Cultured Skin Substitutes Reduce Requirements for Harvesting of Skin Autograft for Closure of Excised, Full-Thickness Burns

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Background: **Rapid and effective closure of full-thickness burn wounds remains a limiting factor in burns of greater than 50% of the total body surface area (TBSA). Hypothetically, cultured skin substitutes (CSS) consisting of autologous cultured keratinocytes and fibroblasts attached to collagen-based sponges may reduce requirements for donor skin, and morbidity from autograft harvesting and widely-meshed skin grafts.**

Methods: **To test this hypothesis, CSS were prepared from split-thickness skin biopsies collected after enrollment of 40 burn patients by informed consent into a study protocol approved by the local Institutional Review Boards of three participating hospitals. CSS and split-thickness skin autograft (AG) were applied in a matched-pair design to patients with full-thickness burns involving a mean value of 73.4% of the TBSA. Data collection consisted of photographs, area measurements of donor skin and healed wounds after grafting, qualitative outcome by the Vancouver Scale for burn scar, and biopsies of healed skin.**

Results: **Engraftment at postoperative day (POD) 14 was 81.5 ± 2.1% for CSS and 94.7 ± 2.0 for AG. Percentage TBSA closed at POD 28 was 20.5 ± 2.5% for CSS, and 52.1 ± 2.0 for AG. The ratio of closed to donor areas at POD 28 was 66.2 ± 8.4 for CSS, and 4.0 ± 0.0 for each harvest of AG. Each of these values was significantly different between the graft types. Correlation of percent TBSA closed with CSS at POD 28 with percent TBSA full-thickness burn generated an r^2 value of 0.37 ($p < 0.0001$). Vancouver Scale scores at 1 year after were not different for erythema, pliability, or scar height, but pigmentation remained deficient in CSS.**

Conclusions: **These results demonstrate that CSS reduce requirements for donor skin harvesting for grafting of excised, full-thickness burns of greater than 50% TBSA with qualitative outcome that is comparable to meshed AG. Availability of CSS for treatment of extensive, deep burns may reduce time to wound closure, morbidity, and mortality in this patient population.**

Key Words: **Burns, Wound healing, Cultured skin, Skin grafts.**

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Permanent wound closure remains a limiting factor in recovery from extensive, full-thickness burn injuries. Recovery from massive burns requires complex critical care that includes, but is not limited to: resuscitation from burn shock, stable respiration, nutritional support of metabolic requirements, restoration of immune function, and management of microbial contamination and infection. However, recovery depends ultimately on closure of the wounds with autologous epidermis and connective tissue to provide stable healing with minimal amounts of scar.[1,2] Furthermore, although wound closure is a requirement for discharge from the hospital, skin pliability and stability are essential for the recovery of range of motion,[3–5] and contribute importantly to long-term quality of life.

Because closure of excised, full-thickness burns is a definitive requirement for recovery, several alternatives have been studied to accomplish more rapid wound closure. Cultured epithelial autografts applied as partially stratified, keratinocyte sheets have been studied extensively, but are reported to blister, ulcerate, and remain mechanically fragile due to poor formation of basement membrane.[6,7] Cultured keratinocytes have also been applied by spraying of cell suspensions over an appropriate wound base, or a dermal substitute,[8,9] but the time to healing may be lengthy due to the slow organization of the cultured cell suspensions into stratified, keratinized epidermis. Replacement of dermal tissue has also been shown to reduce long-term morbidity from scarring. Dermal analogs from natural or engineered sources[10–14] have been reported to provide connective tissue beneath either skin epidermal autograft, or cultured keratinocytes. However, none of these alternatives compares favorably to unmeshed, split-thickness skin autograft, which has been reported to provide superior results in pediatric burns and grafting to the face or genitalia.[15–17]

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Previous reports from this laboratory have reported the design and testing of cultured skin substitutes (CSS) prepared from epidermal keratinocytes and dermal fibroblasts attached to collagen-glycosaminoglycan substrates.[18,19] The epidermal substitute stratifies and keratinizes in vitro to initiate formation of epidermal barrier.[20,21] Proliferating keratinocytes attach directly to dermal fibroblasts on the surface of the biopolymer sponge and initiate development of a basement membrane, which inhibits blistering after healing. Clinical experience with this model has shown rapid healing of burns, surgical wounds, or chronic wounds, but pigmentation has been deficient.[22–26] Addition of cultured epidermal melanocytes in preclinical models has restored skin color, and cultured microvascular endothelial cells have formed vascular analogs after grafting.[27,28] The present study is a prepivotal investigation of autologous cultured skin substitutes to evaluate whether or not this device provides new medical benefits for treatment of burns of greater than 50% of the total body surface area (TBSA). In addition, the present study includes treatment of patients at a distant hospital and evaluates whether skin healed with CSS grows proportionally to the growth of pediatric patients.

MATERIALS AND METHODS

This study was performed with permissions from the Institutional Review Boards of the University of Cincinnati and the University of California Davis, and from the US Food and Drug Administration under an Investigational Device Exemption (IDE) protocol. All patients were enrolled into the study by completion of Informed Consent forms.

The study design consisted of a prospective, randomized, open-label, paired-site comparison of grafting of excised, full-thickness burns with CSS, and split-thickness skin autograft (AG). CSS was meshed at a ratio of 1 to 1.5 and not expanded, and AG was meshed and expanded between 1 to 1.5, and 1 to 4. Application sites were paired by selecting adjacent, contra-lateral or anterior-posterior areas that required skin grafting. Two sites (~150 cm^2 each) were randomized as "A" or "B" before the beginning of the study. Site A was defined as the rightmost, uppermost, or frontmost of the pair, and site B as the leftmost, lowermost, or rearmost. Comparative grafting was performed in one procedure for each patient. If additional applications of CSS were performed, they were evaluated only for quantitative closure of wounds. If additional applications of AG were performed, they were not evaluated.[23] The main hypotheses of the study were that CSS close greater areas of wound than AG per unit of skin autograft harvested and that CSS provide qualitative outcome that is not different from AG.

Two data sets were collected to test these hypotheses. Quantitative measurements consisted of tracings and planimetry of skin biopsies from which CSS were generated and tracings of treated areas on postoperative days (POD) 14 and 28. The tracings were measured for total area and wound tracings were segmented into closed or open areas. Areas were expressed in square centimeters (cm^2). Eleven tracings were also performed in nine patients at time points between 2 to 7 years after grafting to evaluate if there was any change of CSS area associated with the growth of pediatric patients. Differences in CSS area were compared by student's *t* test to changes in TBSA to determine whether CSS were growing proportionally to the individual. TBSA was calculated according to Mosteller,[29] and percent burn by using the Lund-Browder formula.[30] From the area tracings, the following calculations were performed:

(1) Percent area closed at POD 14 and 28 = (closed area/total treated area) × 100
(2) Ratio of closed:donor areas at POD 28 = area closed with CSS/donor area
(3) Percent TBSA closed at POD 28 = (area closed with CSS/TBSA) × 100

Engraftment was defined as the percent of the treated area that was closed at POD 14. For AG, the ratio of closed-to-donor areas was assigned as the maximum value of 4 per harvest, and the percent TBSA closed was calculated as the percent TBSA full-thickness burn minus the percent TBSA closed with CSS. Multiple harvests of donor sites were considered independent events with each harvest of AG expanded by a factor of not greater than 4.

Qualitative data were collected according to the Vancouver Scale for burn scar assessment[31] with a minor modification. The scale for pigmentation in this study was: 0 = none, 1 = hypopigmented, 2 = normal pigmentation, and 3 = hyperpigmented. The individual values for erythema, pigmentation, pliability, and scar height of the Vancouver Scale were added, and expressed as a composite score.

Enrollment criteria included patients with greater than 50% TBSA full-thickness cutaneous burns. Between February 1998 and December 2003, 70 patients were enrolled into the IDE protocol, of which 62 were acute burns. Of those 62, 49 were treated, of which five expired and four were excluded from evaluation (Table 1). Of the 40 patients evaluated, there were 26 males and 14 females. The age (mean ± SEM) was 7.5 ± 0.9 years (range 0.6–17 years), the percent TBSA burns were 75.8 ± 1.7% (range 53–95%), and the percent TBSA full-thickness burns of 73.4 ± 2.2% (range 34–95%). The percent TBSA treated with CSS per patient of 27.8 ± 3.1% (range 5–88%), and the days to initial CSS treatment were 32.8 ± 1.1 (range 24–56; Table 2). Thirty-seven patients were treated at the Shriners Burns Hospital in Cincinnati, OH, and three at the Shriners Hospital for Children in Northern California (Sacramento, CA).

Table 1 Enrollment and Treatment Data

Parameter	Enrolled	Treated
Totals	70	49
Survived	55	44
Expired	15	5
Excluded		9
Evaluated		40

Table 2 Demographic Data of the Patient Population

Parameter	Mean ± SEM	Range
Age (years)	7.5 ± 0.9	0.6–17
Male/female	26/14	
TBSA burn (%)	75.8 ± 1.7	53–95
TBSA FT burn (%)	73.4 ± 2.2	34–95
TBSA CSS/patient (%)	27.8 ± 3.1	5–88
Days to first CSS	32.8 ± 1.1	24–56

Biopsy samples of split-thickness skin were collected as early as possible after injury, usually during the first week of the hospitalization. The absolute areas (cm^2) to be treated with CSS, and for CSS biopsy for each patient were estimated with the following formulae[23]:

(4A) % TBSA eligible for CSS = (% TBSA of full-thickness burn)–(40% TBSA treated with AG)

(4B) Absolute area (cm^2) to be treated with CSS = (% TBSA eligible for CSS) × TBSA (cm^2)

(5) Absolute area (cm^2) of CSS biopsy = Absolute area (cm^2) to be treated with CSS × 0.01

Formula 4A assumed that about 40% TBSA would be treated with AG during the time of CSS preparation. This assumption was based on performance of two skin grafting operations during about 4 weeks covering about 20% TBSA per operation. In cases of very extensive burns (e.g., >80% TBSA), the value of 40% TBSA coverage with AG was revised downward upon the advice of the medical staff, with a consequent increase in biopsy area (Formula 5). Split-thickness skin samples for preparation of CSS were collected with a dermatome set at a depth of 0.010 to 0.012 inches and transferred to the laboratory for cell culture. Keratinocytes were isolated from epidermis; fibroblasts were isolated from dermis of each biopsy and placed into selective cell cultures as described previously[21,32,33] in 5% CO_2/95% air atmosphere with saturated humidity at 37°C. During primary culture, human keratinocytes were incubated in coculture with lethally-irradiated murine 3T3 fibroblasts in serum-free medium. At near-confluence of the primary culture, or first subculture, part of each population was cryopreserved by controlled-rate freezing and part was continued in culture. After sufficient populations of keratinocytes and fibroblasts were available, fibroblasts were harvested and inoculated at an approximate density of 3.75 to 5.0 × 10^5 cells/cm^2 onto collagen-glycosaminoglycan substrates[34] and incubated at least 18 hours to allow cell attachment. Next, keratinocytes were harvested and inoculated at an approximate density of 0.75 to 1.0 × 10^6 cells/cm^2, which was defined as incubation day 0 for CSS. CSS were incubated at the air-liquid interface to stimulate keratinization and formation of epidermal barrier.[21] CSS were usually scheduled for surgical application on incubation days 10 to 14 (~28–35 days after biopsy collection), subject to patient condition. In preparation for grafting, CSS (approximately 36 cm^2 each) were meshed at a ratio of 1:1.5, but not expanded, placed in petri dishes with sufficient medium to avoid desiccation, and transported to the operating room. For patients treated in Sacramento, CSS were packaged in sealed jars, immobilized with sterile gauze packing that was kept moist with irrigation solution (see below), and sent by express delivery for application in the operating room the following day. After initial training of staff at the distant hospital, CSS were delivered without the attendance of laboratory staff en route. Typically, 600 to 1200 cm^2 of CSS were applied weekly at each operative procedure until wound closure was completed. Usually, multiple procedures for grafting of CSS were required for each patient.

Quality assurance standards were applied to CSS before transfer to the operating room. Two parameters of assessment were evaluated: light microscopy by standard histology and surface hydration of the epithelium by surface electrical conductivity/impedance.[33] Histologic evaluations consisted of examination of 18 of 32 CSS prepared for each surgical procedure. CSS epithelia were scored as excellent (well organized and keratinized epithelium), good (organized and stratified epithelium), fair (multilayered, continuous epithelium), or poor (discontinuous, heterogeneous epithelium), and scores of excellent, good and fair were considered acceptable for transplantation. Epithelial surface hydration was measured with a Nova 9003 Dermal Phase Meter (DPM; Nova Technology Corporation, Portsmouth, NH), which reports a high value on a wet surface or a low value on a dry surface. For clinical use, DPM values for each CSS demonstrated a decrease in DPM values on two successive readings of 2 or more days apart.

Burn eschar was excised as early as possible after completion of resuscitation, and sites planned for treatment with CSS were covered with cadaveric allograft or the dermal replacement, Integra Dermal Regeneration Template (Integra LifeSciences Corp, Plainsboro, NJ).[22,35] For excised burns covered with allograft, it was usually excised 1 day before grafting of CSS and AG, and irrigated at alternating 2-hour intervals with 5% wt/vol solution of mafenide acetate in water, and a solution of 40 μg/mL neomycin and 700 U/mL polymyxin B in saline, delivered through perforated red rubber catheters into bulky gauze. The following day, dressings were removed, hemostasis was obtained, and prepared wounds were irrigated with a solution of nutrients and antimicrobials. For excised burns covered with the dermal replacement, Integra Dermal Regeneration Template, the outer silastic layer was removed to expose the vascularized wound bed and prepared wounds were irrigated as above. The irrigation solution consisted of a modified formulation of Dulbecco's Modified Eagle's nutrient medium that was supplemented with 5 μg/mL human recombinant insulin, 0.5 μg/mL hydrocortisone, 40 μg/mL neomycin, 700 U/mL polymyxin B, 20 μg/mL mupirocin, 20 μg/mL ciprofloxacin, and 1 μg/mL amphotericin B.[36–38] After irrigation of the prepared wound beds, CSS were grafted using a backing of N-terface (Winfield Laboratories, Richardson, TX) dressing and stapled in place. Split-thickness AG, meshed at ratios between 1 to 1.5 and

1 to 4 was expanded and stapled to wounds. CSS and AG were dressed with fine mesh gauze, and covered with bulky gauze containing perforated red rubber catheters that were secured with Spandex (De Royal, Powell, TN) that was stretched to apply gentle pressure and to immobilize the grafted sites. If CSS and AG were under the same dressing, the irrigation solution for CSS was used.

Postoperatively, CSS were irrigated with the solution of nutrients and antimicrobials described above, at a dosage of 1 mL per cm^2 CSS three times per day for 5 to 7 days. Dressing changes for CSS and AG were routinely performed on postoperative days (POD) 2 and 5, and all staples and N-terface were removed on POD 5. CSS were treated with an ointment (NBN) consisting of equal parts NeoSporin (Pfizer; New York, NY), Bactroban (Glaxo-SmithKline; London, UK), and Nystatin (Wyeth Pharmaceuticals; Madison, NJ), and covered with dry bulky gauze. Dry, keratinized areas were treated with Curel (Kao Brands Co., Cincinnati, OH) lotion and wet areas were treated with NBN ointment on Adaptic (Johnson & Johnson; New Bruswick, NJ) until healing was complete. Daily dressing changes were performed from POD 6 to 7, after which dressings were changed twice daily. If healing was not complete by POD 15, routine wound care for AG was performed on CSS sites. AG was usually irrigated for 5 days with alternating solutions of 5% wt/vol mafenide acetate in water and a solution of 40 μg/mL neomycin and 700 U/mL polymyxin B in saline. Dry dressings for AG routinely consisted of Adaptic coated with an ointment consisting of either 3 parts bacitracin and 1 part silver sulfadiazine if no yeast species were cultured from the grafts, or equal parts silver sulfadiazine, bacitracin, and nystatin if yeast were cultured.

Statistical Analysis

Primary analyses of data were performed on POD 28 for quantitative and qualitative endpoints, and at 1 year $\pm$ 1 month for qualitative outcome. Qualitative data sets were analyzed for overall significance by the Kruskal-Wallis test. If overall significance was found, then differences were subjected to Wilcoxon's rank sum test. Data from positive/negative scoring of site regrafting was subjected to Fischer's exact test. For the endpoint, ratio of closed-to-donor areas, which is defined in the IDE protocol as the primary medical benefit, a statistical power analysis was performed based on preliminary data. For an alpha value of 0.05, and beta values ranging from 0.95 to 0.80, the estimated size of the population to determine a statistical difference between the CSS and AG treatments was 13–21 patients. For that endpoint, a single-value *t* test was applied to compare CSS to a maximum value of 4 per harvest of AG. Values for expansion of AG were most often less than 3, but were not recorded for all AG applied to all patients in this study. This statistical approach minimizes the benefit of CSS for this endpoint, and therefore was considered the most conservative statistical analysis.

RESULTS

Figure 1 shows microscopic anatomy of AG (left panel) and CSS (right panel) before grafting. Both have dermal and epidermal components with a total thickness of less than 400 μm. The dermal component of CSS consists of reticulations of collagen-glycosaminoglycan (GAG) biopolymer populated with cultured fibroblasts to which the epidermal component is attached biologically. The epidermal component consists of cultured keratinocytes that stratify and differentiate to form an analog of stratum corneum, which is a precursor of functional epidermal barrier. The CSS generally resemble the anatomy of split-thickness skin but lack blood vessels. Therefore, CSS develop vascular perfusion entirely by angiogenesis, rather than by inosculation of blood vessels in the wound to those in the graft as occurs in AG. Most CSS in this study were approximately 6 $\times$ 6 cm in area, but a limited number of CSS of larger area ($\sim$12 $\times$ 12 cm) were also applied.

Surgical application and healing during the first year after surgery are shown in Figure 2. Not surprisingly, it was found that overlapping of the edges of CSS suppressed formation of granulation tissue between grafts and reduced linear scars after healing. In this patient, CSS were applied over excised, full-thickness burns and accomplished more than 90% wound closure at POD 14. At POD 28, the closed wounds are stable and use of pressure garments was begun. The healed CSS was stable, pliable, and hypopigmented at POD 69 and remained pliable and hypopigmented at POD 479. By 1 year after grafting in this patient, the autograft had developed greater areas of hypertrophic scar than the CSS.

Epithelial engraftment and wound closure at POD 14 (Fig. 3*A*) was 81.5% for CSS compared with 94.7% for AG which was statistically significant ($p < 0.05$). A need for regrafting of CSS (13 of 40) was observed, but not for AG (0 of 40). Nonetheless, the magnitude of regrafting was usually small and the quantitative closure of wounds was much greater for CSS than AG (Fig. 3*B*). The ratio of closed wound area to donor skin area for CSS was 66.2 versus a maximum of 4 per harvest of AG. This difference is highly significant ($p < 0.01$), represents a reduction of donor skin harvesting of more than an order of magnitude by use of CSS, and defines the medical benefit of this alternative therapy for burn pa-

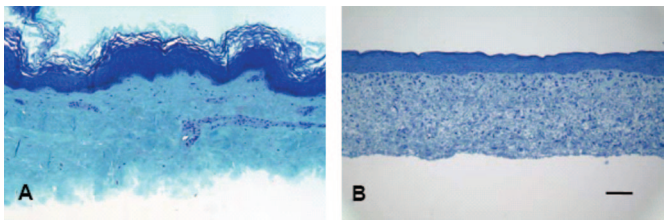

Fig. 1. *Histologic anatomy of split-thickness skin and cultured skin substitutes before surgery. (**A**) Split-thickness skin has a fully keratinized epidermis and vascularized dermis. (**B**) Cultured skin substitute has partially keratinized epidermis and dermal substitute without a vascular network. Scale bar = 0.1 mm.*

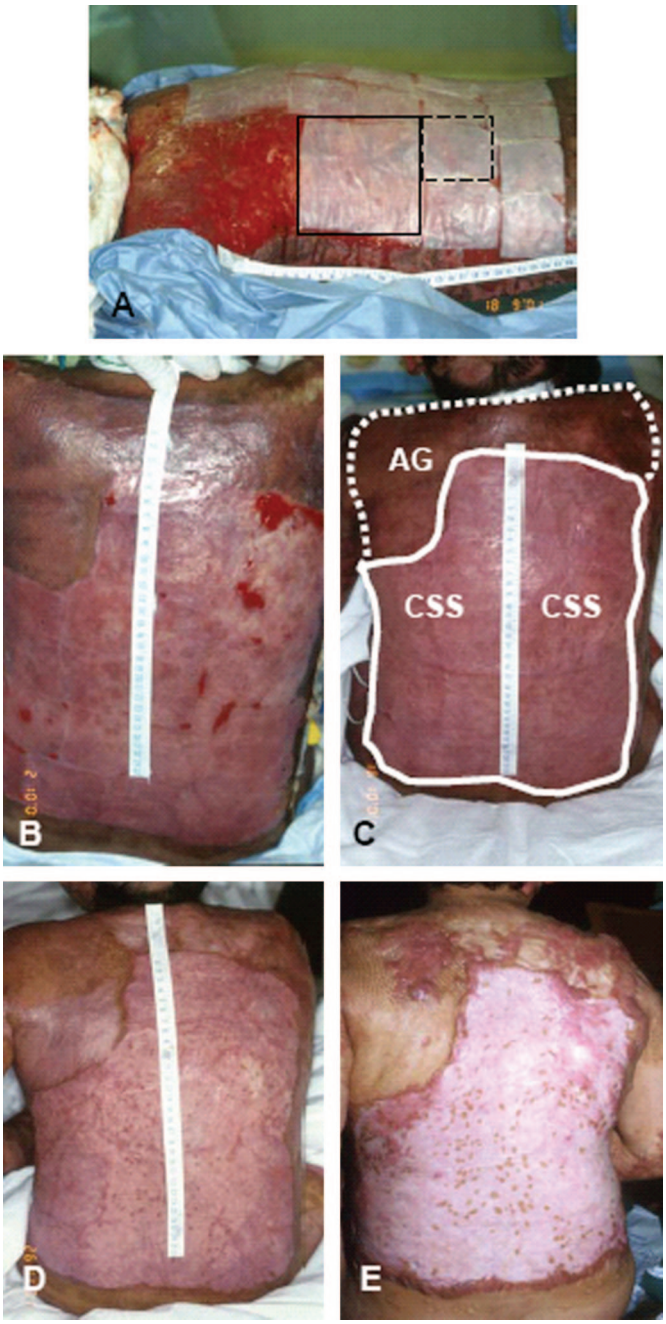

Fig. 2. *Clinical observations at surgery and healing during the first postoperative year. (**A**) Surgical application of large (solid box) and small (dotted box) formats of cultured skin substitutes (CSS). (**B**) Postoperative day (POD) 14. (**C**) POD 28 shows areas treated with split-thickness autograft or CSS. (**D**) POD 69. (**E**) POD 479. Scales in cm.*

tients. This is the primary benefit that is reported to the US FDA for measurement of the efficacy of this device.

A positive correlation was found between the percent TBSA of wound closure with CSS at POD 28, and the percent TBSA full-thickness burn (Fig. 4*A*). Importantly, the range of percent TBSA closed extended to 60% or greater in selected

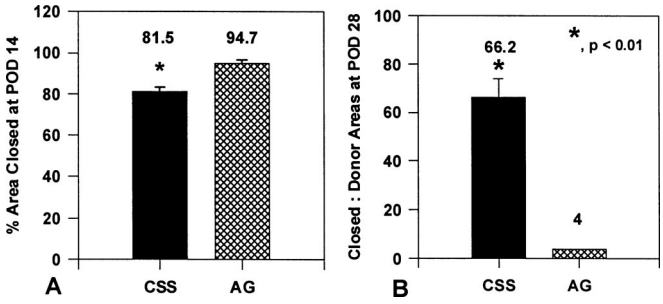

Fig. 3. *Engraftment and donor skin reduction. (**A**) Percentage areas (mean ± SEM) closed at POD 14 were 81.5 ± 2.1 for CSS and 94.7 ± 2.0 for AG. (**B**) Ratios of closed-to-donor areas at POD 28 were 66.2 ± 7.6 for CSS and 4.0 ± 0.0 for AG.*

cases, emphasizing the therapeutic impact of this device in life-threatening burns. On average, CSS covered 20.3% TBSA, and AG covered 52.5% (Fig. 4*B*).

No differences in qualitative outcome between CSS and AG were found at 1 year after grafting (Fig. 5) according to ordinal scoring by the Vancouver Scale for scar assessment. Significant differences were found between CSS and AG at time points of 6 months and earlier. Application of CSS without the expanded mesh of AG generated a smoother surface and CSS were consistently hypopigmented. Both of these factors contributed to lower scores for CSS before 6 months.

Histologic anatomy of CSS and AG is shown in Figure 6. At 5 months after grafting, the epidermis had matured and remained stable and tightly-adhered to connective tissue. Neither healed AG (Fig. 6*A*) nor CSS (Fig. 6*B*) developed glands or follicles. Vascularity had decreased and collagen distribution was orthogonal, not linear as in scar. The epidermal surface and dermal-epidermal junction remained relatively linear indicating the absence of rete peg formation. At 25 months after grafting, AG (Fig. 6*C*) had developed a well interdigitated dermal-epidermal junction, and CSS (Fig. 6*D*) showed a nonlinear basement membrane zone.

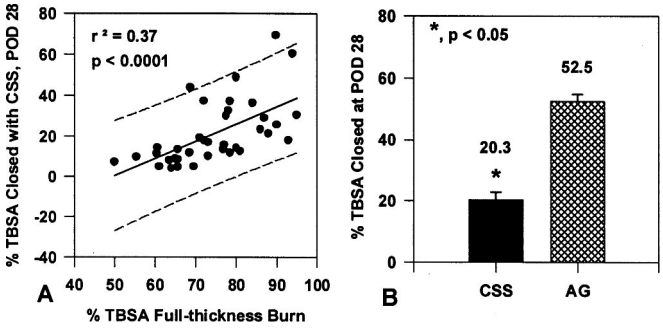

Fig. 4. *Correlation of percent total body surface area (TBSA) closed with percent TBSA full-thickness burn and percent TBSA closed. (**A**) Positive correlation ($r^2 = 0.37$, $p < 0.0001$) was detected between percent TBSA closed with CSS and percent TBSA burned. (**B**) TBSAs (mean ± SEM) closed at POD 28 were 20.3 ± 2.0 for CSS and 52.5 ± 2.0 for AG.*

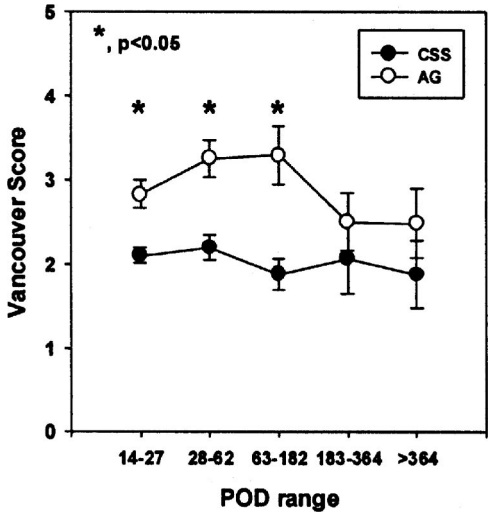

Fig. 5. *Modified Vancouver Scale of qualitative outcome. Cultured skin substitutes (CSS) have statistically lower scores than autograft (AG) during the first 6 months after grafting. By 1 year or longer after grafting, no differences are found in the Vancouver Score.*

Similar clinical results have been obtained with CSS treatment of patients at the Shriners Hospital for Children in Northern California. Figure 7 shows complete healing at POD 28 of the anterior torso of patient 64 in Sacramento. These results demonstrate that autologous CSS can be prepared and delivered unattended to hospitals in distant locations.

Because this study was performed in a pediatric population, it was possible to follow the long-term outcome as the patients grew. Figure 8 shows anecdotal data from 11 measurements of change of CSS area in nine patients, compared with increases of TBSA. CSS area increased at least as much as TBSA (61.7% versus 50.5%) demonstrating that CSS grew proportionally with these children over 2 to 7 years after treatment.

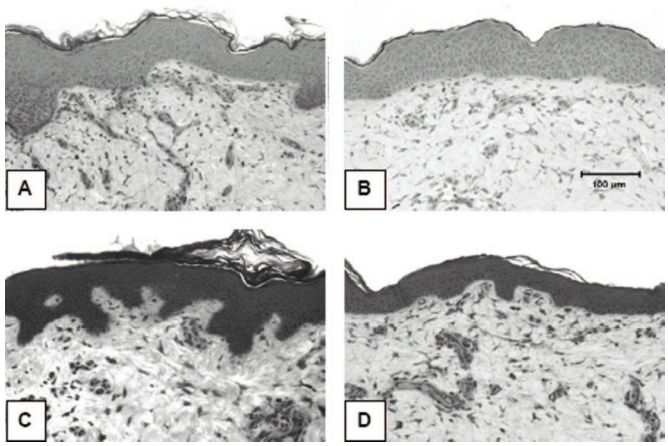

Fig. 6. *Histologic anatomy of closed wounds. Autograft (**A**) and cultured skin substitute (**B**) shown 5 months after grafting. Autograft (**C**) and cultured skin substitute (**D**) shown 25 months after grafting. Both tissue sources have a mature epidermis, a well-vascularized dermis with orthogonal distributions of collagen, and lack epidermal adnexi. Scale bar = 0.1 mm.*

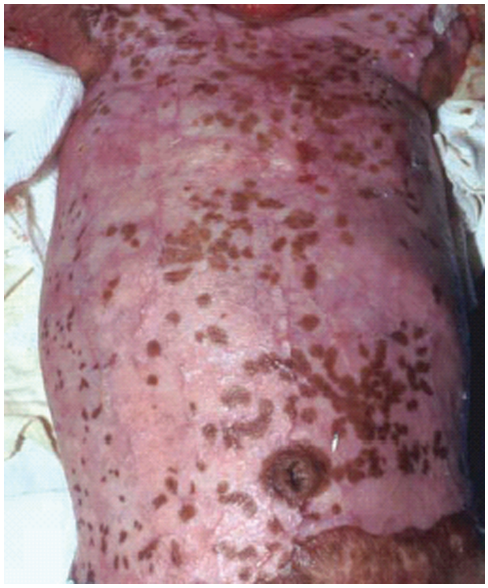

Fig. 7. *Qualitative outcome in Sacramento, California, with CSS from Cincinnati, Ohio. A skin biopsy was harvested from this patient in Sacramento and sent by express delivery to Cincinnati, where CSS were prepared. They were returned to Sacramento where they were applied to a 4 year-old patient with 85% TBSA burns. At POD 99, the healed skin from CSS on the anterior torso is smooth, soft, strong and hypopigmented. These results are directly comparable to treatments with CSS in Cincinnati and demonstrate feasibility for distribution of CSS within the continental United States.*

DISCUSSION

Data from this study support the hypothesis that autologous CSS reduce harvesting of donor skin for closure of burn injuries involving greater than 50% TBSA. This reduction in donor site harvesting represents a new medical benefit in the treatment of extensive, full-thickness burn injuries. The reduction in donor skin requirements implies reductions in donor site morbidity, numbers of skin-grafting operations, and intensive care days, but those data were not collected in this study. The reduction in donor site harvesting is interpreted to result from qualitative and quantitative advantages provided by CSS.

Because the epithelium of CSS forms partial barrier and basement membrane in vitro,[20,21] epithelial closure occurs rapidly after grafting. Effectively, the keratinized epithelium provides a biological closure to the wound at the time of grafting and the basement membrane anchors the epithelium to the connective tissue. Engraftment of CSS occurs between connective tissue in the wound and in the graft in analogy to AG. Upon vascularization of the dermal component of CSS, which occurs by POD 5, the CSS begins to stabilize as barrier function and basement membrane are restored. By POD 7, engrafted CSS have closed the wounds with a permanent,

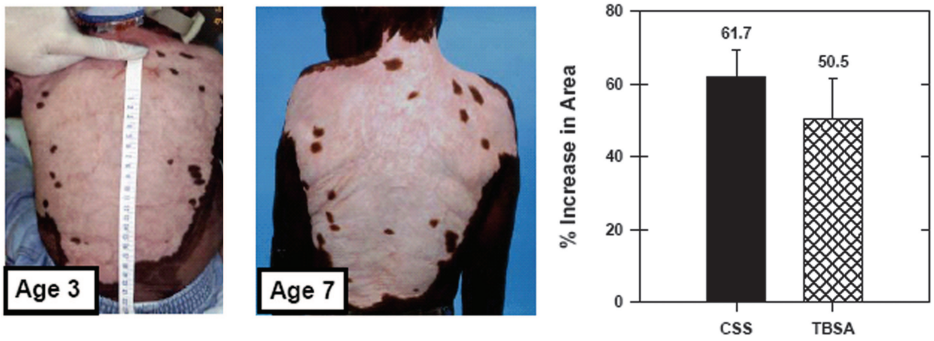

Fig. 8. *Increase in area of TBSA and CSS in pediatric patients. Serial examinations were performed over 2 to 7 years of 11 sites in nine patients. Left and center panels, patient 3 at age 3 and age 7 years. Increase of CSS area in these 11 sites averaged 61.7%, and increase of TBSA averaged 50.5% demonstrating that CSS grow proportionally with pediatric patients. Scale in cm.*

natural tissue. By POD 14 (Fig. 2*B*), healed CSS has sufficient mechanical strength to allow physical therapy to begin. By POD 28, pressure garments, which help to control burn scar, can be worn without loss of CSS. Furthermore, application of CSS without expansion of the mesh that was used in AG may suppress the initiation of scar formation. Unmeshed AG applied as sheet grafts on the hands and face has been reported to reduce scar formation and improve functional and cosmetic outcomes.[15,39,40] In this patient population, engraftment (Fig. 3*A*) was greater than 80% but remained statistically lower than AG. This difference introduced a requirement for minor regrafting of CSS sites at a higher frequency than AG, despite a reduction in donor site harvesting.

The primary medical benefit of CSS is defined by a ratio of closed areas to donor areas of greater than 65. This value was compared statistically to a maximum expansion of 1:4 for AG, but the actual expansion of AG was not measured in this study. In most cases, the usual expansion of AG was 1:2 at the performance site in Cincinnati. Therefore, the conservation of donor skin with CSS compared with AG may actually have been as much as 30-fold. The factor of donor skin expansion of greater than 65-fold by CSS suggests hypothetically that less than 2% TBSA of donor skin is sufficient to resurface the body completely with CSS. This benefit has been realized in selected cases of greater than 90% TBSA full-thickness burns, in which excised burns of greater than 50% TBSA were closed with CSS from a biopsy of less than 1% TBSA. In addition to reduction of donor skin harvesting, this conservation of donor skin offers a definitive benefit for closure of life-threatening burns. Based on these selected cases, it may be possible that broad use of CSS could increase the LD_{50} for burns, which is estimated to be 70% to 80% TBSA in healthy adults, but is much lower in the elderly and the very young.[36] The positive correlation of percent TBSA closed with CSS to percent TBSA full-thickness burn demonstrates that CSS remain effective even as the magnitude and complexity of the burn injury are at their greatest. This was shown not to be true for cultured epithelial autografts, in which effectiveness correlated inversely with burn magnitude.[41]

Despite conservation of donor skin, less average area (20.3% TBSA) was covered with CSS than AG (52.5% TBSA). This apparent anomaly was attributed to greater frequencies of burns between 50% and 80% TBSA in which lower percent TBSA is treated with CSS, and limited capacity to generate the cultured grafts. Due to limited laboratory facilities, about 1000 cm^2 of CSS was applied each week, which decreased the rate at which wounds were treated with CSS and allowed more grafting with AG. Nonetheless, the range of areas closed with CSS extended to about 70% TBSA. It was also observed that because of the sparing of donor skin by CSS, the mesh ratio for AG for most cases could be reduced to 1:2 or less, compared with as much as 1:4. This reduction of mesh ratio resulted in faster healing and less scarring of wounds closed with AG. This indirect benefit is also believed to contribute to improved functional outcome and long-term recovery.

Qualitative outcome by a modified Vancouver Scale was not different between treatments at 1 year after grafting. However, subjective differences between CSS and AG can be accounted for by lower pigmentation and less raised scar in CSS. Reduced pigmentation is understood to result from dilution of epidermal melanocytes during selective culture of keratinocytes, and poor survival during cryopreservation.[42] As discussed above, reduction in raised scar may result from application of CSS without expanded mesh and AG with expanded mesh. Histologic anatomy of healed CSS is consistent with the general process of scar maturation observed in AG. These results suggest that skin tissue generated from CSS is regulated by the same physiologic mechanisms of healing as AG. However, CSS respond somewhat differently than AG because of anatomic differences such as fewer melanocytes and absence of a vascular plexus or immune cells at the time of grafting. Data for growth of CSS was collected anecdotally from a subset of patients after clinical examinations over several years. Proportional increases of areas of CSS and body surface area suggests normalization of tissue anatomy and physiology and limited scar formation in sites treated with CSS. Considerable effort was made during

the development of surgical and nursing protocols to manage CSS as similarly as possible to AG. Therefore, training of the staff at the Sacramento Shriners Hospital consisted of one inservice before the initial application of CSS and provision of detailed written protocols for postoperative care. Successful use of CSS with this limited training provides feasibility for performance of a multicenter study of this medical device which is required before premarket approval can be received.

Remaining anatomic limitations of CSS compared with AG include (but are not limited to) hypopigmentation and absence of blood vessels, glands, or follicles. Operational limitations include the time to first application, compromise of tissue biopsies or CSS grafts during transport, microbial contamination in skin samples that may be carried into the cell cultures, or variability in materials used in CSS fabrication. Among these limitations, the time to first application may be reduced somewhat by more efficient culture processes. However, the delivery of a cultured graft with a keratinized epidermis, basement membrane, and dermal substitute requires time for these biological structures to form. The formation of these epidermal structures is required ultimately for stable wound closure and this model controls the formation of these structures in the laboratory rather than on the wound. Therefore, medical efficacy must consider not only time of preparation and delivery of a cultured cell graft to the patient, but also the total time to complete healing and the long-term outcome. Together, these operational limitations are expected to be reduced greatly as this technology moves from the research laboratory to a process of current good manufacturing practices for medical devices. If sufficient facilities were available and greater amounts of CSS were generated, this therapeutic approach would allow coverage of wounds of virtually any magnitude in approximately 6 weeks after initiation of the process for CSS preparation. Hypopigmentation and lack of a vascular plexus have been addressed in preclinical studies from this laboratory.[27,28] Pigmentation has been regulated by the addition of epidermal melanocytes to CSS and the addition of dermal microvascular endothelial cells has resulted in formation of vascular analogs that form tubular structures after grafting. Hypothetically, hair follicles and sweat and sebaceous glands may be regenerated in vitro, but accomplishment of these goals will require regulation of developmental signals in vitro, which is beyond the scope of the present studies. However, it is important to recognize that split-thickness skin AG also does not regenerate glands or follicles. Therefore, regeneration of hair and/or glands in CSS would offer anatomic structures found only in full-thickness skin.

CSS has also been used successfully in treatment of congenital giant hairy nevus and postburn scar reconstruction. For these elective applications, the time of preparation does not limit the time of recovery because there is not an emergent need for treatment. Also, if patients for burn scar reconstruction had been treated with CSS during their acute care hospitalization, then cryopreserved cells may be used to prepare CSS and eliminate the need for donor site harvesting for these procedures. Together, these advantages offer new alternatives for reduced harvesting of donor skin to patients with needs for closure of extensive full-thickness burns or with limited donor site availability for skin grafting. These therapeutic advantages may be realized for faster recovery with improved outcome for patient populations with burns, burn scars, chronic wounds, and congenital skin diseases.

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