We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Surgical Treatment of Wounds Using Stem Cells in Epidermolysis Bullosa (EB)

Magdalena Nita, Jacek Pliszczynski, Andrzej Eljaszewicz, Marcin Moniuszko, Tomasz Ołdak, Katarzyna Woźniak, Sławomir Majewski, Cezary Kowalewski, Artur Kamiński, Dariusz Śladowski, Zbigniew Zimek, Maciej Kosieradzki and Piotr Fiedor

Abstract

Epidermolysis bullosa (EB) is a group of hereditary skin diseases, or genodermatoses, characterized by the formation of severe, chronic blisters with painful and life-threatening complications. Despite the previous and ongoing progress in the field, there are still no effective causative treatments for EB. The treatment is limited to relieving symptoms, which—depending on disease severity—may involve skin (blisters, poorly healing wounds caused by the slightest mechanical stimuli, contractures, scarring, pseudosyndactyly) and internal organ abnormalities (esophageal, pyloric, or duodenal atresia; renal failure; and hematopoietic abnormalities). The last decade saw a series of important discoveries that paved the way for new treatment methods, including gene therapy, bone marrow transplantation, cell therapy (allogenic fibroblasts, mesenchymal stem cells [MSCs], and clinical use of induced pluripotent stem cells. Tissue engineering experts are attempting to develop skin-like structures that can facilitate the process of healing to promote skin reconstruction in injuries that are currently incurable. However, this is incredibly challenging, due to the complex structure and the many functions of the skin. Below, we characterize EB and present its potential treatment methods. Despite the cure for EB being still out of reach, recent data from animal models and initial clinical trials in humans have raised patients', clinicians', and researchers' expectations. Consequently, modifying the course of the disease and improving the quality of life have become possible. Moreover, the conclusions drawn based on EB treatment may considerably improve the treatment of other genetic diseases.

Keywords: biological dressing, human skin allograft, allogenic human skin equivalent, Advanced Therapy Medicinal Product, Epidermolysis Bullosa, Rare Diseases

1. Introduction

Epidermolysis Bullosa (EB) is a group of heterogeneous genetic conditions (genodermatoses) characterized by skin fragility and blister formation. These blisters, or bullae, may form spontaneously or as a result of slight mechanical injuries. EB is estimated to occur in 1 person per 50,000 live births.

EB constitutes a group of conditions with diverse clinical courses. Depending on the type of abnormalities in the specific genes, the course, severity, and location of lesions may vary. EB is a result of abnormal connection between the epidermis and dermis. The epidermis, which is the most superficial layer of the skin, constitutes an important barrier between the body and its external environment. The epidermis prevents the loss of water and protects the body against ultraviolet radiation and pathogens. The dermis contains blood vessels, nerve endings, and skin appendages. Under normal conditions, the epidermis and dermis are tightly connected via protein molecules [1–6].

2. The epidermis – structure and functions

The epidermis is the outermost part of the skin and serves as a barrier protecting the body against pathogens, ultraviolet radiation, and excessive loss of water. The epidermal layers, listed from the deepest to the most superficial, include the basal, spinous, granular, and cornified layers. The basal layer is composed of keratinocytes, which undergo intense cell divisions. The newly formed cells differentiate as they progress towards the epidermal surface, eventually becoming dead, anuclear cells (corneocytes) that have no mitochondria. Since they are surrounded by a lipid layer, corneocytes form an impermeable barrier. The epidermis is strongly and permanently connected to the dermis via a cytoskeleton and hemidesmosomes. (Figure 1) [7–9].

The course of EB may be severe if the condition is due to a lack of key adhesion proteins, for example as a result of loss-of-function mutations in laminin 332 or collagen VII genes. Conversely, isolated amino acid substitutions typically lead to a mild fragility of the skin. The genetic and allelic heterogeneity of EB is due to

Figure 1.
Skin structure. (from private sources MN).

pathological gene variants in 20 different genes. The genes associated with EB encode intracellular, transmembrane, or extracellular proteins that constitute structural components of the cytoskeleton (keratin 5 and 14), extracellular matrix (integrin $\alpha 6 \beta 4$, collagen XVII, laminin 332, collagen VII, $\alpha 3$ integrin, kindlin-1), or intercellular adhesions (desmoplakin, plakophilin, plakoglobin).

3. Epidermolysis Bullosa

The key clinical manifestation of EB is a tendency to develop skin lesions in response to mechanical stimuli, even those of a very low magnitude. The most common lesion types include blisters, milia, pigmented lesions, erosions, epidermal defects, and scars. Other characteristic features of the condition are nail plate changes, ranging from dystrophy to a complete loss. Another common symptom is hair loss and—in severe cases—alopecia. Blisters, erosions, and scars developing near joints may result in contractures and tissue adhesions due to scarring. The lesions that develop on hands and feet (which are most prone to mechanical injuries) may result in pseudosyndactyly. Contractures exacerbate hand and foot deformities, leading to disability (“cocoon hand”, or “mitten hand” deformities).

Figure 2.
Ultrastructural sites of blister formation in major forms of epidermolysis bullosa EB. 1. In intact skin, the ultrastructural regions of the epidermal basement membrane zone consist of basal keratinocytes and the hemidesmosomal plaque, the lamina lucida, the lamina densa, the upper papillary dermis 2. in eb simplex (EBS), blisters arise within the lower portion of basal keratinocytes 3. In junctional EB (JEB) blisters form within the lamina lucida 4. In dystrophic EB (DEB), blisters develop below the lamina densa. Anchoring fibrils are reduced in number in dominant DEB (DDEB) and absent or rudimentary in recessive DEB (RDEB). KRT5, KRT14 and keratin 5 and keratin14 respectively (s. <https://plasticsurgerykey.com/epidermolysis-bullosa/>).

Severe forms of EB additionally involve internal anomalies in the oral cavity, esophagus, trachea, lungs, urinary catheter, or urinary bladder. Intestinal tract erosions, ulcerations, and scarring lead to strictures, which may result in difficulty swallowing (dysphagia) and necessitate a feeding jejunostomy to provide enteral nutrition. Oral manifestations of EB may include the tongue adhering to the floor of the mouth (ankyloglossia); a narrowed oral opening (microstomia); and difficulties in chewing and swallowing, which result in malnourishment, osteopenia, osteoporosis, growth retardation, and eating disorders, leading to cachexia. Oral lesions may cause oral hygiene problems, which leads to caries. Perianal erosions and ulcerations cause severe pain during defecation, which contributes to constipation. Possible ocular manifestations involve marginal blepharitis, eyelash loss, ectropion, adhesions between the palpebral and bulbar conjunctivae (symblepharon), and corneal blistering, which may lead to blindness. Other manifestations include treatment-refractory anemia, iron deficiency, and hypoalbuminemia. Due to chronic ulcerations and an impaired protective function of the epidermis, EB patients may develop skin cancer (squamous cell carcinoma [SCC]) in their thirties or forties (**Figure 2**) [10–15].

3.1 Classification

EB is a result of mutations in approximately 20 genes that encode structural and enzymatic proteins responsible for forming and maintaining the connections between the epidermis and dermis. The most common mutations occur in one of three genes: *KRT5*, *KRT14*, or *TGM5*.

- **KRT5:** The protein encoded by this gene is a member of the keratin gene family. The type II cytokeratins consist of basic or neutral proteins which are arranged in pairs of heterotypic keratin chains coexpressed during differentiation of simple and stratified epithelial tissues. This type II cytokeratin is specifically expressed in the basal layer of the epidermis with family member KRT14. Mutations in these genes have been associated with a complex of diseases termed epidermolysis bullosa simplex. The type II cytokeratins are clustered in a region of chromosome 12q12-q13. (RefSeq, Jul 2008)
- **KRT14:** This gene product, a type I keratin. At least one pseudogene has been identified at 17p12-p11.
- **TGM5:** This gene encodes a member of the transglutaminase family. The encoded protein catalyzes formation of protein cross-links between glutamine and lysine residues, often resulting in stabilization of protein assemblies. This reaction is calcium dependent. Mutations in this gene have been associated with acral peeling skin syndrome (RefSeq, Oct 2009). [<https://www.genecards.org/>]

EB can be classified into three main types, which can be further divided into subtypes. This classification is based on anomalies in various protein molecules and each of the resulting EB types has a different clinical course.

- simple epidermolysis bullosa (SEB) involves epidermal anomalies
- junctional epidermolysis bullosa (JEB) involves basement membrane anomalies

- dystrophic epidermolysis bullosa (DEB) involves anomalies of the dermis

The diagnosis is made based on a thorough microscopic examination of a skin sample. The examination helps determine the exact layer of the skin where tissue separation causes blister formation. There are several layers that can be identified under a microscope in a skin cross-section. If the blisters form within the epidermis, the patient is diagnosed with SEB; if they form within the lamina lucida, the patient is diagnosed with JEB, and if they form just underneath the lamina densa, the patient is diagnosed with DEB (**Table 1**).

Overview of EB classification			
Subtype	Phenotype	Inheritance	Gene affected
<i>EB simplex — Intraepidermal</i>			
EB simplex, localized	Palmoplantar blistering from birth or early infancy, with subsequent keratoderma in affected areas	AD	<i>KRT5 or KRT14</i>
EB simplex, severe	Early generalized blistering at or soon after birth; congenital areas of denuded skin may be present; can be life threatening in first year of life; classically, tense clustered ‘herpetiform’ blisters arise with minimal trauma or spontaneously; development of confluent palmoplantar keratoderma; nail dystrophy common	AD	<i>KRT5 or KRT14</i>
EB simplex, intermediate	Generalized, although less severe blistering than EB simplex, severe	AD	<i>KRT5 or KRT14</i>
EB simplex with mottled pigmentation	Blistering from birth of intermediate severity; additional mottled or reticulate macular pigmentation typically of the neck, upper trunk and acral skin; punctate keratoderma; nail dystrophy may develop	AD	<i>Predominantly KRT5; less frequently KRT14</i>
EB simplex, migratory circinate	Vesicles from birth, on a background of inflammatory migratory circinate erythema that fades to leave post-inflammatory hyperpigmentation; nail dystrophy possible	AD	<i>KRT5</i>
EB simplex, intermediate with cardiomyopathy	Marked erosions in limbs at birth, healing with dyspigmentation and atrophic burn-like scars; keratoderma, nail-thickening and onychogryphosis possible; diffuse alopecia has occasionally been reported; dilated cardiomyopathy develops later in young adulthood	AD	<i>KLHL24</i>
<i>EB simplex, intermediate with PLEC mutations</i>	<i>Autosomal dominant disease is mild with mainly acral blistering; autosomal recessive has an intermediate presentation</i>	AD or AR	<i>PLEC</i>
EB simplex, intermediate with muscular dystrophy	Generalized blistering with variable-onset myopathy including possible cardiomyopathy; focal plantar	AR	<i>PLEC</i>

Overview of EB classification			
Subtype	Phenotype	Inheritance	Gene affected
	keratoderma and nail dystrophy; mucosal involvement is common; upper respiratory tract stenosis has been reported		
EB simplex, severe with pyloric atresia	More severe, widespread generalized blistering or loss of skin at birth with pyloric atresia; early mortality within a few months of birth	AR	<i>PLEC</i>
EB simplex, autosomal recessive, KRT5 or KRT14	Generalized blistering, intermediate or severe; keratin 5 abnormalities tend to have a more severe phenotype; absence of keratin 5 associated with widespread skin disease and early mortality; improvement of blistering with age is not expected	AR	<i>KRT5 or KRT14</i>
EB simplex, localized or intermediate with BP230 deficiency	Early-onset blistering, relatively mild, usually with acral predominance; plantar keratoderma	AR	<i>DST</i>
EB simplex, localized or intermediate with exophilin 5 deficiency	Generalized intermittent blistering and skin fragility; mild mottled pigmentation may be evident	AR	<i>EXPH5</i>
EB simplex, localized with nephropathy (CD151 deficiency)	Early blistering, with pretibial predominance; poikiloderma may be seen; early alopecia; extracutaneous involvement manifests as oesophageal webbing and nephropathy	AR	<i>CD151</i>
Junctional EB, severe	Blistering may be mild at birth and localized to periungual, buttock and elbow regions; overgranulation develops, particularly on orofacial and periungual regions, with development of bulbous nail folds; alopecia is common; dental enamel defects are usual; a hoarse cry is often a feature; usually fatal within the first 2 years of life	AR	<i>LAMA3, LAMB3 and LAMC2</i>
Junctional EB, intermediate	Less severe than above, with a reduced tendency to develop exuberant granulation tissue; elevated risk of SCC in adulthood	AR	<i>LAMA3, LAMB3, LAMC2 and COL17A</i>
Junctional EB with pyloric atresia	Extensive areas of skin loss seen at birth with severe cutaneous fragility; early-onset pyloric atresia, a frequent cause of early mortality, within days or weeks of birth; duodenal and anal atresia may also feature; milder non-lethal variants often show genitourinary involvement	AR	<i>ITGA6 and ITG84</i>
Junctional EB, localized	Limited cutaneous fragility, often acral; variable nail and dental defects; normal hair	AR	<i>LAMA3, LAMB3, LAMC2, COL17A1, ITGB4 and ITGA3</i>

Overview of EB classification			
Subtype	Phenotype	Inheritance	Gene affected
Junctional EB, inversa	Flexural blistering from birth; dental abnormalities and nail loss	AR	<i>LAMA3, LAMB3 and LAMC2</i>
Junctional EB, late onset	Onset in childhood, with often acral fragility; skin fragility is progressive and loss of dermatoglyphs may be seen owing to scarring; variable dental enamel and nail defects	AR	<i>COL17A1</i>
Junctional EB–laryngo-onycho-cutaneous (LOC) syndrome	Skin fragility from birth with marked exuberant granulation tissue (greater than that in junctional EB, severe), particularly on face and neck; nail dystrophy and loss with granulation tissue of nail beds; laryngeal granulation can lead to respiratory compromise and death; conjunctival and eyelid granulation with consequent symblepharon, scarring and visual loss	AR	<i>LAMA3</i>
Junctional EB with interstitial lung disease and nephrotic syndrome	Variable degree of cutaneous involvement; fatality in early childhood is common; nail dystrophy possible; hair loss may occur	AR	<i>IGTA3</i>
Dystrophic EB — sublamina densa			
Intermediate DDEB ₁	Generalized skin fragility, scarring and milia presenting from birth or early infancy, with prominence over acral sites, elbows and knees; involvement of the mucous membranes may lead to microstomia, ankyloglossia and oesophageal stenosis, although less commonly than in severe RDEB	AD	<i>COL7A1</i>
Localized DDEB ₁	Predominantly acral blistering, scarring and milia seen from birth or early infancy; occasional nails-only presentation, with progressive dystrophy and eventual nail loss; rarely, cutaneous features may predominate over pretibial skin alone (and can present as late-onset disease)	AD	<i>COL7A1</i>
DDEB, pruriginosa ₁	Profoundly pruritic linear cords of papules associated with fragility, scarring and milia on the shins, and occasionally progressing to arms; may present in childhood or adulthood; nail dystrophy is usual	AD	<i>COL7A1</i>
DDEB, self-improving _{1,2}	Blistering evident at or shortly after birth, usually on extremities where there may be aplasia cutis, whilst scarring and milia may occur; spontaneous resolution of cutaneous fragility within the first 2 years of life	AD	<i>COL7A1</i>

Overview of EB classification			
Subtype	Phenotype	Inheritance	Gene affected
Intermediate RDEB ₃	Phenotype similar to that of intermediate DDEB, although greater severity with flexion contractures, limited digital fusion and occasional striate keratoderma	AR	COL7A1
Severe RDEB ₃	Widespread blistering from birth, with extensive scarring and development of microstomia, ankyloglossia, oesophageal stenosis, flexion contractures of limbs and pseudosyndactyly; nails are often lost early in disease course; high risk of cutaneous SCC arising in EB wounds.	AR	COL7A1
RDEB, inversa ₃	Generalized blistering from birth, of intermediate severity; subsequently, fragility tends to be displayed on flexural sites	AR	COL7A1
RDEB, localized ₃	Skin fragility and blistering typically at birth or neonatal period, limited to acral sites such as hands and feet, or occasionally only to pretibial skin, where it may manifest as late-onset disease during adulthood; nail dystrophy and loss usual	AR	COL7A1
RDEB, pruriginosa ₃	As for DDEB, pruriginosa	AR	COL7A1
RDEB, self-improving ₃	As for DDEB, self-improving	AR	COL7A1
DEB, severe ₄	Clinically indistinguishable from severe RDEB, with severe mucocutaneous fragility from birth	Dominant and recessive compound heterozygosity	COL7A1
Kindler EB — variable and mixed			
None	Generalized blistering and variable photosensitivity from birth or early childhood, with mucosal fragility; blistering gives way to progressive poikiloderma, initially most marked over dorsal hands and neck; confluent palmoplantar keratoderma and adermatoglyphia may occur; gingivitis and dental disease is a feature; oesophageal narrowing and colitis has been reported; mucocutaneous SCC has been reported, with poor prognosis	AR	FERMT1
AD, autosomal dominant; AR, autosomal recessive; DDEB, dominant dystrophic epidermolysis bullosa; DEB; dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; EM, electron microscopy; ER, endoplasmic reticulum; RDEB, recessive dystrophic epidermolysis bullosa; SCC, squamous cell carcinoma. 1Major type is DDEB. 2Previously known as transient bullous dermolysis of the newborn baby. 3Major type is RDEB. 4Major type is DEB (dominant and recessive compound heterozygosity). Adapted from consensus guidelines3.			

Table 1.
Overview of EB classification [16].

3.2 Heredity

SEB primarily shows an autosomal dominant pattern of inheritance, with the most common mutations in genes *KRT5* and *KRT14*. Autosomal recessive inheritance is less common and caused by mutations in genes *KRT14*, *ITGA6*, *ITGB4* (*this gene encodes a member of the integrin alpha chain family of proteins. Integrins are heterodimeric integral membrane proteins composed of an alpha chain and a beta chain that function in cell surface adhesion and signaling. The encoded preproprotein is proteolytically processed to generate light and heavy chains that comprise the alpha 6 subunit. This subunit may associate with a beta 1 or beta 4 subunit to form an integrin that interacts with extracellular matrix proteins including members of the laminin family. The alpha 6 beta 4 integrin may promote tumorigenesis, while the alpha 6 beta 1 integrin may negatively regulate erbB2/HER2 signaling. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2015]*).

DSP (This gene encodes a protein that anchors intermediate filaments to desmosomal plaques and forms an obligate component of functional desmosomes), or *PKP1* (*Plakophilin proteins contain numerous armadillo repeats, localize to cell desmosomes and nuclei, and participate in linking cadherins to intermediate filaments in the cytoskeleton. This protein may be involved in molecular recruitment and stabilization during desmosome formation*). SEB caused by a *PLEC1* (*Plakins, with their multi-domain structure and enormous size, not only play crucial roles in maintaining cell and tissue integrity and orchestrating dynamic changes in cytoarchitecture and cell shape, but also serve as scaffolding platforms for the assembly, positioning, and regulation of signaling complexes (reviewed in PMID: 9701547, 11854008, and 17499243)*) mutation may show an autosomal recessive or autosomal dominant pattern of inheritance.

JEB is primarily caused by mutations in genes *LAMB3*, *LAMC2*, *LAMA3* (*this gene is a laminin that belongs to a family of basement membrane proteins*), *COL17A1* (*This gene encodes collagen XVII is a structural component of hemidesmosomes, multiprotein complexes at the dermal-epidermal basement membrane zone that mediate adhesion of keratinocytes to the underlying membrane*), *ITGA6* or *ITGB4* and is characterized by autosomal recessive inheritance. A recent study (2009) showed a possible autosomal dominant inheritance pattern in the case of a mutated *COL17A1*.

DEB is caused by mutations in only one gene, *COLA1*. The location and type of mutation determine the inheritance pattern (autosomal recessive or dominant).

3.3 SEB subtypes

- Koebner type: mutated genes for keratin 5 and 4 (*KRT5*, *KRT4*); lesions are often present at birth or in infancy; characteristic features are hyperkeratotic lesions, hemorrhagic bullae, and erosions.
- Dowling-Meara type: mutated *KRT14* and *KRT5* genes, which encode keratin 14 and 5, respectively; autosomal dominant inheritance; lesions are located primarily on the feet, less commonly in other locations; a relatively mild course.
- Weber-Cockayne type: associated with mutated *KRT5* (region 12q13.13) and *KRT14* (region 17q21.2) genes; characterized by a severe course and herpetiform blisters. Poorly healing blisters and erosions lead to scarring and contractures.
- SEB with muscular dystrophy: a mutated plectin-encoding *PLEC1* gene.

3.4 JEB subtypes

- JEB with pyloric atresia: this rare type of EB results from mutated *ITGB4* and *ITGA6* genes that encode $\alpha 6 \beta 4$ integrin. Skin lesions are accompanied by esophageal, pyloric, and/or duodenal atresia. Enamel hypoplasia is common.
- Herlitz type: mutations in the *LAMA3*, *LAMB3*, and *LAMC2* genes, which encode the polypeptide subunits of laminin 5 (α -3, β -3, and γ -2, respectively). Fatal type of EB, characterized by blisters and erosions over the entire body, which causes multiple infections that may lead to sepsis, loss of proteins (malnourishment), scarring, contractures, defects of large areas of skin.
- Non-Herlitz type: mutations in genes *COL17A1*, *LAMB3*, *LAMC2*, or *LAMA3* encoding laminin 5 and collagen XVII.

3.5 DEB subtypes

- Hallopeau-Siemens type: a mutated *COL7A1* gene, which encodes collagen VII. This type of EB is characterized by scarring, erosions, pseudosyndactyly of the hands and feet; nail plate involvement, esophageal atresia, and corneal ulcers are common.
- non-Hallopeau-Siemens type: mutated *COL7A1* gene, encoding collagen VII.
- Cockayne-Tourelle type: autosomal dominant inheritance; mutated *COL7A1* (collagen VII); skin lesion on the limbs.
- Pasini type: possible nail plate involvement; oral and mucosal lesions.

3.6 Diagnostic investigations

A primary diagnosis of EB is based on the clinical presentation. The definitive diagnosis is established after skin samples are examined via immunofluorescence antigen mapping and transmission microscopy.

Diagnosis is confirmed via genetic analysis that determines the type of mutation.

3.7 Differential diagnoses

The differential diagnoses should include congenital dermatoses, herpes simplex virus infections, epidermolytic hyperkeratosis with erosions and blisters, staphylococcal scalded skin syndrome, bullous pemphigoid, neonatal pemphigoid, and gestational pemphigoid.

3.8 Treatment

Management is primarily symptomatic. Surgical treatment mainly involves skin grafting. Importantly, the use of autologous skin grafts is ineffective due to poor healing and chronic wound formation at the donor sites. Plastic surgery procedures play an important role in repairing contractures and pseudosyndactyly of the hands and feet. In the case of esophageal, pyloric or duodenal atresia, various surgical procedures are used to overcome the effects of gastrointestinal strictures (e.g. feeding jejunostomy, endoscopic balloon dilatation).

EB management involves primarily local care of chronic wounds, ulcers, erosions, and blisters. Treatment challenges involve frequent bacterial infections, due to their chronic character, and factors that inhibit healing, such as malnutrition, anemia, itching, or repetitive wound irritation with regular dressing changes, all of which disturb epithelialization. Moreover, wounds may cause severe pain, exacerbated by regular, frequent dressing changes. Importantly, the condition requires life-long care, with the cost of monthly treatment often exceeding several hundred dollars. Therefore, the process of selecting the optimal dressing should include the following parameters: the price, availability, effectiveness, and safety. Other important complementary treatments include physiotherapy, genetic counselling, aggressive treatment of infections, nutritional supplementation, and skin cancer monitoring [17–31].

Despite the enormous advances in our understanding of molecular genetics and EB physiopathology that have taken place over the last several decades, a definitive cure is yet to be discovered. There are many ongoing studies aiming to develop an effective treatment. These studies focus on several potential lines of treatment, including disease modifying treatments to diminish disease severity. Gene therapies, bone marrow transplants, and tissue engineering are receiving the most attention.

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells. They offer groundbreaking new opportunities for the treatment of disease and injury. ATMPs can be classified into three main types: gene therapy medicines, somatic-cell therapy medicines, tissue-engineered medicines. In addition, some ATMPs may contain one or more medical devices as an integral part of the medicine, which are referred to as combined ATMPs. An example of this is cells embedded in a biodegradable matrix or scaffold.

Gene therapy involves cultures of keratinocytes (obtained from patients with recessive DEB [RDEB]) that have been transduced with a retroviral vector containing full-length cDNA of the *COL7A1* gene (for collagen VII). These cultures are, subsequently, placed onto the patient's wounds in the form of epidermal grafts [32]. Treatment efficacy and collagen VII expression were demonstrated; however, the response lasted up to 12 months. Nonetheless this therapy is safe. One disadvantage of this method is the fact that it can be used in limited areas (at chronic wound sites). This method has been also used in a patient with JEB, in whom the placement of genetically corrected keratinocytes onto chronic wound sites led to successful wound healing. Based on the available reports, gene therapies are promising treatment modalities with a potential therapeutic effect in genodermatoses.

Bone marrow transplant (BMT) and allogeneic stem cell transplantation (ASCT) are other very promising treatment strategies. In 2010, Wagner et al. performed ASCT in children with RDEB. Although the patients were not completely cured, their skin blisters were reduced, and skin regeneration was accelerated. BMT in RDEB patients has been reported to improve the clinical status, despite the lack of collagen VII growth in the skin. BMT is an experimental therapy, which is used as part of clinical studies, and currently is not an approved treatment. The risk of death and the uncertain degree and mechanism of the clinical response should be viewed in light of the results of the most recent translational research in RDEB, which reports ASCT to be currently the only therapeutic approach that shows systemic effects in what essentially is a systemic disease. There is a clear need for reports presenting data from extensive clinical studies to establish guidelines and warnings for the use of ASCT in EB treatment.

As pluripotent cells, MSCs have a potential to differentiate into many different types of skin cells, including keratinocytes, endothelial cells, and monocytes. Due to

their immunomodulatory and anti-inflammatory effects, MSCs may play a significant role in wound healing and tissue regeneration. Moreover, MSCs do not trigger an immune response in the recipient, hence there is no need to match the donor's and recipient's human leukocyte antigen (HLA) types [33]. Due to their multi-directional differentiation potential, MSCs have been shown to regenerate collagen VII, which has a beneficial effect on the healing of wounds (including chronic wounds) and improves skin stability. These effects were observed with intradermal administration, which—apart from presenting fewer challenges—does not require as many MSCs as intravenous administration. Most studies have focused on bone marrow-derived MSCs (BM-MSCs). However, their harvesting from the bone marrow is a relatively invasive procedure. Moreover, the multipotent differentiation potential of BM-MSCs diminishes with age. Therefore, MSCs are currently obtained from alternative sources, such as the umbilical cord [34], which can provide up to a billion cells in 30 days, obtained non-invasively. The umbilical cord consists of umbilical vessels surrounded by a connective tissue, referred to as Wharton jelly (WJ). WJ-derived MSCs have a higher proliferative potential and are more homogeneous than those derived from the bone marrow. WJ-MSCs are similar to BM-MSCs in their fibroblast-like phenotype, non-hematopoietic surface markers [35], low immunogenicity [36], multipotent plasticity, and the expression of CD90, CD73, CD105 markers [37]. Moreover, WJ-MSCs seem to have more pronounced pro-angiogenic properties than BM-MSCs; they promote neovascularization and perfusion by releasing paracrine factors and by playing the role of perivascular precursor cells [38]. WJ-MSCs are a highly efficient source of young, non-carcinogenic, and non-immunomodulatory cells [39]. All these properties and the fact that WJ-MSCs are easily available make these cells a promising strategy for treating wounds in EB patients.

Sebastiano et al. propose an innovating cell therapy for RDEB treatment, by developing a state of the art protocol of genetically repaired induced pluripotent stem cells (iPSCs) as to generate sheets of normal skin tissue to treat affected skin areas [40]. Moreover, as numerous stem cells are needed in order to cover the affected surface area, authors outline the necessity for creating personalized iPSCs banks as to provide a constant long-term iPSCs source. Generally, human iPSCs can be generated by reprogramming differentiated somatic cells into pluripotent embryonic stem cells (ESCs) capable of differentiating into ectoderm, mesoderm or endoderm cells. Reprogramming involves the introduction of a known set of genes into the somatic cells, using integrating viral and non-integrating non-viral methods. Following successful reprogramming, somatic cells will express genes and surface proteins similar to ESCs in vitro and will be able to differentiate into any of the three embryonic germ layers.

Tissue engineering: Not unlike patients with extensive burns, patients with EB do not qualify for autologous skin grafts. One solution available to these patients involves the use of allogeneic grafts, which serve to temporarily cover the wound (after 7 days the graft is rejected by the recipient; [41]). Therefore, tissue engineering seems to be a promising solution, as it helps create biopolymer scaffolds to cover the wounds. The idea is to create skin substitutes, which can then be seeded with keratinocytes, fibroblasts, or stem cells. Such polymer materials constitute a micro-environment and provide adequate scaffolds for cell colonization and epithelial cell migration during wound epithelialization. The multi-disciplinary nature of tissue engineering has helped develop many bioengineered skin substitutes, with potential applications as a suitable dressing for treating refractory wounds, such as those in EB patients. The field of tissue engineering has been rapidly transferring from the realm of basic research to commercial applications. There are many in

vitro-generated skin substitutes. They are available in various forms, which include epidermal, dermal, and dermo-epidermal analogs or complex skin analogs, and can be composed of cellular or acellular scaffolds [42–51].

High-quality, safe skin analogs should be cost-effective, biocompatible, biodegradable, and noncarcinogenic, carry no risk of infectious disease transfer, and provoke no activation of the recipient's immune system. Despite a whole spectrum of bioengineered products currently available on the market, there are scarcely any that meet all the requirements of natural skin. Natural skin is composed of the epidermis, dermis, and subcutaneous tissue. It contains appendages, such as sweat glands, nails, and hair, as well as nerve endings and blood vessels. Additionally, natural skin protects the body against the external environment via its thermoregulatory function and its role in maintaining water–electrolyte balance. It also facilitates the perception of pain, heat, and touch; manufactures vitamin D; and shields the body against ultraviolet radiation by the means of melanin-producing melanocytes responsible for skin pigmentation [52, 53]. Due to the wide range of functions performed by human skin, creating its analog is a challenge for tissue engineers.

The first product that has transferred the potential of bioengineering into real-life EB applications is an autologous cultured epidermal substitute (CES). The pioneering study by Rheinwald and Green demonstrated that epidermal keratinocytes from a single-cell suspension can be cultured in the form of sheets, and the resulting multi-layered sheets have proven to be very effective in the treatment of burns and wounds in EB patients. There are many commercially available skin substitutes composed of both epidermal and dermal components. Bell et al. developed a cultured skin substitute (CSS) (an equivalent of living skin) composed of keratinocytes and fibroblasts in a collagen gel. Boyce and Hansbrough developed a CSS composed of a collagen-glycosaminoglycan composite scaffold populated with keratinocytes and fibroblasts. Kuroyanagi et al. developed another CSS, composed of a spongy collagen matrix with keratinocytes and fibroblasts. Such two-layered CSSs are intended to permanently cover full-thickness skin defects. There have been studies on wound healing in EB with the use of OrCel™, Biobrane, and Apligraf dressings. OrCel™ is a bilayer dressing composed of a bovine-collagen I matrix populated with neonatal foreskin keratinocytes and fibroblasts [54–56]. Despite the fact that OrCel™ exhibits beneficial wound healing properties in RDEB patients—via cytokines and growth factors, such as tumor growth factor alpha (TGF α), fibroblast growth factor 1 (FGF-1), and keratinocyte growth factor 1 (KGF-1)—its bovine collagen component increases the risk of graft rejection and transfer of diseases to the donor [57]. Another bilayer skin substitute is Biobrane, which is composed of a 3D nylon fiber scaffold and an ultrathin semipermeable epidermis-mimicking silicone layer that controls fluid loss [56–59]. The nylon fibers are surrounded by porcine collagen type 1. Jutkiewicz and Noszczyk [60] were the first to report the use of Biobrane in the postoperative hand care in a group of RDEB patients. Apligraf is another bilayer skin substitute composed of dermal and epidermal analogs. The epidermal and dermal layers contain cultured keratinocytes and neonatal foreskin fibroblasts. The dermal layer additionally contains bovine collagen type 1, which facilitates cell growth and differentiation. Apligraf has a short life span, and its use is associated with high costs [57]. Nonetheless, this dressing was reported to be effective in treating EB wounds [61, 62].

Safe and Effective Therapy in the Light of Clinical Trials - New Approach to Treatment by Innovative Method (BIOOPA-ATMP) grant no. STRATERMED2/269807/14/NCBR/2015.

Alternative promising product for the treatment of chronic wounds that occur in EB and other genodermatoses, as well as in burns, is an allogeneic, acellular human skin equivalent sterilized with radiation, and seeded with Wharton's jelly-derived mesenchymal stem cells- WJ-MSCs about the acronym in polish BIOOPA (biological dressing) is an advanced therapy medicinal product composed of a decellularized matrix of the superficial layers of cadaveric human skin (10 cm × 10 cm). Acellular dermal matrix (ADM) is a Chemically/enzymatically processed allograft. This processing removes all epidermal and dermal cells while preserving the molecular and physiological structure of collagen fibers. The scaffold is sterilized via radiation and then seeded with 30 million WJ-MSCs. As a result of decellularization, this skin substitute does not induce an immune response in the recipient and poses a lower risk of transmitting any diseases. In order to assess the safety and efficacy of the BIOOPA dressing, the relevant study was conducted in two stages. During the first stage, in vitro experiments showed BIOOPA viability. All examination techniques demonstrated graft infiltration by host cells and neovascularization of the biological dressing. Moreover, BIOOPA is characterized by low immunogenicity, which was confirmed in histopathology examinations and in vitro T-cell proliferation tests. The second stage of the study was conducted in a group of qualified volunteers with EB and approved by an ethics committee. The 6-month follow-up indicates the safety and efficacy of the BIOOPA dressing, with no infections or necrosis at the graft implantation site observed over the follow-up period. The subjects reported decreased pain and improved quality of life **Figures 3–8** [63, 64].

Figure 3.
BIOOPA- Advanced Therapy Medicinal Product (ATMP) acellular human skin equivalent sterilized with ultraviolet radiation.

Figure 4.
Day 0, procedure: chronic wound in the knee area covered with prepared graft in the 20-years old patient with EB (allogenic,acellular, human skin equivalent).

Figure 5.
Bioopa dressing: The scaffold is seeded with 30 million WJ-MSCs in 5 mL of a 5% human albumin solution covered with chlorhexidine-impregnated dressings and collagen gel. The same 20-years old patient with EB (chronic wound in the knee area).

Figure 6.
Results after 30-day follow-up in this patient with EB: All examination techniques revealed host-cell infiltration and neovascularization of the biological dressing. They are characterized by low immunogenicity, as confirmed by histopathology and in vitro T-cell proliferation assays.

Figure 7.
Hematoxylin and eosin stain of scaffold populated with mesenchymal cells from Wharton's jelly. After 72 hours of culture mesenchymal stem cells create a multilayer structure on the scaffold resembling human epithelium.

Figure 8.
Laser scanning confocal microscopic study using second-harmonic generation technique reveals the structure of collagen fibrils in acellular dermal matrix after decellularization and X-ray radiation 35 kG (Bar 1/4 50 μ m).

4. Conclusion

To date, there is no causative treatment of EB, despite multiple ongoing studies involving gene therapy and bone marrow transplantation. The standard of EB management still involves symptomatic conservative treatment. There is immense hope in therapies with the use of stem cells of various origins (bone marrow, umbilical cord, etc.). Advanced applications of various types of cells (embryonic, prenatal, and adult stem cells, endothelial cells, and melanocytes) and the rapid development of biomedical engineering, which contributes to refining biocompatible materials, such as collagen, hyaluronic acid, elastin, polylactic acid (PLA), poly lactic-co-glycolic acid (PLGA), and polyethylene glycol (PEG), bring hope of effective treatment for chronic wounds of various origin. The most recent developments allow for the manufacture of progressively better skin substitutes, which in the future may exhibit the fundamental characteristics of natural human skin (including sweat glands and hair follicles), more homogeneous pigmentation, and allow for the healing of scars [65]. Thus, further studies and efforts are crucial for creating skin substitutes truly mimicking natural skin. Despite the enormous progress in the treatment of EB, the current treatments are clearly not a definitive cure for this debilitating disease, and the risk associated with some of these procedures must be weighed against their potential benefits. Effective treatment of this, currently incurable, group of diseases requires advanced and innovative strategies with an improved safety profile, such as the ones that are currently being developed [66–77].

The BIOOPA dressing is easily available, safe, and relatively inexpensive, all of which make it a promising therapy for EB-associated wounds. Preliminary results of the BIOOPA study indicate the dressing to be safe and effective to improve the quality of life in study subjects. Currently BIOOPA is evaluated as part of a phase

I/II clinical study during the second year of observation. Our preliminary results of clinical trial strongly suggest, that our innovative dressing is a promising strategy and a tool for clinicians in the search for new opportunities of treatment for this rare condition.

Acknowledgements

This is unpublished Original Article. The study has been approved by an ethics committee (KB/ 2019 14/01/2019; KB/177/2015). Supported by The National Center for Research and Development in terms of the project “Prevention Practices and Treatment of Civilizations Diseases-STRATEGMED” (grant no. STRATERMED2/269807/14/NCBR/2015).

EudraCT Number: 2018-003890-91.

Protocol number BIOOPA DBL.474.317.2020.

IntechOpen

IntechOpen

Author details

Magdalena Nita¹, Jacek Pliszczynski¹, Andrzej Eljaszewicz², Marcin Moniuszko^{2,3}, Tomasz Oldak⁴, Katarzyna Woźniak⁵, Sławomir Majewski⁶, Cezary Kowalewski⁵, Artur Kamiński⁷, Dariusz Śladowski⁷, Zbigniew Zimek⁸, Maciej Kosieradzki¹ and Piotr Fiedor^{1*}

1 Department of General and Transplantation Surgery at Medical University of Warsaw, Infant Jesus Clinical Hospital, Warsaw, Poland

2 Department of Regenerative Medicine and Immune Regulation at Medical University of Białystok, Białystok, Poland

3 Department of Allergology and Internal Medicine at Medical University of Białystok, Białystok, Poland

4 PBKM, Polish Stem Cells Bank, Warsaw, Poland

5 Department of Dermatology and Immunodermatology at Medical University of Warsaw, Warsaw, Poland

6 Department of Dermatology and Venereology at Medical University of Warsaw, Warsaw, Poland

7 Department of Transplantology and Central Tissue Bank at Medical University of Warsaw, Warsaw, Poland

8 Institute of Nuclear Chemistry and Technology, Warsaw, Poland

*Address all correspondence to: piotrfiedor@wp.pl

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Epidermolysis bullosa – pęcherzowe oddzielanie się naskórka. Etiopatogeneza, dziedziczenie, diagnostyka, leczenie. Katarzyna Wertheim-Tysarowska, Cezary Kowalewski, Katarzyna Woźniak, Jerzy Bal, Wyd. Continuo;
- [2] Genetyczne uwarunkowanie choroby skóry-przegląd wybranych genodermatoz, Medycyna Wieku Rozwojowego, 2012, XVI, 3, © IMiD, Wydawnictwo Aluna.
- [3] Tantcheva-Poór I, Oji V, Has C (2016) A multistep approach to the diagnosis of rare genodermatoses. *J Dtsch Dermatol Ges* 14: 969-986 2. www.genodermatoses-network.org
- [4] Schaffer JV (2016) Practice and Educational Gaps in Genodermatoses. *Dermatol Clin* 34: 303-310
- [5] Smith FJD, McLean WHI (2011) Genodermatoses: Inherited Disease of the Skin, W: Murphy MJ (red) Molecular Diagnostics in Dermatology and Dermatopathology, Estados Unidos : Humana Press, str 379- 410
- [6] Jabłońska S, Majewski S (2006) Budowa i czynności skóry W: Jabłońska S, Majewski S (red) Choroby skóry i choroby przenoszone drogą płciową PZWL, str 15-28
- [7] Tsuruta D, Hashimoto T, Hamill KJ, Jones JC (2011) Hemidesmosomes and focal contact proteins: functions and cross-talk in keratinocytes, bullous diseases and wound healing. *J Dermatol Sci* 62: 1-7
- [8] Green KJ, Simpson CL (2007) Desmosomes: new perspectives on a classic. *J Invest Dermatol* 127: 2499-2515
- [9] Coulombe PA, Kerns ML, Fuchs E (2009) Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. *J Clin Invest* 119: 1784-1793
- [10] Fine JD, General cutaneous manifestations (2009), W: Fine JD, Hintner H (red) Life with Epidermolysis Bullosa, SpringerWienNewYork, str 99-106
- [11] Fine JD, Johnson LB, Weiner M, Li KP, Suchindran C (2009) Epidermolysis bullosa and the risk of life-threatening cancers: the National EB Registry experience, 1986-2006 *J Am Acad Dermatol* 60: 203-211
- [12] Fine JD, Other internal complications (2009), W: Fine JD, Hintner H (red) Life with Epidermolysis Bullosa, SpringerWienNewYork; str 185-196
- [13] Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, Heagerty A, Hintner H, Hovnanian A, Jonkman MF, Leigh I, Marinkovich MP, Martinez AE, McGrath JA, Mellerio JE, Moss C, Murrell DF, Shimizu H, Uitto J, Woodley D, Zambruno G (2014). Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. *J Am Acad Dermatol* 70: 1103-1126 14.
- [14] Has C (2018) Advances in understanding the molecular basis of skin fragility. *F1000Res* 7: 279
- [15] Wertheim-Tysarowska K, Ołdak M, Giza A, Kutkowska-Kaźmierczak A, Sota J, Przybylska D, Woźniak K, Śniegórska D, Niepokój K, Sobczyńska-Tomaszewska A, Rygiel AM, Płoski R, Bal J, Kowalewski C (2016) Novel sporadic and recurrent mutations in KRT5 and KRT14 genes in Polish epidermolysis bullosa simplex patients: further insights into epidemiology and genotype-phenotype correlation. *J Appl Genet* 57: 175-81
- [16] Bardhan, A., Bruckner-Tuderman, L., Chapple, I. L. C., Fine, J.-D., Harper, N., Has, C., ... Heagerty, A. H. (2020).

Epidermolysis bullosa. *Nature Reviews Disease Primers*, 6(1). doi:10.1038/s41572-020-0210-0

[17] Blount AL, Foster S, Rapp DA, et al. The use of bioelectric dressings in skin graft harvest sites: a prospective case series. *J Burn Care Res*. 2012;33:354–357.

[18] Goertz O, Abels C, Knie U, et al. Clinical safety and efficacy of a novel thermoreversible polyhexanide-preserved wound covering gel. *Eur Surg Res*. 2010;44:96–101.

[19] Hasatsri S, Angspatt A, Aramwit P. Randomized clinical trial of the innovative bilayered wound dressing made of silk and gelatin: safety and efficacy tests using a split-thickness skin graft model. *Evid Based Complement Alternat Med*. 2015;2015:206871.

[20] Metelmann HR, Brandner J, Schumann H, et al. Accelerating the aesthetic benefit of wound healing by triterpene. *J Craniomaxillofac Surg*. 2012;40:e150–e154.

[21] Potocká D, Kevická D, Koller J. Clinical trial of the temporary biosynthetic dermal skin substitute based on a collagen and hyaluronic acid named Coladerm H/HM, first part. *Acta Chir Plast*. 2012;54:31–38.

[22] Baser NT, Yuksel A, Unzile B, et al. High-valve vapor-permeable film dressing versus fine mesh gauze dressing on skin graft donor areas in diabetic patients: a prospective randomized controlled trial. *European J Plast Surg*. 2008;31:219–228.

[23] Siritientong T, Angspatt A, Ratanavaraporn J, et al. Clinical potential of a silk sericin-releasing bioactive wound dressing for the treatment of split-thickness skin graft donor sites. *Pharm Res*. 2014;31:104–116.

[24] Junker JP, Kamel RA, Caterson EJ, et al. Clinical impact upon wound

healing and inflammation in moist, wet, and dry environments. *Adv Wound Care (New Rochelle)*. 2013;2:348–356.

[25] Rakel BA, Bermel MA, Abbott LI, et al. Split-thickness skin graft donor site care: a quantitative synthesis of the research. *Appl Nurs Res*. 1998;11:174–182.

[26] Wiechula R. The use of moist wound-healing dressings in the management of split-thickness skin graft donor sites: a systematic review. *Int J Nurs Pract*. 2003;9:S9–17.

[27] Moher D, Shamseer L, Clarke M, et al.; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4:1.

[28] Fine JD. Musculoskeletal deformities. In: Fine JD, Hintner H: *Life with epidermolysis bullosa (EB): Etiology, diagnosis, multidisciplinary patient care, and therapy*. Wien, New York: Springer, 2009: 178–84.

[29] Rees TD, Swinyard A. Rehabilitative digital surgery in epidermolysis bullosa. *Plast Reconstr Surg* 1967;40(2):169–74.

[30] Vozdivzhensky SI, Albanova VI. Surgical treatment of contracture and syndactyly of children with epidermolysis bullosa. *Br J Plast Surg* 1993; 46:314–6.

[31] Ciccarelli AO, Rothaus KO, Carter DM, et al. Plastic and reconstructive surgery in epidermolysis bullosa: clinical experience with 110 procedures in 25 patients. *Ann Plast Surg* 1995;35:254–61.

[32] Siprashvili, Z., Nguyen, N. T., Gorell, E. S., Loutit, K., Khuu, P., Furukawa, L. K., ... Marinkovich, M. P. (2016). Safety and Wound Outcomes Following Genetically Corrected Autologous Epidermal Grafts in Patients With Recessive Dystrophic

- Epidermolysis Bullosa. JAMA, 316(17), 1808. doi:10.1001/jama.2016.15588
- [33] Kühl, T., Mezger, M., Hausser, I., Guey, L. T., Handgretinger, R., Bruckner-Tuderman, L., & Nyström, A. (2016). Collagen VII Half-Life at the Dermal-Epidermal Junction Zone: Implications for Mechanisms and Therapy of Genodermatoses. *Journal of Investigative Dermatology*, 136(6), 1116–1123. doi:10.1016/j.jid.2016.02.002
- [34] Nekanti, U., Rao, V. B., Bahirvani, A. G., Jan, M., Totey, S., & Ta, M. (2010). Long-Term Expansion and Pluripotent Marker Array Analysis of Wharton's Jelly-Derived Mesenchymal Stem Cells. *Stem Cells and Development*, 19(1), 117–130. doi:10.1089/scd.2009.0177
- [35] Karahuseyinoglu, S., Cinar, O., Kilic, E., Kara, F., Akay, G. G., Demiralp, D. Ö., ... Can, A. (2007). Biology of Stem Cells in Human Umbilical Cord Stroma: In Situ and In Vitro Surveys. *Stem Cells*, 25(2), 319–331. doi:10.1634/stemcells.2006-0286
- [36] Tipnis, S., Viswanathan, C., & Majumdar, A. S. (2010). Immunosuppressive properties of human umbilical cord-derived mesenchymal stem cells: role of B7-H1 and IDO. *Immunology and Cell Biology*, 88(8), 795–806. doi:10.1038/icb.2010.47
- [37] Shen, Y., Zhu, Y.-M., Fan, X., Shi, J.-Y., Wang, Q.-R., Yan, X.-J., ... Chen, S.-J. (2011). Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. *Blood*, 118(20), 5593–5603. doi:10.1182/blood-2011-03-343988
- [38] Choi, S.-E., & Kemper, J. K. (2013). Regulation of SIRT1 by MicroRNAs. *Molecules and Cells*, 36(5), 385–392. doi:10.1007/s10059-013-0297-1.
- [39] Nekanti, U., Mohanty, L., Venugopal, P., Balasubramanian, S., Totey, S., & Ta, M. (2010). Optimization and scale-up of Wharton's jelly-derived mesenchymal stem cells for clinical applications. *Stem Cell Research*, 5(3), 244–254. doi:10.1016/j.scr.2010.08.005
- [40] Koster MI (2009) Making an epidermis. *Ann N Y Acad Sci* 1170: 7-10
- [41] Chen, H., Wang, W., Xie, H., Xu, X., Wu, J., Jiang, Z., ... Zheng, S. (2009). A pathogenic role of IL-17 at the early stage of corneal allograft rejection. *Transplant Immunology*, 21(3), 155–161. doi:10.1016/j.trim.2009.03.006
- [42] Gough MJ, Page RE. Surgical correction of the hand in epidermolysis bullosa dystrophica. *Hand* 1979; 11(1): 55–8.
- [43] Eastwood DS. Autografting in the treatment of squamous cell carcinoma in epidermolysis bullosa dystrophica. *Plast Reconstr Surg* 1972;49(1):93–5.
- [44] Eisenberg M, Llewelyn D. Surgical management of hands in children with recessive dystrophic epidermolysis bullosa: use of allogeneic composite cultured skin grafts. *Br J Plast Surg* 19 Damanhuri M, Boyle J, Enoch S (2011) Advances in tissue-engineered skin substitutes. *Wounds Int* 2 (1):27–34
- [45] Nicholas MN, Yeung J (2017a) Current status and future of skin substitutes for chronic wound healing. *J Cutan Med Surg* 21(1):23–30
- [46] Nicholas MN, Jeschke MG, Amini-Nik S (2016a) Cellularized bilayer pullulan-gelatin hydrogel for skin regeneration. *Tissue Eng A* 22(9–10): 754–764
- [47] Sheikholeslam M, Wright ME, Jeschke MG, Amini-Nik S (2017) Biomaterials for Skin Substitutes. *AdvHealthcare Mater*

- [48] MacNeil S (2007) Progress and opportunities for tissue- engineered skin. *Nature* 445(7130):874
- [49] Böttcher-Haberzeth S, Klar AS, Biedermann T, Schiestl C, Meuli-Simmen C, Reichmann E, Meuli M (2013) “Trooping the color”: restoring the original donor skin color by addition of melanocytes to bioengineered skin analogs. *Pediatr Surg Int* 29(3):239–247
- [50] Biedermann T, Boettcher-Haberzeth S, Reichmann E (2013) Tissue engineering of skin for wound coverage. *Eur J Pediatr Surg* 23(5):375–382
- [51] Lovett M, Lee K, Edwards A, Kaplan DL (2009) Vascularization strategies for tissue engineering. *Tissue Eng B Rev* 15 (3):353–370
- [52] Catalano E, Cochis A, Varoni E, Rimondini L, Azzimonti B (2013) Tissue-engineered skin substitutes: an over- view. *J ArtifOrgans* 16(4):397–403 98;51:608–13
- [53] Damanhuri M, Boyle J, Enoch S (2011) Advances in tissue-engineered skin substitutes. *Wounds Int* 2 (1):27–34
- [54] Supp, D. M. & Boyce, S. T. 2005 Engineered skin substitutes: practices and potentials. *Clin. Dermatol.* 23, 403–412. (doi:10.1016/j.clindermatol.2004.07.023)
- [55] Halim AS, Khoo TL, Mohd Yussof SJ (2010a) Biologic and synthetic skin substitutes: An overview. *Indian J Plast Surg* 43(Suppl):S23–S28
- [56] Halim AS, Khoo TL, Yussof SJM (2010b) Biologic and synthetic skin substitutes: an overview. *Indian J Plastic Surg* 43(Suppl):S23
- [57] Lepow, B. D., Downey, M., Yurgelon, J., Klassen, L., & Armstrong, D. G. (2011). Bioengineered tissues in wound healing: a progress report. *Expert Review of Dermatology*, 6(3), 255–262. doi:10.1586/edm.11.27
- [58] Varkey M, Ding J, Tredget EE (2015a) Advances in skin substitutes- potential of tissue engineered skin for facilitating antifibrotic healing. *J Funct Biomater* 6 (3):547–563
- [59] Varkey, M., Ding, J., & Tredget, E. (2015). Advances in Skin Substitutes— Potential of Tissue Engineered Skin for Facilitating Anti-Fibrotic Healing. *Journal of Functional Biomaterials*, 6(3), 547–563. doi:10.3390/jfb6030547
- [60] Jutkiewicz, J., Noszczyk, B. H., & Wrobel, M. (2010). The use of Biobrane for hand surgery in Epidermolysis bullosa. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 63 (8), 1305–1311. doi:10.1016/j.bjps.2009.06.038
- [61] Fivenson DP, Scherschun L, Cohen LV. Apligraf in the treatment of severe mitten deformity associated with recessive dystrophic epidermolysis bullosa. *Plast Reconstr Surg* 2003;112 (2):584–8.
- [62] Falabella, A. F., Valencia, I. C., Eaglstein, W. H., & Schachner, L. A. (2000). Tissue-Engineered Skin (Apligraf) in the Healing of Patients With Epidermolysis Bullosa Wounds. *Archives of Dermatology*, 136(10). doi: 10.1001/archderm.136.10.1225
- [63] Nita, M., Pliszczynski, J., Kowalewski, C., Woźniak, K., Eljaszewicz, A., Moniuszko, M., Fiedor, P. (2020). New Treatment of Wound Healing With Allogenic Acellular Human Skin Graft: Preclinical Assessment and In Vitro Study. *Transplantation Proceedings*. doi: 10.1016/j.transproceed.2020.02.115
- [64] Pliszczynski, J., Nita, M., Kowalewski, C., Woźniak, K., Eljaszewicz, A., Moniuszko, M., Fiedor, P. (2020). Transplantation of a New

Biological Product in Rare Diseases, Such as Epidermolysis Bullosa: Response and Clinical Outcome. Transplantation Proceedings. doi:10.1016/j.transproceed.2020.02.119

[65] MacNeil S (2008) Biomaterials for tissue engineering of skin. *Mater Today* 11(5):26–35

[66] Conget P, Rodriguez F, Kramer S, et al. Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin after intradermal administration of allogeneic mesenchymal stromal cells in two patients with recessive dystrophic epidermolysis bullosa. *Cytotherapy* 2010;12:429–31.

[67] Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol* 2001;166: 7556–62.

[68] Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* 2005;11: 1351–4.

[69] Leoni G, Neumann PA, Sumagin R, Denning TL, Nusrat A. Wound repair: role of immune-epithelial interactions. *Mucosal Immunol* 2015;8: 959–68.

[70] Badiavas EV, Abedi M, Butmarc J, Falanga V, Quesenberry P. Participation of bone marrow derived cells in cutaneous wound healing. *J Cell Physiol* 2003;196:245–50.

[71] Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 2008;180:2581–7.

[72] Inokuma D, Abe R, Fujita Y, et al. CTACK/CCL27 accelerates skin regeneration via accumulation of bone

marrow-derived keratinocytes. *Stem Cells* 2006;24:2810–6.

[73] Sebastiano V, Zhen HH, Haddad B, Bashkirova E, Melo SP, Wang P, Leung TL, Siprashvili Z, Tichy A, Li J, Ameen M, Hawkins J, Lee S, Li L, Schwertschko A, Bauer G, Lisowski L, Kay MA, Kim SK, Lane AT, Wernig M, Oro AE; Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. *Sci Transl Med*. 2014 Nov 26; 6(264):264ra163.

[74] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors *Cell*. 2007 Nov 30; 131(5):861–72

[75] S Spiliopoulos, N Davans, Induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. *Ann Transl Med*. 2015 Dec;3 (22):349. doi: 10.3978/j.issn.2305-5839.2015.09.42.

[76] Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyeritz RE, Brenner MK. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood*. 2001 Mar 1;97(5):1227–31.

[77] Dominici M, Marino R, Rasini V, Spano C, Paolucci P, Conte P, Hofmann TJ, Horwitz EM. Donor cell-derived osteopoiesis originates from a self-renewing stem cell with a limited regenerative contribution after transplantation. *Blood*. 2008 Apr 15;111 (8):4386–91. doi: 10.1182/blood-2007-10-115725. Epub 2008 Jan 8.