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Cultured Skin Substitutes Combined With Integra Artificial Skin* to Replace Native Skin Autograft and Allograft for the Closure of Excised Full-Thickness Burns

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Prompt and permanent closure of excised full-thickness burns remains a critical factor in a patient's recovery from massive burn injuries. Hypothetically, Integra Artificial Skin (Integra) may replace the need for allografts for immediate wound coverage, and cultured skin substitutes (CSS) that contain stratified epithelium may replace the need for autografts for definitive wound closure. To test this hypothesis, 3 patients with full-thickness burns of greater than 60% of their total body surface areas had their eschar excised within 14 days of admission. Integra was applied, and a skin biopsy was collected from each patient for the preparation of CSS. At 3 weeks or more after the application of the Integra and the collection of skin biopsies, the outer silastic cover of the Integra was removed and CSS were grafted. The CSS were irrigated with nutrients and antimicrobials for 6 days and then dressed with antimicrobial ointment and cotton gauze. Treated wounds were traced on days 14 and 28 after the grafting of CSS for determination of engraftment and wound closure, respectively. Cost analysis was not performed. Engraftment on postoperative day (POD) 14 was 98% \pm 1% (mean \pm standard error of the mean), the ratio of closed:donor areas on POD 28 was 52.3 \pm 5.2, and no treated sites required regrafting. The histology of the closed wounds showed stable epithelium that covered a layer of newly formed fibrovascular tissue above the reticulated structure of the degrading Integra. The clinical outcomes of the closed wounds after POD 28 demonstrated smooth, pliable, and hypopigmented skin. Two patients who had received CSS grafts over Integra on their backs were positioned supine on air beds from POD 8 or POD 9 with minimal graft loss because of mechanical loading. One patient with a full-thickness burn of 88% of the total body surface area was covered definitively at 55 days postburn. These results demonstrate that the combination of CSS and Integra can accomplish functionally stable and cosmetically acceptable wound closure in patients with extensive full-thickness burns. This combination of alternatives to the conventional grafting of split-thickness skin permits the substitution of cadaveric allograft with Integra and the substitution of donor autograft with CSS. This approach to the closure of excised full-thickness burns is expected to reduce greatly the time to definitive closure of burn wounds and to reduce the morbidity associated with the harvesting of donor sites for split-thickness skin autografts. (*J Burn Care Rehabil* 1999;20:453-61)

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Restoration of the skin barrier remains a definitive criterion for survival and recovery from large total body surface area (TBSA) full-thickness burn injuries. The conventional standards for treatment and closure of full-thickness burns include early excision to reduce toxicity from degradation of necrotic tissue and to minimize microbial growth in wounds.¹⁻³ If sufficient donor skin for grafting is not available, excised wounds are covered temporarily to reduce fluid loss and infection.⁴ Allograft skin from cadaveric donors provides genuine epidermal barrier, and it may be revascularized from the wound bed and last for weeks or months, especially if it has not been cryopreserved.⁵⁻⁸ Despite these advantages, cadaveric allograft has inherent variability from donor to donor, and it is not uniformly available to all burn surgeons. Allograft also carries the potential risk of disease transmission, but current standards for pathogen screening virtually eliminate this risk. For patients with full-thickness burns of greater than 50% TBSA, the availability of donor autograft and cadaver allograft may be rate-limiting factors in the accomplishment of permanent wound closure.

Alternatives for skin autografts and allografts include cellular and acellular materials and their combinations. Epidermal substitutes include cultured keratinocytes,⁹⁻¹² thin epidermal autografts,^{13,14} suction blisters of epidermis,¹⁵ epidermal cell suspensions,¹⁶ minced epidermis,^{17,18} and polyurethane membranes.¹⁹ Dermal substitutes include collagen-based sponges and gels with or without fibroblasts,^{14,20-22} acellular cadaveric dermis,²³ polyglycolic/polylactic acid mesh with fibroblasts,²⁴ hyaluronic acid substrates,²⁵ and polyethylene-oxide/polybutylene-terephthalate²⁶ matrices. Each of these materials has the prospective advantage of greater availability for wound coverage and closure, but none contains as complete an anatomy and physiology as native skin. Therefore the comparison of alternative materials to conventional standards is critical to validate the therapeutic benefits of such materials. It is important to appreciate that alternative materials for wound coverage that are available commercially may be marketed under regulatory guidelines for medical devices or banked tissues. A categorical distinction between these two regulatory pathways is a requirement for the demonstration of both safety and efficacy of medical devices, but requirements predominantly to assure the safety of the banked tissues, without demonstration of efficacy.

Previous studies performed in the laboratory where the current study was performed have included the design and testing of cultured skin substitutes

(CSS) that consist of cultured autologous keratinocytes and fibroblasts that are attached to collagen-based sponges.²⁷ These CSS have been shown to regenerate epidermal barrier *in vitro*,²⁸ to restore pigmentation after the addition of melanocytes and transplantation to athymic mice,²⁹ to express angiogenic and inflammatory cytokines,^{30,31} and to heal burn wounds after excision and temporary coverage with cadaveric allograft.^{32,33} On the basis of the clinical success of these CSS in combination with cadaveric allograft, it was hypothesized that the CSS may be combined with Integra Artificial Skin (Integra; Integra LifeSciences Corp, Plainsboro, NJ), which would serve as a substitute for allograft.¹³ In this preliminary study, autologous CSS were combined with Integra to test whether definitive wound closure could be accomplished and quantified by measurement of epidermal closure and donor site conservation to demonstrate medical benefits to patients.

MATERIALS AND METHODS

Experimental Design. This study was performed with approval of the local institutional review board and the informed consent of the legal representatives of the patients. The study design followed the recommended uses for Integra Artificial Skin and CSS. A schematic schedule for the performance of the study is shown in Figure 1. Burn eschar was excised as early as possible after the injury occurred, and Integra was applied to the excised wound. At the same time or during the first autografting procedure, a biopsy of split-thickness skin (< 1% TBSA) was harvested and transferred to the laboratory for the culturing of keratinocytes and fibroblasts. After the CSS were prepared, the silastic layer of the Integra was removed and the CSS were applied. Grafted wounds were evaluated by means of tracings for epithelial engraftment at postoperative day (POD) 14 and for total area closed at POD 28. Photographs were taken at periodic intervals, and biopsies were collected for the histology of the CSS before and after it was grafted onto the Integra.

Patient Demographics. Three male patients (MP, JT, EM) were included in this study. Their ages at the time of injury were 7 years (MP), 4 years (JT), and 2 years (EM), and their percentages of TBSA that were covered with full-thickness burns were 63% (MP), 88% (JT), and 65% (EM). All of the injuries were caused by flames.

Preparation of CSS. CSS were prepared from collagen-glycosaminoglycan substrates that were inoculated with cultured fibroblasts and ke-

ratinocytes after isolation from a biopsy of autologous split-thickness skin. Skin biopsies were collected aseptically at the time of the first scheduled operative procedure. Keratinocytes and fibroblasts were isolated as previously described,³⁴ propagated in primary and secondary culture, cryopreserved, and recovered into culture for the inoculation of the CSS.¹² Acellular collagen-glycosaminoglycan substrates were sterilized by gamma-irradiation and stored dry until use. Substrates were rehydrated in N-2-hydroxyethylpiperazine-N-2-ethanesulfonic (HEPES)-buffered saline followed by Dulbecco's modified Eagle's medium on the day that the fibroblasts were inoculated at a density of 5×10^5 cells/cm². One or two days later, keratinocytes were inoculated at a density of 1×10^6 cells/cm² onto substrates previously inoculated with fibroblasts. On incubation day 3 after the inoculation of keratinocytes, the CSS were lifted to the air-liquid interface and incubated with daily medium changes until the day of grafting.

Wound Care. The wounds of the patients in this study were excised within 14 days of injury, and Integra was applied at the time of the excision of eschar. Integra was meshed at a ratio of 1:1 and not expanded. The grafted Integra was covered with coarse gauze that contained catheters for irrigation, and the outer dressings were changed twice per day. The dressing covering the artificial skin was irrigated at 2-hour intervals with alternating solutions of genitourinary irrigant (40 mg/L neomycin and 200,000 units/L polymixin B) and either 5% wt/vol mafenide acetate (MP), 5% wt/vol silver nitrate (EM), or both (JT). At the time of surgery, CSS (approximately 6 cm × 6 cm each) were delivered to the operating room, and the outer silastic cover was removed from the Integra. Surface swab cultures were collected from the connective tissue under the silastic cover, and the tissue was irrigated with a solution of nutrients and antimicrobial agents as previously described.^{35,36} After this irrigation procedure, the CSS were transferred to the wounds with the use of a porous nonadherent dressing and stapled at the corners and the midpoints of the sides. The CSS were dressed with fine-mesh cotton gauze and coarse cotton gauze (ie, standard burn dressings) with red rubber catheters for irrigation, and they were held in place with a stretch fabric that was stapled or sutured to the perimeter of the treatment site. The CSS were irrigated with the nutrient-antimicrobial solution at a dose of 30 cc/CSS 3 times per day. Gauze dressings were changed on POD 2, 4, and 5. On POD 6, all of the staples, the stretch fabric, and

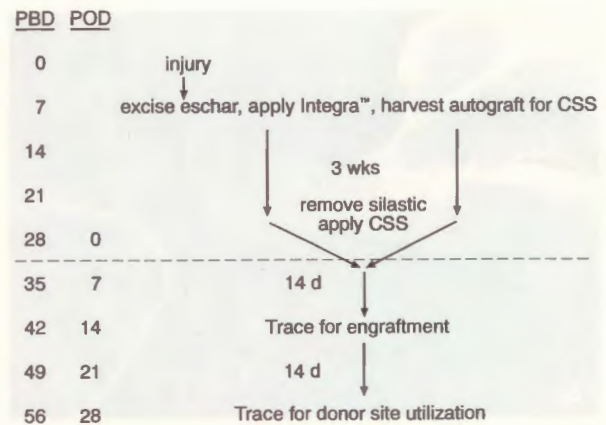


Figure 1. Schematic diagram of schedule for grafting CSS onto Integra. Wounds are grafted with Integra during first month postburn, and remaining wounds closed with CSS during second month postburn. If patient is eligible for surgery, all skin wounds, regardless of magnitude, may be closed during second month postburn. PBD, Postburn day; POD, CSS postoperative day.

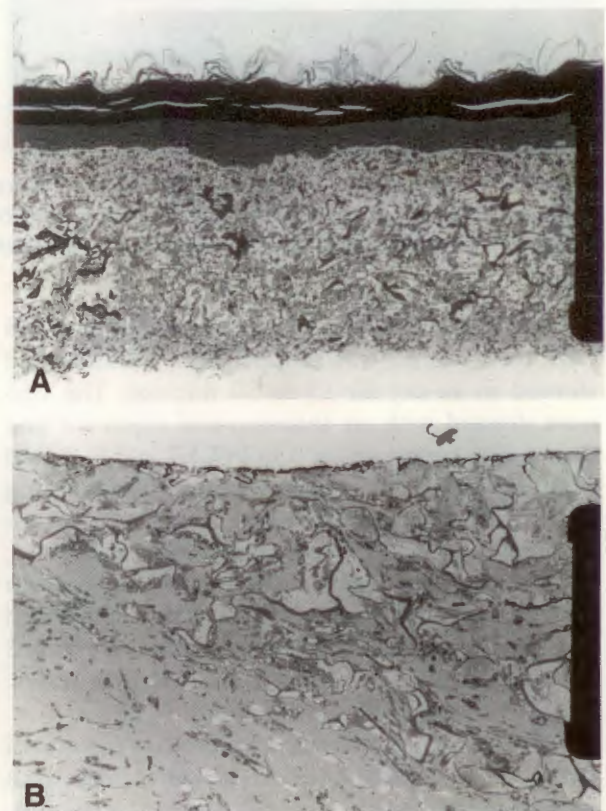


Figure 2. Histologies of components as combined at approximately 1 month postburn. Scale bars: 320 μm between inset notches. A, CSS have well-stratified epidermal substitute attached biologically to dermal substitute. B, Integra Artificial Skin in wound bed has developed fibrovascular tissue to support epidermal substitute.

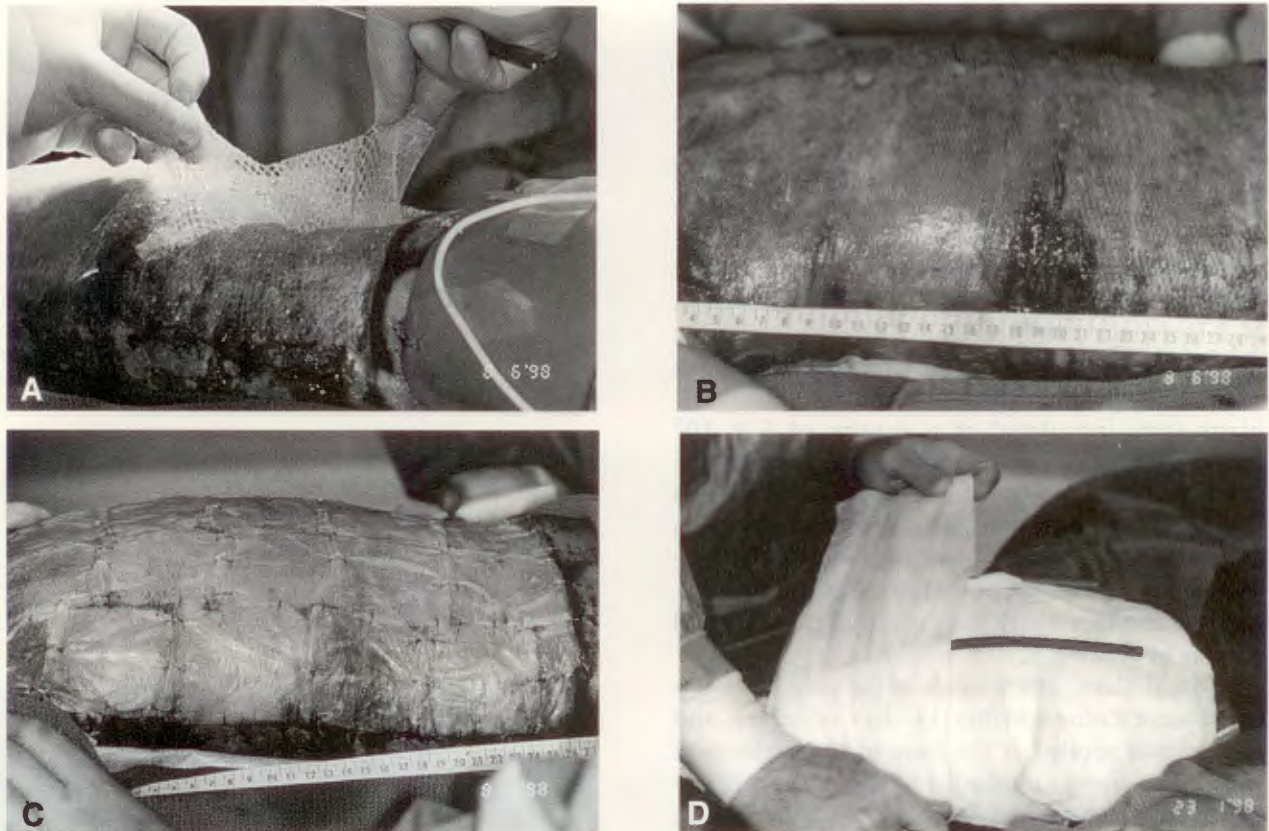


Figure 3. Intraoperative photographs. Tape measures in centimeters. **A**, Removal of meshed silastic layer from Integra artificial skin. **B**, Prepared wound bed. **C**, CSS grafted, covered with nonadherent dressing, and stapled to wound beds. **D**, Wet dressings with placement of catheters for delivery of irrigation.

the nonadherent dressing were removed. The CSS were then washed with the irrigation solution and allowed to air-dry for 15 to 30 minutes. The CSS were dressed with an antimicrobial ointment and dry, coarse, cotton gauze. On POD 7 and 8, closed areas were treated once per day with moisturizing lotion, and open areas were treated with antimicrobial ointment on a porous, nonadherent dressing, dressed with coarse cotton gauze, and wrapped with rolled gauze. Starting on POD 9, CSS were washed twice per day with a nondetergent cleanser, rinsed 3 times with saline, air-dried, and dressed as above. Elastic bandages were not used until POD 14.

Two of the 3 patients in this study received grafts of CSS onto the Integra that had been applied to their backs. Those 2 patients were maintained in a prone position through POD 8, after which time physical therapy was initiated and each patient was placed in a supine position on an airbed through POD 14. Starting on POD 21, all standard nursing protocols for postgrafting care were resumed.

DATA COLLECTION

Photography and Histology Before and After Grafting. Qualitative assessment of the CSS and the Integra was performed by standard photography and light microscopy. Photographs were taken with a Nikon 8008S camera (Nikon Corp, Tokyo, Japan) on Kodak Ektachrome 400 ASA film (Eastman Kodak Co, Rochester, NY). Biopsies for histology were fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer. Punch biopsies (3 mm) were collected from wound beds before grafting and as soon as possible after grafting. Biopsies were then embedded in glycol methacrylate and sectioned at a thickness of 2 μ m. Photomicrography was performed on a Nikon FXA equipped with a 35-mm camera back and Kodak TMAX 400 ASA film.

Quantitative Analysis of Engraftment and Donor Site Use. On the day of the primary culture of the skin cells for the preparation of CSS, the perimeter of the biopsy of split-thickness skin was

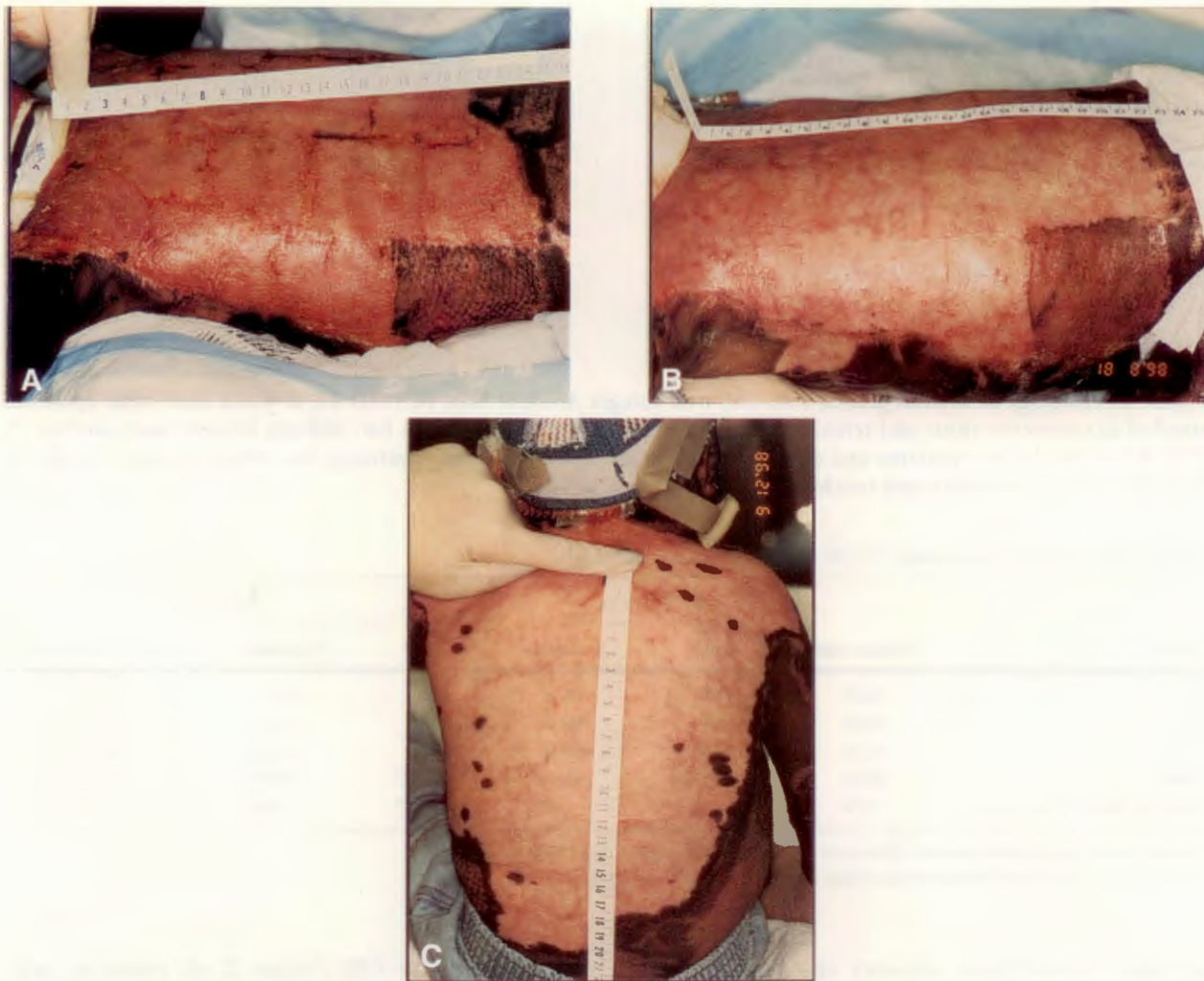


Figure 4. Postoperative photographs. Tape measures in centimeters. **A**, POD 7, 1 day after discontinuation of wet dressings. Grafted wounds dry, keratinized, and clinically closed. **B**, POD 28, at which time ratio of closed:donor areas calculated. Healed skin tolerates physical therapy and pressure garments. **C**, Cosmesis at 5 months postgrafting. Pliability excellent and pigmentation mostly absent.

traced onto clear acetate. On PODs 14 and 28, wound closure with CSS was determined by direct tracings of the treated areas. Tracings were subjected to computer-assisted planimetry to determine the area of the skin biopsy for cell culture and to determine the area of the closed and open subsets of treated sites on PODs 14 and 28. Closed areas were expressed as a percentage of the total treated area. *Engraftment* was defined as the percentage of closed area on POD 14. *Donor site utilization* was defined as the absolute area closed by CSS on POD 28 divided by the absolute area of the biopsy for cell culture. The dimensionless quotient is the ratio of closed-to-donor areas. Because one biopsy for cell culture was used to prepare CSS to cover wound beds whether

they were treated with Integra or not, the values presented represent all of the areas treated with CSS in each patient. A comparative analysis of CSS on wound beds that were treated with Integra and those that were not was not performed. Cost analysis was also not performed.

Instrumental Assessment of Pliability with the Dermal Torque Meter. The pliability of the healed skin was assessed with the use of the Dermal Torque Meter (model DTM 300; Dia-Stron Ltd, Andover, England).³⁷ A fixed torque (10 milli-Newton-meters) was applied to the healed skin by the rotating probe of the instrument. The stretch and recovery of skin was recorded during a loading interval of 10 seconds and during an unloaded interval of 10

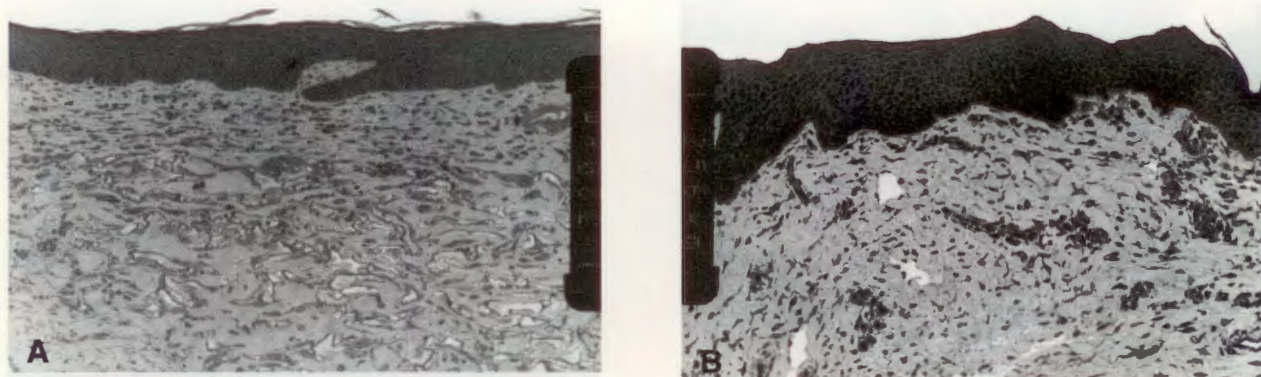


Figure 5. Histology of wounds grafted with CSS over Integra Artificial Skin. **A**, POD 21, at which time stable epidermis attached to connective tissue and reticulations of Integra remain in wound. Scale bar: 320 μ m between inset notches. **B**, POD 63, at which time epidermis and dermis very stable and small fragments of artificial skin remain in connective tissue. Scale bars: 256 μ m between inset notches.

Table 1. Quantitative outcome of CSS on Integra Artificial Skin*

Patient	Donor cm ²	POD 14		POD 28		
		cm ²	% closed	cm ²	% closed	cm ² closed:donor*
MP	13.7	188	96.6	124	100.0	55.9
JT	30.4	911	98.8	1025	100.0	58.8
EM	11.6	458	99.4	488	100.0	42.1
Mean	18.6	519.0	98.3	545.7	100.0	52.3†
Standard error of the mean	5.9	210.9	0.9	261.7	0.0	5.2

*Values for cm² closed:donor represent all wounds treated with CSS whether over Integra or not.

† $P < .02$ by 1-sample t test compared with a value of 4 for 4:1 meshed autograft.

seconds. Stretch and recovery are expressed as degrees of rotation from baseline. Parameters of stretch include elastic (U_e), viscous (U_v), and total ($U_f = U_e + U_v$). Parameters of recovery include elastic (U_r), total (U_a), and not recovered (R), which corresponds to skin plasticity. Measurements were collected for the CSS that were grafted onto the Integra (4 time points \times [3 values/time point] = 12 values from 3 patients) between days 29 and 141 after grafting. Measurements were collected for the meshed autograft that was applied to the Integra (3 time points \times [3 values/time point] = 9 values from 3 patients) between days 32 and 141 after grafting. Each component of the pliability of the CSS and the autograft was compared statistically by t test to uninjured skin on the volar forearms of healthy adult volunteers ($n = 13$).

RESULTS

Light micrographs of a CSS graft and an Integra wound bed are shown in Figure 2. The microscopic

anatomy of the CSS (Figure 2, *A*) resembles split-thickness skin; it consists of a well-stratified epidermal substitute and a dermal substitute that has a total thickness of less than 0.5 mm. Anatomic deficiencies of CSS include its lack of a vascular plexus, glands, follicles, nerves, and immune cells. Figure 2, *B* shows the reticulations of collagen-glycosaminoglycan in the Integra that contained ingrowth of fibrovascular tissue from the wound bed at the time of grafting.

Operative procedures are shown in Figure 3. After the removal of outer dressings, the silastic component of the Integra was removed from the vascularized collagen-glycosaminoglycan component (Figure 3, *A*). The prepared artificial skin wound bed is shown in Figure 3, *B*. The uniformity and smoothness of the surface are notable. CSS were transferred to the wound and stapled in place (Figure 3, *C*). Wet dressings (as described above) were applied to the grafted wounds, and perforated red rubber catheters were included to dispense the irrigation solution (Figure 3, *D*).

Postoperative photographs are shown in Figure 4. On POD 7, one day after the discontinuation of the

Table 2. Assessment of skin pliability with the Dermal Torque Meter: parameters for meshed autograft (AG) or cultured skin substitutes (CSS) on Integra artificial skin as compared with native human skin (NHS)

Parameter	AG	CSS	NHS
Elastic stretch (Ue)	2.33 ± 0.39*	2.76 ± 0.53	3.02 ± 0.13
Viscous stretch (Uv)	3.99 ± 0.61*	3.10 ± 0.25	2.85 ± 0.07
Total stretch (Uf)	6.32 ± 0.55	5.86 ± 0.68	5.87 ± 0.18
Elastic recovery (Ur)	1.93 ± 0.25	1.93 ± 0.33	2.15 ± 0.11
Total recovery (Ua)	3.70 ± 0.49	3.75 ± 0.49	3.74 ± 0.13
Stretch not recovered (R)	2.61 ± 0.61	2.10 ± 0.27	2.13 ± 0.07

**P* < .05 versus NHS.

wet dressings, grafted wounds were dry because a fully-keratinized epithelium had developed (Figure 4, A). The two patients (EM, JT) that had received CSS grafts over the Integra on their backs were positioned supine on air beds on POD 8 (EM) or POD 9 (JT). Graft loss from the mechanical loading of CSS was minimal. On POD 28, all of the wounds of all 3 patients were closed completely (Figure 4, B). Cosmesis at 5 months after grafting is shown in Figure 4, C. The pliability of the healed skin was excellent, and there was virtually no hypertrophic scarring, even between the edges of adjacent grafts. However, the skin had pronounced hypopigmentation and a few discreet foci of pigmentation.

Table 1 presents the data about the percentage of engraftment that had occurred by POD 14 and for closed:donor ratios on POD 28. The percentage of engraftment on POD 14 was more than 95% in these 3 cases, and complete healing had occurred in all cases by POD 28. Patient JT, who had 88% TBSA full-thickness burns, had definitive wound closure by postburn day 55. Closed:donor ratios that included all areas (both those that were covered with Integra and those that were not) treated with CSS were 52.3 ± 5.2. According to a 1-sample *t* test comparison with a maximum closed:donor ratio of 4:1 for meshed split-thickness autograft, values for CSS were significantly greater (*P* < .02). Blistering or breakdown of the grafted area was negligible, and none of the grafted sites required regrafting. Percentages of TBSA that were covered with CSS were 8.4% (MP), 21.3% (JT), and 8.7% (EM); these percentages included the area covered with Integra Artificial Skin and all other wound beds.

Histologic assessments of wounds treated with Integra and grafted with CSS are shown in Figure 5. On day 21 after grafting of CSS (Figure 5, A), fully keratinized epidermis covered a uniform and thin layer of connective tissue. Under the thin layer of connective tissue, reticulations of Integra were

observed. The Integra was grafted 48 days before tissue was collected for this biopsy. On day 63 after grafting of CSS (Figure 5, B), stable epidermis was tightly attached to connective tissue that did not contain the parallel banding pattern of scar tissue. Small fragments of artificial skin remained in the tissue at this time point (not shown). The Integra was grafted 90 days before the collection of this biopsy.

Assessments of skin pliability that were determined with the use of the Dermal Torque Meter are shown in Table 2. No parameters of stretch or recovery were different between CSS on Integra and native human skin in this limited sample. Meshed autograft on artificial skin had statistically less elastic stretch (Ue) and greater viscous stretch (Uv) than native human skin, but total stretch (Uf) of autograft was not different from native human skin. These results are consistent with the clinical observations that both types of grafts, when placed over Integra Artificial Skin, had pliability similar to that of uninjured skin.

DISCUSSION

Data from this preliminary study demonstrate that the utilization of autologous split-thickness skin may be increased by grafting of CSS. Previous studies have demonstrated that the requirements for cadaveric allograft may be decreased with the use of a dermal-epidermal skin substitute such as Integra.¹³ Prospective benefits to patients from reduced requirements for autograft and allograft include the more consistent availability of temporary wound coverage after early excision²¹ and reduced need for the donation of autograft for the completion of wound closure.^{38,39} However, reduced requirements for donor autograft do not provide inherent justification not to harvest donor sites that are available. Both of the tissue substitutes used in this study require 2 or more weeks for recommended prepara-

tion. Therefore burns that can be excised and grafted within 3 weeks of injury are not appropriate candidates for these kinds of tissue substitutes. The combined use of CSS and Integra Artificial Skin is justified in proportion to the magnitude of full-thickness burn injury. In this study, the combination of CSS and Integra was qualified for patients who had full-thickness burns that covered greater than 50% of the TBSA.

Although the time between injury and grafting of CSS ranged from 31 to 36 days in the patients in this study, there was still an important conservation of donor autograft. Also, because no regrafting was required, the harvesting of donor sites was reduced during the second month after injury as definitive wound closure was accomplished. High rates of engraftment and low rates of regrafting contributed to a reduced time to wound closure (55 days) in a patient with 88% TBSA full-thickness burns. This amount of time compares favorably to data from the Burn Registry of the American Burn Association⁴⁰⁻⁴² and suggests that total time of hospitalization for patients with extensive burns may decrease with the implementation of the approach to wound treatment described here. However, end points including length of hospitalization, number of surgical procedures, number of donor skin harvests, qualitative outcome, and number of reconstructive procedures must be studied prospectively with appropriate controls.¹ Together with the evaluation of medical effectiveness, an analysis of cost is essential to the introduction of this kind of approach to wound closure. Without question the costs of materials for the combined use of CSS and Integra greatly exceed the costs of native skin autograft and cadaveric allograft. However, if savings in total treatment costs from this approach are comparable to or greater than the increase in materials costs, then the introduction of this approach will be justified, and it will provide medical and fiscal incentives to both the patient and the practitioner.

CSS was also used successfully in other cases in which Integra had failed because of microbial overgrowth and had to be excised. Engraftment of CSS subsequent to the microbial overgrowth of Integra was attributed to the application of topical antimicrobial agents³⁶ until the graft had vascularized. Conversely, Integra has no antimicrobial activity and vascularizes more slowly than split-thickness skin grafts. Therefore it is not surprising that the Integra provides a greater opportunity for microbial growth than grafts which vascularize rapidly and can be treated with topical antimicrobial agents.

The results of this exploratory study demonstrate an important prospect for significant reductions in the requirements for native skin autografts and allografts for the effective and permanent closure of catastrophic burn injuries. This prospect offers new possibilities for reductions in morbidity and mortality from traumatic skin loss and increased quality of life for survivors of major burns. Principles demonstrated by this study may be applied specifically to burns and more broadly to burn reconstruction, congenital skin defects, and the treatment of chronic cutaneous wounds.

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