Clinical Applications of Skin Substitutes

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**ANATOMY AND FUNCTION OF SKIN**

The skin is the largest and one of the most vital organs in the human body. It serves as a protective barrier to the outside world and plays a key role in thermoregulation. At a more basic level, the skin is a bilayer composed of an avascular epidermal layer interdigitating with and overlying a vascularized dermal layer. The epidermis is composed of primarily of keratinocytes that undergo a constant cycle of proliferation at the

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stratum germinativum and gradual apoptosis at the stratum granulosum. A scaffold of anuclear keratinocytes forms the most superficial stratum corneum. During migration from the stratum germinativum to the stratum corneum, keratinocytes are connected by cell junctions called desmosomes that contribute to the mechanical strength of the epidermis.

The dermis is composed of collagen, glycosaminoglycans, and elastin fibers that provide elasticity and tensile strength to the skin. Within the dermis, there is a region of loose areolar connective tissue with papillae that extends toward the dermis. Deep to this is the reticular dermis, which contains larger collagenous fibers that are multidirectional. Between the dermis and epidermis lies the basement membrane, which regulates transport. Many interesting aspects of skin anatomy, immunology, and physiology play a role in the maintenance of this vital organ. A unique understanding of the components of mammalian skin has led to the development of numerous skin substitutes. These skin substitutes attempt to compensate for functional and physiologic deficits present in damaged tissue.

A wound is present when areas of skin are missing. Without covering, humans are subject to local and systemic infections. For small areas of skin loss, wound closure occurs by wound contraction and ingrowth of cells. Larger skin loss needs to be covered with skin grafts. The twin problems of skin graft donor site scarring and lack of skin graft in large burn patients motivated the development of skin substitutes.

CURRENT PRODUCTS

**Allografts: Gammagraft, GraftJacket**

An allograft is material derived from a genetically nonidentical donor of the same species. Allografts are clinically indicated when donor source of autografts is limited. Allografts for wound care were first described in the literature in the early 1800s. In 1871 George David Pollock grafted a piece of his own skin to one of his patients. Throughout the 1900s allografts continued to gain popularity and today are used in most burn centers throughout the world. Cellular elements in allografts undergo immunologic rejection but structural elements, such as the dermal scaffold, can remain without rejection.

Allografts are effective in preventing loss of water, electrolytes, proteins, and heat from the wound bed. They also provide a mechanical barrier that reduces microbiologic infiltration and contamination. In patients with large total body surface area (TBSA) burns, allografts can serve as a temporary skin substitute. The literature demonstrates that patients treated with allografts have shorter in-hospital stays and more favorable wound beds for secondary autografting. Despite these advantages, concerns remain regarding rejection, disease transmission, sensitization of the recipient, and cost of allografts. Before implementation of more stringent screening methods, rare transmission of hepatitis B and C was reported. To date, there is at least one documented transmission of HIV from an improperly screened donor to recipient. The addition of gamma irradiation sterilization likely eradicates allograft disease transmission but may cause damage to the underlying protein structure of the skin.

Commercially available allografts previously required storage at 4°C with an effective shelf-life of 7 to 10 days. More commonly, allografts are cryopreserved and stored for prolonged time periods. Today, ready-to-use gamma-irradiated allografts, such as Gammagraft (Promethean LifeSciences, Inc, Pittsburgh, PA), can be stored at ambient temperature with a shelf-life of 2 years.

Gamma-irradiated skin allograft can be used to treat venous stasis ulcers, chronic wounds, diabetic foot ulcers, and burn patients. The graft is applied dermis-side facing the wound, and care must be taken not to disturb the bilayer structure. Following
application, a nonadherent dressing and gauze is placed to protect the graft. The graft is evaluated in 24 to 48 hours to ensure take. There remains a lack of randomized prospective data on gamma-irradiated human skin allograft, although several case series are reported in the management of chronic wounds.9

GraftJacket (Wright Medical Technologies Inc, Arlington, TN) is a human skin-derived acellular dermal equivalent product available in sheet (GraftJacket regenerative tissue matrix [RTM]) and micronized (GraftJacket Xpress flowable soft tissue scaffold) forms. Donor human skin is decellularized using a proprietary process, preserving structure and bioactive agents within the dermal matrix that may support host cell repopulation. GraftJacket has been reported in the management of a wide range of wounds, from diabetic foot ulcers to rotator cuff repairs.10

GraftJacket RTM is shipped freeze dried and should be stored at 1°C to 10°C for a maximum shelf-life of 2 years. It comes prefenestrated in a 1:1 ratio. At time of use, GraftJacket RTM should be rehydrated in normal saline or lactated rings solution for 10 to 15 minutes and must be used within 4 hours of rehydration. GraftJacket is then applied to the wound site dermal side down (basement membrane side up). Notably, the basement membrane side can be distinguished by its dull appearance, rough texture, and ability to repel blood. The matrix can then be sutured or stapled in place with a moist secondary dressing or alternatively bolstered with negative pressure wound therapy device.11

A multicenter prospective randomized trial enrolled 86 patients with diabetic foot ulcers and assigned patients to a single application of GraftJacket RTM with nonadherent secondary dressing or standard-of-care moist-wound therapy. At 12-week follow-up, patients treated with GraftJacket had higher rate of complete wound healing compared with control subjects (70% vs 46%; P = .03) with odds of healing 2.7 times higher than control group.12

Epidermal Equivalents: Epicel

Epidermal equivalent substitutes seek to restore the epidermal layer of skin. Early excision and grafting of extensive burns has consistently demonstrated improved functional and aesthetic outcomes. In patients with adequate donor site availability, split-thickness skin grafting (STSG) remains the gold standard. However, patients with large TBSA burns may lack sufficient donor tissue surface area. In these patients, epidermal equivalents, such as cultured epidermal autografts (CEAs), provide a valuable alternative to conventional skin grafting.

The ability of the skin to continuously regenerate relies on a subpopulation of rare stem cells and a larger pool of short-lived progenitor cells known as transit amplifying cells.13 In 1975 Rheinwald and Green developed a reliable in vitro protocol for expansion of donor keratinocytes into stratified epithelium with adequate integrity for grafting.14,15 By using this protocol, epidermal cells can be isolated from a 3-cm² area of uninjured donor skin. These cells are then plated on irradiated, inactivated feeder cells. Keratinocytes with colony-forming capacity are allowed to proliferate until the stratified squamous epithelium is approximately 8 to 10 cells thick. This sheet of epithelium is detached with an enzymatic agent, such as dispase or thermolysin, and reattached to a carrier material, such as petroleum gauze. The entire process of CEA growth occurs over the course of 3 to 4 weeks. During that time a 3 to 4 cm² donor tissue can be expanded 5000- to 10,000-fold.16–18

Although CEA is Food and Drug Administration (FDA) approved for greater than 30% TBSA burns, it is most beneficial in the management of patients with burn wounds greater than 75% TBSA. Widespread application of CEA is limited by its fragile nature, inability to withstand infection, variable take, and high cost of production. Because of
its thin structure, CEA demands meticulous and precise application technique. Even when optimally applied, CEA can fail because of shear forces unless the patient is immobilized.19,20 When applied to an inadequately prepared wound bed, CEA has a high susceptibility to bacterial cytotoxins that severely decrease graft-take. Over time, improved clinical outcomes have been observed with CEA use in conjunction with cadaveric allograft.

**Allogenic Skin Equivalents: Apligraf, Dermagraft**

The pathophysiology of chronic wounds poses a particular challenge in their management. Normal wound healing is a complex, concerted process involving parenchymal cells; extracellular matrix; and soluble mediators, such as growth factors and cytokines.21 Chronic wounds can demonstrate several biologic derangements including impaired fibroblast replicative abilities and keratinocyte migration abilities.22,23 The pathophysiology of impaired wound healing has been particularly well-described in diabetic ulcers, in which compromised epidermal barrier function, loss of cellular response to growth factors, and decreased collagen accumulation have been observed.24–26 Living cellular allogenic skin equivalent products may address these biologic deficits. Compared with the large number of acellular wound healing products, few cellular skin substitutes are commercially available today. These cellular constructs are the closest substitutes to living skin and offer theoretical advantages including production of growth factors and cytokines to actively recruit host cells and stimulate tissue regeneration. The high costs of cellular skin substitutes pose a major constraint. Products of this class may be structurally monolayer or bilayer (epidermal and/or dermal equivalent), and the cellular components may be autologous or allogenic in origin.

Apligraf (Organogenesis Inc, Canton, MA) is a bilayer allogenic skin equivalent product. Human neonatal fibroblasts cultured with bovine type I collagen produce and condense human matrix proteins. The resulting extracellular matrix impregnated with fibroblasts serves as the dermal equivalent layer. Human neonatal keratinocytes are then cultured on top of the dermal layer, and subsequent incubation in an air-liquid interface stimulates cornification. This produces a stratified monolayer of keratinocytes similar to that of the stratum corneum.27,28 The final bilayer product is thought to offer the epidermal protective barrier function and the dermal layer’s growth factors and cytokines that promote tissue regeneration. However, the exact mechanism through which Apligraf promotes wound healing remains poorly understood.29,30

Apligraf is shipped with a shelf-life of 10 days and must be stored at 20°C to 23°C. It is 0.75 mm thick and has physical properties compatible with meshing or fenestration. Before use, adequate debridement of the wound bed to prevent infection or ischemia is critical. Apligraf is then placed dermal-side down over the wound; fixed in place with sutures, Steri-Strips, or glue; and covered with nonadherent dressing. Apligraf can be reapplied as needed every 4 to 6 weeks depending on wound type, location, and physician preference.

The effectiveness of Apligraf has been most closely studied in the management of chronic venous and diabetic foot ulcers. In one multicenter prospective randomized study, 240 patients with chronic venous ulcers were assigned to compression therapy with Apligraf or compression therapy alone. Among patients with venous ulcers greater than 1 year in duration, Apligraf treatment was three times more likely to achieve wound closure by 8 weeks (32% vs 10%; P = .008) and two times more likely by 6 months (47% vs 19%; P = .002).31 Similarly, another multicenter prospective randomized study assigned 208 patients with diabetic foot ulcers to Apligraf or standard saline-moistened gauze treatment. Notably, this trial used weekly application of Apligraf for up to 4 weeks. At the 12-week follow-up visit, patients treated with Apligraf had
higher rates of complete wound closure (56% vs 38%; \( P = .0042 \)) and shorter median time to wound closure (65 vs 90 days; \( P = .0026 \)). The odds ratio for complete wound closure in the Apligraf group compared with control group was 2.14 (95% confidence interval, 1.23–3.74) with no increased rate of adverse events. An international multicenter study with similar experimental parameters also supported these findings.

Dermagraft (Advanced Biohealing Inc, La Jolla, CA) is a monolayer allogenic dermal equivalent product indicated for treatment of full-thickness diabetic foot ulcers greater than 6 weeks in duration. Non–FDA-approved uses have been reported in the management of chronic venous ulcers, fasciotomy wounds, buccal fat pad graft donor site healing, pediatric postsurgical abdominal wound healing, and vestibuloplasty. Human fibroblasts cultured in polyglactin mesh scaffold produce dermal matrix proteins, collagen, growth factors, and cytokines. The resulting dermal matrix, containing metabolically active fibroblasts, serves as the dermal equivalent substrate.

Dermagraft is cryopreserved (must be stored at \(-75^\circ C\)) and shipped with a shelf-life of 6 months. It is available in 5 cm × 7.5 cm sheets intended for single-use application. Ongoing wound bed infection and hypersensitivity to bovine proteins are contraindications for most skin substitutes, including Dermagraft and Apligraf. At time of application, the wound bed should be debrided and prepared to a condition acceptable for living skin graft. Dermagraft is then thawed in 34°C to 7°C water bath for 2 minutes and rinsed in normal saline. The product must be used within 30 minutes of thawing. Dermagraft is then cut, implanted into the wound, and covered with nonadherent moist dressing. In clinical trials, up to eight applications have been used over a 12-week period.

A large multicenter prospective randomized trial investigated the use of Dermagraft in the treatment of chronic diabetic foot ulcers. The study enrolled 314 patients who were randomized to Dermagraft plus conventional saline-moistened gauze dressings or conventional therapy alone. During the 12 week follow-up, patients in the Dermagraft treatment arm received up to eight applications of Dermagraft. At the 12-week follow-up visit, patients in the Dermagraft treatment group had a higher rate of complete wound closure compared with control subjects (30.0% vs 18.3%; \( P = .023 \)). Furthermore, median percent wound closure was greater in the Dermagraft group compared with control subjects (91% vs 78%; \( P = .044 \)). Incidence of adverse events was comparable for both treatment groups, but Dermagraft patients had fewer ulcer-related adverse events (local wound infection, cellulitis, and osteomyelitis) compared with control subject (19% vs 32.5%; \( P = .007 \)).

**Dermal Templates: Integra, Terudermis (Japan), Pelnac (Japan), Matraderm (Europe)**

Collagen is the major structural protein of mammalian connective tissue. In 1954 Ramachandran published his work on the triple helical structure of collagen. Despite extensive publications on the molecular structure of collagen, its application in biomaterials remained limited. In the 1970s Yannas and Burke jointly designed a collagen scaffold for dermal replacement. When type I collagen was exposed to acid at a pH of 3, the collagen scaffold developed increased swelling and porosity. The optimum parameters to produce a polymeric matrix that could support cellular ingrowth, revascularization, and neodermis formation led to the development of dermal templates, such as Integra (Integra LifeSciences Corporation, Plainsboro, NJ), PELNAC (Gunze Co, Kyoto, Japan), and Matriderm (Dr Suwelack Skin & Health Care AG, Billerbeck, Germany). Integra is composed of cross-linked type I bovine collagen coprecipitated with chondroitin-6-sulfate, which is covered with a silicone elastomer. PELNAC and Terudermis (Olympus Terumo Biomaterials Corp, Japan) are similarly composed of a collagen matrix with silicone top layer commercially available in Japan, South Africa,
Australia, and New Zealand. Matriderm, a thin (1 mm) single-layer dermal matrix composed of collagen I, III, and V, has been marketed as a single-stage dermal template for reconstruction. Integra has recently released a thin single-layer dermal template.

The use of dermal templates to successfully manage wounds is predicated on adequate debridement. Because dermal templates have limited capacity to fight infection, application of dermal templates on contaminated wounds results in a high risk for infection. After application, fibroblasts, endothelial cells, and inflammatory cells migrate into and repopulate the dermal template, eventually replacing the scaffold. After template integration, in large wounds, a thin STSG can be applied for wound coverage. When a thin scaffold, such as Matriderm or thin Integra, is used, the dermal template can be grafted at the time of dermal template application in a well-vascularized wound.

Although dermal templates were initially used to manage extensive burns, uses continue to expand. Most recently the FDA extended dermal template indications to include chronic lower and upper extremity wounds and traumatic wounds. In one prospective trial, patients with bilateral acute full-thickness burns on the dorsum of hands were randomized to receive conventional STSG on one hand and a one-step dermal template with STSG on the contralateral hand. There was no difference in graft-take between the two groups (P = .02), and the dermal template group demonstrated superior active range of motion (P = .02). In another study, Integra application in lower extremity wounds with exposed bone demonstrated a 91% overall implant-take rate and 80% skin graft success rate. Additional studies have demonstrated similarly positive outcomes for other complex wounds.

**Xenografts: EZ Derm, Mediskin**

Xenografts are biologic material transplanted from one species to a different species. Over the years, xenograft skin substitutes have been sourced from various species, including frog, dog, and pig. Today, porcine xenografts are most commonly used. When compared with cadaveric skin or autografts, xenografts offer the advantage of readily available supply. Furthermore, xenografts may be preferred in settings where access to human dermis is limited because of cultural beliefs.

Porcine xenografts are indicated for temporary coverage of wounds, such as partial-thickness burns and autograft donor sites. It has also been reported in the management of exfoliative diseases, including Stevens-Johnson syndrome and toxic epidermal necrolysis. Allergy to porcine materials is the major contraindication to use of porcine xenografts. EZ Derm (Mölnlycke Health Care AB, Gothenburg, Sweden) and Mediskin (Mölnlycke Health Care) are examples of commercial porcine xenograft products. However, many medical centers opt to process their own xenografts on site. Porcine xenografts can be used fresh or processed and preserved for reduced antigenicity and convenient storage. After standard wound bed preparation, the xenograft is applied directly to the wound site and covered with nonadherent dressing. With proper application the xenograft adheres to the wound in approximately 1 or 2 days. The xenograft can be left in place until the wound re-epithelializes, eventually causing the porcine skin to separate and fall off. Alternatively, the xenograft is exchanged at regular intervals (every 1–4 days have been reported). Studies have shown that temporary coverage of partial-thickness burns with xenograft decreases healing time, reduces pain, and decreases bacterial overgrowth.

**Clinical Products Not Currently Available: TransCyte, OrCel**

The skin substitute market is dynamic, and several products not currently on the market are worth noting. TransCyte (Advanced BioHealing) is an allogenic dermal
equivalent product. Production of TransCyte halted when the previous manufacturer declared bankruptcy, and although production of its sister product Dermagraft has resumed, TransCyte remains commercially unavailable. TransCyte begins as a nylon mesh bonded to silicone membrane, with the latter serving as a protective, semipermeable epidermal layer. Neonatal fibroblasts are then cultured on the nylon mesh covered with porcine dermal collagen and produce growth factors, fibronectin, proteoglycans, and human dermal collagen. The resulting matrix is frozen, halting cellular metabolic activity while preserving the bioactive extracellular matrix and growth factors. TransCyte was indicated for temporary coverage of full and deep partial-thickness burns before autografting or definitive management of middermal burns not requiring autografting.

A randomized, controlled, within-patient paired comparison study enrolled 66 patients with full or deep partial-thickness burns and treated two comparable wounds on the same patient with TransCyte and frozen human cadaver allograft. Both sites subsequently received split-thickness autografts when clinically indicated. Results showed that wounds treated with TransCyte and cadaver allograft had equivalent rates of autograft take on postautograft day 14. TransCyte demonstrated additional benefits of being easier to remove with no epidermal sloughing and less bleeding when compared with cadaver allograft.53 Another prospective randomized trial enrolling 21 patients with mid-partial thickness facial burns showed that TransCyte application resulted in shorter wound care time, shorter re-epithelialization time, and decreased pain levels compared with open wound care with bacitracin ointment.54 When studied in pediatric burn patients, TransCyte application resulted in decreased rate of autografting (1 vs 7 children) and decreased length of hospital stay (5.9 ± 0.9 vs 13.8 ± 2.2 days; \( P = .002 \)) when compared with standard therapy.55

OrCel (Ortec International Inc, New York, NY) is a bilayer allogenic skin equivalent product. OrCel begins as a bovine type I collagen sponge that is porous on one side and nonporous gel-coated on the opposite side. Human neonatal fibroblasts are cultured on the porous side and infiltrate the collagen matrix. Human keratinocytes from the same donor are cultured on the nonporous side, thus giving rise to an epidermal layer.

The fresh form of OrCel has been FDA approved for the treatment of STSG donor site and mitten hand deformity after epidermolysis bullosa. When compared with Biobrane (Smith & Nephew, London, UK), Orcel application was shown to accelerate STSG donor site healing and enabled earlier recropping.56 However, the manufacturer discontinued sales of this product to focus on FDA approval of cryopreserved OrCel, which boasts a shelf-life of 6 months compared with 3 days for fresh Orcel. As of 2007, the company completed pivotal clinical trials for the use of cyropreserved OrCel in management of chronic venous ulcers and filed a premarket approval application with the FDA for this indication.

DISCUSSION

The diversity of skin substitutes on the market today represents the culmination of modern advancements in wound healing biology and bioengineering technologies (Fig. 1). These products range from acellular synthetic dermal templates to living cellular bilayer skin equivalents and offer unique combinations of mechanical and biologic properties that support wound healing. Given that every skin substitute has different sets of strengths and weaknesses, it is critical that clinicians select optimal products for specific wound indications.
For example, such products as Integra are readily available, have a long shelf-life, and offer barrier protection against evaporative losses and infection. As a result, Integra is a good candidate for temporary wound coverage for large TBSA burn patients who lack sufficient autograft donor sites. However, such products as Apligraf offer a metabolically active cellular composite structure that more closely recapitulates normal skin architecture. These properties have proved to be effective in the treatment of difficult chronic wounds, such as venous ulcers or diabetic foot ulcers. However, Apligraf is expensive, has a short shelf-life, and may require multiple applications to achieve wound closure.
Looking to the future, the ideal skin substitute should strive to encompass the following properties:

- Improved host tissue regeneration
- Barrier protection and resistance to local infection
- Resistance to shear forces
- Improved cosmesis
- Easy handling and application
- Long shelf-life
- Cost-effectiveness
- Control pigmentation
- Enable regeneration of adnexal structures, such as hair follicles and sweat glands

Of note, increasing costs associated with new technologies continues to be an area of concern and scrutiny in the healthcare landscape. Several cost analyses suggest that despite high initial costs, skin substitute use can shorten time to wound closure and decrease wound-associated morbidities, resulting in overall cost reduction when compared with standard-of-care therapy.\(^{57,58}\)

Finally, the success of skin substitutes is ultimately predicated on the basic principles of wound management. Before application, clinicians must address any underlying causes of poor wound healing, perform adequate debridement, achieve infection control, and restore perfusion as needed. Skin substitutes, when appropriately applied in optimized settings, offer a promising solution to difficult wound management.

The body of literature on skin substitutes increases as the understanding of tissue engineering and molecular biology expands. Although more studies are published annually, studies conducted to date are limited by their small sample size, variability in outcome measurements, and methodologic inconsistencies. Furthermore, the proprietary nature of products available may lead to some degree of bias toward reporting positive outcomes. Given the high cost of these products, future randomized large prospective studies are needed to guide the clinical applications of skin substitutes.

REFERENCES


