Tissue-Engineered Skin

Why do we need tissue-engineered skin?

Application of tissue-engineered skin can be crucial to close deep second- and third degree burns, which usually do not heal without any grafts.
Another example:

A neuropathic diabetic ulcer persistent for 2 years (a) and complete healing after eight applications of TranCell (b).
The Structure of Human Skin

Shier D et al., in Hole’s Human Anatomy and Physiology, (1999) 160-183
Skin Structure and Function

The skin is a **physical barrier** b/w the body and the external environment. The passive and active functions of skin are carried out by specialized cells and structures located in the **two main layers of skin**: the **epidermis** and the **dermis**.

- **Epidermis**
  - The **outermost layer** of skin must be tough and impermeable to toxic substances or harmful organisms. It must also control the loss of water from the body.
  - The epidermis is composed primarily of **keratinocytes**, which form a stratified squamous (鱗狀的) epithelium.
Skin Structure and Function (cont.)

- Epidermis (cont.)
  - Proliferating cells in the basal layer of epidermis anchor the epidermis to the dermis and replenish the terminally differentiated epithelial cells. These cells stop proliferating and **terminally differentiate** as they move from the basal layer through the suprabasal layers to the surface of epidermis. The most superficial keratinocytes in the epidermis form **stratum corneum**, the dead outermost structure that provides the physical barrier.

  - *In the last stages of differentiation*, epithelial cells extrude **lipids** into the intercellular space to form the permeability barrier. The cells break down their nuclei and other organelles and form a highly cross-linked **protein envelope** beneath the cell membranes. The envelop connects to a dense network of intracellular **keratin filaments** to provide further physical strength of epidermis. (cf. brick and mortar)
Skin Structure and Function (cont.)

- **Dermis**
  - **Papillary dermis** lies immediately beneath the epidermis
  - **Reticular dermis** is more acellular and has a denser meshwork of thicker collagen and elastic fibers. Loss of reticular dermis can often lead to excessive scarring and wound contraction.
  - **Fibroblasts**, the major cell type of the dermis, produce and maintain most of the ECM.

- **Immunology and the skin**
  - Antigen presentation by keratinocytes and fibroblasts does not result in T cell activation due to the deficiency of the necessary co-stimulatory molecules. Therefore, it is OK to use allogeneic cells.
  - The primary mode of skin rejection is likely mediated via an attack on the vasculature present in a normal skin graft.
Skin Wound Healing

Generally, wound healing includes (1) reepithelialization, (2) remodeling of granulation tissue (a highly vascularized and cellular wound connective tissue, 肉芽組織), and (3) formation of scar tissue.

Recent advances in our understanding of fetal wound healing, the concerted action of growth factors, the role of ECM in regulating the healing process, and the ability of epidermal sheet grafts to close severe wounds raise the possibility of intervening therapeutically in tissue repair by providing lost epithelial, stimulating dermal regeneration, and reconstituting full-thickness skin.
Engineering Skin Tissue

Although the epidermis is capable of healing itself, there are situations in which it is necessary to replace large areas of epidermis or in which normal regeneration is deficient.

The dermis has very little capacity to regenerate. The scar tissue that forms in the absence of dermis lacks the elasticity, flexibility, and strength of normal dermis.

Engineered tissues that not only close wounds but also stimulate the regeneration the dermis would provide a significant benefit in human wound healing.
Engineering Skin Tissue (cont.)

Tissue engineering has not focused on the regeneration of less important structures, like hair follicles and sebaceous glands (皮脂腺). It has primarily focused on providing or imitating structural and biological characteristics of dermis, epidermis, or both. The key features to be replicated in an engineered skin construct:

- A dermal or mesenchymal element capable of aiding appropriate dermal repair and epidermal support.
- An epidermis capable of easily achieving biologic wound closure.
- An epidermis capable of rapid reestablishment of barrier properties.
- A permissive environment for the components of the immune system, nervous system, and vasculature.
- A tissue capable of achieving normalization of structure and additional function such as reduction of long-term scarring and reestablishment of pigmentation.
Epidermal Regeneration

Reepithelialization of the wound is a paramount concern. The approaches to reestablishing epidermis are numerous:

- An integrated sheet such as Epicel. A biopsy of the patient’s cells is grown into an integrated sheet and enzymatically detached for delivery to the patient.
- Subconfluent cells on a carrier such as Myskin (CellTran). Cells are delivered to the patient before they reach confluence on a chemically defined carrier dressing.
- Small sheets cultured from a patient’s hair follicles such as Epidex.
- A spray such as CellSpray. Subconfluent cells are expanded in the laboratory and delivered to the patient as a spray.
Dermal Replacement

The dermis is needed to prevent wound contraction and scar formation.

- **Donor skin**: skin from screened skin donors (temporary or permanent)
- **Integra**: it composed of bovine collagen and chondroitin sulfate, with a silicone membrane covering.
- **Alloderm**: freeze-dried human donor dermis
- **Dermagraft**: a synthetic material conditioned with donor fibroblasts
- **Transcyte**: similar to Dermagraft but with a silicone membrane to act as a temporary epidermal barrier.
- **Permacol**: porcine sin that provides a temporary wound dressing
Epidermal/Dermal Replacement

The epidermis and dermis act synergistically to maintain homeostasis. Cultured epidermal grafts are more likely to “take” when the dermal bed is relatively intact.

- **Apligraf**: this combines allogeneic keratinocytes and fibroblasts with bovine collagen to provide a temporary skin-replacement material suitable for use in chronic wounds but not major burns.

- **Orcel**: similar to Apligraf.

- **Cincinnati skin substitute**, or **Permaderm**: comprises autologous keratinocytes and fibroblasts crafts into reconstructed skin with bovine collagen. Can provide a permanent skin substitute for burns patients.

http://www.apligraf.com/patient/what_is_apligraf/how Works/how_apligraf_works.html
History

Timeline

- Keratinocyte culture established in 1970
- Clinical use of CEA in burns patients in 1975
- Culture of keratinocytes into small sheets for grafting in 1980
- Development of a synthetic dermal alternative (Integra) in 1985
- Use of CEA with donor skin as a dermal alternative in 1990
- Determination of the replicative capacity of keratinocytes
- Understanding of the co-dependence of keratinocytes and fibroblasts in 1995
- Reconstructed skin using autologous keratinocytes and fibroblasts in bovine collagen for use in patients with severe burns in 2000

CEA: cultured epithelial autografts
The engineering of skin tissue has been at the forefront of tissue engineering for many years and has now yielded some of the first medical products to emerge from this field of work.

This is the result of the hard work and dedication of many individuals over 25 years in the areas of keratinocyte cell biology, extracellular matrix biology, collagen scaffolds, polymer scaffolds, and tissue equivalents.

The success of tissue-engineered skin demonstrates that making a broadly available, effective medical device through tissue engineering is possible!