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Applicability of bioengineered human skin

From preclinical skin humanized mouse models to clinical regenerative therapies

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Ongoing progress in the field of regenerative medicine, in combination with the development of tissue-engineered skin products, has opened new possibilities for the treatment of certain diseases in which current treatments are aimed at alleviating symptoms but are not able to get a permanent cure. Our laboratory has developed a fibrin-based bioengineered human skin that has been successfully used for permanent regenerative therapies in different situations in the clinic. Moreover, we have been able to stably regenerate human skin by orthotopic grafting of this skin equivalent onto the back of immunodeficient mice. The so-called skin-humanized mouse model system has permitted us to model several monogenic skin diseases, when keratinocytes and fibroblasts harboring the genetic defect were used. In most cases different gene therapy approaches for ex vivo correction of cells have proved effective in reverting the phenotype using this model. More importantly, the feasibility of the system has allowed us to generate a skin humanized mouse model for psoriasis, a common chronic inflammatory disease where the immune component has a pivotal role in the pathogenesis. Establishing reliable humanized animal models for skin diseases is necessary to gain a deeper knowledge of the pathogenesis and to develop novel therapeutic strategies. In this sense, the skin humanized mouse model developed in our laboratory meets the needs of this field of research.

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Introduction

Skin is the largest organ of the body and accounts for about 15 percent of adult body weight. It confers protection against trauma and foreign chemical and biological substances and provides important functions contributing to maintain homeostasis. Loss of skin integrity can compromise a patient's quality of life and in severe cases can even lead to further medical complications resulting in major disability or death.

The accessibility of skin has made this organ an interesting target for replacement therapies over the past decades. Tissue expansion and development of substitutes have been commonly used in reconstructive surgery to restore skin structure and function in patients suffering traumatic injuries with significant skin losses. Moreover, tissue engineered skin may result in multiple benefits for patients showing several skin conditions such as chronic non-healing wounds associated with diabetes or vascular disorders.

In some monogenic skin diseases, such as severe cases of Epidermolysis Bullosa (EB), the skin integrity is severely compromised and patients present a high risk of developing skin cancer. Intensive multidisciplinary care and surgical procedures constitute the only alternative for these patients to relieve symptoms and prevent malignant transformation. Gene therapy in combination with tissue engineering represents a promising therapeutic option for these patients.

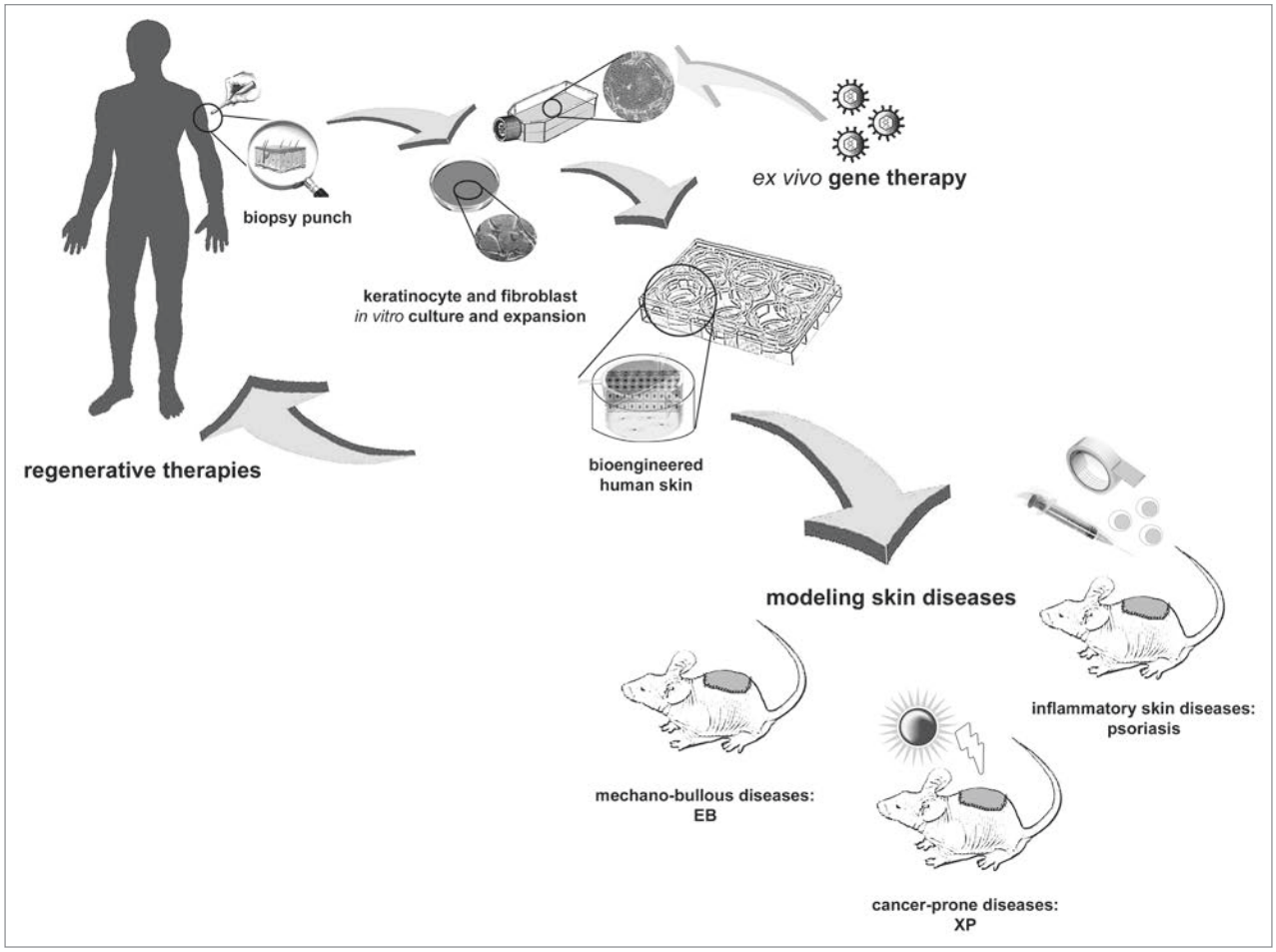

Figure 1. Schematic representation of the different pre-clinical and clinical options for the bioengineered human skin.

Therapeutic management and drug development in other common chronic inflammatory skin disorders, such as psoriasis and atopic dermatitis (AD), largely depend on the characterization of the molecular pathways contributing to the pathogenesis of these complex diseases.

Modeling skin diseases in a humanized context is fundamental to acquire basic knowledge about mechanisms of disease and enables preclinical studies that will have a potentially positive impact on the clinical outcome.

Bioengineered Skin

Present culture technologies enable optimal in vitro expansion of cells obtained from skin biopsies that can be assembled in three-dimensional matrices to engineer skin equivalents suitable for clinical use. A large list of natural scaffolds and synthetic materials have been developed to date for skin regeneration.[1] In patients with severe skin losses there is an unavoidable need for the production of large-scale composite skin equivalents. We have developed an improved and autologous bioengineered skin based on the use of clotted human plasma as a three-dimensional dermal scaffold in which fibroblasts are embedded. We have demonstrated that this plasma-based dermal equivalent provides excellent keratinocyte growth support.[2] As a result, experimental grafting in immunodeficient mice yielded a healthy and mature skin with human architecture that persisted even after several epidermal turn-overs took place, as demonstrated immunohistochemically during long-term follow-up periods.[3,4] Preservation of the epidermal cell stemness is a requisite for permanent skin regeneration. We have successfully used this bioengineered human skin (World Patent WO/2002/072800) in the clinics for permanent coverage in several situations, such as extensive burns, necrotizing fasciitis, removal of giant nevi and graft-versus-host disease[3,5] (**Fig. 1**).

Some of the advantages of autologous cell sourcing include the absence of immune reaction in the host and eliminating the need for immunosuppressive therapies. However, chimerical bioengineered skin containing autologous keratinocytes and allogeneic fibroblasts has been shown to be clinically valuable in asisting wound healing in Dystrophic Epidermolysis Bullosa (EBD). This new product has been granted the Orphan Drug designation by the EMEA (orphan designation number EU/306/369).

Completely allogeneic bioengineered skin has been shown to be benefical in the treatment of chronic wounds, where permanent engraftment is not needed but the skin behaves as a healing device that contributes to enhance the patient's own repair process.

To favor the efficacy of autologous bioengineered skin substitutes in engraftment, it is necessary to improve wound bed conditioning. Infection is one of the major causes of graft rejection and this is critical in immunocompromised patients presenting with severe and extensive burns, leading to further complications and even death. Antimicrobial peptides (AMPs) appear as an attractive alternative to the use of conventional antibiotics, as they present microbicidal activity against a broad spectrum of pathogens and low-level resistance. Some of them have also been shown to increase the repair process. We have shown efficient antimicrobial activity of AMPs gene-transduced keratinocytes forming part of the epidermal component in a human skin equivalent.[6] Cutaneous tissue engineering in combination with antimicrobial gene therapies emerges as a promising strategy to improve wound coverage and combat infection at the same time.

Modeling Human Skin Diseases

We have generated a skin-humanized mouse model based on the permanent engraftment of a bioengineered human skin onto the back of immunodeficient mice.[3,7] It consists of a chimeric model in which a skin of human origin is regenerated, vascularized and innervated by mouse vessels and nerves. One of the main advantages of this method is that the generation of a large number of engrafted mice containing a significant area of homogeneous single donor-derived human skin would be available in a relatively short period of time. The functionality of this mature and quiescent human skin has been also demonstrated, with the main features of a human physiological process, such as wound healing having been accurately recreated in the context of physiologic acute human excisional wounds.[8] This model also offers the possibility of using in vitro genetically manipulated human keratinocytes and/or fibroblasts during the amplification procedure, either to overexpress or silence specific genes, thus generating transgenic or KO humanized-skin respectively.

One of the main challenges in regenerative medicine is to create smart bioengineered skins capable of delivering, in a time-controlled fashion, critical growth factors and/or cytokines for scar-free tissue repair such as those involved in embryonic skin regeneration. As a first attempt, the combination of cell- and genetic-based therapies has made it possible to evaluate the promoting or detrimental wound-healing activities of specific factors using different model systems, such as transgenic mice, KO-mice or xenograft models.[9-11] As a proof of concept, we have selected KGF, a well-characterized factor that is differentially regulated in normal and impaired healing, to compare the efficacy of different transient gene transfer strategies aimed at delivering smart factors to promote cutaneous repair in the wound healing skin-humanized model.[12,13]

Developing new in vivo models of impaired wound healing may better provide reliable platforms resembling human chronic wounds to test the healing-promoting activities of different molecules. Diabetic foot ulcerations constitute a major public health concern. Leptin-deficient ob/ob mice have been widely used as a model of diabetes impaired-wound healing. Thus, we took advantage of this model system in order to test the repair-promoting activities of the pleiotropic factor LL-37 antimicrobial peptide, which has been shown to improve the repair process as stated above, in addition to its role in defense processes. We used an adenoviral-mediated gene transfer approach to overexpress this factor around wound margins of full-thickness wounds generated in this animal model, and demonstrated that LL-37 increased the re-epithelialization rate and granulation tissue formation in a healing-impaired situation.[14]

Our work is currently focused on generating a humanized animal model of impaired wound healing, by inducing experimental diabetes in the well-characterized skin-humanized mice using streptozotocin injection (Martínez-Santamaria et al. manuscript in preparation).

The skin-humanized mouse model has also been very useful in recreating different human monogenic skin diseases (**Fig. 1**), such as the mechano-bullous disease,[15,16] the Netherton Syndrome (NS), an epidermal differentiation disorder caused by mutations in the SPINK5 gene,[17] and the cancer-prone disease Xeroderma Pigmentosum (XP).[18] Regenerated human skin obtained by orthotopic grafting of bioengineered cutaneous equivalents containing collagen VII-null RDEB keratinocytes showed histological evidence of extensive blistering as observed in skin biopsies from RDEB patients. A complete and permanent reversion of this phenotype was achieved when RDEB epidermal stem cells used in the generation of the skin equivalents were genetically modified by using retroviral vectors encoding human collagen type VII.[15] Characteristic histological features of NS were also demonstrated after grafting human NS bioengineered skin onto immunodeficient mice. Reversal of the skin abnormalities was attained after using a lentiviral vector to direct SPINK5 expression in keratinocytes.[17] We have also established a photosensitive humanized skin model through the grafting of bioengineered skin containing XP-C patient cells. The XP keratinocyte deficiency in nucleotide excision repair (NER) was evidenced in vivo after acute UVB irradiation.[18]

Gene therapy approaches in combination with the bioengineered skin-humanized mouse model constitute a robust platform to conduct preclinical studies. However, the use of spontaneous immunocompetent animal models for genodermatoses will be relevant as a complementary tool to evaluate the potential adverse immune response that can be mounted by the host after introducing foreign therapeutic genes. This is particularly critical in the case of null-mutation carriers.[16,19,20] In other cutaneous disorders presenting dominant negative mutations that lead to aberrant protein expression, an alternative to a replacement gene therapy option must be sought, either by preventing mutant gene expression or by overexpressing the normal gene product.

Animal Models of Human Inflammator Skin Diseases

Inflammatory and autoimmune cutaneous disorders constitute a major health and social problem around the world. Sometimes they may be disfiguring and disabling and have a direct impact on patient's psychological distress. Skin

infiltrating T lymphocytes play a fundamental role in both the initiation and maintenance of common chronic inflammatory skin diseases, such as psoriasis and atopic dermatitis. These disorders present a clear deregulation in the Th1/Th2/Th17 balance that accounts for the pathogenesis. In psoriatic skin this equilibrium is skewed towards Th1 whereas a Th2 phenotype is mainly observed in atopic dermatitis and increased numbers of Th17 cells can be observed in both pathologies.[21]

Establishing reliable animal models for inflammatory cutaneous pathologies will allow for a deeper and comprehensive understanding of the basic mechanisms underlying the epidermal-immune cell interactions and more importantly, will allow for the development of new therapeutic strategies. Clinical success will be largely dependent on the appropriate choice of a suitable animal model with predictive value. Transgenic and knockout technologies have yielded several animal models resulting in psoriasis- or atopic dermatitis-like phenotypes.[22-26] Differences in both architecture and function between mouse and human skin account for the major limitation of these models, although they serve as important tools that can help to understand how certain molecules contribute to the pathology. Alternatively, xenotransplantation models have been widely employed for psoriasis studies as they closely resemble the human pathology,[27-29] although the number of grafted mice that can be obtained from one patient is limited by ethical and practical issues. In this scenario, the bioengineered-skin humanized mouse model emerges as a powerful tool presenting several potential advantages over other genetically modified or xenotrasplantation animal models, including the possibility to perform studies in a human context on homogeneous and large samples. Based on this approach, we have recently reported the generation of a bona-fide skin-humanized mouse model for psoriasis.[30] An intrinsic limitation of this method would be the loss of specific immune populations of the skin after the isolation and in vitro culture expansion of keratinocytes and fibroblasts. This becomes manifest when the bioengineered grafted skin is generated using keratinocytes and fibroblasts that are obtained from psoriatic skin biopsies, as the regenerated human skin presents a normal phenotype. However, the practicality of the system enables us to re-introduce activated specific lymphocyte subpopulations from the same patients by subcutaneous injection (autologous approach). These cells are able to trigger the psoriatic response in conjunction with a mild alteration of the epidermal barrier function when tape-stripping is used (**Fig. 1**). More importantly, we have demonstrated that a healthy normal human skin regenerated in immunodeficient mice by bioengineering approaches might develop a psoriasiform phenotype in the presence of adequate signals provided by a wounding stimulus and of the appropriate cytokines produced by specific lymphocyte subpopulations (Th1/Th17) obtained from unrelated healthy donors (allogeneic approach), that have been previously shown to present a key role in the formation of the psoriatic plaque. We believe that these results have contributed to shed some light on the immunopathogenesis of psoriasis. Although several genetic association studies have shown that a wide variety of susceptibility factors play an important role in the acquisition and/or severity of the disease,[31] a specific spatiotemporal combination of cytokines/factors can directly affect the normal lymphocyte-keratinocyte interacting pathways and can give rise to the disease. Nonetheless, the easy-to-handle genetic manipulation of the individual cellular components of the bioengineered humanized skin will make it possible to test the contribution of potential susceptibility factors to the pathogenesis of psoriasis by using this model. Finally, merging of these technologies will be relevant to assess the potential therapeutic effectiveness of novel compounds.

Conclusions and Future Prospects

Acquisition of basic knowledge concerning wound healing mechanisms together with the development of robust biomaterial-derived skin equivalents has been pivotal in the progress of the skin tissue engineering field. In fact, skin bioengineering has successfully coped with different clinical conditions, ranging from severe skin losses to chronic wounds of different origin. Nowadays, a merge of skin bioengineering and gene therapy is envisioned as the ultimate regenerative medicine advance towards treatment of some monogenic inherited skin disorders. Our group has contributed to the skin bioengineering discipline through the development of a new skin substitute based on the use of fibroblasts embedded in a three-dimensional dermal scaffold made of clotted human plasma. The special characteristics of this plasma-based dermal equivalent, in terms of keratinocyte growth support and stem cell preservation, have allowed its successful use in the clinics for permanent skin replacement in several situations. However, future challenges remain concerning the clinical outcome from a cosmetic and performing point of view. Some important issues need to be solved, such as the regeneration of a fully functional skin containing appendages (hairs, sweat glands and sebaceous glands) and to attain scar-free remodelling features as seen in embryonic wound repair. At the preclinical level, we have been able to develop a skin humanized animal model that recreates various skin conditions of genetic origin by grafting patient-derived bioengineered skin onto immunodeficient mice. More recently, we have also been able to model a complex inflammatory skin disease, such as psoriasis, by introducing specific cytokines and/or immune cell subpopulations in this model. The versatility of the system, which includes the possibility of human skin regeneration with genetically modified cells, greatly expands the range of possibilities for studying innovative therapeutic approaches and the contribution of different factors/signalling pathways to specific dermatological entities.

Acknowledgments

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