa (RDEB), gene therapy holds significant potential. This review aims to evaluate the role of new therapeutic approaches in the treatment of EB. The review includes findings from studies conducted on humans. While early studies and clinical trials have shown promising results, further research and trials are necessary to establish the safety and efficacy of these innovative approaches for EB treatment.

Keywords Epidermolysis bullosa · Stem cell therapy · Gene therapy · Tissue engineering

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Introduction

Epidermolysis bullosa (EB) represents a diverse group of rare hereditary dermatological disorders typified by mucocutaneous fragility and blistering induced by minor trauma. A wide phenotypic spectrum exists with potentially serious extracutaneous impacts, morbidity, and mortality (specially through squamous cell carcinoma) [1].

EB covers an array of inherited blistering ailments impacting skin and sometimes mucosa/organs. Currently categorized into four key subtypes based on cleavage level (EB simplex, junctional EB, dystrophic EB, and Kindler syndrome), over 30 clinical variants exist with pathogenic mutations in at least 21 unique genes [2]. The preferred diagnostic approaches involve a panel of next-generation sequencing screening all EB variants alongside certain specialized immunofluorescence and electron microscopy skin biopsies in unique situations [3]. EB's root cause lies in genetic alterations impacting proteins maintaining skin

structural integrity. These proteins include collagen, laminin, integrins, and other components of the dermal-epidermal junction. Mutations disrupt anchoring fibrils linking epidermal/dermal layers, rendering skin fragile and prone to blistering. Management primarily prevents/treats skin fragility complications. Strategies center on wound care, pain control, nutrition support, and physiotherapy to enhance quality of life, alleviate symptoms and prevent issues like infection [4–6].

Additionally, the emerging fields of stem cell-, particularly mesenchymal stem cell (MSCs)-, therapy, gene therapy, protein replacement, and tissue engineering offer potential avenues for future treatments [7]. MSCs, as multipotent cells, possess the ability to undergo differentiation into diverse cell lineages, encompassing skin cells among their potential differentiation outcomes [8]. They have shown promise in preclinical and early clinical trials for promoting wound healing and reducing inflammation in EB [9]. Gene therapy also aims to correct the underlying genetic defect responsible for EB by delivering functional copies of the mutated genes or modifying the existing genes to restore protein function. This approach holds great potential for treating EB, especially for severe subtypes such as Recessive Dystrophic Epidermolysis Bullosa (RDEB). Early studies have shown promising results in animal models and initial human trials, but further research and clinical trials are needed to establish the safety and efficacy of gene therapy for EB [10, 11].

Regarding above information, the primary objective of this review is to evaluate the role of new therapeutic approaches, particularly stem cell and gene therapy, in the treatment of EB taking into account the findings from studies conducted on both animals and humans.

Epidemiology, Symptoms, and Various Types of Disease

EB is considered a rare disease, with varying prevalence rates depending on the subtype and geographical regions [12, 13]. For example, approximately 1 of 1,000,000 newborns in the United States suffer from EB [14]. One of the largest studies of epidermolysis bullosa patients in England and Wales reported a prevalence of 34.8 per million and an incidence of 67.8 per million. This analysis of over 2,500 patients represents one of the largest EB cohorts examined to date for the population of England and Wales [15]. EB affects both males and females, and there is no known predilection for any particular ethnic group [16].

The trademark sign of EB is delicate skin, making it prone to blistering and skin breakdown even from minor trauma. Symptom seriousness differs from mild to severe depending on the EB type [17]. Common issues include:

Blisters and raw areas on the skin caused by friction or little injury. Blisters may happen anywhere on the body like skin, mouth lining, and internal organs. Blisters are painful and skin breakdown remains after bursting, healing slowly and potentially causing scars. Over time, repeating blistering and healing results in scarring. Scarring can lead to tightening and deformities of the skin and below tissues, restricting joint movement and reducing functioning. Many experience nail issues like changes in shape, splitting or lack of nails while hair problems like sparse growth or fragility may also occur. EB impacts the mouth liner, causing blisters and skin breakdown in the mouth and throat. This causes difficulties eating, swallowing and speaking. Some types involve the stomach and gut, leading to symptoms like difficulty swallowing, acid reflux and nutritional deficiencies [1, 18, 19]. EB may divided into the following four subtypes: Epidermolysis Bullosa Simplex (EBS), Junctional Epidermolysis Bullosa (JEB), Dystrophic Epidermolysis Bullosa (DEB), and Kindler Syndrome.

Figure 1 illustrates a schematic diagram showcasing the cutaneous basement membrane zone and mutated proteins caused by gene defects in various subtypes of EB disease.

Therapeutic Strategies

Epidermolysis bullosa presents a challenging condition requiring a multidimensional treatment approach. While current methods may ameliorate some clinical manifestations of EB, they clearly do not cure this devastating disease. Advanced regenerative medicine strategies, like those involved in precision medicine, are needed to design treatments for these presently intractable disorders. Pharmacotherapy including pain control, wound dressings and topical medications helps relieve symptoms and promote healing. Maintenance therapies such as proper skincare, nutritional support and physical/occupational therapy are essential for long-term management. Additionally, emerging approaches like stem cell and gene therapy show excellent promise for transforming EB's treatment landscape. Ongoing exploration and clinical trials are paving the path for innovative interventions that may ultimately deliver a cure for this disabling condition [20, 21]. These strategies are summarized below:

Pharmacotherapy

Pain caused by EB-associated wounds and blisters can be alleviated through the use of nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, or local anesthetics [22]. These

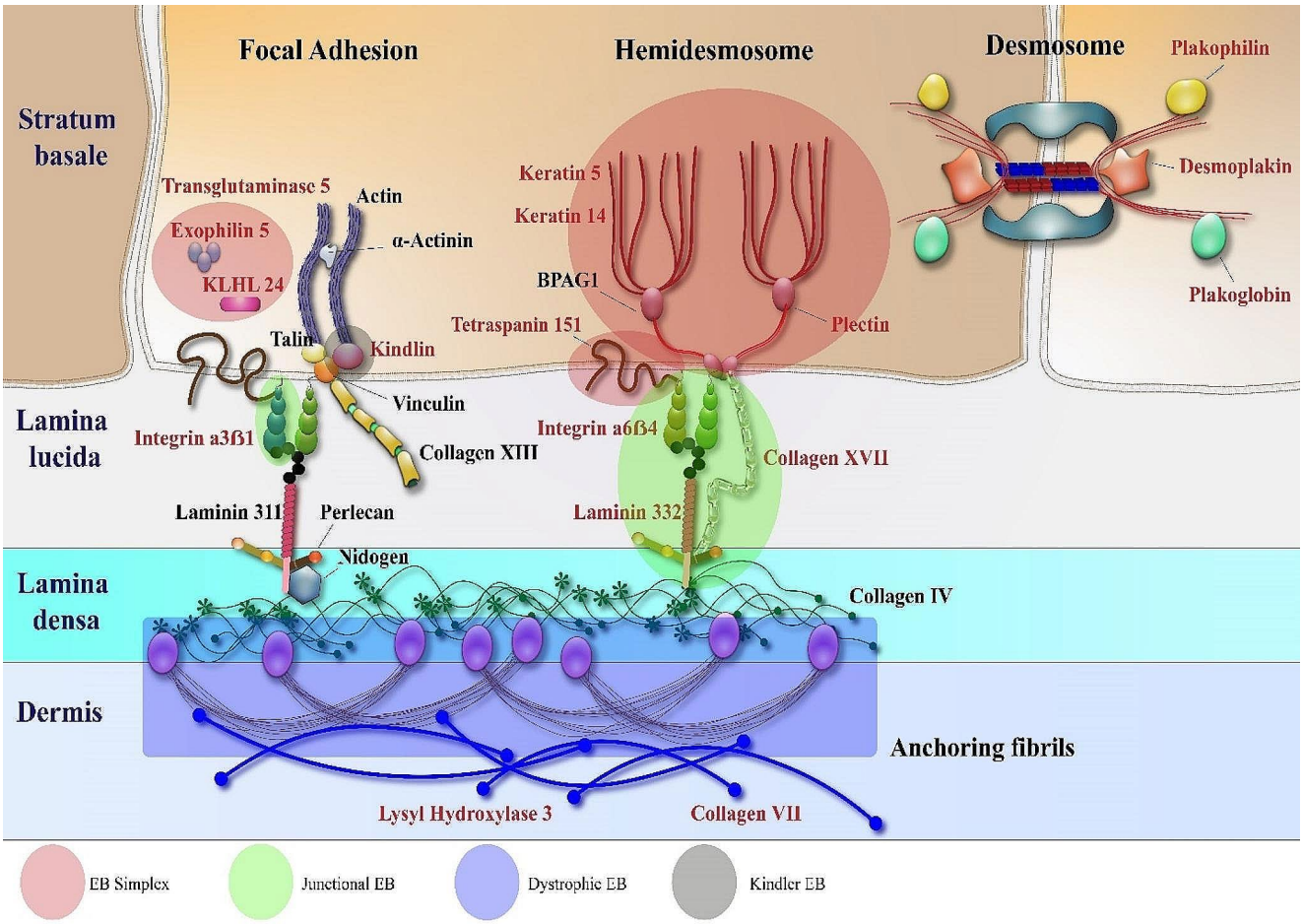

Fig. 1 Schematic diagram of cutaneous basement membrane zone and mutated proteins in various subtypes of EB disease (the figure is created using Adobe illustrator 2019). This diagram highlights the crucial attachment complexes responsible for establishing stable cell-to-cell contacts and connecting the epidermis with the underlying dermis. The proteins highlighted in red, originating from 21 unique genes, undergo mutations in various manifestations of EB

medications help to provide temporary relief and enhance the patient's overall comfort. Topical corticosteroids and immunosuppressants, such as tacrolimus (a calcineurin inhibitor) and pimecrolimus, can also be prescribed to reduce inflammation and manage symptoms associated with EB [23, 24]. These medications help to alleviate itchiness and prevent the progression of the disease.

Maintenance Treatments

Regular and meticulous skin care is essential to prevent skin breakdown and minimize the risk of infection. Measures include gentle cleansing, moisturizing, and the application of barrier creams or ointments. Bathing in lukewarm water and using mild, fragrance-free products are recommended. Besides, maintaining adequate nutrition is crucial for individuals with EB, as they often have increased nutritional needs due to chronic inflammation and impaired nutrient absorption [25]. A balanced diet, often requiring the involvement of a registered dietitian, can help optimize the patient's overall health and wound healing. Physical and occupational therapy play a vital role in managing EB. These therapies focus on maintaining joint mobility, preventing contractures, improving muscle strength, and optimizing functional independence. They also provide education on safe positioning and activities of daily living [26, 27].

Emerging Therapies

Stem Cell Therapy

Stem cell-based interventions show tremendous therapeutic promise for epidermolysis bullosa. This regenerative approach aims to replenish aberrant cell populations through transplantation of healthy progenitor populations. Indeed, bone marrow-derived mesenchymal stromal cells and human-induced pluripotent stem cell-derived keratinocytes represent leading candidate graft sources under exploration.

Several early phase clinical investigations are currently in progress to evaluate the safety and preliminary efficacy of this novel cellular regenerative modality in various diseases like GVHD [28], Covid-19 [29–31], and neurodegenerative diseases like ALS [32, 33]. As outlined in Table 1, these pioneering works seek to elucidate optimal cell dosage and formulation parameters while monitoring for potential adverse events. Methods such as allogeneic transplantation via intradermal injection or autologous grafting employing Bio advanced skin substitutes are among strategies under assessment.

Transplants of bone marrow stem cells for patients afflicted by EB started in 2007. Results from the first batch of 7 RDEB recipients who underwent this process appeared in a 2010 publication. Regrettably, two from this initial group passed away. However, the remaining five reportedly exhibited reductions in vesicle formation and erosions. Enhanced type VII collagen expression at dermal foundational barriers was documented in 4 of the 5, yet one amongst them with no such collagen presence post-transplant nevertheless showed clinical improvement. This pioneer study provided early signals that bone marrow transplants may benefit EB management by potentially fixing type VII collagen insufficiencies and mitigating side effects, while highlighting the necessity for additional work to augment outcomes [34].

As shown in Table 1, subsequent clinical trials confirmed the safety and effectiveness of this treatment method. Although most of these studies lacked a control group and had a small sample size, the initial clinical results were promising. Moreover, when it comes to genomic editing studies utilizing CRISPR-Cas9, human pluripotent stem cells (hPSCs) and induced pluripotent stem cells (iPSCs) offer a clear advantage over other cell types, including somatic cells and adult stem cells. This advantage stems from their unlimited capacity for proliferation, which is particularly beneficial in cases where clonal selection becomes a requirement [42]. Several reports have described methods for developing genome-changed cells from tissues not typically accessible to acquire [43, 44]. For instance regarding EB, one investigation grafted keratinocytes prompted from gene-treated induced pluripotent stem cells onto immunodeficient rodents. Two months subsequent to transplantation, standard manifestation of the COL7A1 gene was visible. This shows the potential of utilizing corrected induced cells to cure EB at the genomic level through restorative transplantation approaches [45]. More exploration utilizing comparable strategies could help advance remedies for this complicated disorder.

Mechanism of Action of Stem Cells in EB Treatment

Hematopoietic and mesenchymal stem cells given during bone marrow transplantation have the key ability to travel from the infusion area in the bloodstream to their intended tissues. Engraftment involves integration into the local microenvironment. Hematopoietic stem cells engraft in bone marrow, while mesenchymal stem cells have been found in skin, lung, and other organs post-bone marrow transplantation. After migrating and homing in the damaged skin, these cells can be effective in the treatment of the EB through the following mechanisms:

Differentiation of Stem Cells Once in the intended skin tissue, stem cells must successfully engraft by lodging, surviving, and differentiating into fibroblasts and skin cells [46].

Stem cells exhibit multipotency, or the ability to differentiate into various phenotypes of the cells [47]. In EB treatment, mesenchymal stem cells mainly mature into fibroblasts within dermal tissue. Differentiation is induced by cues from the microenvironment such as growth factors and the extracellular matrix [48]. During differentiation, stem cell genes shut off and genes for fibroblast markers like type VII collagen turn on. The newly formed fibroblasts now synthesize the functional protein lacking in EB patients. Stem cell plasticity to take on required cell phenotypes allows corrected functional cells to be produced from transplanted stem cells [49]. This halts the pattern of vesiculation and allows wound repair.

Paracrine Effects of Stem Cells Beyond serving as direct cellular grafts undergoing lineage commitment, stem cell therapies exert paracrine influences of significant therapeutic impact [50]. Through secreted factors including cytokines like prostaglandin E2, indoleamine 2, 3-dioxygenase, VEGF and TGF-β, chemokines and extracellular vesicular cargo, transplant-derived cells activate downstream signaling cascades in the local microenvironment. By modulating target cell behavior, stem/progenitor (an intermediate state between stem cells and fully differentiated cells) recruitment is optimized, angiogenic revascularization enhanced, and anti-inflammatory modulation promoted [51, 52]. The paracrine effects of adult stem cells have been found to be a substantial contributor to the positive outcomes observed in various clinical trials [53].

Immunomodulatory Effects of Stem Cells Some soluble factors released by stem cells exert a suppressive effect on the activation and proliferation of Th1 and Th17 cells, thereby resulting in a reduction of inflammatory cytokine produc-

Table 1 Clinical trial studies on stem cell therapy for epidermolysis bullosa

Trial design	Sample size	EB type	Type of stem cells	Route of injection	Follow-up period	Main results	Ref
Phase I clinical trial	7	NM	Allogenic BM-HSCs	IV	799 days	2 patients did not survive. However, the other five patients experienced clinical improvements with less blistering and fewer erosions observed.	[34]
Phase I clinical trial	2	RDEB	Allogenic BM-MSCs	Intradermal	4 months	Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin was observed	[35]
Phase I clinical trial	14	RDEB	NHBMSC	IV	1-year follow-up period	Significant decrease in the number of new blisters and faster healing was observed.	[36]
phase I/II clinical trial	20	Generalised severe RDEB	Allogenic Blood- and BM-MSCs	IV	NM	Many, but not all patients, had significant benefit from allogeneic blood and marrow transplantation	[37]
phase I/II clinical trial	10	RDEB	Allogenic BM-MSCs	IV	9 months	Improved wound healing and reduced skin erythema was reported.	[38]
Case report	1	RDEB	Allogenic BM-HSCs	IV	700 days	Skin lesions reduced and healing improved	[39]
phase I/II clinical trial	10	RDEB	Allogenic BM-MSCs	IV	12 months	A transient reduction in disease activity scores and significant reduction in itch was reported.	[40]
Phase I/II clinical trial	6	RDEB	Allogenic UC-MSCs	IV	8–24 months	Improvement in blister count, pain, quality of life and decrease in inflammation was observed.	[41]

Abbreviations; RDEB: recessive dystrophic epidermolysis bullosa/ BM-HSCs: bone marrow hematopoeitic stem cells/ IV: intravenously/ NM: Not mentioned/ BM-MSCs: bone marrow mesenchymal stem cells/ NHBMSC: bone marrow non-hematopoeitic stem cells/ UC-MSCs: Umbilical cord mesenchymal stem cells

tion. Through another mechanism, MSCs induce remodeling and modulate the composition of T-cell subsets towards T-regulatory cells, subsequently elevating the levels of anti-inflammatory cytokines. Numerous studies have provided evidence that MSCs impede the acquisition of the M1 macrophage phenotype while promoting M2 polarization [54, 55]. These paracrine signatures likewise stimulate resident progenitor proliferation and differentiation integral to regenerative tissue remodeling [56, 57]. MSCs also dampen the functions of immune effector cells like natural killer cells, cytotoxic T lymphocytes, B cells and neutrophils that might identify donated stem cells as alien [58, 59]. The ensuing immuno-tolerant microenvironment allows donor cells to establish long-term engraftment and exert therapeutic effects without being rejected. Such properties are important for conferring enduring therapeutic grafts capable of long-term disease modification.

Rebuilding the Skin Epithelial Stem Cell Niche Besides, stem cell therapies may contribute to rebuilding the skin epithelial stem cell niche compromised by epidermolysis bullosa. These niches govern progenitor fate by modulating signals from extracellular matrix components, surrounding supportive cell types, and paracrine factors [60]. Transplanted stem cells can secrete matrix proteins like fibronectin, laminin and collagen, along with paracrine signals, to aid stabilization of progenitor adhesion and restoration of structural and molecular niche properties. This influences hematopoietic and epidermal progenitor behavior in native skin to advance wound healing. Reconstructed niches support lineage commitment of normal keratinocytes from resident progenitors, thereby facilitating engraftment and functionality of donor stem cell grafts [61].

Regenerative Effects of Extracellular Vesicles/Exosomes Released from Stem Cells Extracellular vesicles are small membrane-bound structures released by cells, including stem cells. Exosomes, a specific type of extracellular vesicle, are nanosized particles involved in intercellular communication. They contain proteins, lipids, nucleic acids, and other bioactive molecules that can modulate cellular functions [62]. In the context of epidermolysis bullosa, studies have demonstrated that extracellular vesicles/exosomes derived from stem cells can improve the integrity of the epidermis, promote collagen synthesis, and enhance the migration and proliferation of keratinocytes [63, 64].

Figure 2 provides a schematic representation of the mechanism by which stem cells exert their therapeutic effects in EB.

Mesenchymal stem cells, utilized in the treatment of EB disease, can be obtained from various sources such as bone

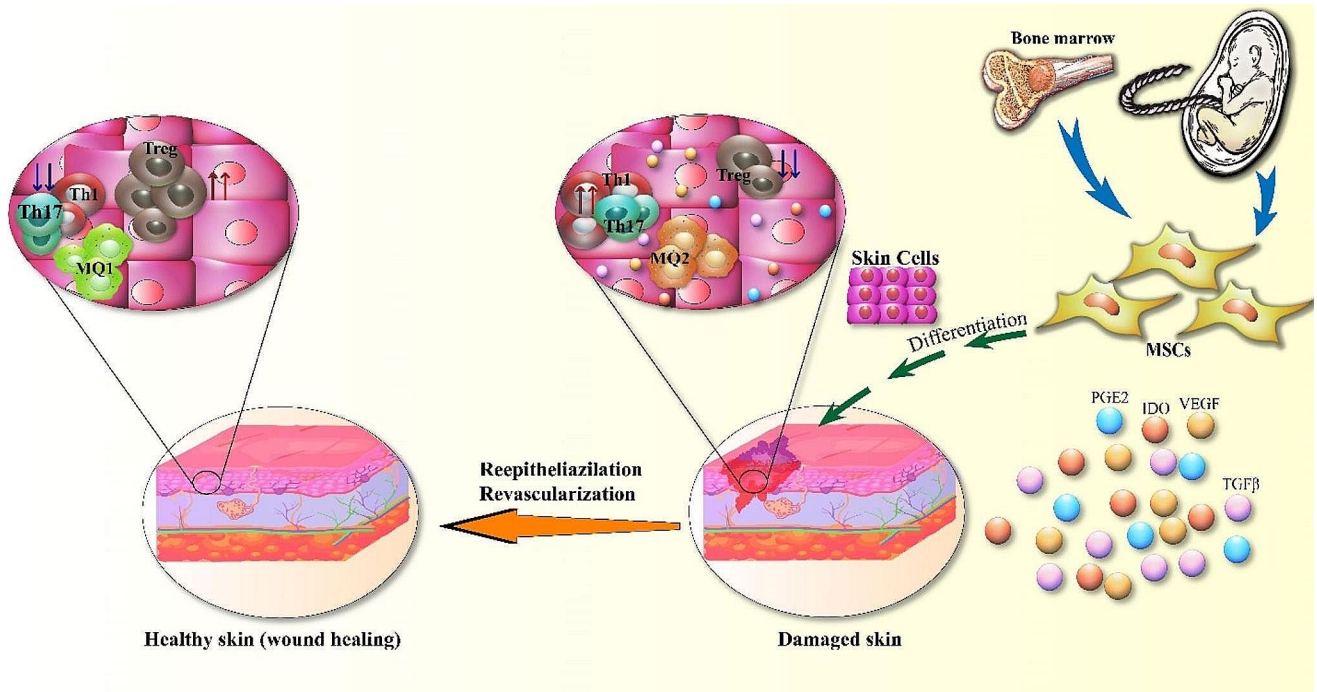

Fig. 2 Mechanism of action of Mesenchymal stem cells in EB treatment (the figure is created using Adobe illustrator 2019). Abbreviations: MSCs: Mesenchymal Stem Cells/ EB: Epidermolysis bullosa/ PGE2: prostaglandin E2, IDO: indoleamine 2, 3-dioxygenase/ TGFβ: Transforming growth factor β/ VEGE: Vascular endothelial growth factor/ MQ: Macrophage/ TH1: T Helper1/ TH17: T Helper17/ Treg: T regulatory cell

marrow or umbilical cord and subsequently transplanted. Upon transplantation, these cells possess the ability to migrate towards the site of damaged tissue in response to signals received from the injured area, facilitated by chemoattractants present along the migratory path. Moreover, they have the capacity to differentiate into specific target cells within the desired tissue. In the context of EB disease, following transplantation, these cells migrate towards the affected skin region and undergo differentiation, contributing to the process of re-epithelialization crucial for skin regeneration. Furthermore, mesenchymal stem cells play a pivotal role in tissue improvement through the paracrine secretion of various cellular cytokines, including cytokines VEGF, TGFβ, PGE2, and IDO. These secreted factors elicit several changes within the tissue microenvironment, such as promoting revascularization, modulating immune processes, and reducing inflammation at the site of the wound. These alterations consequently result in the transition of macrophages from an inflammatory phenotype (MQ1) to an anti-inflammatory phenotype (MQ2). Additionally, transplantation leads to a reduction in the number of inflammatory cells (TH1 and TH17) and an increase in the number of regulatory and anti-inflammatory cells (Treg). The collective impact of these changes ultimately enhances tissue condition and facilitates wound healing in individuals afflicted with EB disease.

Gene Therapy

Gene therapy aims to remedy the genetic mutations responsible for EB by introducing functional genes into the patient's cells. This approach poses immense prospective for delivering a long-lasting cure for EB. Multiple gene therapy tactics, such as gene substitution and gene modifying utilizing CRISPR-Cas9, are being investigated in preclinical and clinical experimentation. Though still in the exploratory stage, gene therapy provides hope for future breakthroughs in managing EB [65]. Several preclinical and limited clinical investigations have demonstrated the potential of gene therapy for EB.

Genetic factors play a defining role in the pathogenesis of numerous cutaneous pathologies. Monogenic disorders exhibit clear genetic determinism, as seen with epidermolysis bullosa resulting from homozygous COL7A1/LAMB3 mutations. However, inter individual variation also surfaces in multifactorial diseases like ichthyosis vulgaris (IV), where FLG homozygosity increases risk [66, 67]. Most research utilizing CRISPR-Cas9 in primary Keratinocytes (KCs) is focused on EB using patient-derived EB KCs, as reviewed recently [68].

In EB, the dermal-epidermal junction proves inherently fragile, clinically manifested as constant vesiculation and resultant infections that compromise quality of life. Through employing CRISPR-Cas9 mediated homology-directed repair, investigators have restored COL7A1 expression in patient keratinocytes [69, 70], and fibroblasts, renewed at the genetic level [69]. Keratinocytes modified thusly demonstrated restored capacity to organize robust skin grafts in murine xenotransplants. Separately, a dual-guide RNA approach also validated capacity to reframe COL7A1 reading and reinstate protein production in recessive dystrophic epidermolysis bullosa keratinocytes, enabling long-term regeneration of properly adherent epithelium. Such findings offer proof-of-concept that precision editing may reverse disease pathogenesis at its roots. Continued methodological refinement brings hope that genomic resolutions may someday eliminate suffering for these patients [71]. Additionally, variations in the LAMB3 gene have been linked to junctional types of epidermolysis bullosa. The LAMB3 gene provides directions for generating a protein called laminin-332, which plays a vital role in preserving the structure of the skin. Investigations have examined employing gene modifying tactics to remedy mutations in the LAMB3 gene and reinstate biosynthesis of functional laminin-332 [72].

When pursuing genomic interventions for epidermolysis bullosa, generating immortalized keratinocyte lines maintains research potential. However, not all cell models faithfully mimic native epithelial behavior or accommodate gene editing with equal ease. HaCaT cells frequently serve as surrogates given their proliferation potential ex vivo [73]. . Yet abnormal stratification, erratic marker expression profiles, and absent cornified layers compromise translation. Aneuploidy introduces further variability. Together, these factors render HaCaT cells suboptimal for elucidating genomic effects on differentiation. Immortalized populations retain value for fundamental mechanistic and target screening studies. Yet direct clinical use necessitates alternatives exhibiting physiology unhindered by culture stresses. Induced pluripotent stem cell-derived keratinocytes may represent a more relevant regenerative approach through preserving normal structural and functional attributes amenable to precision modifications aimed at durable, restorative outcomes for patients [74]. As gene therapy is a novel and developing approach for EB, preclinical investigations remain the current focus in this area with only sparse early-phase clinical studies conducted thus far. Some representative published clinical efforts in this nascent field are concisely summarized in Table 2. Considerable additional research is still warranted to fully assess the therapeutic potential and safety profile of gene-based interventions for diabetes prior to broader clinical translation.

Table 2 Clinical trial Studies on Gene Therapy for Epidermolysis bullosa

Disease Subtype	Gene Therapy Approach	Sample size	Treatment Dosage	Applied intervention	Rout of injection	Main outcomes	Follow-up Period	Ref
RDEB	Lentiviral vector mediated gene therapy	4	1×10^6 cells/ cm^2 of intact skin	COL7A1-modified autologous fibroblasts	3 intradermal injections	Significant increase in C7 MFI in the injected skin	1-year	[75]
RDEB	Retroviral virus mediated gene therapy	4	0.8 proviral genome copies (containing full-length COL7A1 sequence) per cell	COL7A1-modified autologous keratinocytes	Cutaneous injection	Significant (%75) wound healing	1-year	[76]
JEB	Retroviral vector mediated gene therapy	NM	NM	LAM5-β3-modified autologous epidermal stem cells	Topical	Full functional correction of the disease during follow-up period	1-year	[77]
RDEB	Viral vector mediated gene therapy	9	$2–8 \times 10^8$ P.F.U / wound/day	Beremagene geperpavec (B-VEC), an engineered, non-replicating COL7A1 containing herpes simplex virus type 1 (HSV-1) vector	Topical	Significant promoted wound healing	12 weeks	[78]

Abbreviations; RDEB: Recessive dystrophic epidermolysis bullosa/ NM: Not Mentioned/ MFI: mean fluorescence intensity/ JEB: junctional epidermolysis bullosa/ P.F.U: plaque-forming unit /

Tissue Engineering and Scaffold-Base Therapy

The treatment of wounds, including those associated with epidermolysis bullosa, represents a significant unmet clinical need. Tissue engineering experts are actively engaged in the development of skin-like structures to facilitate the healing process and promote the reconstruction of skin in currently untreatable wound injuries. However, this pursuit is exceptionally challenging due to the intricate nature of the skin, encompassing its complex structure and multifaceted functions [79]. Developing safe and high-quality engineered skin necessitates careful consideration of various factors, such as biocompatibility, biodegradability, non-carcinogenic cross-linking, cost-effectiveness, prevention of infectious diseases, and mitigation of immune system activation [80]. A range of biodegradable protein-based natural polymers, such as collagen, fibrin, and hyaluronic acid, have found extensive application in the fabrication of scaffolds for tissue regeneration [81, 82]. These scaffolds have been utilized for the regeneration of various tissues, including bone, neural tissues, skin, skeletal muscle, and blood vessels [83, 84]. The primary approach in engineering skin substitutes involves cultivating primary skin cells, including stem cells, fibroblasts, and keratinocytes within scaffolds that emulate the three-dimensional (3D) structure of normal cells, either through natural or biosynthetic means. Despite notable advancements, many wound treatments remain unmet clinical needs, demanding a multidisciplinary approach to devising effective solutions [85, 86]. Recent clinical trials have shown that modern dressings and skin substitutes offer a convenient, easily accessible, and cost-effective approach for treating chronic wounds. Consequently, the ultimate objective is to develop readily available off-the-shelf wound dressings that can be promptly employed by patients as needed [77, 87]. In order to achieve this objective, there is considerable potential in developing cost-effective manufacturing techniques that utilize non-mammalian sources of collagen or extracellular matrix (ECM) components. These alternative sources, in combination with synthetic scaffolds, offer a promising avenue for producing materials with favorable properties. Such materials would provide an optimal structure for cellular ingrowth and enable the modulation of the chronic wound microenvironment, thus facilitating the healing process. Moreover, these bioengineered materials can be customized to incorporate mechanisms for controlled release of bioactive molecules or drugs. This customization can be based on factors such as the scaffold's degradation rate or specific signals emanating from the wound, thereby enhancing their therapeutic capabilities [85]. . However, it is important to note that while these novel treatment methods exhibit promise, they are still in the preliminary and pre-clinical stages

of development, with limited application in human studies. Table 3 provides a comprehensive summary of the current state of this technology in human research, encapsulating the progress achieved thus far.

Limitations and Feature Directions

Despite significant advancements in the treatment of Epidermolysis Bullosa, there are persistent limitations associated with pharmacotherapy, stem cell therapy, gene therapy, and tissue engineering approaches. However, ongoing research and progress in these fields offer promising avenues for overcoming these limitations.

In terms of pharmacotherapy, its capacity to address the underlying genetic abnormalities responsible for EB is limited. Future directions in this area involve the development of targeted therapies that aim to correct or modulate the genetic defects associated with EB. This entails the exploration of novel drugs, including gene-editing agents or small molecules that specifically target the pathways involved in the pathogenesis of EB. Also, moving on to stem cell therapy, several challenges need to be surmounted. These challenges include the scarcity of suitable cell sources, such as autologous cells with a healthy gene profile, as well as the risk of immune rejection. Future directions in stem cell therapy aim to enhance the availability and successful engraftment of stem cells through innovative strategies. This can encompass the utilization of iPSCs generated from patient-derived cells, the development of gene-edited stem cells with corrected genetic defects, and the exploration of alternative stem cell sources like amniotic fluid-derived stem cells. In addition, Skin organoids derived from patient-specific induced pluripotent stem cells offer a promising platform for studying the pathogenesis of epidermolysis bullosa and evaluating potential therapeutic strategies. By incorporating extracellular vesicles/exosomes derived from stem cells into skin organoid models, it is possible to enhance their regenerative potential and better mimic the physiological microenvironment of the skin.

Regarding to gene therapy, although it has demonstrated promising results in preclinical and early clinical trials, there are still challenges that need to be addressed. These challenges include the efficient delivery of therapeutic genes to target cells, long-term gene expression, and potential immune responses triggered by viral vectors employed for gene delivery. Future directions in gene therapy involve refining gene delivery techniques, such as viral vectors or non-viral methods, to enhance efficiency and safety. Furthermore, advancements in genome editing technologies, such as CRISPR-Cas9, hold considerable potential for precise gene correction in EB. Besides, with regard to tissue

Table 3 Clinical trial studies on scafold-base therapy for epidermolysis bullosa

Disease Subtype	Sample size	Scaffold Type	Scaffold material	Surgical Procedure	Wound Healing Assessment	Follow-up Period	Ref
RDEB	7	Biological (amniotic membrane)	Fibroblast	Cell-seeded scaffold implantation	Improved wound closure	12 weeks	[88]
NM	1	Biological (acellular epidermal scaffold)	acellular	allogenic acellular epidermal sheet engraftment	Partial healing	6 months	[89]
RDEB	7	Biological (COL7A1-modified autologous epidermal sheets)	Type VII collagen	Topical engraftment	Significant promoted wound healing	2–5 years	[90]
RDEB	7	Synthetic	Type I collagen	Collagen-based scaffold engraftment	Complete healing (Full wound reepithelialization)	6 months	[91]

Abbreviations; RDEB: Recessive dystrophic epidermolysis bullosa/ NM: Not mentioned

engineering, while it holds promise, there are limitations that persist. These limitations encompass the need for optimized scaffold designs that accurately mimic the intricate structure and functionality of the skin, as well as challenges associated with long-term integration and vascularization of engineered tissues, along with scalability of production. Future directions in tissue engineering involve integrating advanced biomaterials, bioactive molecules, and growth factors into scaffolds to augment tissue regeneration. Additionally, the development of vascularized tissue constructs and the application of 3D bioprinting technologies present exciting prospects for the treatment of EB.

Conclusion

Epidermolysis bullosa represents a class of genetically inherited disorders characterized by fragile skin and mucous membranes prone to blistering. Significant morbidity arises from cutaneous fragility and associated complications such as wound infections and squamous cell carcinoma development. Precise subclassification relies on clinicopathological correlation and genetic screening to identify causal mutations in genes encoding structural proteins of the dermal-epidermal junction. Delineating molecular etiologies informs prognosis and targeted therapeutic development. Current management prioritizes preventative wound care, pain control and nutrition support to mitigate injury sequelae. However, regenerative strategies offer hope to address underlying defects. Stem cell therapies, particularly in combination with tissue engineering strategies, show promise to promote healing through paracrine signaling and skin cellular differentiation ability. Gene therapy also demonstrates potential to achieve durable correction by delivering functional gene copies or modifying mutations. Initial animal and early-phase human studies yield encouraging outcomes, yet rigorous evaluation in well-powered clinical trials is required to establish long-term safety and effectiveness profiles particularly for recessive dystrophic epidermolysis bullosa. Continued mechanistic investigations and controlled human subject research hold the key to fully realizing the curative capacity of these innovative approaches. Realizing their full potential may ultimately transform the prognostic landscape for this challenging condition.

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Author Contributions ES & RR & HRR were involved in search strategy and drafting. All authors supervised in all steps of the project, and revised and edited the manuscript. All authors read and approved the final manuscript.

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Declarations

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Consent for publication All authors consent to publish the article.

Competing Interests None.

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