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-
plex, Dowling-Meara variant, was treated with tissue-engineered skin.

**REPORT OF A CASE**

A female newborn was seen at birth on November 12, 1998, with extensive bullae and erosions over her face, thorax, abdomen, buttocks, and extremities. The erosions involved approximately 60% of her total body surface. A clinical diagnosis of EB was made. Skin and blood cultures were obtained. She was managed topically with daily dressing changes using gauze covered with petroleum jelly, non-adherent pads, and elastic gauze bandages.

Skin biopsies were performed, and hematoxylin-eosin staining showed intraepidermal cleavage with minimal cellular infiltrate, consistent with the clinical diagnosis of EB. Electron microscopy showed clumped tonofilaments, confirming the diagnosis of EB simplex, Dowling-Meara variant (Figure 1). This was further verified by immunohistochemical staining.

The patient continued to develop widespread blisters and erosions, involving up to 80% of her body surface. In addition, she lost her fingernails and toenails. After discussing potential risks and benefits, her parents gave consent to treat the newborn with the tissue-engineered skin (Apligraf).

At 8 days after birth, tissue-engineered skin was applied to the medial aspect of the left leg and knee, the sole of the left foot, the left buttock, and the right elbow. These areas were selected because of the presence of significant erosions (Figure 2, A). No sutures, staples, or other devices were used to hold the tissue-engineered skin to the erosions. Before placement, several incisions were made in all but 1 of the grafts to create slits through which fluid could drain. Since the erosion on the buttock was not as exudative, slits were not done on that graft (Figure 2, B). The grafts were then covered with gauze containing petroleum jelly, non-adherent pads, and elastic gauze bandages. Three days later, the erosions on the left leg, knee, and foot and the erosion on the right elbow were completely healed. The healed sites were thick and opaque, consistent with graft “take,” rather than thin and translucent, as seen in early reepithelialization. Most of the area on the left buttock was healed (Figure 2, C). There were no signs of infection, and no other adverse effects were noted. Lesions treated only by conservative measures on the right knee, right buttock, and trunk showed no notable improvement.

Despite conservative therapy, the patient continued to develop blisters and erosions over much of her body, with the exception of the sites treated with tissue-engineered skin. Following the successful application of the initial grafts, tissue-engineered skin was used to treat other eroded sites, including the right buttock, the dorsal aspect of the hands, and the dorsum of the right foot. By the time she was discharged from the hospital at the age of 20 days, tissue-engineered skin grafts had been applied to approximately 40% of her total body surface.

Following discharge from the hospital, the patient continued to develop lesions, including clustered vesicles in a herpetiform pattern, bullae, and erosions at sites treated by conservative management alone. While she developed lesions at the periphery of several of the sites treated with tissue-engineered skin, no new lesions developed within treated sites on the buttocks, the dorsum of the right foot and right elbow, and the dorsum of the hands and knees. Several small blisters have developed in tissue-engineered skin–treated sites on the left leg and the sole of the left foot; however, these blisters are much smaller than blisters that have developed on the contralateral, untreated sites. She has tolerated all of the grafts well, without developing infection or other adverse effects. Six weeks after the initial treatment, electron microscopy was performed on tissue obtained from a tissue-engineered skin–treated site, and it did not show clumped tonofilaments.

Molecular genetic testing was performed on tissue samples obtained from treated and untreated areas. HLA class II antigen (HLA-DQB1) was used as a specific marker for tissue-engineered skin cells. The results showed that HLA-DQB1 antigen was present in the treated but not in the untreated areas. These results are consistent with the clinical observation of primary graft take of the tissue-engineered skin grafts. Further evaluations are under way at later time points for the detection of both HLA class II antigens, and genetic testing is being performed to determine the persistence of male tissue from the skin grafts.

**COMMENT**

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 bioengineered human 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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REFERENCES