Yonsei Medical Journal
Vol. 41, No. 6, pp. 774−779, 2000

Tissue-engineered Human Living Skin Substitutes: Development and Clinical Application

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Abstract

The skin acts as a barrier to exogenous substances, pathogens, and trauma. Skin defects caused by burns, venous ulcer, diabetic ulcer, or acute injury occasionally induce life-threatening situations. Tissue engineering provides an alternative for autologous or allogeneic tissue transplantation, which is required because of donor site limitations and the risks of transmitting infection. Currently, skin substitutes are made of only extracellular matrix, mainly cells, or combination of cells and matrices. New biotechnological approaches have led to the development of the skin equivalent, the closest match yet to native human skin in terms of histological and functional properties. This review article focuses upon the development of the *in vitro* and *in vivo* epidermis and dermis and their clinical applications.

Key Words: Tissue engineering, skin, skin substitutes

INTRODUCTION

The skin is composed of three layers; epidermis, dermis, and subcutaneous fat. The epidermis is directly contiguous with the environment, is formed by keratinocytes, and acts as a barrier to exogenous substances, chemicals, the loss of moisture, and pathogens. Melanocytes and Langerhans cells also reside in the epidermis. At the junction between the epidermis and dermis is the basement membrane, which anchors the epidermis to the dermis. The dermis consists primarily of connective tissues that protect against trauma, and its major structural component is collagen. There are at least 9 types of connective tissue collagen that have been detected in human skin. Type I collagen, which accounts for 80% of the total collagen, is present throughout the dermis. The main cellular constituents are fibroblasts, which synthesize collagen.

Skin defects caused by burns, venous ulcer, diabetic ulcer, or acute injury induce water, electrolytes, and protein loss from the wound site and may allow bacteria to invade. Some of these processes can be prevented if the wound is covered or the lost skin is replaced by a dressing or skin substitute. Skin grafts have been used for both acute and chronic large and slow healing wounds.[1] Autologous skin grafts, which involves harvesting skin from one area and transplanting it to another area on the same person, and allogeneic skin grafts, which are harvested from one person and transplanted onto a genetically non-identical person,[2] are the methods currently used to replace defective skin. Autologous skin grafts are effective, but they are often limited by the availability of donor tissue. Allogeneic grafts are subject to rejection, as antigens present in the donor tissue may elicit an immune reaction in the recipient, and are further limited by pathogens found in the donor tissue.[3] Many temporary or permanent skin substitutes that are readily available, have been developed to be nonimmunologic and to mimic the characteristics of normal human skin. The ideal skin substitute would interrupt water and electrolyte loss and infection, but relieve pain and enhance wound healing.

The phrase "tissue engineering" was adopted by the Washington National Science Foundation bioengineering panel meeting in 1987.[4] It refers to the application of the principles and methods of engineering and the life sciences toward the development of biological substitutes to restore, maintain, or im-

Received November 17, 2000
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prove function.

OVERVIEW OF BIOENGINEERED SKIN SUBSTITUTES

The use of living cells together with natural or synthetic extracellular components is essential for tissue engineering. Currently, skin substitutes are made of only extracellular matrix, mainly cells, or a combination of cells and matrices (Table 1).[5] The development of tissue engineered skin products has been triggered by advances in research, which have focused on cell culture and the cryopreservation of living cells. A fundamental advance was made when it was discovered how to grow human keratinocytes in culture.[6] The development of the techniques of culturing keratinocytes is wholly attributable to the efficient production and reproducible yield of cells from very small pieces of donor skin. Cell banks can be developed, and when needed cells can be thawed and propagated exponentially in culture.

Since the pioneering work in which autologous keratinocyte sheets were used to treat burns,[7] improved methods have been established for the production of epidermal cell sheets in vitro.[8-11] A disadvantage of cultured keratinocytes had always been that they are very fragile. Keratinocyte sheets are difficult to handle, a more structural dermal component was necessary to increase the handling properties of cultured cells. These were produced by the introduction of floating[12-15] and anchored dermal substitutes,[16-18] using collagen hydrated gels or lattices. The first floating skin equivalent was produced in 1981,[12] by adding multilayered epidermis to the dermal equivalent. However, its severe contraction restricted its use for wound coverage *in vivo*.[19] Living fibroblasts contract a floating collagen gel matrix, *in vitro,* the extent of which depends on the number of cells, the collagen concentration and the contractile properties of the cell population seeded in the gel.[13-16,18] Such surface contraction of floating dermal substitutes produced by hydrated collagen gels can he prevented using anchorage methods *in vitro*.[16-24]

CHARACTERISTICS OF TISSUE-ENGINEERED SKIN PRODUCTS AND THEIR CLINICAL APPLICATION

Tissue-engineered skin substitutes can be broadly categorized into epidermal components alone, mainly dermal components, or composite grafts (Table 2).[25]

Table 1. Tissue-Engineered Skin Substitutes

Product name	Company	Materials	
		Epidermis	Dermis
Epicel[32]	Genzyme tissue repair	Living cultured autologous keratinocytes	None
Alloderm[44,45]	Life cell	None	Salt processed, human cadaveric skin with acellular dermis
Integra[46,47]	Integra life sciences	Silastic membrane	Bovine tendon collagen and shark glycosaminoglycan
Dermagraft-TC[49]	Advanced tissue sciences	Silicone polymer	Nylon-mesh with non-viable cultured foreskin-derived dermal fibroblasts and their products
Dermagraft[48]	Advanced tissue sciences	None	Living human neonatal foreskin-derived dermal fibroblasts on a bioabsorbable polyglactin mesh without silastic layer
Apligraf[50]	Organogenesis	Living human neonatal foreskin-derived keratinocytes	Living human neonatal foreskin-derived dermal fibroblasts and bovine tendon-derived collagen plus the fibroblast-produced matrix and growth factors

Table 2. Advantages and Disadvantages of Tissue-Engineered Skin Substitutes

Product name	Type	Advantages	Disadvantages
Epicel[32]	Epidermal Autograft	Coverage of large areas; permanent wound coverage; minimal risk of disease transmission	2−3 wks intervals for cultivation; fragile, difficult to handle
Alloderm[44,45]	Dermal Allograft	Immediate wound coverage; no immunological reaction	Allograft supply, preservation; virus screening; two steps procedure
Integra[46,47]	Dermal Replacement	Immediate wound coverage; allow ultra-thin split-thickness skin autografts	Complete wound excision; two steps procedure; susceptible to infection
Dermagraft[48]	Dermal Replacement	Immediate availability	Multiple applications
Apligraf[50-54]	Composite graft	Immediate availability; single step (no requirement for subsequent skin grafting); easy handling	Limited viability

Cultured epidermal grafts

In 1975, Rheinwald and Green[26] described a method of *in vitro* cultivation and expansion of human keratinocytes. This method has been used clinically to cover burns and other acute and chronic wounds.[27-31] One product, Epicel, developed by the Genzyme Tissue Repair Corporation, has been commercially available since 1988.[32] This product relies on a small skin biopsy sample to generate a large area of cultured epidermis. It also provides permanent wound coverage, with acceptable cosmetic results and minimal risk of disease transmission. However, it takes a 2−3 weeks to cultivate practical quantities of epidermis after the initial biopsy.

Cultured allogeneic keratinocyte grafts were developed to avoid this 2−3 week delay. However, cultured epidermal allografts serve only as a temporary wound covering that is eventually replaced by the host's epidermis.[33-38] Both keratinocyte autografts and allografts are thin, fragile and lack a dermal component. Several authors have also suggested that dermal elements play an important role in the wound healing process.[39-43]

Dermal replacements

Cadaver allograft skin has been used for many years, primarily in patients with full-thickness burns, as a temporary wound coverage. Replacement of the antigenic epithelium with a split-thickness skin graft has been limited for reasons of availability and safety.[32] Alloderm, developed by the Life Cell Corporation, is made of salt processed human cadaveric skin to form an acellular dermal matrix and an intact basement membrane complex without an epidermis.[25,44] Alloderm is decellularized, freeze-dried and biochemically stabilized, and has been used successfully alone and in combination with cultured autografts for the treatment of burn wounds and dermal defects.[45]

A composite graft, Integra, developed by the Integra Life Science Corporation, is composed of a bilaminated membrane consisting of a silastic outer covering bonded to a collagen based dermal analog (bovine tendon collagen and shark glycosaminoglycan).[46,47] The dermal component is designed to be slowly biodegradable and a split thickness autograft is applied to the wound surface after the silicone layer is removed. This composite graft was used in a multicenter study in burn patients and received FDA approval, but its use is limited to practitioners that have undergone a company sponsored training program.

Another product, Dermagraft, developed by Advanced Tissue Sciences Inc., uses a mesh of bioabsorbable polyglactin (Vicryl) as a platform on which dermal fibroblasts from neonatal foreskin are grown in a sterile bag with circulating nutrients. These fibroblasts multiply and secrete collagen, fibronectin, glycosaminoglycans, and growth factors over the course of 14 to 17 days, thereby producing a dermis-like matrix.[48] Dermagraft-TC (Dermagraft-Transitional Covering) is composed of an outer silicone polymer "epidermal layer" and a "dermis", which is synthesized from human neonatal fibroblasts cultured

on polyglactin mesh.[49] Dermagraft-TC has been used as temporary wound coverage for excised burn wounds[49] and has been approved by the FDA. The product is also eventually removed and replaced with autologous skin grafts. A modification of this graft, Dermagraft, which does not have a silastic layer, is a living dermal equivalent, contain allogeneic neonatal fibroblasts on a bioabsorbable polyglactin mesh that disappears after 3 to 4 weeks. Dermagraft fibroblasts remain viable and the absence of a silastic layer allows a single step procedure.

Composite grafts

Apligraf (Grafts skin, Living Skin Equivalent: LSE), developed by Organogenesis, is the only bilayered skin equivalent approved by the FDA.[50] Apligraf is composed of living cells. Keratinocytes and dermal fibroblasts are derived from neonatal foreskin and propagated in culture. Cultured cells are extensively screened for pathogens and tumors.[51] The initial step involves mixing fibroblasts with purified bovine type I collagen and heating in order to trap the fibroblasts as a loose matrix.[44,52] Within this organizational collagen, new collagen and matrix protein are produced, which are placed in a mold to limit lateral contraction. After 2 weeks, the mixture forms a dense fibrous network. A suspension of keratinocytes is added to the surface of the collagen fibroblast layer and these are allowed to proliferate. After several days of growth whilst submerged in a tissue culture medium, the surface of the skin equivalent is exposed to air (the air-liquid interface), to promote keratinocyte differentiation, and the formation of a stratum corneum, in the presence of an increased calcium concentration in the culture medium and the absence of epidermal growth factor. Apligraf, despite containing allogeneic proteins and cells, has as yet not led to an adverse host response. In part, this may be because the tissue-engineered products do not contain antigen presenting cells, such as, Langerhans cells and endothelial cells.

Apligraf can be thought of as a "smart tissue". It has been shown to have the ability to produce a number of cytokines and growth factors, and it acts very much like human skin. It is hypothesized that the cells of these tissue-engineered skins can serve the environment into which they are placed, and take "corrective action" by producing the appropriate soluble factors including, interleukin 1 (IL-1), IL-3, IL-6, IL-8, transforming growth factors α and β, and basic fibroblast growth factors.[5]

Apligraf is approved in the United Sates for the treatment of venous ulcers, and has been studied for the treatment of venous ulcers in a parallel group, multicenter, randomized, controlled trial, which compared Apligraf therapy to compression therapy.[50] Two hundred and seventy-five patients were evaluated in this study. 63% (92 of 146) of the Apligraf cases, compared with 49% (63 of 129) of the compression-treated cases were completely healed at 6 months. The median time to complete wound closure was significantly faster with Apligraf patients (61 days vs 181 days). The product was especially effective in the most refractory venous ulcers. There was no clinical or laboratory evidence of rejection or response sensitization to Apligraf application. Up to five applications of Apligraf were allowed in the trial during the first three weeks, depending on whether there was a 50% take of the previously applied graft. Now Apligraf is available commercially, one application is usually sufficient to either obtain graft take and healing or to stimulate the wound to heal.[53]

Apligraf has also been studied for the treatment of acute wounds. In a study, a single application of Apligraf was placed onto excision sites of 15 patients that had undergone surgical excisions for skin cancer. Twelve of the 15 patients had graft take.[52] The product proved safe and offered clinical advantages over autografting and secondary healing. In another study involving acute wounds, Apligraf was evaluated in a parallel comparison trial of donor site wounds,[54] and was compared to a polyurethane film for healing time, pain, and cosmesis.

The best skin equivalent is the closest to native human skin in histological and functional properties. Apart from their clinical applications, skin substitutes arouse a high level of interest with the pharmaceutical and cosmetic industries for the research and development of new commercial products. Tissue-engineered skin constitutes have been developed that contain melanocytes[55] and Langerhans cells.[56] Apligraf and other tissue-engineered skins have also been transfected with viruses.[57,58] In the near future, skin substitutes containing a superabundance of selected cells or selected functions by genetic engineering will be used clinically for selected purposes.

CONCLUSION

Tissue engineering provides an alternative for autologous or allogeneic tissue transplantation, which circumvents problems associated with the limited number of sites and donors, and the risks of transmitting infection. Recently developed skin equivalents are superior to compression therapy for refractory venous ulcers, and acute wounds. These products behave in a similar way to autografts. The development of a new tissue-engineered skin, which contain cells possessing selected functions is a target made possible by genetic engineering.

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