

Autologous Cultured Skin Substitutes Conserve Donor Autograft in Elective Treatment of Congenital Giant Melanocytic Nevus

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Giant congenital melanocytic nevi, or bathing trunk nevi, have challenged reconstructive surgeons for many years. These large lesions can cover more than 50 percent of the total body surface area and have a lifetime risk of malignant degeneration of approximately 5 percent.¹⁻⁴ Histologically, nests of melanocytes may be found extending into the deep dermis and the subcutaneous layer. To completely eradicate these potentially malignant cells, surgeons must excise the lesions to the level of the muscle fascia.^{5,6} Traditional modalities used to manage large nevi include serial excision and skin grafting. Serial excision, although an effective technique that minimizes scarring, is limited by finite skin elasticity and scar spread. Alternatively, skin grafts provide a readily available, autologous tissue for wound coverage. Donor sites from split-thickness skin graft harvesting, however, are prone to hypertrophic scarring in the pediatric population and are also very painful. Skin grafts can typically be expanded two to three times by meshing to reduce donor-site size, but the cosmetic result is poor.^{2,5,7} Even if expanded grafts are used, extensive donor sites are required to treat some of the larger congenital nevi. In an attempt to avoid such large, unsightly, and painful donor areas, surgeons adopted tissue expansion technology. Tissue expanders are prosthetic devices that gradually stretch the surrounding normal skin to provide tissue for

wound coverage.^{8,9} The expanded skin is full-thickness, well-vascularized tissue that provides a good color and texture match. It is more aesthetically appealing than split grafts because scarring is limited to linear incisions as opposed to large areas of donor sites. The expanded flaps also contain subcutaneous adipose tissue and therefore provide a more natural contour and appearance. Tissue expansion does, however, have its limitations. Skin can be stretched up to approximately five times its area, and it cannot always reach distant sites. Skin expanders also require multiple procedures under general anesthesia and carry the significant risks of expander exposure or infection. Frequent office visits are needed during expansion for prosthesis inflation. Closure of the excised lesion may be performed effectively with tissue expanders for small lesions. The time required to inflate tissue expanders and the limited area of expansion reduce their effectiveness and increase risks of morbidity in large lesions.^{6,8,9} Expanders also have unacceptably high complication rates when used on the extremities or in the perineum. Because of these limitations, traditional split-thickness skin grafting techniques remain an acceptable alternative despite the resultant donor-site morbidity.^{8,9} Although attempts have been made in the past to use cultured keratinocytes in monolayer for wound coverage, the results were clinically unacceptable. These monolayer

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DOI: 10.1097/01.PRS.0000138250.41268.41

ers of cells have poor long-term durability, a propensity to hypertrophic scarring and inferior cosmesis relative to traditional autografts.^{10–13} By comparison, autologous cultured skin substitutes resemble split-thickness skin in anatomy by using a bilaminate design that contains autologous cells in both dermal and epidermal components. Cultured skin substitutes offer a novel surgical approach that significantly decreases donor-site size and provides results similar to skin grafting.^{14,15} A patient's own keratinocytes and fibroblasts are harvested and expanded in culture. They are combined with a degradable, biopolymer matrix and grafted onto the patient's wounds, similarly to split-thickness autograft. Cultured skin has been used successfully to treat large burns, which present problems similar to those of congenital nevi. Both conditions have large full-thickness defects with limited donor-site availability. The donor skin for extensive burns can be expanded by 60 to 70 times,¹⁵ significantly more than with meshed grafting. This report describes two cases in which cultured skin substitutes were used to treat giant congenital nevi with results comparable to split-thickness skin grafting while greatly reducing the area of donor skin.

PATIENTS AND METHODS

Two girls, ages 2 years (patient 1) and 8 years (patient 2), were enrolled by informed consent into a study protocol approved by the University of Cincinnati Institutional Review Board.

Patient 1 had a contiguous, full-thickness giant nevus covering 22 percent of the total body surface area. The lesion extended from the neck to the waist on the back, and over the flanks to the front of the torso. She was treated previously by partial excision of the lesion on her back, harvesting of split-thickness autograft from the scalp, and application as unmeshed sheets to the excised area. Complete removal of the lesion would have required four or more harvests of autograft and would have caused substantial morbidity at the donor sites from pain and from either alopecia of the scalp or scarring at non-hair-bearing sites. To avoid these forms of morbidity, the patient's representatives consented to treatment with autologous cultured skin substitutes as an investigative alternative.

Patient 2 had a contiguous, full-thickness giant nevus covering 24 percent of the total body surface area that extended from the shoulders

to the knees involving the perineum. She was treated in numerous procedures with combinations of tissue expanders and split-thickness skin grafts. After depletion of available donor sites for tissue expanders and skin grafts, the family was informed about the availability of autologous cultured skin substitutes as an alternative for treatment of remaining lesional skin on the left buttocks and abdomen. After consideration of relative morbidity factors, the patient's representatives consented to treatment with autologous cultured skin substitutes as an investigative alternative.

Cultured Skin Substitute Preparation

Patients were hospitalized overnight and donated small biopsies ($\sim 19 \text{ cm}^2$ for each patient) of split-thickness skin and were discharged home the next day. Keratinocytes and fibroblasts were isolated from the skin, cultured, and cryopreserved as previously reported.¹⁶ Approximately 1 month before excision of the lesion, autologous skin cells were recovered into culture and cultured skin substitutes were prepared.¹⁵ Collagen-glycosaminoglycan substrates were inoculated sequentially with $5 \times 10^5/\text{cm}^2$ fibroblasts and $1 \times 10^6/\text{cm}^2$ keratinocytes and incubated at the air-liquid interface for 14 days or longer. Incubation at the air-liquid interface promoted development in vitro of a keratinized epidermal surface with a stratum corneum analog.¹⁷ One day before surgery, cultured skin substitutes were meshed 1:1 to avoid fluid collection under the grafts and to promote delivery of the irrigation solution in the immediate postoperative period. The irrigation solution consisted of nutrients and antimicrobials as previously reported.¹⁸

Wound Treatment

At the time of excision of the lesion, the wounds were grafted with cadaveric human skin to stimulate vascularization in the wound bed. One week later, allograft skin was removed and hemostasis was obtained. Meshed cultured skin substitutes covered with a nonadherent dressing (N-terface, Richardson, Texas) were applied but not expanded, attached with standard surgical staples, and dressed with bulky gauze held in place with Spandex. Cultured skin substitutes were irrigated with a solution of nutrients and antimicrobial agents^{18,19} for 5 days, at which time staples were removed and dry dressings were applied. Open areas were treated with Adaptic (Johnson & Johnson,

New Brunswick, N.J.) and coated with equal parts of Neosporin, Bactroban, and Nystatin. The lesion for patient 1 extended over the entire back, bilateral flanks, and parts of the anterior trunk. This lesion was replaced with cultured skin substitutes in two procedures approximately 1 year apart using the same supply of cryopreserved cells.

Data Collection

Donor biopsies were traced at the time of isolation of cells for culture. Fourteen days after cultured skin substitute application, the grafted wounds were traced and areas of closed and open wounds were determined by computerized planimetry to determine percentage engraftment. After complete wound closure was obtained, the areas were traced to determine the expansion ratio of area closed to donor skin. Qualitative outcome of wound closure was assessed subjectively by clinical examination and recorded photographically.

RESULTS

Histologic anatomy of the cultured skin substitute grafts closely resembled the anatomy of split-thickness skin (Fig. 1). The keratinized epidermal analog (Fig. 1, *below*) provided immediate protection to the wound. The epidermal component of cultured skin substitutes was fully stratified and attached to the dermal analog by a well-formed precursor of the basement membrane. Consequently, after grafting virtually no blistering of the epidermis was observed. Complete replacement of the lesions was obtained with no requirement for conventional autografting of the sites treated with cultured skin substitutes (Fig. 2). Patient 1 had engraftment of 98 percent at postoperative day 14 and was discharged home without complications on postoperative day 15. Patient 2 had engraftment of 65 percent at postoperative day 14, was regrafted with additional cultured skin substitutes at postoperative day 15, and was discharged home at postoperative day 24 from the first procedure. Healed areas at day 28 after surgery were 654 cm² for patient 1 and 412 cm² for patient 2. Expansion ratios were 31 and 21, respectively, with a mean value of 26. Pliability and cosmetic outcome were comparable to other sites treated previously with split-thickness skin autografts. Importantly, virtually no epidermal blistering occurred after healing of the cultured skin substitutes, and the healed skin has not required regrafting. Pigmentation

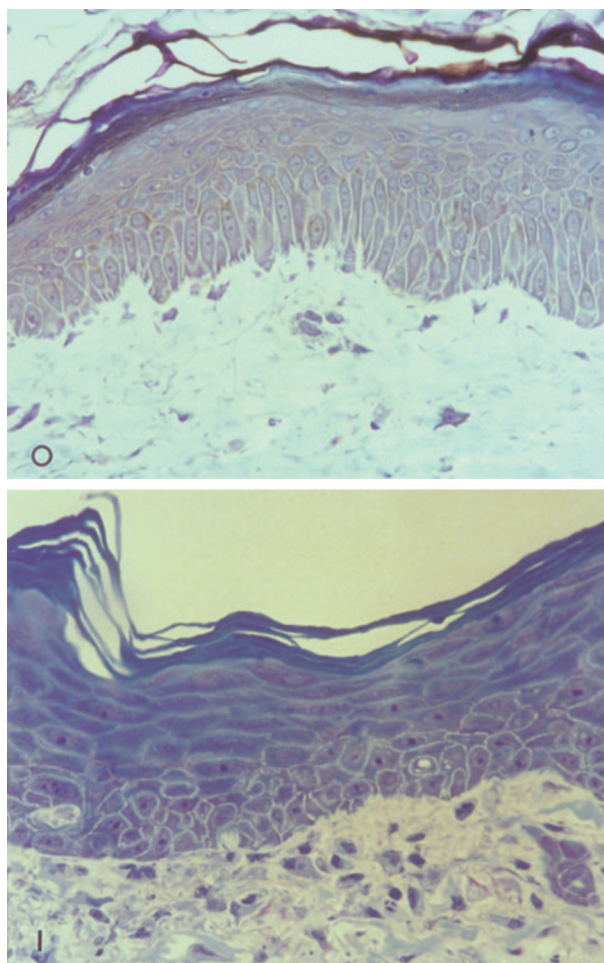


FIG. 1. Histological anatomy of cultured skin substitutes and split-thickness skin graft. (*Above*) Split-thickness skin graft; (*below*) cultured skin substitutes.

was irregular in both patients and included hypopigmented skin in areas treated with cultured skin substitutes. Pigmented areas of healed skin substitutes resulted from normal melanocytes from donor skin used to prepare the grafts. The intensity of pigmentation in cultured skin substitutes was consistent with hyperpigmentation of normal skin at the wound margin (Fig. 2, *below, right*).

DISCUSSION

The medical benefits of reduced donor-site requirements together with comparable functional and cosmetic outcome define a reduction in morbidity for the treatment of giant congenital nevi. These benefits may be extended to future patients with these lesions as an alternative to conventional therapy. Serial excision and tissue expansion, when possible, yield superior cosmetic and functional results to cultured skin substitutes and are routinely



FIG. 2. Photographs of a patient treated with cultured skin substitutes and split-thickness skin graft. (Above, left) Giant congenital melanocytic nevus on the back treated previously with split-thickness skin autograft from the scalp. (Above, right) Postoperative day 15, cultured skin substitute is 98 percent closed, and patient is discharged home. (Below, left) Postoperative day 156, cultured skin substitute is smooth, soft, and strong but has irregular pigmentation. (Below, right) Postoperative day 356, healed cultured skin substitutes remain stable and pliable and has grown with the patient. Above, left and below, right panels reprinted with permission from Boyce, S. T., and Warden, G. D. Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. *Am. J. Surg.* 183: 445, 2002.

used in our practice. Many patients, however, cannot be adequately treated with these techniques because of lesion size and location. It is

for these patients who would otherwise be treated with split-thickness skin grafting that cultured skin substitutes provide the greatest

advantages. In the patients reported here, donor skin was effectively expanded by greater than 25 times, which significantly exceeds either tissue expanders or meshed skin grafts.

Disadvantages of the techniques described here include multiple surgical procedures and high cost. To develop a recipient bed that will accept the cultured skin substitutes, the lesions are excised and the wounds covered with cadaveric skin for 1 week. This process currently requires one more week of hospital stay with considerable wound and patient care and associated expenses. Culturing skin cells and inoculation of the biopolymer matrix is also labor intensive and requires trained staff and laboratory facilities with additional costs. In the future, as the techniques are improved and simplified, it may be possible to reduce these fixed costs and make cultured skin substitutes a cost-effective alternative to current treatment modalities.

Although this cultured skin substitute has been found to have equivalent durability and cosmesis to split-thickness skin grafts, it does not achieve the uniform contour, color, or pliability of uninjured skin or full-thickness skin grafts. Deficiencies in pigmentation have been corrected in preclinical models of wound closure with cultured skin substitutes by addition in vitro of melanocytes to the grafts.²⁰⁻²² The thickness and pliability of healed cultured skin substitutes can be improved in burn patients by the application of Integra before placement of the cultured skin substitutes.¹⁴ After the Integra has vascularized, it provides a thicker, collagen-rich graft bed more similar to dermis. In burn patients this has significantly increased the qualitative outcome of the areas grafted with cultured skin substitutes. In the future, the use of Integra for wound preparation in patients with giant congenital nevi will be explored for the prospective improvement of the aesthetics and pliability of the healed cultured skin substitutes.

Autologous cultured skin substitutes have proven to be an effective treatment for both burns and giant congenital nevi. In the future, the uses of cultured skin could be expanded to include chronic wounds, scar contractures, and reconstructive procedures.²³ For each medical indication, the significant reductions in donor-site area and consequent patient morbidity may provide important advances in patient care.

SUMMARY

This report presents a series of two patients having giant congenital nevi (400 to 650 cm²) treated by excision and grafting with autologous cultured skin substitutes prepared from less than 20 cm² of donor skin autograft. Nevi were excised to fascia and grafted with cadaveric allograft for 1 week, followed by removal of the allograft and grafting with cultured skin substitutes. The mean ratio of closed to donor areas was 26. In comparison to sheet split-thickness skin grafting, cultured skin substitutes exhibited comparable cosmesis, pliability, and durability while reducing the donor-site area by approximately one order of magnitude.

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