Regenerative medicine in burn wound healing: Aiming for the perfect skin

Summary
The healing of full thickness wounds such as burn wounds remains complicated by hypertrophic scar formation and contraction. The standard treatment is transplantation with autologous split thickness skin grafts. For extensive burns, these grafts are widely meshed due to limited donor sites, which often results in a poor functional and cosmetic outcome. The application of cultured autologous keratinocytes may enhance wound closure and improve scars.

The first epidermal substitute, a confluent epithelial sheet, was developed in 1979. These cultured epidermal autografts (CEA) have been used in burn patients with variable success. Due to the variation in the efficacy of CEAs, however, new strategies have been employed. Currently, the application of preconfluent proliferating keratinocytes is considered a better strategy for burn wound treatment.

In addition to improvements in epidermal grafts, the healing outcome may improve with the application of dermal substitutes. Over the past several decades, several scaffolds have been developed to mimic the dermis. These substitutes can be supplemented with growth factors and cells. In particular, the application of mesenchymal stem cells (MSCs) is thought to be a promising perspective for cell-based tissue engineering.

DERMAL SUBSTITUTION
Although transplantation with a meshed split thickness autograft is the gold standard, healing of the transplanted burn wound is not optimal and results in scar formation, which is thought to be due to the lack of sufficient dermal material in the thin autograft and a delayed re-epithelialisation of the wound. The interstices of the meshed graft especially are prone to hypertrophic scarring. Several skin substitutes have been explored over the past several decades to improve the healing process. These substitutes comprise epidermal, dermal, or full skin constructs.

Epidermal substitutes consist mainly of cultured keratinocytes, which can be supported by a carrier system. The main component of the dermis is the extracellular matrix (ECM), which provides support for different cell types and skin appendages such as hair, sebaceous glands, and sweat glands. The ECM plays an important role in the regulation of the different cells in the dermis. The lack of dermal ECM in full-thickness burn wounds is thought, therefore, to be an important cause of scar formation. Several scaffolds have been developed to create an environment that mimics the dermal ECM. Many of these constructs are based on the natural components of the dermal ECM, with collagen as the most abundant component of the skin as a base.

One of the first skin substitutes used in burn patients was an acellular bilayered construct that consists of a collagen matrix with a silicon top layer to mimic the epidermis. In addition to collagen, this scaffold also contains chondroitin 6-sulfate, a glycosaminoglycan (GAG), which provides more elasticity and better tensile strength to the scaffold and protects the scaffold against fast biodegradation. The porous structure of the collagen/GAG scaffold allows the migration of various cell types and structures such as blood vessels into the substitute. This construct, which is currently available as Integra®, is used in a two-step procedure. During the first operation, the burn wound is excised and the skin substitute is applied. In a second operation...
after revascularization of the collagen matrix, the silicon top layer is removed and an autologous meshed split skin autograft is applied on top of the substitute. This two-step procedure is the main drawback of this material, which has led to the development of a skin substitute that can be applied in a one-step procedure where application of the dermal substitute and the meshed split skin autograft occurs during the same operating procedure. Matriderm® is one of these products. This scaffold is also composed of porous collagen sponge, which is supplemented with elastin hydrolysate. The elastin coating of the collagen has been shown to improve scaffold stability and reduce granulation tissue formation as well as fibrosis and contraction and stimulate collagen deposition by fibroblasts. Although the take rate of the autologous meshed skin graft was somewhat diminished, a statistically significant improvement in scar elasticity was observed at the 3-month follow-up in patients undergoing scar reconstructive surgery. Furthermore, although the differences did not reach significance at the 12-month follow-up, most patients considered the scars of the substituted sides as better. In a 12-year follow-up, a significant improvement was observed in elasticity, especially in patients in which a large extension of the autograft was used. In this latter study, surface roughness also was evaluated, and this study reported that the substituted wounds were smoother than wounds in which the standard treatment with an autograft alone was applied. In a more recent multicentre clinical trial, the treatment of acute burns with a skin substitute was combined with topical negative pressure (TNP) therapy with the aim of improving the graft take rate. Although the latter goal was not accomplished, the application of TNP did improve elasticity.

EPIDERMAL SUBSTITUTION

The first cellular substitute was the cultured epidermal autograft (CEA), which is an in vitro cultured and differentiated epidermis. The first clinical application of this construct for burn wounds was performed during the 1980s. Although the results appeared promising at first, problems with the use of this technique became apparent over the years. Unpredictable take rates, the fragility of cell sheets, and long culture times quickly reduced the enthusiasm towards this product. Blistering often occurred due to the destruction of anchoring molecules from the tissue culture disc during the CEA harvesting procedure. In addition, the wounds remained open for a long period due to the long culture time, which increased the risk of infection and sometimes led to the inability to transplant the cultured keratinocytes back to the patient.

New methods have been developed in more recent years. These new techniques make use of proliferating keratinocytes. After transplantation to the wound bed, these cells start to differentiate and form an epidermis in vivo. This technique has the advantage that the culture times are reduced and that anchoring molecules are not destroyed during the process, which results in a better take rate. The cultured cells can be applied either to the wound bed on a carrier or sprayed as a suspension. Several clinical studies have been performed and show promising results. However, although these new techniques restore most of the disadvantages of the CEAs, the product may still be improved. For example, the culturing conditions of keratinocytes often include animal (xenobiotic) products, which potentially increase the risk of transmission of animal-derived disease components such as viruses or prions to the patient.

CELLULAR DERMAL SUBSTITUTES

Although the clinical requirements for the function of dermal substitutes have been defined, the translation of these requirements into physical and mechano-biological properties of scaffolds is difficult. The materials should not only provide the correct mechanical properties of the dermis, such as tensile strength and flexibility, but also provide a template for cells. The materials also should create a molecular microenvironment in which the different cell types migrate, grow, and acquire the proper phenotype to allow the regeneration of a new dermis.

References

Scaffolds such as the products Integra® and Matri-derm® are typically based on biomolecules present in the dermis, such as collagen, elastin, and GAGs. However, although the clinical use of these scaffolds shows promising results, healing is still not optimal.

To further improve the healing process, cells have been included in the scaffolds. Several skin substitutes that use allogeneic or autologous fibroblasts and keratinocytes (e.g., Tiscover®, Apligraf®, and OrCel®) have been described. Their limited clinical use worldwide, however, is probably due to the high expenses for production of these constructs as well as possible immunological reactions to the allogeneic cells and the long production time for autologous cell constructs.

Recently, interest in the field has shifted to the use of mesenchymal stem cells (MSC) in skin substitutes. MSCs are thought to play an important role in tissue homeostasis and in the facilitation of the repair of damaged tissue to restore the function of injured organs. Over the past several decades, stem cells have been isolated from various tissues. Stem cells are considered to be promising for tissue engineering purposes due to their multi-lineage differentiation capacity as well as their immune-modulating effects.

**MESENCHYMAL STEM CELLS FOR SKIN REGENERATION**

MSCs have been shown to improve healing in different wound models15, 16. The exact mechanism by which this improvement in healing is accomplished is not known. Initially, it was thought that MSCs were incorporated into damaged tissues and differentiated into tissue-specific cells. However, it is now becoming clear that these cells exert their main therapeutic effect through paracrine actions and their immune regulatory features, and to a lesser extent through their incorporation into damaged tissue.

Several studies have shown that MSCs reduce fibrosis. MSCs are able to reduce the immune response by suppressing the activation of T cells, B cells, and natural killer cells via a reduction in the maturation of dendritic cells. MSCs reduce the expression levels of MHC class I and II molecules, and they do not express co-stimulatory molecules (CD80, CD86, and CD40)17. Due to these features, the MSCs are immune privileged and therefore can be used in an allogeneic setting, which is a major benefit that allows MSCs to be used as an off-the-shelf product.

There remain concerns with the use of MSCs. For example, MSCs may lose their immune suppressive and immune privileged status during differentiation18. MSCs also may differentiate into the incorrect phenotype. MSCs also have the potential to differentiate into tumour cells due to their high capacity for self-renewal.

Despite the criteria postulated by the International Society for Cellular Therapy (ISCT) for defining multipotent mesenchymal stromal or stem cells19, real discriminating markers for MSCs are missing. The criteria formulated by the ISCT state that these cells have to express a specific CD marker pattern in which they are positive (≥ 95%) for CD73, CD90, and CD105 and negative (≤ 2%) for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. These criteria suggest that the cell isolates represent a homogeneous cell population. The MSC populations from various tissues, however, are a very heterogeneous population of cells, of which only a small percentage possess multi-lineage differentiation potential20. Until proper discriminating markers have been found to identify MSCs and other cell types, it is unknown what the different cell types in these populations contribute to the healing process. Currently, most papers that describe MSCs confine their characterization of the cell population to the different markers that have been defined by the ISCT.

Only a few studies have shown that the cell population containing stem cells also contains alpha smooth muscle actin (α-SMA) positive cells. It is unclear whether the MSCs themselves express α-SMA or whether these cells are differentiated myofibroblasts, which is the cell type responsible for scar formation and fibrosis. Recently, we have shown that MSCs migrate into the wound during the first 10-14 days post-burn21. We hypothesized that these cells contribute to scar formation. The microenvironment created by these myofibroblast-like

**References**

cells is distinctly different from normal dermal tissue, and because cell function and tissue performance is largely dependent on the cellular microenvironment, the healing process is trapped in a vicious circle. Ideally, tissue engineering could play an important role in the development of a scaffold that is able to guide the stem cells into the proper phenotype. Although various preclinical (animal) studies have been performed and show promising results, translation into clinical trials remains limited (reviewed in\textsuperscript{22}).

**FUTURE PERSPECTIVES**

Despite the substantial progress in skin tissue engineering over the past several decades, the regeneration of fully functional skin following a burn wound still has not been achieved. Elucidating the signals that are required to guide the cells into the desired phenotype and away from the myofibroblast phenotype will be useful for scaffold design. Such scaffolds should create the microenvironment necessary for skin regeneration instead of scar formation.

In addition to the aesthetic problems and functional impairments due to diminished joint mobility of scars, other problems may occur from scarring as a result of the absence of skin appendages. For example, the lack of sweat glands may impair the thermoregulatory function of the skin. In addition, scars often lack hair follicles and sebaceous glands. Hair transplantation and skin expansion techniques have been used to restore the lack of hair follicles in scars\textsuperscript{23, 24}. In vitro and in vivo experiments have shown that a tissue engineering approach may be successful to reconstitute hair follicles\textsuperscript{25}. The use of stem cells in skin substitutes have also shown promising results with respect to hair follicle induction\textsuperscript{26}. Further research to elucidate the mechanisms involved in the development of sebaceous glands and sweat glands is needed to address the lack of these structures in the healing wound.


\textsuperscript{22} Jackson WM, Nesti LJ, Tuan RS. Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. Stem cells translational medicine. 2012;1(1):44-50.


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