# Strategies of Gene and Cell Therapy for the Epidermolysis Bullosa Treatment and Future Perspectives

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## 1. Introduction

**Epidermolysis Bullosa** (EB) is a group of genetically transmitted and chronic skin disorders without effective treatment. It affects 1 in 17.000 children and is characterized by spontaneous mucocutaneous blistering and ulcers caused by minor trauma.

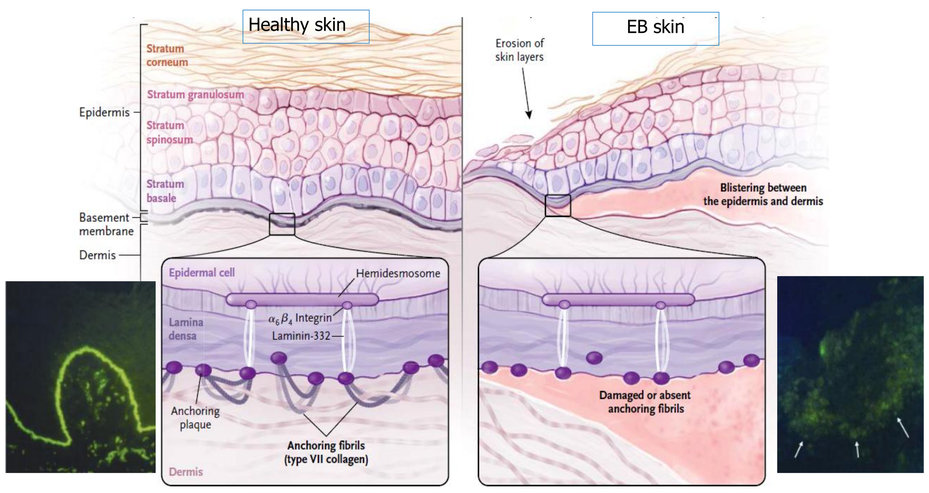



***Figure 1**. Structures of healthy and EB skin and molecules affected with blister formation. It is shown also an immunofluorescence where collagen VII is marked in green -normal skin- [Tolar et al, 2015]*

About 18 genes have been identified to be involved in EB's etiology, encoding structural proteins and adhesive molecules of the epidermal basement membrane, resulting in over a thousand mutations that may occur *de novo* or follow a pattern autosomal dominant or recessive inheritance. The level of expression of these genes in the different skin layers and different types of mutations explain the phenotypic variability of the disease. So the pathophysiology consists in abnormal separation of skin layers due to genetic defects that alter one or more essential dermo-epidermal junction molecules.

There are three EB classic types (*Table 1*) which are differentiated by type of injury, histological characteristics, anatomical location, pattern of inheritance and gene mutation. The clinical presentation varies according to the type of disease.

| Types | Main Gene and Protein affected | Features |
|---|---|---|
| **EB simplex** (92%) | KRT5 (keratin 5)<br>KRT14 (keratin 14) | Intraepidermal shapes (basal keratinocytes); Autosomal recessive or dominant |
| **Junctional EB** (1%) | LAMB3 (laminin 322)<br>COL17A1 (collagen XVII) | Anchoring fibrils (basal membrane); Autosomal recessive |
| **Dystrophic EB** (5%) | COL7A1 (collagen VII) | Dermal shapes; Autosomal recessive or dominant. Severe |

***Table 1**. Classic types of EB*

## 2. Objectives

The aims of this study are:

- ❖ Present a review of Epidermolysis Bullosa's research, understanding the rare disease's context.
- ❖ Expose the protein, gene and cell therapies trials published, in order to discuss their treatment potential in humans.
- ❖ Understand the tissue regeneration and purpose a future strategy through 3D skin bioprinting.

## 3. Methodology

- ❖ Search articles in PubMed database published between 2008 and 2015 using the following terms: *Epidermolysis Bullosa, rare diseases, orphan drugs, EB treatment, gene therapy for EB, cell therapy for EB, skin regeneration*.
- ❖ Search scientific literature in conferences, Gene and Cell Therapy books and courses related with rare diseases.
- ❖ Consult reference web pages like *CIBERER, EURORDIS, ORPHANET, UpToDate* and *GeneGraft*.
- ❖ Contact to Marcela del Rio, principal investigator for Epidermolysis Bullosa in CIBERER, and contact the association *DEBRA* in Spain.

## 4. Treatment Strategies for Epidermolysis Bullosa

**1. Allogenic Fibroblasts**. Intradermal injection leads to increased C7 expression in dermal-epidermal junction (ulcer's healing). Limitations:

- ❖ C7 expression just for 28 days
- ❖ Multiple local injections (effectiveness?)

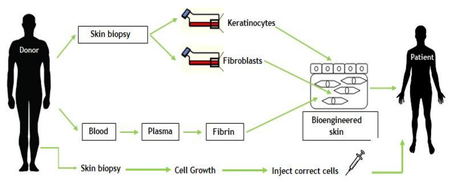



***Figure 3**. Two possible administration: bioengineered skin (top) and intradermal injections (bottom) [Adapted from Robinson, 2010]*

**2. Allogenic Blood and Bone Marrow Cells**. Increased fully functional lympho-haemopoietic system which develops different cellular shapes.

- ❖ Cellular capacity to secrete C7 in lesions
- ❖ Long-term heal internal lesions
- ❖ Security and effectiveness?

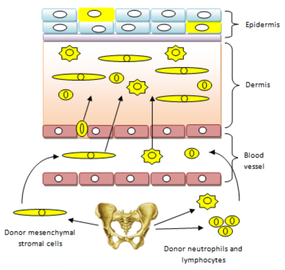



***Figure 4**. Normal collagen VII secreted by donor cells and potentially keratinocytes [Adapted from Tolar et al, 2013]*

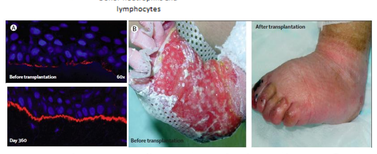


***Figure 5**. Increased C7 expression and clinical benefit after transplantation [Adapted from Tolar et al, 2013]*

**3. Mesenquimal Stem Cells**. Currently the most important therapy for ulcer's healing. Cells can be isolated from bone marrow or adipose tissue and their potential characteristics are:

- ❖ Antiinflammatory
- ❖ Source of C7
- ❖ Pro-angiogenic
- ❖ Antimicrobial

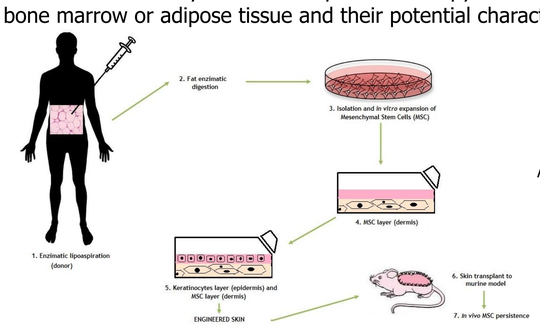



***Figure 6**. MSC cell therapy process. Transplanted MSC remain in dermal layer [Created by author]*

**4.Natural Therapy: revertant mosaicism**. Second-site somatic mutation that corrects the inherited one and produce a normal patch skin (auto-corrected cells).

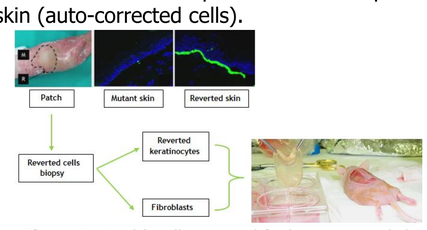



***Figure 7**. Patch's cells are used for bioengineered skin [Adapted from M. Del Rio, 2015]*

**5. Induced Pluripotent Stem Cells (iPSC)**. Dedifferentiated keratinocytes and fibroblasts to create genetically corrected cells.

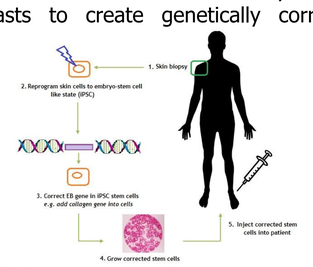



***Figure 8**. Cellular reprogramming [Adapted from Robinson, 2010]*

Systemic infusion of recombinant absent protein. There are good results in pre-clinical trials with murine model, that suggest it could be a possible treatment in humans.

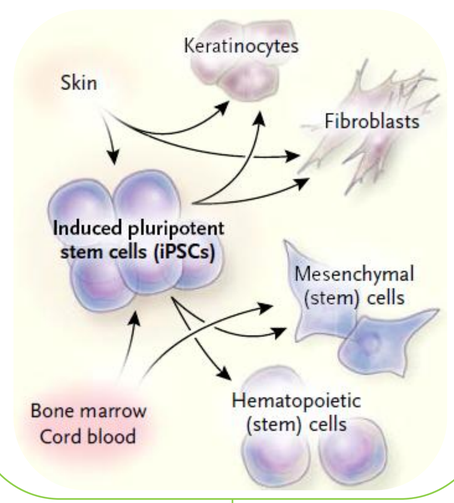
1) Intradermal injections

2) Intravenous injections

Both are safety/effective systems that improve the EB phenotype accelerating wound healing. However, necessary dosing levels has yet to be determined.

**Protein Therapy**

**Cell Therapy**




**Local Therapy**
- Intradermal injection
- Skin grafts

→ Skin healing effects

**Systemic Therapy**
- Intravenous injection
- Intraarterial injection

→ Healing effects on skin and mucosa

***Figure 2**. Combined therapies for EB [Adapted from Tolar et al, 2015]*

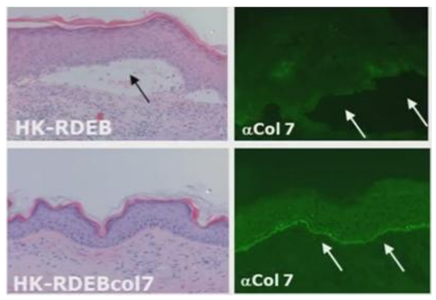



***Figure 9**. Images above: RDEB patient skin, fragile and non expressed collagen VII. Images below: Genetically corrected skin in laboratory with collagen VII expression. [Conference Marcela del Rio, 2015]*

**Gene Therapy**

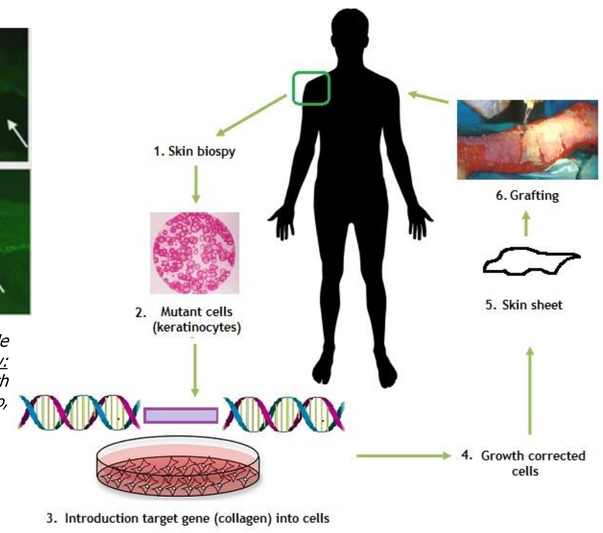



***Figure 10**. Ex vivo therapy. From patient's mutated cells the gene correction is performed in the laboratory by entering the target gene through a vector and transplanted the corrected skin layer. [Adapted from conference Dr Robinson from DEBRA International]*

Most promising therapies based on keratinocytes and epidermal stem cells can correct due to the insertion of a normal copy of the gene (encouraging results both *in vivo* and *ex vivo*). The research develops slowly because of the delivery vector (must be secure without causing immunogenicity), although it is believed it could be one of the best strategies for the future.

*Gene therapy methods:* viral vectors, non viral vectors, zinc finger nucleases and TALENs, and siRNA.

**Future Perspectives**

Innovative technique in regenerative medicine to treat dermatological diseases like EB through skin creation with 3D bioprinter.

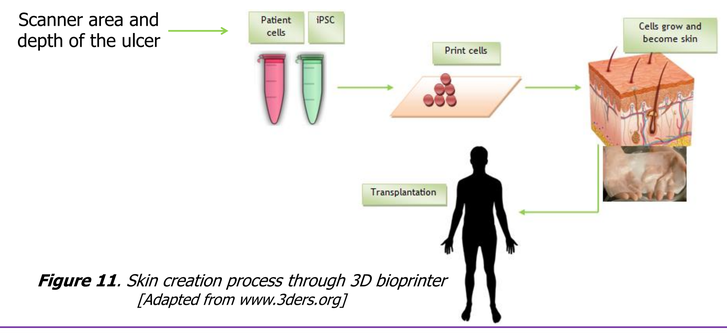



***Figure 11**. Skin creation process through 3D bioprinter [Adapted from www.3ders.org]*

## 5. Conclusions

- ❖ Epidermolysis Bullosa is a complex rare disease with many clinical forms, that challenges the biomedical investigation.
- ❖ The patient's associations role, like DEBRA, are very important as a support to these and for investing in research. It's also important to have the world biobank where many EB samples can facilitate the study.
- ❖ The main treatment for EB is symptomatic and dealt with the care of wounds and blisters on a daily basis, as well as performing a small prophylaxis avoiding friction that can cause damage to the patient. Despite this, current research in this area is very active, which tries to develop new treatment strategies based on the therapies combination, and bioengineering.
- ❖ Although the best potential therapy would be gene therapy, currently there are more developed cell therapies, especially using mesenchymal stem cells and reversed epidermal stem cells.
- ❖ A possible hopeful future strategy could be the skin creation through 3D bioprinters.
- ❖ The main limitation in EB treatment's research is the health care cost (orphan drugs), as this is a minority disease that affects relatively few patients. This is the principal weakness in biomedical research as it also involves an issue of ethics.

## 6. References

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