Skin bioengineering with *Mus musculus*

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1. Introduction

In the last 30 or more years, there has been a great advance in the knowledge and understanding of the cellular processes that take place in acute wound healing and scarring in chronic wounds. In this period of time, big efforts have been done to create skin substitutes *in vitro*. Tissue engineering has been the approach used for this purpose, considering that skin wounds, scars and burns spend a big amount of the healthcare system's budget.

In vertebrate organisms, the skin is the largest organ of the body, being composed of the epidermis and the dermis. The epidermis is the surface epithelium of the skin that overlies the dermis. This layer is constantly being regenerated with the epidermal stem cells' action. When developing new possible therapeutic strategies for clinical application, it is necessary to properly understand the basic epidermal stem cell biology, the mechanisms that drive their multipotency and the genetic base of skin disease. In the last decade, there has been a great advance in the identification, isolation and characterization of epidermal stem cells.

A possible technique for skin regeneration could be the generation of keratinocytes, the most common type of skin cells, using somatic cells derived from embryonic stem cells. However, there is still controversy on the use of embryonic cells, so this approach is still under developing conditions. In the following paragraphs, the newest approach in skin regeneration techniques will be explained: the creation of human dermoepidermal equivalents and skin-humanized mouse models. Two methods that, when combined, serve for multiple applications in clinical dermatology.

2. Bioengineering in medicine

Nowadays, the most common treatment used with patients with severe and extensive burns is autografting. In this procedure, healthy skin from an uninjured area of the patient's body is grafted in the damaged area, with the objective of regenerating the skin. However, this technique presents several drawbacks: it is only effective in small/medium injuries (less than 50-60% of the body surface area damaged) and if there is enough healthy skin available. It is also important to remark that the procedure is slow and painful. In addition, there are numerous functional parameters that have to be addressed in order to obtain a successful operation: an adequate attachment of the tissue-engineered construct to the wound bed, a support neovascularization and the graft acceptance by the patient's immune system.

Bioengineered skin has been developed as a more suitable option to achieve enough permanent skin cover. This technique presents advantages such as no pain, possible donor-site complications and the necessity of finding enough healthy skin available in the patient. The bioengineered skin is used to solve acute skin loss, to treat chronic wounds, and major burns among other, and is made of unique sheets of human fibroblasts (the major cell type present in the dermis) with keratinocytes (the major cell type present in the epidermis) and a scaffold that acts as a connective tissue. This still needs improvement to meet all the requirements that a skin substitute should have, but the results in the clinical trials have been promising.

3. Skin-humanized mouse model

In dermatology, *Mus musculus* is the preferent model organism used. This is because it is a cheap resource, easy to manipulate and has a skin organization similar to the human one. However, they also present some important differences that have to be taken into account when investigating, like the number of layers of the epidermis, being 6-10 in humans and of around 3 layers in mice. In addition, the mouse's skin has a high quantity of hair follicles.

To develop this new skin regeneration technique, the investigators used immunodeficient mice generated with modern genetic manipulation techniques. This skin-humanized mouse model is prepared as follows:

1) Extraction of the patient's fibroblasts and keratinocytes and expansion *in vitro* (**Figure 1**).
2) Placement the expanded fibroblasts in a matrix (made of fibrine, a component of the blood that has factors that allow the cells to maintain the adequate conditions). The keratinocytes then seeded in this artificial bioengineered dermis in order to form the epidermal component (**Figure 1).**
3) Creation of a wound in the back of the mouse that will bear the human dermoequivalent. The mouse's skin obtained from the wound is devitalized with cycles of freezing in liquid N2 and defrosting by immersion in boiling water (**Figure 2**).
4) Grafting of the equivalent in the back of the mouse. Covering of the dermoequivalent with the mouse's devitalized skin. This devitalized will serve as a protection layer that will fall off in 2-3 weeks (**Figure 2**).

If everything goes as planned, after 4 weeks the epidermal layers must have differentiated correctly and, therefore, the patient's skin must have been regenerated. If this happens, this would mean that the epidermal stem cell function is preserved in the human skin regenerated on the animal.

This model has multiple advantages, such as the homogeneity of the acquired cells (because it has been amplified *in vitro)*, the possibility of producing a high number of mice with regenerated human skin using a small amount of the patient's healthy skin and the possibility of studying the characteristics of the healing process of the skin. In addition, *M. musculus* can also be used to investigate cell and gene therapies. This is because when the patient's cells are transplanted to the mice, the obtained skin shows the patient's clinical and histologic phenotype. This approach has served to model rare monogenetic hereditary diseases with no treatment, like epidermolysis bullosa or xeroderma pigmentosum (pathologies grouped, among others, as genodermatoses). The first disease presents blisters that appear with no reason or with minimal trauma, whereas the second is characterized by mutations in DNA repair enzymes that may lead to cancer. In addition, this mouse models have been used in the study of genetic diseases that affect proliferation and differentiation patterns like the Netherton syndrome.

4. Conclusions

The clear advantages that bioengineered skin present cannot be ignored. This technique allows to permanently replace an area of mouse skin with human skin. Called humanized skin mouse, this model is generated by transplanting a skin equivalent that carries a keratinocyte population that includes epidermal stem cells. The humanized mouse allows the study of cutaneous physiological processes, as well as the modeling of different

genodermatoses. In addition, the use of this humanized animal models allows studies on human skin in vivo without the need to recruit volunteers or healthy patients and can also be used as a platform to evaluate the therapeutic potential of new gene or cell therapy strategies.

Dermoepidermal substitutes developed in the laboratory, as well as some of the validated therapeutic strategies in the humanized mouse skin model, have been used successfully in the clinic. This has allowed the development of a preclinical platform for humanized model used in gene and cell therapies among others and the appearance of a new a promising alternative for the traditional treatments of skin replacement and healing. In the future, it is necessary to increase the knowledge in this area in order to develop new and better treatments for skin diseases.

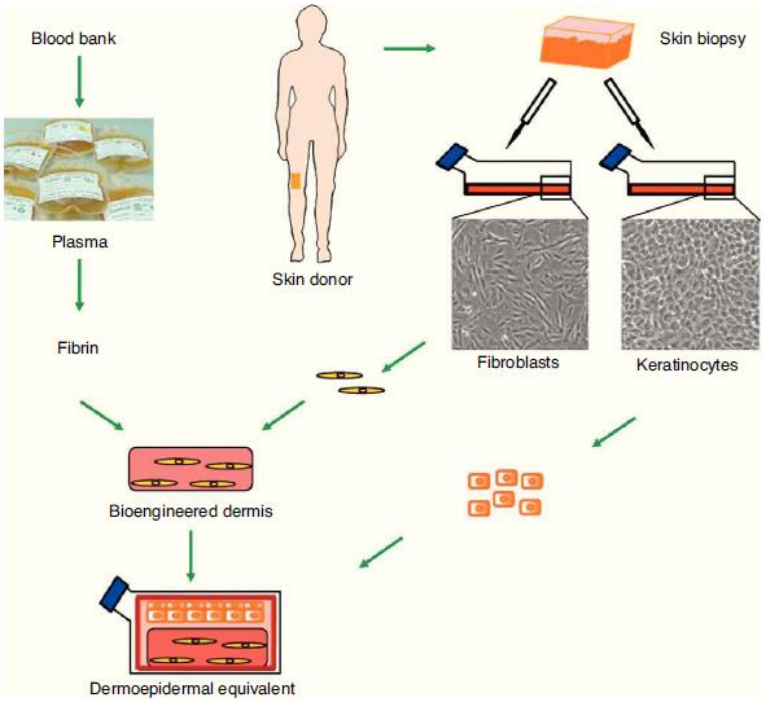

Figure 1: general scheme of the generation of a human dermoepidermal equivalent, where fibroblasts and keratinocytes are cultivated. Then the fibroblasts are placed in a fibrin matrix and the keratinocytes, in order to form the epidermal component, are seeded in the artificial bioengineered dermis. Material adapted from Martínez-Santamaría L., Guerrero-Aspizua S., Del Río M (2012).

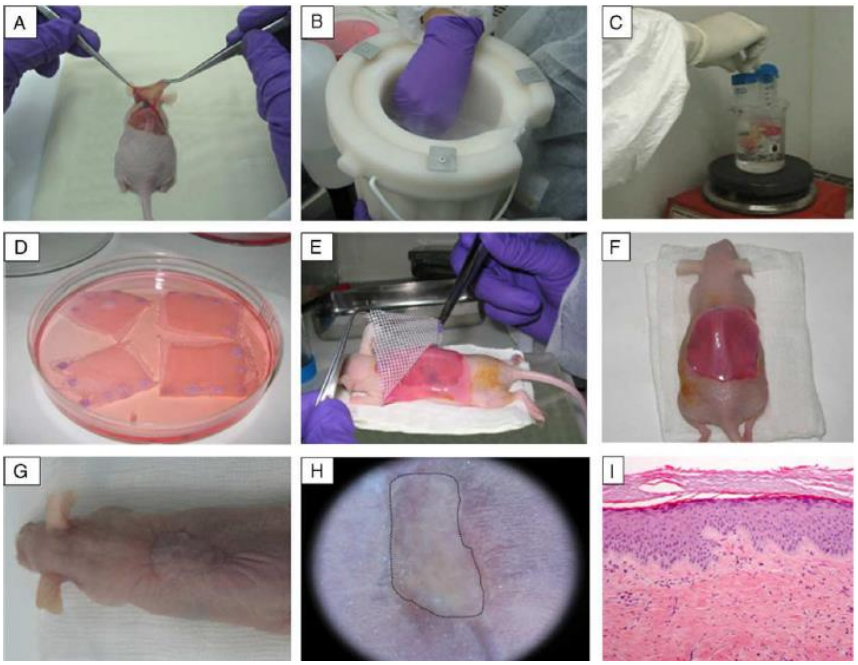

Figure 2: scheme of the process of generation of a skin-humanized mouse model. (A-C) The skin of the back's mouse is devitalized with cycles of freezing in liquid N2 and immersion in boiling water. The devitalized skin is then placed over the dermoepidermal equivalent that was previously placed in the mouse's back, in order to serve as a protection layer. (D-H): After 4 weeks, the dermoepidermal equivalent is placed on the patient's wound. (G-I): 14 weeks later, it is possible to see regenerated human skin (delimited by dots) in mice. Material adapted from Martínez-Santamaría L., Guerrero-Aspizua S., Del Río M (2012).

5. Bibliography

Anthony D. M., Mark W.J. F. Bioengineering skin using mechanisms of regeneration and repair. Biomaterials 28 (2007) 5100-5113.

Larcher F., Espada J., Días-Ley B., P. Jaén, Juarranz A., Quintanilla M. New experimental models of skin homeostasis and diseases. Actas Dermosifiliogr. 2015; 106(1): 17-28.

Martínez-Santamaría L., Guerrero-Aspizua S., Del Río M. Skin bioengineering: preclinical and clinical applications. Actas Dermosifiliogr. (2012); 103(1): 5-11