The Use of Tissue-Engineered Skin (Apligraf) to Treat a Newborn With Epidermolysis Bullosa

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Background: Inherited epidermolysis bullosa (EB) is a mechanobullous disorder. The Dowling-Meara variant, a subtype of EB, is characterized by widespread blister formation that may include the oral cavity and nails. Many patients with the Dowling-Meara phenotype are at increased risk of sepsis and death during infancy. The treatment of EB is generally supportive. The tissue-engineered skin used (Apligraf) is a bilayered human skin equivalent developed from foreskin. It is the only Food and Drug Administration–approved skin equivalent of its kind. It is approved for the treatment of venous ulcers of the lower extremities. It has also been used to treat acute wounds, such as graft donor sites and cancer excision sites.

Observation: To our knowledge, we describe the first case in which a newborn with EB, Dowling-Meara variant, was treated with bilayered tissue-engineered skin. The areas treated with the tissue-engineered skin healed faster than the areas treated with conventional therapy. Most of the areas treated with tissue-engineered skin have remained healed, without developing new blisters. These areas appear to be more resistant to trauma.

Conclusions: Our early success with tissue-engineered skin in this patient may have a significant impact on the future treatment of neonates with EB simplex. Future studies are needed to determine if the beneficial effects of tissue-engineered skin are reproducible in other neonates with EB simplex and in patients of all ages with different subtypes of EB.

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Epidermolysis bullosa (EB) is a mechanobullous disorder characterized by blister formation as a result of minor trauma to the skin. Although more than 20 subtypes have been described, EB can be grouped into 3 major types according to the level of blister formation. In EB simplex, the blister occurs within the epidermis; junctional EB refers to blister formation at the dermoepidermal junction, and EB dystrophica refers to blister formation below the basement membrane in the dermis. Onset is early in life, often during the neonatal period or infancy. With the exception of the acquisita type, EB is an inherited disorder, and the disease may be mild or severe, localized or generalized. The blisters may heal with disfiguring scarring or with no scarring at all. Epidermolysis bullosa simplex is characterized by mechanical fragility of the basal cells of the epidermis, and this fragility has been linked to mutations in the keratin K5 or K14 genes. Epidermolysis bullosa simplex, Dowling-Meara variant, is characterized by widespread blister formation that may include the oral cavity, airway, esophagus, and nails. The blisters usually occur as a result of trauma, pressure, or even slight friction. Many patients with the Dowling-Meara variant are at an increased risk of sepsis and death during infancy. The treatment of EB is generally supportive. Sterile dressings, antibiotics, nutrition, and analgesics have been the mainstays of therapy. Other treatments reported include systemic psoralen–UV-A (PUVA) therapy, a neuroleptic agent (pipamperone), corticosteroids, and prolonged exposure to a warm environment.

Tissue-engineered skin (Apligraf, developed and manufactured by Organogenesis Inc, Canton, Mass), is a bilayered human skin equivalent developed from neonatal foreskin. The skin equivalent contains living foreskin-derived keratinocytes and fibroblasts and bovine collagen. It is the only Food and Drug Administration–approved skin equivalent of its kind. It is approved for the treatment of venous ulcers. It has also been used to treat acute wounds, such as graft donor sites and cancer excision sites. Its characteristics are similar to those of normal human skin morphologically, biochemically, and metabolically. However, it does not contain blood vessels or appendages. Although it is an allogenic graft, tissue-engineered skin is believed to be immunologically inert because it does not contain Langerhans cells, endothelial cells, and certain cell surface markers. It also has a high proliferative potential.

To our knowledge, we describe the first case in which a newborn with EB sim-
Epidermolysis bullosa simplex, Dowling-Meara variant, is characterized by generalized blister formation and is usually present at birth. It may include oral and nail involvement. Patients with this disease are at a high risk of death during the first year of life because of widespread denudation of the skin and an increased risk of infection and sepsis. Later in life, they develop herpetiform clusters of blisters, minor scarring with milia, and palmoplantar keratoderma.

There is no known cure for EB, so the treatment is supportive. Sterile dressings, topical and systemic antibiotics, nutritional support, and analgesics have been the mainstays of therapy. To our knowledge, no one has ever attempted to treat a newborn with EB with a tissue-engineered skin equivalent. There have been 2 published clinical reports of keratinocyte allografts being used to treat children with EB. In 1 report, keratinocyte
autografts were used to successfully treat 3 boys with facial erosions caused by junctional EB. However, only 2 of the 3 boys actually had complete reepithelialization, and this was achieved only after a period of 7 to 10 months. A later study tested the effectiveness of keratinocyte allografts on 10 patients with recessive EB dystrophica. This treatment provided little clinical benefit.

Tissue-engineered skin, Apligraf (developed and manufactured by Organogenesis Inc) is the only Food and Drug Administration–approved bilayered human skin equivalent. It is approved for the treatment of venous ulcers. It contains neonatal foreskin keratinocytes, fibroblasts, and bovine collagen and is morphologically, biochemically, and metabolically similar to normal human skin. It has not produced acute rejection reaction clinically, and because it contains living neonatal keratinocytes and fibroblasts, it has a high proliferative potential.

It has been suggested that tissue-engineered skin promotes wound healing in 2 ways: (1) through stimulation of healing of the host wound probably by producing numerous cytokines that promote healing, including interleukins 1, 3, 6, and 8, transforming growth factor α, transforming growth factor β, granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, and basic fibroblast growth factor, or (2) by graft take. In venous ulcers, it most often functions by stimulating host wound healing, while in superficial acute wounds, such as graft donor sites, it most often appears to function by graft take. It is unclear if tissue-engineered skin persists permanently or if it is replaced by host skin.

In our patient, our primary short-term goal was to accelerate the wound healing process to decrease the risk for infection, sepsis, and death. Important secondary goals were to provide comfort and improve the ability of her

Figure 2. A, Wound treated with tissue-engineered skin (Apligraf). B, Tissue-engineered skin applied to a wound site. C, Wound site 3 days after treatment with tissue-engineered skin. Most of the treated area healed by graft take. D, Wound site 21 days after treatment with tissue-engineered skin. Eleven weeks after treatment, the area remains healed, without development of new blisters, and appears the same as in this picture taken 3 weeks posttreatment.
parents to care for her without causing further blistering. We believed that tissue-engineered skin could satisfy these goals based on our positive experience treating patients with venous ulcers and acute wounds. The results thus far have been promising. The patient tolerated all of the grafts and showed no evidence of developing infection or sepsis, even after discontinuation of intravenous and oral antibiotics. Interestingly, healing in most of the tissue-engineered skin–treated sites occurred more rapidly than expected and faster than in the sites treated with gauze covered with petroleum jelly. We have seen no evidence of tissue rejection.

The lack of an immune response against this allogenic graft may be due to the absence of immunogenic antigens and immune response–mediating cells in the tissue-engineered skin. Our patient received the first graft 8 days after birth. Recent studies suggest that this may be an immunologically critical period. These studies have shown that, unlike adults, neonatal mice develop lifelong tolerance to allogenic skin grafts when exposed during the first 3 to 4 weeks of life to allogenic cells of the same donor strain. The reasons for graft tolerance appear to be 2-fold. First, the neonatal immune system has different trafficking patterns for lymphocytes than the system found in adults. Large-scale migration of naive CD8+ T lymphocytes into the skin brings them in contact with skin-specific antigens. By preventing T-lymphocyte migration into the neonatal skin, researchers were able to prevent the development of tolerance to these antigens. Second, exposure of naive T lymphocytes to antigens expressed by peripheral tissue confers tolerance to the host. This may be due to characteristics of the target tissues in neonatal mice.

In the case we describe, even in sites where the living skin equivalent was applied and did not take, it may have induced healing. As an example, the perianal region and part of the buttocks region were selected as initial sites for treatment. Most of that area healed by graft take, but healing occurred more slowly in the perianal region, possibly because of frequent diaper changes and wiping of the perianal area. However, 100% healing was ultimately achieved.

Our long-term goals are to observe the behavior of the grafted skin, to determine if new blister formation will occur at treated sites, and to study the impact of tissue-engineered skin on the patient’s own skin. Early findings lead us to believe that the tissue-engineered skin healed by graft take, at least in the short-term. Healing was evident 3 days after treatment, because the graft sites looked thick and had a mature feel. In contrast, sites that reepithelialized on their own took longer to heal and passed through a translucent–appearing phase. In addition to the clinical appearance of graft take, the biopsy specimens of the treated sites revealed chromosomal markers identical to the tissue-engineered skin, and electron microscopy revealed a normal epidermis with normal tonofilaments in the treated sites at 6 weeks following treatment. Eleven weeks after treatment, the patient has not developed new lesions in most of the grafted sites (Figure 2, D). These findings and observations further support our belief that tissue-engineered skin functioned like traditional autografts. However, it is conceivable that tissue-engineered skin may have induced changes that allowed the patient’s epidermal cells to make normal tonofilaments. There are little data available regarding the survival time of the foreskin-derived cells in treated patients. Future biopsy specimens will help us determine if treated sites lose tissue-engineered skin markers and revert back to the patient’s chromosomal pattern. If this does occur, it will be interesting to see if the patient’s skin continues to produce normal tonofilaments. This could indicate that tissue-engineered skin, in the long-term, “teaches” native cells how to “behave” properly.

Our early success with tissue-engineered skin in this patient may have a significant impact on the future treatment of neonates with EB simplex. Future studies are needed to determine if the beneficial effects of tissue-engineered skin are reproducible in other neonates with EB simplex and in patients of all ages with different subtypes of EB.

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