Tissue-Engineered Skin (Apligraf) in the Healing of Patients With Epidermolysis Bullosa Wounds

Anna F. Falabella, MD; Isabel C. Valencia, MD; William H. Eaglstein, MD; Lawrence A. Schachner, MD

Background: At present, wound treatment of inherited epidermolysis bullosa (EB) is only supportive.

Objective: To determine the safety and clinical effects of tissue-engineered skin (Apligraf; Organogenesis Inc, Canton, Mass) in the healing of wounds of patients with different types of EB.

Design: An open-label uncontrolled study of 15 patients with EB treated with tissue-engineered skin. Each patient received tissue-engineered skin on up to 2 wounds on each of 3 clinic visits: day 1, week 6, and week 12. They were evaluated 7 (±3) days and 6 weeks after each round of treatment. A quality-of-life survey was administered during week 6.

Setting: University of Miami, Miami, Fla.

Patients: Volunteers with EB.

Main Outcome Measure: Safety and wound healing.

Results: A total of 69 different acute wounds received tissue-engineered skin at the day-1 (24 wounds), week-6 (23 wounds), and week-12 (22 wounds) visits. Overall, 63 wounds (79%) were found healed at the day-7 visit. Of the acute wounds, 82% (51/62) were healed 6 weeks after being treated, 75% (27/36) after 12 weeks, and 79% (11/14) after 18 weeks. Nine chronic wounds were also treated. Four were healed at 6 weeks; however, 7 were still open at the last clinic visit (week 18). There were no signs of rejection or clinical infection and no adverse events related to the tissue-engineered skin. The quality of life for most patients improved after treatment. Compared with patients’ recollection of wounds treated with standard dressings, healing was faster and less painful.

Conclusion: In this series of patients, tissue-engineered skin induced very rapid healing, was not clinically rejected, and was devoid of adverse effects. It was felt by the patients and families to be more effective than conventional dressings for EB wounds.

Arch Dermatol. 2000;136:1225-1230

Epidermolysis bullosa (EB) comprises a group of genetically determined skin fragility disorders characterized by blistering of the skin and mucosae following mild mechanical trauma. Most forms of inherited EB are characterized by a lifetime of blister and wound formation. Although some forms of inherited EB are associated with normal longevity, several forms of severe inherited EB are associated with significant morbidity and increased mortality as either a direct or indirect result of the EB. Death may occur from the first days of life to the first 3 decades in severely affected patients. Death usually occurs because of infections in patients who are nutritionally compromised and anemic. Organ dysfunction and metastatic squamous cell carcinoma are other important causes of mortality.

There is no specific treatment for any form of EB, and the mainstay of clinical management is based on protection and avoidance of provoking factors. At present, wound treatment of inherited EB is only supportive. Good topical therapy is fundamental. However, these wounds tend to heal slowly and on some occasions fail to heal, becoming chronic wounds. There is a pressing need for an effective means to halt and possibly reverse the relentless progression toward chronic wound formation in some forms of the disease.

Apligraf (graftskin, living skin equivalent; Organogenesis Inc, Canton, Mass) is the only bilayered skin approved by the Food and Drug Administration. It is indicated for the treatment of venous ulcers. This human skin equivalent is an allogenic cultured bilayer of human skin origin containing both epidermal and dermal components. The human keratinocytes and dermal fibroblasts are

From the Departments of Dermatology and Cutaneous Surgery (Drs Falabella, Valencia, Eaglstein, and Schachner) and Pediatrics (Dr Schachner), University of Miami School of Medicine, and the Department of Dermatology, Veterans Affairs Medical Center (Dr Falabella), Miami, Fla.
PATIENTS AND METHODS

VOLUNTEERS

The authors applied for and received an Investigative Device Exemption from the Food and Drug Administration to treat patients suffering from EB with a tissue-engineered skin device, Apligraf. The Institutional Review Board at the University of Miami School of Medicine, Fla, approved the use of the tissue-engineered skin to treat patients with EB. Patients were recruited from a list of patients with EB requesting treatment for acute and chronic wounds. Volunteers of any age with the clinical and histologic diagnosis of EB could participate. Patients were excluded who (1) were pregnant or were able to become pregnant and not practicing a medically proven form of contraception, (2) had clinical evidence of infection, (3) were receiving immunosuppressive agents or systemic medications used for the treatment of EB, or (4) had a history of cancer developing in an EB chronic wound.

GENERAL PROTOCOL

After giving their informed consent, the patients received up to 3 rounds of tissue-engineered skin therapy: day 1, week 6, and week 12. The patients were evaluated 7 (±3) days after the application of each round of treatment. Earlier treatments were evaluated on the day of new treatment. Six weeks after the application of the third round of treatment, patients returned for the last visit, for a total of 7 clinic visits. The patients had a complete physical examination performed by a pediatrician on day 1 (visit 1) and at week 6 (visit 3). The percentage of skin involvement, the type of EB lesions, and sequelae (eg, blisters, wounds, mitten deformities, scars, syndactyly, milium cysts) were recorded. A blood sample for complete blood cell count and blood chemistry tests, scars, syndactyly, milium cysts) were recorded. A blood sample for complete blood cell count and blood chemistry tests was collected at these clinic visits. Skin biopsy specimens were taken before treatment and 6 weeks after treatment at visit 1 (day 1) and visit 3 (6 weeks) to test for the persistence of the tissue-engineered skin. For this purpose, the expression of specific HLA genes by the tissue-engineered cells was used (Apligraf’s HLA phenotypes have been previously determined). This information was used to create sequence-specific primers directed toward the DNA of the tissue-engineered skin. An HLA class II antigen (HLA-DBQ1) was used as a specific marker for the tissue-engineered skin cells. Specific primers were used and amplified using polymerase chain reaction. A quality-of-life survey form was administered at week 6 (Figure 1).

Up to 2 spontaneously blistered or wounded areas were selected for tissue-engineered skin treatment at day 1 (visit 1), week 6 (visit 3), and week 12 (visit 5). The wounds were chosen in conjunction with the patients’ and parents’ advice. Acute wounds located in areas often subject to continued trauma and some chronic, difficult-to-heal wounds were selected for treatment. Treated wounds were traced on an acetate sheet, and photographs were taken. Blisters located on the oral mucosa, face, genitalia, or scalp were excluded. The tissue-engineered skin was used within 2 days of receipt. Each tissue-engineered skin sample measured 7 cm². After fenestration of the sample with a No. 15 blade, it was applied to the wound dermis side down as per the package insert. Vaseline gauze, Telfa nonstick dressing (Kendall Health Care Products, Mansfield, Mass), Comform elastic bandage (Kendall Health Care Products), and a stocking net (standard dressing) were used to keep the tissue-engineered skin in place. Treated sites that did not completely heal received a second and third reapplication during the following treatment-clinic visits.

FOLLOW-UP EVALUATIONS

Patients returned for clinical evaluation 7 (±3) days after each round of treatment. Dressings were removed to evaluate wounds for the presence of infection, assess for adverse events, and measure healing. The wounds were then photographed and, if still open, traced. The treated wounds were wrapped with the standard dressing. The patients were instructed to change the dressings once a week until the next clinic visit or until the wounds were healed. At 6 weeks (visit 3) and 12 weeks (visit 5), the treated areas were evaluated and the next round of therapy was given.

RESULTS

SAFETY

There were no clinical signs or symptoms of acute graft rejection or immune reaction at any of the sites treated.

There were no clinically significant changes during the course of the study in serum chemistry results or complete blood cell counts from baseline pretreatment levels.

Seven adverse events were detected during the study period. None was related to the application of tissue-engineered skin. One patient with a poor nutritional status was diagnosed with an upper respiratory tract infection and had to receive a blood transfusion because of iron-deficiency anemia. He had received transfusions many times in the past for the same reason. Two patients had to receive antibiotics for streptococcal pharyngitis. Three patients had to receive antibiotics (2 oral and 1 topical) because of skin infection of wounds other than those treated.

GENERAL

We previously reported the successful treatment of a female infant with EB simplex by applying tissue-engineered skin to her wounds. Following reports of our
success in the popular news, we received about 200 telephone calls from EB patients or their parents requesting the same treatment. We enrolled the first 16 patients who at least one of the treated wounds were located on the extremities, please answer the following question. Otherwise, go to question 5.

4. If the treated wounds were located in places easily exposed to trauma, the wound sites treated with Apligraf allowed you/your child to:

- Play/walk more without developing blisters in those treated wounds:
  - 1. Yes
  - 2. No

- Let you do vigorous activities such as running, strenuous sports, without developing blisters in those treated wounds:
  - 1. Yes
  - 2. No

5. Regarding the dressing changes in the wounds treated with Apligraf, you would say:

- You have spent more time in dressing changes than when using your regular dressings:
  - 1. Yes
  - 2. No

6. Regarding pain/discomfort in the treated sites:

- You have had:
  - More pain or discomfort than when using regular dressings
  - Less pain or discomfort than when using regular dressings
  - Pain/discomfort is about the same

7. Regarding healing in the treated wounds:

- Your wounds healed:
  - Faster than when using regular dressings
  - Slower than when using regular dressings
  - About the same
  - You don’t know

8. Have you observed formation of new blisters at the treated sites?

- 1. Yes
- 2. No

9. Regarding resistance of the treated wounds to trauma, you would say your treated wounds are:

- More resistant to trauma
- Less resistant to trauma
- About the same

10. After receiving the treatment with Apligraf:

- You are feeling less depressed or less anxious with your disease
- You are feeling more depressed or more anxious with your disease
- The application of Apligraf has not changed your feelings about the disease

11. After the treatment with Apligraf, how much time has your physical health or emotional problems interfered with your daily activities as compared with before the Apligraf application:

- Interfered more
- Interfered less
- You observed no changes

12. After this experience with Apligraf, are you willing to receive Apligraf again in the future?

- 1. Yes
- 2. No

Review Board–approved informed consent form and participated in this study. Nine patients had a diagnosis of dystrophic EB, 5 EB simplex, and 1 junctional EB. Diagnoses were confirmed by light microscopy, electron microscopy, or immunomapping. Patient characteristics are summarized in Table 1. All 15 patients were evaluated at 6 weeks, 14 were evaluated at 12 weeks, and 8 were evaluated at 18 weeks.

A total of 69 different acute wounds were treated with tissue-engineered skin at day 1 (24 wounds), week 6 (23 wounds) and week 12 (22 wounds). Some patients did not return for all of the clinic evaluations. The following data correspond to the wounds we were able to follow-up. Cumulative experience showed that 51 (82%) of the 62 treated acute wounds were healed at the 6-week evaluation. At the 12-week evaluation, 27 (75%) of 36 treated acute wounds remained healed. Eleven (79%)
of 14 treated acute wounds were still healed after 18 weeks (Table 2). Nine chronic wounds were treated with tissue-engineered skin during the study, 4 at day 1, 5 at week 6, and 2 at week 12. Two chronic wounds were retreated, but failed to heal. At the 6-week evaluation, 4 of the wounds showed complete healing. However, by week 18, only 2 of these 4 wounds remained completely healed (Table 2).

FIRST TREATMENT ROUND

On day 1 (visit 1), 28 EB wounds were treated with the tissue-engineered skin. Four of the wounds were considered chronic, while the other 24 were considered acute. The healing results for the acute wounds are shown in Table 2. Six weeks later, at visit 3, 24 (22 acute, 2 chronic) of the 28 wounds were totally healed and 4 (2 acute, 2 chronic) were still open. At week 12, 21 wounds (19 acute, 2 chronic) treated on day 1 remained healed (Figure 2 and Figure 3) and 5 (3 acute, 2 chronic) were not healed. We were able to follow up 18 of these wounds to the last visit on week 18. Eleven (acute) were totally healed and 7 (4 chronic, 3 acute) were not healed. Six of the healed wounds had blistered after complete healing during the 18-week follow-up period. Eight of the 28 wounds blistered after total healing at their tissue-engineered skin–treated areas, but only 5 of these wounds remained open at the last clinic visit.

SECOND TREATMENT ROUND

At week 6 (visit 3), 28 wounds (23 acute, 5 chronic) received treatment with tissue-engineered skin. One of the previously treated chronic wounds received a second application because it had not healed. No acute wounds were retreated. Six weeks later, at visit 5, 20 wounds (18 acute, 2 chronic) were healed and 8 wounds (5 acute, 3 chronic) were not healed. Six weeks after the second round of treatment (visit 7), 10 (8 acute, 2 chronic) of 17 wounds were healed and 7 (3 chronic, 4 acute) were not healed. Ten of the wounds treated in the second round blistered after total healing.

THIRD TREATMENT ROUND

On week 12 (visit 5), 24 wounds (22 acute, 2 chronic) were treated with the tissue-engineered skin. A nonhealing chronic wound was retreated during this visit. No acute wounds were retreated. We were able to follow up 16 wounds 6 weeks later at the last clinic visit (visit 7). We found that 11 acute wounds were completely healed and 5 (1 chronic, 4 acute) were not healed. During the 6-week follow-up, 6 of these wounds relapsed after complete healing, but only 3 were still open at the time of the examination.

DAY 7 VISITS

The patients were examined 7 (±3) days after the application of each round of treatment and at visit 2 (day 6), visit 3 (week 6, ±4 days), and visit 5 (week 12, ±7 days). No signs of clinical infection on the grafted sites were detected during these clinic visits. Sixty-three (79%) of the 80 wounds (58 acute, 5 chronic) were healed at these clinic visits.

PERSISTENCE OF TISSUE-ENGINEERED SKIN

We were able to obtain pretreatment (day 1) and posttreatment (week 6) skin biopsy specimens from 12 of 15 patients. It was possible to positively identify the persistence of the tissue-engineered skin in 2 patients (patients 10 and 14), as pretreatment samples tested negative for the tissue-engineered skin, but posttreatment sites tested positive. One patient (patient 7) tested positive on a sample of skin taken before the application of the tissue-engineered skin. In this case, it was not possible to positively identify the persistence of the tissue-engineered skin, as the patient had the same HLA type. The results for the other 9 patients were inconclusive.
QUALITY OF LIFE EVALUATION

Fourteen of the 15 patients/parents answered the quality-of-life form administered at week 6 (visit 3). When answering the quality-of-life survey form, 12 patients (or their parents) reported that the quality of daily activities had improved after the application of tissue-engineered skin. Ten patients reported that their wounds had healed more rapidly compared with treatment with standard dressings, and 12 reported less pain or discomfort compared with standard dressings. All 14 respondents requested more treatment with the tissue-engineered skin.

Although it was not part of the quality-of-life evaluation form, patients were asked about blistering of the treated sites after complete healing. While 22 treated wounds (28%) experienced reblistering, neither duration nor severity of the blister was as severe as usual.

COMMENT

This is the first series of EB patients treated with a tissue-engineered skin product, Apligraf. Importantly, there were no acute rejection reactions or any other treatment-related adverse effects during the course of the study. While most of the patients (9/15) had the dystrophic type of EB, 1 patient with junctional EB and 5 patients with EB simplex were also treated. Treatment results seemed to be independent of the EB type. The tissue-engineered skin was only applied to spontaneous open wounds or spontaneous blisters with their roofs removed. No wounds were induced nor skin removed, although such manipulations would allow evaluation of comparable wounds and replacement of more widespread areas of genetically compromised skin in future studies.

Twenty-two treated sites (28%) experienced blistering after healing for a variable period. It is likely that the blistering occurred because the patient’s skin had largely replaced the tissue-engineered skin. Although for most patients (9/15) the 6-week biopsy specimen could not be evaluated, we found molecular evidence of persistence of tissue-engineered skin in 2 patients. Further molecular and electron microscopy studies will be needed to determine the persistence and duration of tissue-engineered skin. Such studies could clarify the basis for reblistering.

Overall evidence showed that 82% (51/62) of the acute wounds were healed at the 6-week evaluation. Seventy-five percent (27/36) remained healed at 12 weeks, and 79% (11/14) remained healed at week 18, the last clinic visit. These findings illustrate that, with this therapy, most acute wounds heal and remain healed for some weeks and do not become chronic wounds. These results were consistent throughout the study and confirm the efficacy of tissue-engineered therapy for acute wound healing. It seems reasonable to conclude that more rapid healing of acute wounds prevents them from becoming chronic wounds. In addition, based on the quality-of-life evaluation form, the acute wounds healed more rapidly than usual. However, these data depend upon patient and parent recollection of wound-healing times and need to be evaluated in a prospective fashion.

Nine chronic wounds were treated in 3 different patients. Two required an additional graft because of non-healing. As noted, 4 of the 9 wounds were healed at 6 weeks; however, 7 wounds were still open at the last clinic visit (week 18).

In summary, this experience indicates that therapy with tissue-engineered skin may be safe and effective, at least in the short term, in inducing rapid healing in most acute EB wounds and some chronic wounds. The pain and tenderness of the wounds is relieved almost immediately by tissue-engineered skin therapy, and the treatment itself does not require sutures.

Although the mechanism of action of the tissue-engineered skin is unknown and likely multifactorial, tissue-engineered skin therapy can promote faster healing and has the potential to prevent the development of chronic wounds.
chronic wounds and to reduce the overall morbidity and mortality caused by infection and sepsis. It also has the potential to reduce scarring and deformity. The vast majority of patients also expressed the additional and important benefit of decreased pain.

In this series, the patients’ quality of life improved over the short-term, and if the benefits we noted are sustained, a longer-term improvement in quality of life is also likely.

Accepted for publication April 27, 2000.

Organogenesis Inc, Canton, Mass, provided the Apligraf device and grant support for this study. The Dermatology Foundation of Miami, Miami, Fla, also provided financial support.

We thank Jan Young, PhD, for testing the persistence of tissue-engineered skin in our samples. We also thank Tori Sullivan, MD, Lourdes Forster, MD, and Jeffrey Bosco, MD, for their assistance and cooperation during the clinic visits and Lisa Pell for contacting and recruiting the patients.

Corresponding author: Anna Falabella, MD, VAMC Department of Dermatology, 1201 NW 16th St (165), Miami, FL 33125 (e-mail: annafalabella@hotmail.com).

REFERENCES


News and Notes

The Committee on International Affairs of the American Academy of Dermatology is seeking to develop a clearinghouse of opportunities for dermatologists to volunteer in clinics in developing countries for short periods of a few weeks to a month. If you know of such opportunities, please send them to Deborah Kroncke, American Academy of Dermatology, PO Box 4014, Schaumburg, IL 60168-4014; fax: (847) 330-1123 (e-mail: dkroncke@aad.org).