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Comparative Assessment of Cultured Skin Substitutes and Native Skin Autograft for Treatment of Full-Thickness Burns

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Objective

Comparison of cultured skin substitutes (CSSs) and split-thickness autograft (STAG) was performed to assess whether the requirement for autologous skin grafts may be reduced in the treatment of massive burns.

Summary Background Data

Cultured skin substitutes consisting of collagen-glycosaminoglycan substrates populated with autologous fibroblasts and keratinocytes have been demonstrated to close full-thickness skin wounds in athymic mice and to express normal skin antigens after closure of excised wounds in burn patients.

Methods

Data were collected from 17 patients between days 2 and 14 to determine incidence of exudate, incidence of regrafting, coloration, keratinization, and percentage of site covered by graft ($n = 17$). Outcome was evaluated on an ordinal scale (0 = worst; 10 = best) beginning at day 14, with primary analyses at 28 days ($n = 10$) and 1 year ($n = 4$) for erythema, pigmentation, epithelial blistering, surface roughness, skin suppleness, and raised scar.

Results

Sites treated with CSSs had increased incidence of exudate ($p = 0.06$) and decreased percentage of engraftment ($p < 0.05$) compared with STAG. Outcome parameters during the first year showed no differences in erythema, blistering, or suppleness. Pigmentation was greater, scar was less raised, but regrafting was more frequent in CSS sites than STAG. No differences in qualitative outcomes were found after 1 year, and antibodies to bovine collagen were not detected in patient sera.

Conclusions

These results suggest that outcome of engrafted CSSs is not different from STAG and that increased incidence of regrafting is related to decreased percentage of initial engraftment. Increased rates of engraftment of CSSs may lead to improved outcome for closure of burn wounds, allow greater availability of materials for grafting, and reduce requirements for donor skin autograft.

Skin substitutes for permanent closure of acute and chronic wounds include keratinocyte sheets applied to fascia or allodermis,[1–4] or cell-biopolymer composites.[5–9] Wound closure with autologous or allogeneic skin cells after full-thickness excision of skin has been reported anecdotally for treatment of burns, giant congenital nevi, excised tattoos, and chronic skin ulcers.[10–16] However, comparative assessment of the safety and effectiveness of skin substitutes to the prevailing standard of skin repair, split-thickness skin autograft (STAG) is lacking.

Guidelines for assessment of wound repair and regeneration in skin have only recently become established for chronic wounds.[17] Although rating of the burn scar has been reported,[18] consensus opinion has not been formed for standards of assessment of burn wounds, graft "take," or healing. Biologic factors that require consideration include, but are not limited to: rates of engraftment, functional outcome, and cosmesis. Expected results for STAG used to treat large burns include 85% to 95% engraftment, recovery of function, and limited numbers of reconstructive procedures. Cultured keratinocyte sheets have failed to accomplish similar rates of clinical engraftment,[19] and rigorous comparative data are lacking for skin substitutes and autograft regarding other fundamental parameters of recovery. Consequently, socioeconomic impacts of skin substitutes on recovery[20,21] are not understood sufficiently well to provide justification for their routine use.

Preclinical studies have described principles and practices for fabrication of cultured skin substitutes (CSSs) from autologous epidermal keratinocytes and fibroblasts attached to glycosaminoglycan substrates.[5,22,23] Rates of engraftment of CSSs in athymic mice[6] approach 100%,[24] and skin pigmentation may be regulated by addition of melanocytes.[25] Cultured skin substitutes also promote repair of connective and epithelial tissues and restoration of skin pigment[26] on excised burns.[10,11] This report presents a comparative study of autologous skin substitutes with split-thickness skin autografts in excised, full-thickness burns. Semiquantitative scales are used to evaluate parameters of engraftment during days 0 to 14 after grafting of CSSs, and outcome parameters are assessed at day 14 and later. The design of this study may serve as a guide for comparative assessment of skin and tissue substitutes. Favorable comparison of CSSs to STAG may allow introduction of an experimental alternative as a routine therapy.

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MATERIALS AND METHODS

Experimental Design

The paired-site comparison format was used to evaluate cultured cell-collagen—glycosaminoglycan (GAG) skin substitutes and STAG for closure of full-thickness, excised burns. An unblinded study was performed in a prospective, randomized design, with each pair in the same patient on wounds of similar area and depth. After selection, site A was defined as the right-most, uppermost, or front-most location. Site B was defined as the left-most, lower-most, or rear-most location. Treatment of site A or B with CSS or STAG was randomized according to enrollment number by a computer-generated schedule before initiation of the study. Wherever possible, contralateral sites were used. Comparative sites did not include joints, hands, or face.

Patient Population

Patients hospitalized at the Shriners Burns Institute, Cincinnati Unit, or University of Cincinnati Burn Special Care Unit were enrolled. Informed consent was obtained and the protocol was approved by the University of Cincinnati Institutional Review Board. The protocol required that patients were expected to require skin grafting at least 3 weeks after hospital admission. Therefore, only patients with >50% total body surface area (TBSA) were petitioned for enrollment. Patient demographics are presented in Table 1. Patients with enrollment numbers that are not reported did not receive CSSs, either because the medical staff elected not to apply CSSs or because the patient died before CSSs were prepared.

Preparation of Cultured Skin Substitutes

During the first operative procedure, an area of the body was selected as the donor site for STAG (0.008–0.012 inches), approximately 10–20 cm^2 in size (Table 2), from which skin cells were isolated for culture. Samples were harvested under sterile conditions, and the skin was taken immediately to the laboratory, where epidermal keratinocytes and fibroblasts were isolated by standard methods.[27–29] Primary cultures were harvested by trypsinization after 7 to 10 days and used to prepare subcultures to greatly expand cell number. Secondary cultures were used to prepare multiple skin substitute grafts by inoculation at high-cell density onto collagen-GAG substrates in special culture vessels.

Collagen-GAG substrates[22] (10 × 10 cm) were transferred from dry storage after sterilization, or from wet storage in 70% isopropanol, into 150-mm Petri dishes containing HEPES-buffered saline solution,[28] and washed 3 × 10 minutes. The substrates were next washed 3 × 10 minutes in medium for human fibroblasts: Dul-

Table 1. PATIENTS ENROLLED (n = 17)

Enrollment No.	Age (yrs)	% TBSA	% FT	PBD	
				CSS Bx	CSS Applied
5	4	57	54	2	26
7	4	68	63	4	25
8	4	51	50	3	45
11	2	62	62	18	18
13	10	75	75	9	39
14	1	62	51	5	33
15	22	66	31	6	27
17	16	58	56	5	26
18	38	87	81	3	38
22	12	80	77	5	25
23	4	59	52	8	29
27	4	77	77	4	42
31	13	70	67	9	32
33	3	74	74	4	31
35	16	75	75	3	36
37	13	66	61	4	26
38	50	83	75	4	28
Mean ± SEM	12.7 ± 3.3	68.8 ± 2.4	63.6 ± 3.1	5.7 ± 0.9	32.0 ± 1.5
Range	1–50	51–87	31–81	2–18	25–45

PBD = Postburn day; TBSA = total body surface area; FT = full-thickness; Bx = biopsy; CSS = cultured skin substitute.

becco's modified Eagle's medium supplemented with 10% fetal bovine serum, 10 ng/mL epidermal growth factor, 5 μg/mL insulin, 0.5 μg/mL hydrocortisone. Human fibroblasts were inoculated at a density of 5×10^5 cells/cm^2 onto the porous side of the collagen-GAG substrate and allowed to attach for a minimum of 16 hours. Medium was changed daily until human keratinocytes were ready for inoculation, usually 1 to 2 days. For human keratinocyte inoculation (1×10^6 cells/cm^2), collagen-GAG with human fibroblasts were washed 3 × 10 minutes in serum-free molecular, cellular, and developmental biology (MCDB) 153 containing 0.5 mM calcium, 0.5% bovine pituitary extract, 1 ng/mL epidermal growth factor, 5 μg/mL insulin, and 0.5 μg/mL hydro-

Table 2. EXPANSION RATIOS OF HEALED AREAS OF CULTURED SKIN SUBSTITUTES AT 1 MONTH

Enrollment No.	Area (cm^2)		Days Postoperation	Expansion Ratio
	Biopsy	CSS Healed		
17	15	18.5	27	1.2
18	26	152	28	5.8
22	24	152	28	6.3
23	14	64.3	36	4.6
27	7	78.3	28	11.2
31	10	155	28	15.5
33	4	148	28	37.4
35	6	73.5	28	12.3
37	9	92.6	32	10.3
38	23	54.2	28	2.4
Mean ± SEM	13.7 ± 2.5	98.8 ± 15	29.1 ± 0.9	10.7 ± 3.3
Range	4.0–26	18.5–154	27–36	1.23–37.4

CSS = cultured skin substitute.

cortisone. Calcium concentration in this medium was elevated incrementally from 0.2 to 1.5 mM to promote both human keratinocyte stratification and continued fibroblast growth during the first 7 days of skin substitute culture. It has been shown that these conditions allow continued proliferation of both cell types in this medium.[23,30] On culture day 2, medium was replaced with lipid-supplemented medium containing fatty acids (linoleic, palmitic, oleic, arachidonic), α-tocopherol, carnitine, increased serine, 1 ng/mL epidermal growth factor, 5 μg/mL insulin, and 0.5 μg/mL hydrocortisone,[30] but no bovine pituitary extract. On culture day 3, human keratinocyte-human fibroblast collagen-GAG skin substitutes were lifted to the air-liquid interface on stainless steel supports, epidermal growth factor was removed from the culture medium, and cell-biopolymer composites were incubated with daily medium changes until grafting. Keratinocyte confluence and stratification was confirmed by removal of a small sample from the composite that was embedded in glycol methacrylate, cut in 3- to 5-μm sections, stained, mounted with cover glasses, and examined by light microscopy.

Completed skin substitutes were approximately 7 × 7 cm (approximately 49 cm^2). After preparation, CSS grafts were transported to the operating room, removed from the culture vessel by the attending surgeon, and applied to patient wounds under sterile conditions. Paired sites were prepared by identical surgical protocols. Cultured skin substitutes of standard dosage (140–150 cm^2) were applied to experimental wound sites. Paired sites were treated with conventional skin autografts of equal area.

Wound Treatment

Before excision, wounds were treated according to prevailing standards of care at the local burn center. In cases of burns involving very large areas of the body surface, eschar was excised and wounds were covered temporarily with human cadaveric allograft. One day before skin grafting, enrolled patients had eschar or allograft excised to viable tissue that most frequently was subcutaneous fat. Excised wounds were irrigated overnight in a solution of 5% (w/v) mafenide acetate, and grafted the following day.[31] Excised wounds were irrigated thoroughly with saline to reduce the residual concentration of mafenide acetate, which is known to be highly toxic to cultured keratinocytes.[32] After preparation of the wound bed, meshed or unmeshed STAG was applied to one site, CSSs covered with polypropylene mesh (N-Terface; Winfield Laboratories, Richardson, TX) to the other site, and both were secured to wounds with surgical staples. Both grafts were covered with fine mesh gauze, and bulky gauze containing red rubber catheters for delivery of irrigation fluids. Bulky gauze was held in place with spandex fabric that was stretched across the graft site and stapled to surrounding uninjured skin.

Wound dressings were cared for as described in previous studies.[10,11] Dressings for CSSs, soaked with solutions of noncytotoxic antimicrobial agents[33,34] and nutrients,[24] and were maintained for 5 to 10 days over both sites, if they were under a common dressing. Alternatively, if STAG was under a separate dressing, it was irrigated according to prevailing standards which were alternating irrigations at 2-hour intervals with solutions of 40 μg/mL neomycin plus 200 units/mL polymyxin B (Schein Pharmaceutical, Inc., Florham, NJ) and 5% mafenide acetate. Mafenide acetate solutions were not used on CSS sites because of extreme cytotoxicity.[32] At days 5 to 10, or when a blanching keratinized surface developed, wet dressings were discontinued, and nonadherent, salve-impregnated dressings were administered daily. Salve compositions were determined by microbial fauna at each site. Standard salves for STAG were selected from either 1) three parts bacitracin plus one part silver sulfadiazine or 2) equal parts bacitracin, silver sulfadiazine, and nystatin. Because silver sulfadiazine is toxic to cultured keratinocytes, a formulation for CSS consisted of equal parts: bacitracin zinc (400 units/g)—neomycin sulfate (5 mg/g)—polymyxin B sulfate (5000 units/g) (E. Fougera & Co., Melville, NY), 2% mupirocin ointment (Smith-Kline Beecham Pharmaceuticals, Philadelphia, PA), and nystatin ointment (100,000 units/g) (NMC Laboratories, Glendale, NY). Salves were used until dry, keratinized surfaces developed, after which a petrolatum-based cream (Eucerin; Beiersdorf, Inc., Norwalk, CT) was used to moisturize newly formed epidermis. Subsequent therapy and pressure dressings were identical.

Endpoints

Two sets of endpoints were evaluated. Between days 2 and 14, data to determine engraftment were collected that included expression of exudate (% of days), coloration, keratinization, and percent of site covered by graft. On day 14, an "investigators global assessment" was completed, and if any keratinized epithelium formed on the CSS site, that patient continued in the study. After day 14, data were collected to evaluate qualitative outcome for areas that developed keratinized epithelium. Parameters of qualitative outcome included the following: erythema, pigmentation, epidermal blistering, surface texture, skin suppleness, and raised scar. All parameters of engraftment and outcome were assessed on a semiquantitative, ordinal scale, with 0 representing a poor result and 10 representing an optimal result. One exception to this general scoring system included pigmentation, in which a score of 5 represented normal pigmentation of uninjured skin of the patient, 0 represented

hyperpigmentation, and 10 represented hypopigmentation. Individual values for each observation represented the mean of wound percentages × respective ordinal scores, where the sum of all percentages = 100 (*e.g.*, [25% × 4] + [75% × 8] = 7). Percent area of epithelial engraftment was scored for each patient at day 14 and day 28. Incidence of regrafting for graft failure was scored as an absolute event for each patient. Expansion ratio of skin biopsies for CSSs was determined by planimetric measures of epithelialized areas of CSS sites at day 28.

Enzyme-Linked Immunosorbent Assay to Bovine Collagen

To test for antibodies to bovine collagen in CSSs, control sera were prepared by inoculation of rabbits (n = 2) with homogenized collagen-GAG substrates together with Ribi's adjuvant.[35] All sera were tested at a dilution of 1:200. Preimmune sera from rabbits and humans were collected and frozen at −70 C. Sera were collected 28 days after CSS grafting from nine patients who received a single treatment, and two patients who received multiple treatments with CSSs. Enzyme-linked immunosorbent assays consisted of coating microtiter plates with homogenized collagen-GAG substrates, air-drying, reacting with test sera and chromogen formation to bound antibodies with secondary antibodies conjugated with peroxidase, and spectrophotometry. Data were collected as OD_{490} from colorimetric solutions, and expressed as percent control (immunized rabbits).

Data Collection and Biostatistics

Between days 2 and 14, data were collected on each day (n = 17). After day 14, data for qualitative outcome were collected from each paired site on each patient during five observation periods defined as follows: 1) 15 to 30 days (n = 11); 2) 1 to 2 months (n = 10); 3) 3 to 4 months (n = 9); 4) 5 to 12 months (n = 5); and 5) 1 year and later (n = 4). Because return to the clinic was scheduled according to patient needs, grouping of qualitative data into observation periods was more pragmatic than collection of data at specific time points. Data for quantitative expansion were collected from ten patients. Computerized databases (SigmaStat [Jandel Scientific, San Rafael, CA] and Statistical Analysis Software [SAS Institute, Cary, NC]) were used to compile, extract and analyze collected data.

Primary analyses of data were performed 1 month after surgery for paired sites in which CSSs remained. Two types of analyses were performed on collected data: McNemar's Binomial 2-Sample Test for binomial proportions was used for positive/negative scoring of whether site regrafting was performed; and Wilcoxon's signed rank test was used to evaluate median score differences between treated sites that were scored on ordinate scales. These endpoints included: 1) percent of days exudate; 2) coloration; 3) keratinization; 4) percent of site covered; 5) erythema; 6) pigmentation; 7) epidermal blistering; 8) external surface; 9) skin suppleness; and 10) raised scar.

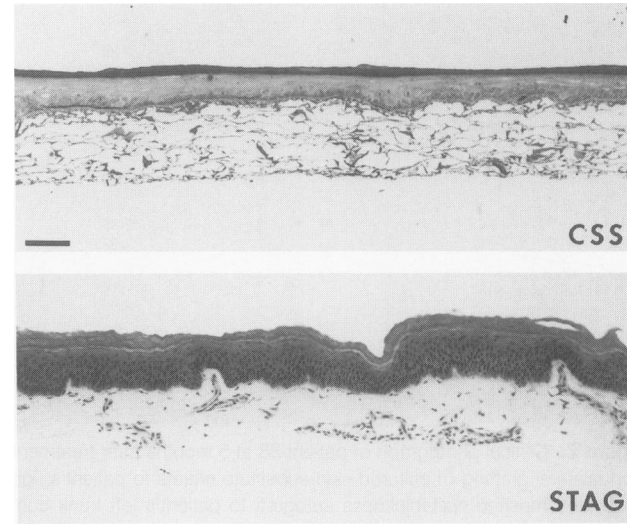

Figure 1. Histologies of a cultured skin substitute and split-thickness autograft. Top panel: cultured skin substitute is organized into epithelial and connective tissue compartments analogous to native skin; epithelium is partially stratified and keratinized, and total graft thickness does not exceed 0.5 mm. Bottom panel: split-thickness autograft is distinguished from cultured skin substitute by fully keratinized epithelium, and presence of a vascular plexus in the dermis. Scale bar = 0.1 mm.

RESULTS

Histology of a CSS and STAG are shown in Figure 1. The skin substitute had general organization into epithelial and connective tissue compartments, epithelium was stratified, and total thickness was similar to STAG. Two anatomic features distinguished the skin substitute from native skin tissue—lack of a vascular plexus and incomplete keratinization of the cultured epithelium. These deficiencies imposed requirements in the skin substitute for longer time of vascularization and resulted in slower keratinization.

Clinical appearance 5 months after contralateral application of CSSs and STAG in patient 33 is shown in Figure 2. Wound closure on both sites was complete and stable. Cultured skin substitutes (patient's right), applied as sheet grafts, developed relatively smooth, supple skin tissue with hyperpigmentation. Split-thickness autograft (patient's left), applied as 4:1 meshed autografts, retained the meshed pattern and was less pigmented than CSSs.

Comparative engraftment of CSSs and STAG at 14 days and 28 days is shown in Figure 3. Patients 5 through 17, who were enrolled earlier in the study, had complete

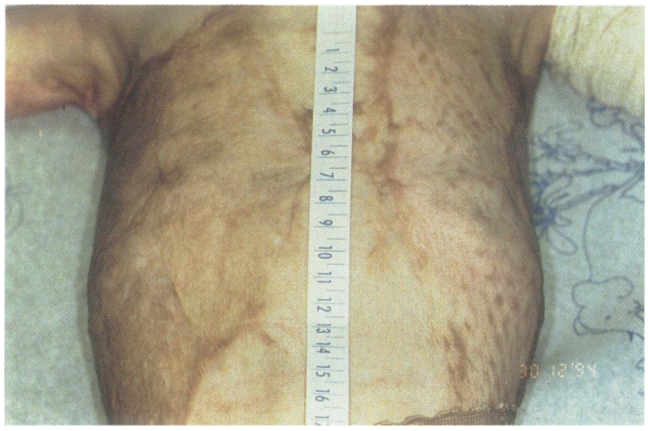

Figure 2. Clinical photograph of patient 33 at 5 months after treatment. Contralateral grafting of cultured skin substitute sheets to patient's right trunk, and meshed split-thickness autograft to patient's left trunk compares gross appearance. General outcome with cultured skin substitutes shows complete wound closure and very favorable functional and cosmetic outcome with hyperpigmentation in healed skin. split-thickness autograft remains mildly erythematous, the mesh pattern is easily perceptible, and healed skin is hypopigmented.

or nearly complete failure of CSSs, and were regrafted (Fig. 3A). However, as experience was gained, engraftment in patients 18 through 38 increased to 50% to 90% of the grafted area. These differences represent the clinical learning curve with the experimental material. Similarly, Figure 3B shows that percentage of engrafted epithelium of CSSs at day 28 in patients 18 through 38 compared very favorably with STAG. Increased percentages of engrafted epithelium in CSSs at day 28 shows secondary coverage of wounds by epithelial outgrowth.

Exudative drainage from CSSs was observed on 40% of days *versus* 23% for STAG ($p = 0.06$). Partial or total regrafting was performed on 11 of 17 patients after grafting of CSSs, and on none after grafting of STAG ($p < 0.05$).

Daily assessments of CSSs and STAG on wounds between days 2 and 14 are shown in Figure 4. Coloration of wounds (Fig. 4A) was more pink to red for CSSs than for STAG, which was more pink to white. Keratinization of treated wounds (Fig. 4B) was less for CSSs than for STAG. Mean percent of sites covered in the first 2 weeks remained greater than 80% for STAG, but declined steadily for CSSs to between 50% and 60% (Fig. 4C).

Qualitative outcome of CSS and STAG was evaluated beginning on day 14 and proceeded as long as patients returned to the hospital for subsequent examination. Erythema in CSSs and STAG (Fig. 5A) gradually resolved in healed areas, except where hypertrophic or keloid scar developed. Pigmentation (Fig. 5B) was relatively constant in STAG and tended to be less than normal. In CSSs, pronounced hypopigmentation was observed for 1 to 2 months, after which a clear tendency of hyperpigmentation occurred. Gradually, hyperpigmented areas of CSSs became lighter, and normalized to each patient's native color. Epidermal blistering (Fig. 5C) occurred with no greater frequency in CSSs than in STAG. Any blisters that occurred has resolved by one month after grafting, and no subsequent ulceration of the epidermis of the healed CSS was observed. Surface texture (Fig. 5D) of healed CSSs was generally smoother than STAG during the first 3 months after grafting. However, by 1 year after grafting, the surface texture of CSSs and STAG was not different. Suppleness of skin (Fig. 5E) was not different between CSSs and STAG during the observation period. Raised scar (Fig. 5F) was less for CSSs at earlier observation periods, but was not different by 1 year.

Table 2 shows the ratio of areas healed with CSS to area of skin biopsies from which CSS were prepared. Expansion ratios ranged from 1.23 to a maximum expansion of area of 37.4 times. The maximum expansion accomplished with CSSs in this study represents a ninefold increase over a 4:1 meshed autograft, and has reduced the requirement for harvesting of autograft in this patient.

To assay for antibodies to bovine collagen that was im-

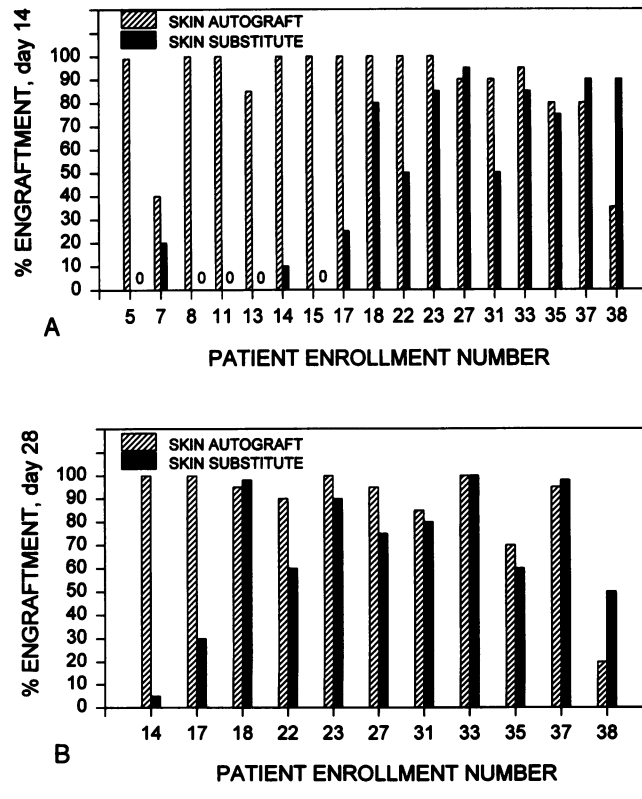

Figure 3. Percentage engraftment *vs.* patient enrollment number. (A) At 14 days after grafting, patients 5 through 17, who were enrolled earlier in the study, had complete failure or very low engraftment of cultured skin substitutes. Patients 18 through 38 had a much greater percent engraftment as laboratory and clinical protocols were improved. (B) At 28 days after grafting, patients 18 through 28 show percent engraftment of cultured skin substitutes that compares more favorably with split-thickness autograft.

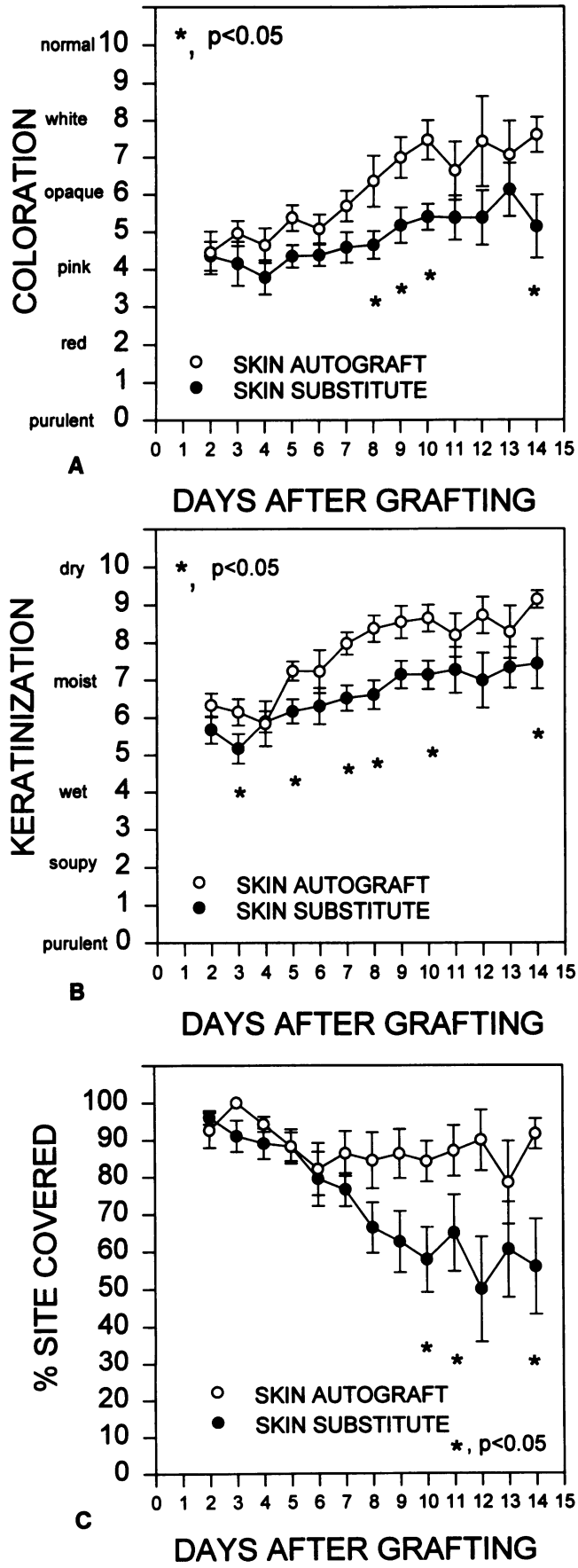

Figure 4. Site assessments, days 2 through 14. (A) Coloration of cultured skin substitutes is more red-pink than split-thickness autograft, which progresses from pink to white with differences at 8, 9, 10, and 14 days of observation. (B) Keratinization of cultured skin substitutes is significantly less than split-thickness 2, 4, 7, 8, 10, and 14 days after grafting. (C) Percent of site covered with cultured skin substitutes is significantly less at days 10, 11, and 14 after grafting.

planted as part of the skin substitute, Enzyme-linked immunosorbent assays were performed. Table 3 presents data from 11 patients from whom serum samples were collected before and 28 days after treatment with CSSs. Enzyme-linked immunosorbent assay values for CSS-treated patients were not statistically different from preimmune sera from patients or rabbits. Control sera from immunized rabbits were statistically different from all other groups.

DISCUSSION

Data reported here support the hypothesis that CSSs prepared from autologous skin cells and collagen-GAG membranes may be a safe and efficacious alternative to skin autograft for life-threatening burns. However, the data also identify multiple deficiencies of this cell-biopolymer graft that occur during the first 2 weeks after grafting compared with skin autograft. These deficiencies include, but are not limited to: slower vascularization, slower keratinization, greater graft loss from microbial contamination, and greater mechanical fragility. Findings of this study also show that epidermis from CSSs stabilizes by 2 weeks, does not blister subsequently, and has outcome that is not different from autograft 6 to 12 months after treatment. Future improvements in composition and use of CSSs are expected to increase rates of initial engraftment.

Although mean rates of engraftment for CSS at 2 weeks are significantly lower than for autograft (Fig. 4C), absolute rates of CSS engraftment have increased during the course of the study (Fig. 3A). Four factors have been introduced that have contributed to improved engraftment of CSS to compensate partially for anatomic and physiologic deficiencies of the cultured graft. First, formulations of noncytotoxic antimicrobial agents for topical use[33,34] provide effective management of gram-negative, gram-positive, and fungal organisms. Second, addition of topical nutrients to irrigation solutions has increased significantly the survival of transplanted cells during vascularization of CSSs.[24] Third, removal of glutaraldehyde from preparation of collagen-GAG substrates[36] is associated with more rapid degradation of the biopolymer substrate. Fourth, culture media for keratinocytes have been optimized to promote development of

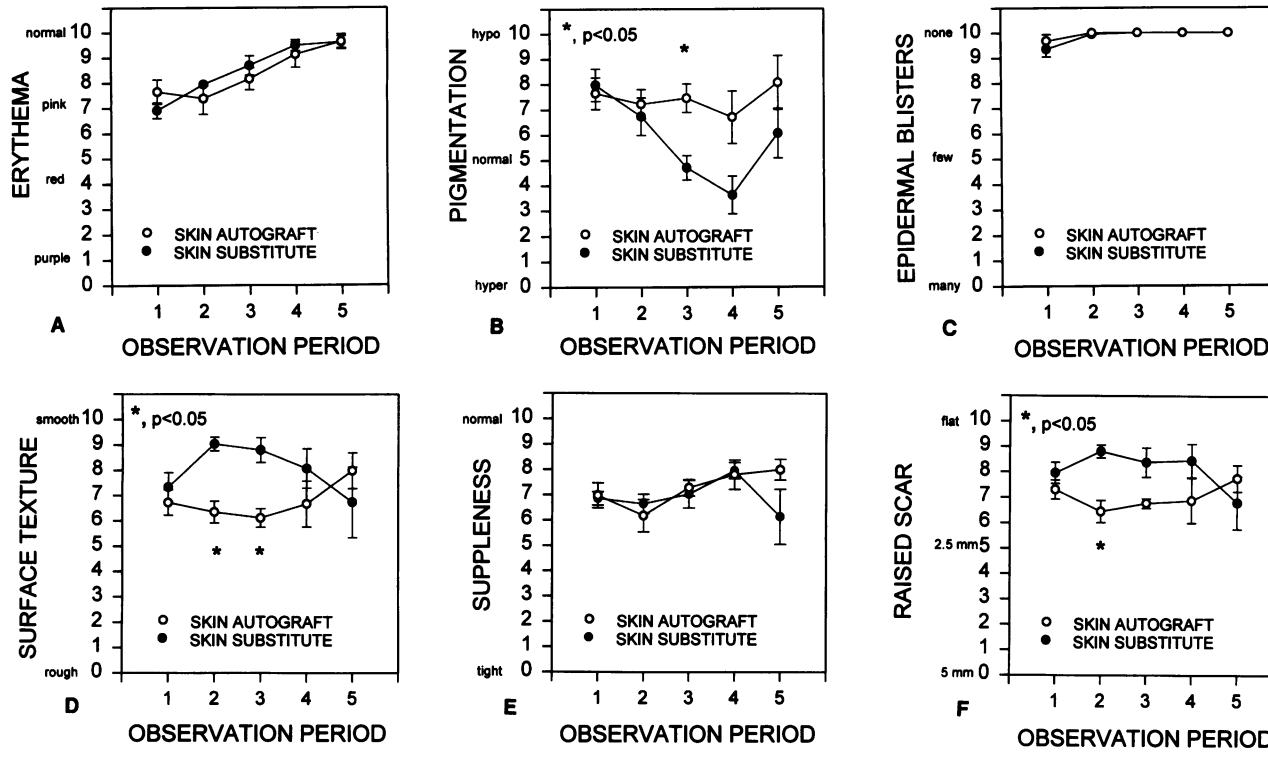

Figure 5. Qualitative outcome, day 14 through 1.5 years. Observation periods for all plots are: 1) 14–30 days; 2) 1–2 months; 3) 3–4 months; 4) 5–12 months; and 5) 1 year and after. (A) Erythema is not different in healed cultured skin substitutes (CSSs) or split-thickness autograft (STAG). (B) Pigmentation is significantly greater in CSSs compared with STAG at 3 to 4 months. (C) Epidermal blistering is not different between CSS and STAG. (D) External surface of CSS is smoother than STAG from 1 to 4 months after grafting, but not different at later time points. (E) Skin suppleness was not different between CSS and STAG in these observation periods. (F) Raised scar was significantly less for CSS in the period of 1 to 2 months after grafting, but not at other observation periods.

epithelial barrier *in vitro*.[30] Together, these factors have resulted in greater survival and engraftment of transplanted autologous skin cells.

Epithelial coverage of excised burns with cultured epithelium occurs by two distinct processes—engraftment and radial outgrowth. Studies with cultured epithelial sheets[2,3,19,37] have reported rates of engraftment of 60% to 90% at 28 days after application. These values may not be compared directly with skin autograft because they include outgrowth between weeks 2 to 4, whereas even 4:1 autograft usually is fully closed by 2 weeks. It is expected that values for engraftment of keratinocyte sheets would be reduced substantially if comparisons with skin autograft were performed in a more direct and contemporary design. Therefore, interpretation of results with keratinocyte sheets alone require additional qualification. Time to closure also is skewed in favor of skin substitutes by the comparison of cultured grafts as sheets with meshed skin autografts. However, even with these factors of bias, rates of engraftment of composite skin substitutes and keratinocyte sheets are significantly lower than native skin autograft. Therefore, increased rates of engraftment may be expected to decrease time to wound closure and reduce requirements for regrafting of wounds treated with skin substitutes.

Replacement of connective tissue in excised, full-thickness wounds has gained consensus as a requirement for restoration of skin function and cosmesis. Native skin autograft applied as sheets[38] provides the highest quality skin repair. Two other general approaches to replacement of dermis

Table 3. ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA) FOR BOVINE COLLAGEN IN SERA FROM BURN PATIENTS (n = 11) TREATED WITH CULTURED SKIN SUBSTITUTES

Sera	No. CSS Inoc	OD_{490}	% Control	n
Rabbit, preimmune	0	0.103 ± 0.008	23 ± 1.8	2
Rabbit, postimmune	3	0.456 ± 0.013*	100 ± 2.9*	2
Patients, pre-CSS	0	0.129 ± 0.006	28 ± 1.3	11
Patients, day 28 CSS	1	0.132 ± 0.006	29 ± 1.3	9
Patients, day 28 CSS	>1	0.110 ± 0.003	24 ± 0.7	2

CSS =; cultured skin substitute; Inoc = inoculations.
* $p < 0.05$.

have been described. The first approach is application of human cadaver skin allograft with subsequent removal of alloepidermis, and grafting of autologous keratinocyte sheets,[4,37] or application of acellular dermal matrix.[39,40] The advantage of cadaveric material is biologic nativity of the biopolymer implant. The disadvantage is the heterogeneous nature of the procurement population and requirements for two surgical procedures.[4,37] The second approach is preparation of a biopolymer implant *in vitro*.[7,40–44] Advantages of this latter approach include control of biochemical and structural composition, and development of reproducible fabrication processes. Disadvantages of biosynthetic approaches include use of non-native polymers, or partially denatured collagen, and lack of a full complement of matrix proteins, particularly those of basement membrane. Because fabrication of dermal replacements *in vitro* currently is exclusive of native processes of biosynthesis, fabricated materials constitute foreign bodies that must be degraded and removed. Therefore, increase of biocompatibility of fabricated biopolymer implants may be expected to increase their ability to vascularize, and decrease their rates of degradation. Conversely, dermal implants harvested *ex vivo* may be made more uniform and available by use of acellular dermis from porcine or bovine sources. The collagen-GAG substrate reported here offers advantages of uniform composition, and high biocompatibility.

Regulatory requirements for use and banking of skin substitutes[45] have gained increasing attention. Depending on the composition of the transplant, it may be classified as a banked tissue or a medical device. Transplantation of autologous cultured skin cells currently is considered a form of tissue banking. However, this classification has not considered factors of safety and efficacy to recipients that have shown increased risks to certain patients.[46] Inclusion of allogeneic cells in chimeric grafts,[47] and composite grafts[48] requires assurances for negative testing of microbial and viral pathogens,[49] and imposes requirements for tracking of patients who receive allogeneic cells. Genetic modification of cells for transplantation[50,51] introduces another set of requirements for safety and efficacy that includes stable integration of the gene into the transplanted cells, stable integration of the cells into tissue, and regulated expression of modified genes. Each of these applications depends greatly on valid assessment of transplanted cells.

Assessment of safety and efficacy in this study was performed in a semiquantitative manner, with introduction of bias from subjective scoring of treated sites.[18] Conclusions of future studies would be strengthened greatly by reduction or elimination of the subjective elements in assessments. Instrumentation for noninvasive and objective measurement of biophysical properties of skin[52] (*e.g.*, epidermal barrier, biomechanical properties, pigmentation, blood flow) continue to emerge for evaluation of skin pathologies in neonatology, dermatology, and pharmaceutical science. Objective, quantitative, and kinetic data of surface electrical capacitance have been collected to evaluate epidermal healing of skin substitutes on full-thickness wounds in athymic mice[53] and humans.[54] Application of biophysical instrumentation to assessment of wound healing is most probable to provide more rigorous data that are needed for regulatory validation of experimental tissue substitutes.

Data included in this report support the use of CSS as an adjunctive treatment for catastrophic burns. Prospective advantages of this material include reduced requirements for donor skin and shorter hospitalization with functional and cosmetic outcome that is comparable to skin autograft. Realization of these advantages will depend most heavily on increased rates of initial engraftment of skin substitutes by further improvements to the composition and clinical application of the cultured grafts. Accomplishment of these few remaining goals is probable to result in reduced mortality and morbidity from burns.

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