Behavior of Tissue-Engineered Skin

A Comparison of a Living Skin Equivalent, Autograft, and Occlusive Dressing in Human Donor Sites

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Objective: To compare the behavior of a tissue-engineered living skin equivalent (LSE) with an autograft in acute donor site wounds.

Design: Paired-comparison, randomized control trial.

Setting: A university dermatology service.

Patients: Three donor sites were created on the anterior thigh of each of 20 patients requiring split-thickness skin grafts.

Intervention: For each patient, the donor sites were randomly assigned to be treated with meshed LSE, meshed autograft, or a polyurethane film (PUF) occlusive dressing. Blood and biopsy samples were taken for immunologic and histological studies.

Main Outcome Measures: Toxic effects or clinically apparent rejection, humoral and cellular immune responses, clinical take, healing time, pain, and 1-month histological appearance.

Results: There was no toxic effect or clinically apparent rejection of LSE. Results of humoral and cellular studies were unchanged from baseline. The average time to healing for LSE with clinical take was 7.3 days (SD, ± 0.8 days); for autograft, 7.6 days (SD, ± 1.1 days); and for PUF, 9.5 days (SD, ± 1.8 days). The difference between LSE or autograft and PUF was statistically significant at the .001 level. Pain was experienced by 1 patient, no patients, and 10 patients at the LSE, autograft, and PUF sites, respectively. Histologically, LSE had the thickest epidermis ($P = .02$), PUF had the greatest degree of fibrosis ($P = .02$), and autograft had the least degree of increased inflammation ($P = .004$) and vascularity ($P = .01$).

Conclusions: In acute donor site wounds, LSE appeared to clinically take and to be a safe and usable form of tissue therapy.

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RECENT ADVANCES in the field of tissue engineering, eg, the development of biologically useful cells, matrices, tissues, and organs, are expected to revolutionize the practice of medicine. Living skin equivalent (LSE), a tissue-engineered skin, is proposed as a form of tissue therapy that has the potential for diverse applications in the management of cutaneous wounds. It is made of type I bovine collagen and cultured allogeneic cells (keratinocytes and fibroblasts) isolated from human neonatal foreskin. In controlled investigational studies, LSE has been shown to provide improved healing in chronic venous ulcers; in open studies, it appears to improve healing in thermal burns and acute wounds created by skin cancer excision. There have been no toxic effects or clinically detectable rejection with the use of LSE. It is thought to mimic the activity of autografts, functioning as a pharmacological agent and a tissue replacement therapy.

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Split-thickness skin grafting (STSG) is a standard reconstructive technique frequently used in patients who require grafts to treat burns, chronic wounds such as venous and decubitus ulcers, and acute wounds following surgery or trauma.

In contrast to chronic wounds, ulcers, burns, and cancer excisions, several donor sites can be made in a standard manner on the same body site, so as to offer the opportunity to compare LSE with autograft in a side-by-side paired-comparison study. We report the clinical immunologic and histo-
PARTICIPANTS, MATERIALS, AND METHODS

LIVING SKIN EQUIVALENT

The LSE (Apligraf; Organogenesis, Inc, Canton, Mass) is a bioengineered product constructed of type I bovine collagen (extracted from bovine tendons and subsequently purified) and cultured allogeneic human fibroblast and keratinocyte cells (isolated from human neonatal foreskin). Using tissue culture conditions previously described, the dermal layer is formed in vitro by combining fibroblasts with collagen, serum, and tissue culture medium. The collagen, the primary component of the dermis, is permitted to self-assemble into fibrils into which the human fibroblasts are interspersed. A bilayer equivalent is then formed by the addition of a suspension of cultured epidermal cells to the surface of the collagen-fibroblast dermal equivalent. After several days of growth and spreading on the dermal equivalent, the surface of the bilayer is exposed to air, the tissue culture medium calcium concentration is increased, and epidermal growth factor is omitted; these conditions favor epidermal differentiation. A “mature” cornified epidermis develops over the dermal equivalent after 7 to 10 days of incubation under these conditions at the air-liquid interface.

The human fibroblasts and keratinocytes used in the LSE have been tested and found to be free of a panel of known human adventitious agents, including human immunodeficiency virus (HIV) and hepatitis. Testing for various agents was performed using the following products: for HIV-1 p24 antigen, an antigen capture enzyme-linked immunosorbent assay (ELISA) technique; for HIV-1/2, hepatitis A, and hepatitis C, polymerase chain reaction; and for hepatitis B surface antigen, monoclonal enzyme immunoassay (Auszyme; Abbott Laboratories, North Chicago, Ill). The LSE is circular, with a diameter of 7.5 cm, and is 0.5 to 0.75 mm thick.

PACKAGING AND SHIPPING

The LSE is shipped by next-day courier delivery service. The LSE is packaged within a clear plastic carrier tray assembly consisting of the tray, a tray insert with porous membrane, a gel support medium, and the tray lid. The entire tray unit is sterile and enclosed within a sterile, sealed polybag surrounded by insulation packets to maintain temperature in the styrofoam box within the shipping case. After receipt at the study site, the polybag-enclosed package is stored in a standard incubator at 35°C to 37°C. The label on the polybag contains the lot number, expiration date, storage instructions, and the statement that it is for investigational use only.

STUDY PARTICIPANTS

Twenty patients presenting with conditions requiring an STSG to treat a primary wound were enrolled. All participants were aged 18 years or older, were willing to comply with the study requirements, and signed an informed consent approved by our investigational review board. Patients with a known history of keloids, uncontrolled diabetes mellitus, collagen vascular disease, hypersensitivity or allergy to bovine collagen or proteins, or clinically significant cardiac, pulmonary, renal, hepatic, neurologic, or immune dysfunction and/or patients receiving medications known to interfere with wound healing were excluded.

BASELINE EVALUATIONS

For each patient, demographic data, medical history including medication use, and photographic documentation of the donor sites were obtained before enrollment.

SAFETY AND IMMUNOLOGIC EVALUATION

To monitor the immune response of patients, tests were conducted to measure the humoral and the cellular responses to components of the LSE from blood samples drawn at baseline and at 1 month after treatment.

Testing for humoral responses to LSE were ELISAs specific for anti–bovine collagen type I antibodies and anti–bovine serum antibodies and a complement-dependent microlymphocytotoxicity assay specific for the class I HLA antigens expressed on LSE cells.

The ELISAs were performed to determine whether bovine type I collagen antibody titers and bovine serum protein antibody titers increase as a result of treatment with LSE. Serial dilutions of patients’ serum were tested in ELISAs specific for bovine type I collagen and bovine serum to determine antibody titers as previously described. Briefly, serum dilutions were added in triplicate to bovine serum– or collagen-coated ELISA plates (Nalge Nunc International, Rochester, NY). After 1 hour, plates were washed, and alkaline phosphatase–labeled goat anti–human IgG (Southern Biotechnology, Birmingham, Ala) was added to the plates. After 1 hour, plates were washed and substrate was added. Developed plates were read on a plate reader (SpectraMax; Molecular Devros Co, Sunnyvale, Calif) at 405 nm. The colorimetric change in each well was recorded in optical density units. Dilutions of commercial pooled human antibody negative serum (Biocell, Rancho Dominguez, Calif) were run on each plate as a control. The appropriate positive control antibody (anti–bovine type I collagen [DMI, Yarmouth, Me] or anti–bovine serum albumin [Sigma-Aldrich Corp, St Louis, Mo]) was also run on each plate.

RESULTS

GENERAL

Twenty patients were enrolled in the study. Patient ages ranged from 30 to 88 years, with a mean of 60 years.
ONE-WAY MIXED LYMPHOCYTE REACTION

The mixed lymphocyte reaction was performed as previously described. Briefly, 6-day proliferation assays were performed in triplicate in 96-well round-bottom plates (Costar, Cambridge, Mass) in a final volume of 200 mL. Density gradients were purified from each patient's heparinized blood samples. The peripheral blood mononuclear cells, \(1 \times 10^6\) cells/well, used as responder cells, were cultured with one of the following cell types as stimulator cells: interferon-γ (IFNγ, Genzyme, Cambridge) and mitomycin-treated fibroblasts (\(2 \times 10^4\) cells/well), or interferon-γ-treated keratinocytes (\(2 \times 10^4\) cells/well). The test stimulator cells (fibroblasts and keratinocytes used in the fabrication of LSE) were treated with interferon-γ to ensure expression of HLA class I and II antigens. After 5 days, proliferative responses were determined by measuring the incorporation of tritiated (\(^3\)H) thymidine during the last 6 to 18 hours of culture (37 MBq/well) (New England Nuclear, Boston, Mass). Cultures were harvested, and \(^3\)H-thymidine incorporation was measured on a beta scintillation counter (LKB Betaplate Reader; Wallac, Gaithersburg, Md).

MICROCYTOTOXICITY ASSAY TO DETECT CYTOTOXIC ANTI–HLA ANTIGENS

A commercial complement-dependent microlymphocytotoxicity assay (One Lambda, Canoga, Calif) was used to detect anti–HLA antibodies (specific for LSE HLA antigens) in patient serum samples. The HLA trays containing lymphocytes expressing different HLA antigens in each well were used according to the manufacturer's instruction. The patient's serum and rabbit complement were added to the trays. If the serum contained antibodies that bound specifically to HLA antigens expressed by the lymphocyte, complement-mediated cytolysis occurred. To control for lymphocyte viability, commercial pooled human serum (negative control) was run along with the patient's serum.

DONOR SITES

For each patient, 3 designated donor sites, each measuring \(2.5 \times 4.0\) cm, were created on 1 anterior thigh to harvest autograft needed to treat a primary wound. The STSGs were harvested under sterile conditions using a dermatome (Zimmer Ortho, Warsaw, Ind) preset at a depth of 0.18 mm using standard methods as previously described. Resultant donor sites were cleansed with sterile isotonic sodium chloride solution and designated as donor sites 1, 2, and 3. Each donor site was then randomly assigned to receive 1 of 3 treatments (Figure 1). Living skin equivalent was removed from its packaging using a septic technique, meshed, expanded, cut to the appropriate size, and then dressed according to the following regimen: petroleum jelly–impregnated gauze directly onto the LSE, followed by a nonadherent dressing pad (Telfa; The Kendall Company, Mansfield, Mass), 4 \(\times 4\) gauze, a bandage (Kerlix; The Kendall Company), and a self-adherent wrap (Coban, 3M, St Paul, Minn). Autograft, surplus from the initial harvesting, was meshed at the same ratio and expanded to the same extent as the LSE, cut to the appropriate size, and then dressed identically to the LSE site as described above. A PUF occlusive dressing (Tegaderm; 3M, St Paul, Minn) was dressed with 4 \(\times 4\) gauze, bandage (Kerlix), and self-adherent elastic wrap (Coban).

DONOR SITE EVALUATIONS

Photographs of each site were taken immediately after grafting and at every visit thereafter. Evaluations were performed by the same investigator for all patients at 1 week posttreatment and every 2 to 3 days thereafter until complete healing of all 3 donor sites occurred. After all 3 donor sites completely healed, no visits took place until 1 and 2 months after treatment. At each return visit, all donor sites were evaluated for reepithelialization and percentage of take of graft, incidence of erythema, exudate, infection, clinical rejection, and pain (based on a scale of 0 to 10), and planimetry was performed indicating graft take and reepithelialization. At 1- and 2-month follow-up visits, cosmetic outcome of each donor site was evaluated based on pigmentation (normal, hyperpigmented, or hypopigmented), vascularity (normal, pink, red, or purple), and height (normal, <2 mm, <5 mm, or >5 mm).

DONOR SITE BIOPSIES

At the 1-month follow-up, a 3-mm punch biopsy specimen from the center of each donor site was obtained for routine histological evaluation. All biopsy specimens were examined by 3 independent evaluators, including a dermatopathologist (M.M., G.W.E., and W.H.E.). Measurements included epidermal thickness from the granular layer to the rete ridge (in millimeters) and the relative degree of fibrosis (increase in the number and thickness of collagen bundles), inflammation (increase in the number of inflammatory cells including neutrophils, macrophages, and eosinophils in the dermis and perivascularity), and vascularity (increase in the number and size of blood vessels in the dermis) based on a scale of 0 to 3 (0 indicates none; 1, mild; 2, moderate; and 3, severe). The relative development of rete ridges and degree of epidermal adherence were also recorded. Clinically, all sites were completely reepithelialized at the time of biopsy.

Seven were men and 13 were women. Race distribution included 1 white patient, 5 black patients, and 14 white Latin patients. Patients enrolled required STSG to treat chronic ulcers secondary to venous hypertension (\(n = 13\)), trauma (\(n = 5\)), chemical burn (\(n = 1\)), and necrotizing infection (\(n = 1\)). One patient was discontinued from the study the day following grafting after pulling the dressing and grafts off the donor sites. Of the 19 remaining patients who completed the study, 18 had clinical take of LSE and all had clinical take of autograft.

TIME TO HEALING

The average time to 100% healing for LSE with clinical take was 7.3 days (SD, 0.8 days); for autograft, 7.6 days (SD, 1.1 days); and for PUF, 9.5 days (SD, 1.8 days) (Figure 2). The calculations exclude the 1 patient whose...
LSE failed to take (n = 18). The donor site that failed to take the LSE healed by day 18. The use of a repeated-measures analysis of variance (ANOVA) and the Tukey procedure indicates that the comparison of LSE and autograft with PUF are significantly different (P < .001). Living skin equivalent and autograft are not statistically different (P > .05).

PAIN

No patients experienced pain at the autograft site. Ten of 18 patients experienced pain at the PUF site, rated as 2 to 5 (average, 4.2), which was greatest on the initial day of evaluation and lasted 9 days in all, except for 1 patient whose pain lasted 10 days. Healing time was not affected by patient age, sex, or race. One patient experienced pain at the LSE site. This was the patient whose LSE did not take. The pain was graded as a 2 and resolved following complete healing.

COSMETIC OUTCOME

In general, all 3 treatments resulted in hyperpigmentation of the donor site regardless of race. With regard to the appearance imparted by the vasculature, LSE was pink, autograft was normal skin color, and PUF was purple. At 2-month follow-up, the autograft and LSE sites were level with the adjacent skin, whereas the PUF site was elevated in 10 of 18 patients (Figure 3).

IMMUNOLOGY

There were no side effects, evidence of infection, or toxic effects observed at any of the donor sites. All patients had baseline anti–bovine collagen type I antibody titers of less than 40 (negative). Titers for all but 1 patient remained negative during the study. Titers in this 1 patient were positive at the 1-month measurement (titer, 1:160), but there were no clinically detectable consequences.

All baseline anti–bovine serum antibody titers that were negative remained negative during the study. All but 1 patient had negative results of testing for the presence of bovine collagen type I antibody titers.
ence of cytotoxic anti–HLA antibodies specific for the HLA antigens expressed on LSE at baseline and at 1 month. This patient had positive test results at baseline, and they remained positive during the study. This was the same patient with positive test results for anti–bovine collagen type I antibodies. This patient had antibodies to most HLA antigens, not just those expressed in LSE cells. All patients entered the study with negative responses to fibroblasts, keratinocytes, and bovine type I collagen, which remained negative throughout the study.

BIOPSIES AND HISTOLOGICAL EVALUATION

Tissue sections from each of the 3 treatment sites were evaluated in 18 of 19 patients who completed the study. The biopsy specimens of 1 patient were damaged in processing. Statistical analysis of the biopsy results used a repeated-measures ANOVA and the Friedman test, a 1-way nonparametric repeated-measures analysis. There were statistically significant differences among the 3 donor site treatments in regard to epidermal thickness, fibrosis, inflammation, and vascularity. The LSE sites had the thickest epidermis (LSE, 0.5 ± 0.04 mm; autograft, 0.06 ± 0.01 mm; \(P = .02\)). The PUF sites had the greatest degree of fibrosis (PUF, 2.0; autograft, 1.3; \(P = .02\)). The autograft sites had the least degree of inflammation (autograft, 1.2; LSE, 2.0; \(P = .004\)) and vascularity (autograft, 1.3; LSE, 2.0; \(P = .01\)) (Figure 4). There were no significant differences in the preservation of rete ridges across all 3 therapies. There was a complete absence of epidermis in 3 of 18 PUF site biopsy specimens and varying degrees of partial detachment of the epidermis from the dermis on 3 additional PUF site biopsy specimens. In contrast, all specimens from LSE and autograft sites had normal-appearing adherence of the epidermis to dermis.

COMMENT

To our knowledge, this is the first controlled study of tissue therapy in acute wounds. The donor site model was chosen because it is a good model for performing a randomized paired-comparison study. Studies of different dressings in the treatment of donor sites have shown a wide range of healing times, eg, 19 days for collagen-grafted nylon velour (Biobrane; Dow-Hickam, Sugarland, Tex), 6.8 to 13 days for hydrocolloid (Duoderm; Convatec, Princeton, NJ), 15.3 days for nonadherent dressing (N-terface; Winfield Labs, Dallas, Tex), 10.5 days for petroleum jelly–impregnated gauze (Xeroform; Sherwood Medical, St Louis, Mo), 10.5 days for dry adherent dressing, and 6.7 to 14.3 days for PUF.7,13-15 In this study, LSE and autograft were 100% reepithelialized by 7.3 days (SD, 0.8 days) and 7.6 days (SD, 1.1 days), respectively (\(P > .05\)). They were compared with a state-of-the-art wound dressing, PUF occlusive dressing.16

The approximately 2-day increase in healing time for LSE and autograft were significantly different from PUF, which took an average of 9.5 days (SD, ± 1.8 days) (\(P < .001\)).

In addition to rapid healing, another advantage of LSE and autograft is their ability to reduce pain associated with healing donor sites. No patients experienced pain with LSE with clinical take or with autograft. In contrast, PUF was associated with pain in 10 of 18 patients. Donor site dressings are generally associated with varying degrees of pain ranging from 4.7 for mesh gauze to 1.6 for PUF, on a scale of 1 to 10. In addition, at 2 months LSE and autograft afforded a more desirable cosmesis in regard to color and elevation.

There was a 95% incidence of clinical take and no signs of toxic effects or clinically detectable rejection of LSE in the patients enrolled in this study. These observations are consistent with those reported for LSE used in the treatment of wounds following Mohs surgery.7 It is not clear whether the LSE take is permanent or if it undergoes a gradual replacement with the patients’ own tissue. Based on experiences with other models, we suspect that the LSE may eventually be replaced by the host tissues. Phillips et al13 reported there was no evidence of survival of cultured allogeneic epidermal cells in biopsy specimens assessed using multilocus DNA analysis in 3 patients studied at 2 months. Although the question of permanent take vs replacement of LSE remains unanswered, the presence of a dermal-like substitute in the LSE may create conditions sufficiently different from cultured epidermal...
allografts to allow long-term or even permanent engraftment. Regardless of take duration, the lack of toxic effects and good clinical results represent an important stride.

To our knowledge, this is the first report of the histological comparison between a tissue-engineered skin and controls. The finding of varying degrees of separation of the epidermis and dermis in processed biopsy specimens from several PUF sites suggests that PUF may not have formed as strong a dermal-epidermal bond at 1 month. However, this observation assumes all biopsy specimens are subjected to equal processing trauma, which may not be the case. If improved adherence proves to be a real clinical finding, it would be an advantage of LSE therapy. The reduced fibrosis seen with LSE compared with PUF suggests that tissue-engineered skin might improve the quality of healing. The reason(s) for LSE having a thicker epidermis and more inflammation at 1 month is unknown; however, these changes might represent a reactivity to the tissue-engineered skin that was not detectable using the immunologic assays.

At present, LSE and other tissue-engineered materials are likely to be costly when viewed on a per unit basis. For this reason, most studies of tissue-engineered materials are aimed at conditions that in their totality are very costly. For example, LSE has been extensively studied for venous ulcer disease, and studies are under way for the treatment of diabetic ulcers, which is considered to have a high global cost. A different tissue-engineered skin product has also been evaluated for diabetic ulcers. Regardless of costs, outcomes such as ours suggest that tissue-engineered materials may be safe and may be beneficially applied to a variety of wounds and skin conditions. Of special clinical relevance is the finding in this study that LSE behaves like autograft, thus providing an off-the-shelf option that avoids creation of a donor site wound with its attendant problems and potential for infection and scarring. This study also suggests that tissue-engineered products may offer a clinically relevant method to speed healing and reduce the pain of acute wounds. The likelihood that LSE functions as a tissue replace-

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