

Wound Treatment with Human Skin Equivalent

Sumit K. De, BA*
Ernane D. Reis, MD†
Morris D. Kerstein, MD‡

Skin grafting provides an effective means of closing chronic wounds. Autografts and allografts are used most often in skin grafting, but Apligraf, a tissue-engineered bilayered human skin equivalent, provides another safe and effective grafting option for treating diabetic, venous, and pressure ulcers. This skin equivalent has an epidermis and dermis similar to human skin, largely due to its derivation from neonatal foreskin. Apligraf is also easily accessible and has shown little immunoreactivity. (J Am Podiatr Med Assoc 92(1): 19-23, 2002)

Chronic wounds represent a special challenge to physicians. A lack of immediate attention to these wounds can severely increase the chance of infection and other complications. Skin grafts are often required for healing such wounds. Except for the use of the bilayered human skin equivalent, Apligraf (Novartis Pharmaceuticals Corp, East Hanover, NJ), almost all skin graft options have risks, including delaying treatment, causing new trauma to the skin of the patient or donor, and immune rejection.¹ Apligraf has many of the characteristics deemed integral in skin-graft therapy, such as availability and accessibility, lack of immunoreactivity, and likeness to human skin.^{2,3}

The function of the human skin depends on the integrity of three layers: the epidermis, the dermis, and a subcutaneous layer. Essentially, the epidermis protects against water loss and infection; the dermis provides mechanical strength and elasticity and its vasculature supplies nutrients to the epidermis; and subcutaneous tissue provides cushion and helps control body temperature. Attempts to manufacture a skin substitute have focused on trying to mimic the properties of these layers.⁴

*Submitted during second year, University of Michigan Medical School, Ann Arbor, MI.

†Assistant Professor, Department of Surgery, Mount Sinai Medical Center, New York, NY.

‡Professor and Vice Chairman, Department of Surgery, Mount Sinai Medical Center, New York, NY. *Mailing address:* 1214 Valley Rd, Villanova, PA 19085-2124.

In acute wound healing, as in surgical incisions, sequential reparative events occur within a relatively short time and ultimately establish a sustained anatomic and functional result.⁵ Initially, platelets attach and aggregate at the site of the insult and release procoagulants and growth factors. Within hours, neutrophils and macrophages drive an inflammatory response. Proliferation then occurs, followed by neovascularization and collagen deposition by recruited fibroblasts. Epithelialization occurs at the wound surface. Concurrently, remodeling and contraction begin, facilitated by collagenases and other proteolytic enzymes.

The Wound Healing Society defines chronic wounds as wounds that have “failed to proceed through an orderly and timely process to produce anatomic and functional integrity, or proceeded through the repair process without establishing a sustained anatomic and functional result.”⁶ Chronic wounds have an increased susceptibility to infection and a prolonged, sometimes interrupted, healing process.⁷ Numerous studies have shown that the growth factors necessary for proper wound healing are not present in chronic wounds; however, chronic wounds treated with Apligraf appear to have these growth factors at levels necessary for proper healing.

Apligraf is a living skin equivalent composed primarily of Type I bovine collagen and keratinocytes and fibroblasts derived from neonatal foreskin and grown in culture. The bilayered structure of the graft

resembles the outer layers of the human skin. The keratinocytes form an epidermis that is similar to the human epidermis and can block water loss and infection. The fibroblasts enmeshed in the collagen matrix form the dermal layer.

Although the healing mechanisms that it induces have yet to be clarified, Apligraf is believed to act as a "smart material" by interacting with adjacent tissue when implanted.³ The immediate action of Apligraf, as in other skin grafts, is to provide coverage to the wound, satisfying an important requisite in wound care. In addition, Apligraf may promote healing by stimulating production of numerous mediators such as interleukins,^{1, 3, 6, 8} transforming growth factors (TGF) α and β , granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, and basic fibroblast growth factor.^{8, 9} These signals are all known to be products of epidermal and dermal fibroblasts. Similarities between the dermal layers of host and graft explain the crossover activity of mediators noted in Apligraf treatments.^{2, 10} The dermal component of the human skin equivalent is thought to respond to signals from both the host and graft dermal cells.

Apligraf has been noted to act through at least three modes of healing: secondary intention, persistent wound closure with underlying healing, and frank graft take. Although Apligraf is regarded as a bilayered construct, the two layers are made up of many layers, including a dermal lattice; multiple layers of live keratinocytes; and a tough, dead stratum corneum similar to natural skin.¹ Whether it is by local production of growth factors, stimulation of growth-factor interactions, matrix deposition and degradation, biologic wound coverage, or direction of responsive cells, the potential of Apligraf to beneficially alter conditions in chronic wounds appears impressive. The skin equivalent has been shown to stimulate cytokines. Chronic wounds are often unable to produce a variety of normal cell signals, and venous ulcers have been shown to be unresponsive to TGF α 1, possibly due to decreased receptor expression.^{9, 11} Apligraf provides a rich environment for tissue differentiation to occur through cell-matrix, cell-cell, and cell-environment interactions lacking in other media that do not use a similar construct.⁹

For tissue transplantation to succeed, donor and recipient antigens must be compatible. At the least, the donor should not contain any new antigens to the host. Cultured epithelial allografts may explain why Apligraf elicits few immunologic responses.² The actual pathways necessary for an immune response may not function properly or may not be present, as is the case with Langerhans' cells, which act as anti-

gen-presenting cells in the skin.^{2, 12} Repeated applications of keratinocyte sheets show treated wounds tend to heal from their edges, supporting the ability of allogeneic grafts to serve as wound-repair stimuli.¹³

Infection, especially with an aerobic microorganism, is one of the most frequent complications of chronic wounds. The significance of these infections should not be underestimated as they often hasten the damage inflicted by a wound. Ulceration provides the micro-organisms with access to microcirculation; therefore, an open wound can lead to systemic infection. Although antibiotics can aid in treating wound infections, reestablishing the skin barrier by using the human skin equivalent is an obvious benefit.^{3, 12} As compared to other grafts, the clear advantage of the human skin equivalent is that it eliminates the pain associated with donor sites as well as the potential scarring and hyperpigmentation on the patient donor site.¹⁴

Edema, pain, venous dilation, and skin ulceration are associated with chronic wounds. The skin may appear brown and thickened due to lipodermatosclerosis, which makes the skin hard and nonpliant. In general, ulcers caused by trauma are observed in the anterior tibial area, whereas most other venous-related ulcers occur in the malleolar regions.¹⁵

The importance of finding an effective way to treat chronic wounds is more evident as the elderly population grows at an unprecedented rate. The number of patients in the United States suffering from venous, diabetic, and pressure ulcers is 3 to 6 million¹⁶ and treatment for these ulcers costs billions of dollars annually. Healing of pressure ulcers at the stage II level (superficial ulcers exhibiting partial-thickness skin loss, including epidermis, dermis, or both) has been noted to be as low as 26% after 6 months.¹⁷ Furthermore, diabetic patients with foot ulcers are prone to lower-extremity amputation, with a mortality rate of at least 39% over 5 years.¹⁸ These facts support the necessity of developing dependable therapies for chronic wounds, and it is clear that education alone cannot halt their rising prevalence.

Pressure ulcers are a common problem affecting an estimated 17% of hospitalized patients.¹⁹ Pressure ulcers are areas of tissue necrosis that result from extended periods of pressure, particularly from compressive and shearing forces.²⁰ Tolerance for pressure and oxygen levels within a given tissue influences the development of these ulcers. Patients are often debilitated and dependent on others for care. Pressure ulcers are directly linked to increased mortality rates in chronic illness. These ulcers develop in elderly patients who are bedridden or have decreased mobility, such as victims of paralysis, and those re-

covering from orthopedic or reconstructive surgery. Unfortunately, relieving pressure often entails major lifestyle adjustments and recommendations to patients on how to avoid pressure ulcers often go unheeded.²¹

Twenty percent of diabetic patients in the United States suffer from foot ulceration, and one-fifth of diabetes-related hospital admissions are related to foot ulcers.^{16, 22} Furthermore, of the more than 50,000 patients who undergo lower-extremity amputation each year, a majority have foot ulcers caused by peripheral neuropathy and peripheral vascular disease, which are common complications of diabetes.^{23, 24} These conditions are associated with sensory loss that facilitates development of painless chronic ulcers. Repetitive foot stress, such as a normal day of standing and walking, aggravates these wounds often without the patient realizing it, allowing development of an equinus contracture at the ankle and clawing of the toes that produces the classic Charcot's foot.²⁵ Peripheral neuropathy also contributes to foot hypoxia and decreases healing ability, resulting in wounds that are highly susceptible to infection.⁷ Diabetes is a known risk factor for vascular disease characterized by arterial wall calcification and thickening of the capillary basement membranes. These changes decrease arterial compliance and increase capillary permeability. High glucose levels can compromise the immune system; nonenzymatic glycosylation alters the chemotaxis of neutrophils. Edema, hyperglycemia, and lack of insulin all contribute to bacterial growth.

The American Diabetes Association recommends treating diabetic ulcers with thorough debridement of necrotic tissue; treatment of infection; nonweight-bearing or offloading techniques to relieve pressure in the lower extremity; and revascularization, if necessary.²⁶ Topical treatments of diabetic ulcers have evolved over the last decade with the introduction of total contact casting, topical antibiotics, enzymatic debridement, hyperbaric oxygen, growth factors, and moisture-maintaining wound dressings that do not disrupt the granulation tissue. None of these topical therapies has gained wide acceptance and the classic treatment of regular application of gauze soaked in saline remains an integral part of most treatments.¹⁵

Venous ulceration is highest among patients older than 65 years of age; and mortality and morbidity are significant in younger patients.²² Thirty percent of the 55- to 74-year-old age group, or an estimated 7 million people, are afflicted with chronic venous insufficiency.²⁷ The cost of caring for venous ulcers was reported to be near \$1 billion a decade ago.²⁸ Venous ulcers are lower-leg skin excavations, produced by

sloughing of inflammatory and necrotic tissues. The abnormality is found in venous drainage of the lower extremity that is associated with stasis and hypoxia. Venous hypertension can be documented in approximately 70% of patients with venous ulcers.^{27, 29} Defective valves in the communicating, superficial, and deep veins, and thrombosis of the deep vein system are the most frequently identified causes of venous ulcers.^{27, 30} Defective valves lead to an increase in venous pressure, dilated veins, and insufficient venous drainage.³⁰ Venous hypertension leads to capillary stasis and increased permeability. The capacity of blood to carry oxygen and nutrients is decreased, which leads to tissue necrosis, cell death, and ulceration. These manifestations are often described as postphlebotic syndrome. Other risk factors for development of venous ulcers include congestive heart failure, obesity, pregnancy, muscle weakness, and occupations that require prolonged standing or sitting.

Clinical Applications

Contraindications for the use of human skin equivalent include clinically infected wounds, allergies to bovine collagen, and hypersensitivity to the agarose shipping medium. Moreover, the effectiveness of Apligraf in skin wounds cannot be obtained in other types of tissue, such as muscle, which are often involved in deep chronic ulcers.

Procedures involved in the application and care of Apligraf do not require extensive training. Pretreatment for Apligraf is similar to other grafts. After thorough debridement and irrigation of the wound, an Apligraf patch can be applied on top of the underlying tissues, including bone and subcutaneous tissue. Adjustments to the size of the graft can be made using scissors and the graft can be sutured in place. Apligraf is usually covered with a transparent film dressing and a nonadherent pad. Visualization of the underlying tissue is not uncommon after placement. For cushioning and maintenance of position, cotton gauze or foam can provide bolstering as a secondary dressing. This dressing can be kept in place using self-adherent elastic wrap or some other form of compression, such as an Unna boot. The grafted wound is usually examined after 3 days, redressed, and checked weekly until full healing is achieved. Depending on the progress of healing, the treated wound may have to be treated by a health-care professional more than once a week. A reapplication of Apligraf is possible for slowly healing ulcers, and it should be considered if less than half of the graft has taken by 6 weeks.^{18, 31} When a reapplication is performed, it is important not to remove any of the skin

that has attached. Nonadherent portions of the human skin equivalent should be removed and their underlying areas cleansed appropriately.

Clinical studies have shown that Apligraf is more efficient at wound healing and closure than other conventional methods, such as compression therapy. Moreover, most of these studies have shown that Apligraf, on average, healed chronic wounds more frequently and at a faster rate (approximately in 60 days) than compression therapy, which required approximately 180 days to achieve the same results.^{10, 18, 21, 26}

Conclusion

The promise of Apligraf, the first effective human tissue-engineered replacement treatment to gain medical acceptance and approval by the US Food and Drug Administration, is extraordinary. In the future, the benefits of Apligraf could be enhanced by combining its use with drug therapies, such as recombinant human platelet-derived growth factor, which has also been shown to hasten the healing process.³² Optimal wound healing should consist of a combination of several therapies and medications, depending on the nature of the wound. Patients with difficult-to-cure chronic wounds are the most likely beneficiaries from such a shift in treatment.

All wounds should be treated as early as possible. Patients suffering from diabetic, venous, and pressure ulcers require special attention because their healing mechanisms are impaired. Removal of infectious agents and stimulation of the healing process are essential to blocking the progression of these wounds. The addition of Apligraf as a treatment for chronic wounds represents a powerful way not only to block the progression and decrease complications of these wounds, but also to accelerate healing time.

Acknowledgment. Gae O. Decker-Garrad for editorial assistance.

References

1. TRENT JF, KIRSNER RS: Tissue engineered skin: Apligraf, a bi-layered living skin equivalent. *Int J Clin Pract* **56**: 408, 1998.
2. PARENTEAU NL, BILBO P, NOLTE CJ, ET AL: The organotypic culture of human skin keratinocytes and fibroblasts to achieve form and function. *Cytotechnology* **9**: 163, 1992.
3. SABOLINSKI ML, ALVAREZ O, AULETTA M, ET AL: Cultured skin as a "smart material" for healing wounds: experience in venous ulcers. *Biomaterials* **17**: 311, 1996.
4. MARTIN P: Wound healing: aiming for perfect skin regeneration. *Science* **276**: 75, 1997.
5. KERSTEIN MD: The scientific basis of healing. *Adv Wound Care* **10**: 30, 1997.
6. LAZARUS GS, COOPER DM, KNIGHTON DR, ET AL: Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol* **130**: 489, 1994.
7. CORNWALL JV, DORE CJ, LEWIS JD: Leg ulcers: epidemiology and aetiology. *Br J Surg* **73**: 693, 1986.
8. NANGIA A, GAMBHIR R, MAIBACH H: Factors influencing the performance of temporary skin substitutes. *Clin Mater* **7**: 3, 1991.
9. TARLTON JF, BAILEY AJ, CRAWFORD E, ET AL: Prognostic value of markers of collagen remodeling in venous ulcers. *Wound Repair Regen* **7**: 347, 1999.
10. FALANGA V, MARGOLIS D, ALVAREZ O, ET AL: Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent: human skin equivalent investigators group. *Arch Dermatol* **134**: 293, 1998.
11. AUGER FA, ROUABHIA M, GOULET F, ET AL: Tissue-engineered human skin substitutes developed from collagen-populated hydrated gels: clinical and fundamental applications. *Med Biol Eng Comput* **36**: 801, 1998.
12. LAING P: The development and complications of diabetic foot ulcers. *Am J Surg* **176** (suppl 2A): 11S, 1998.
13. KATZ AB, TAICHMAN LB: Epidermis as a secretory tissue: an *in vitro* tissue model to study keratinocyte secretion. *J Invest Dermatol* **102**: 55, 1994.
14. FALANGA V: Apligraf treatment of venous ulcers and other chronic wounds. *J Dermatol* **25**: 812, 1998.
15. ANGLE N, BERGAN JJ: Chronic venous ulcer. *BMJ* **314**: 1019, 1997.
16. ALLMAN RM: Pressure ulcer prevalence, incidence, risk factors, and impact. *Clin Geriatr Med* **13**: 421, 1997.
17. ALLMAN RM: Outcomes in prospective studies and clinical trials. *Adv Wound Care* **8** (suppl): 61, 1995.
18. BREM H, BALLEUX J, BLOOM T, ET AL: Healing of diabetic foot ulcers and pressure ulcers with human skin equivalent: a new paradigm in wound healing. *Arch Surg* **135**: 627, 2000.
19. RATLIFF CR, RODEHEAVER GT: Pressure ulcer assessment and management. *Lippincotts Prim Care Pract* **3**: 242, 1999.
20. DEFLOOR T: The risk of pressure sores: a conceptual scheme. *J Clin Nurs* **8**: 206, 1999.
21. DOLYNCHUK K, HULL P, GUENTHER L, ET AL: The role of Apligraf in the treatment of venous leg ulcers. *Ostomy Wound Manage* **45**: 34, 1999.
22. NELZEN O, BERGQVIST D, LINDHAGEN A, ET AL: Chronic leg ulcers: an underestimated problem in primary health care among elderly patients. *J Epidemiol Community Health* **45**: 184, 1991.
23. BOULTON AJ, MENESES P, ENNIS WJ: Diabetic foot ulcers: a framework for prevention and care. *Wound Rep Reg* **7**: 7, 1999.
24. DANIELS TR: Diabetic foot ulcerations: an overview. *Ostomy Wound Manage* **44**: 76, 1998.
25. LEVIN M: Diabetic foot wounds: pathogenesis and management. *Adv Wound Care* **10**: 24, 1997.
26. PHAM HT, ROSENBLUM BI, LYONS TE, ET AL: Evaluation of a human skin equivalent for the treatment of diabetic foot ulcers in a prospective, randomized, clinical trial. *Wounds* **11**: 79, 1999.
27. LANGEMO DK: Venous ulcers: etiology and care of patients treated with human skin equivalent grafts. *J Vasc Nurs* **17**: 6, 1999.

28. RUDOLPH DM: Pathophysiology and management of venous ulcers. *J Wound Ostomy Continence Nurs* **25**: 248, 1998.
29. BLACK SB: Venous stasis ulcers: a review. *Ostomy Wound Manage* **41**: 20, 1995.
30. FITZPATRICK JE: Stasis ulcers: update on a common geriatric problem. *Geriatrics* **44**: 19, 1989.
31. EAGLSTEIN W, IRIONDO M, LASZLO K: A composite skin substitute (graftskin) for surgical wounds: a clinical experience. *Dermatol Surg* **21**: 839, 1995.
32. KNIGHT EV, OLDHAM JW, MOHLER, MA, ET AL: A review of nonclinical toxicology studies of becaplermin (rhPDGF-BB). *Am J Surg* **176** (suppl 2A): 55S, 1998.