# In Vitro Reconstitution of Skin: Fibroblasts Facilitate Keratinocyte Growth and Differentiation on Acellular Reticular Dermis

Niels C. Krejci, Charles B. Cuono, Robert C. Langdon, and Joseph McGuire

Departments of Dermatology (NCK, RCL, JMcG) and Surgery (CBC), Yale University, School of Medicine, New Haven, Connecticut, U.S.A.

Extensive full-thickness burns require replacement of both epidemis and dermis. We have described a method in which allogeneic dermis from engrafted cryopreserved cadaver skin was combined with cultured autologous keratinocytes. In the present study we combined human keratinocytes and fibroblasts, and acellular human dermis in vitro and transplanted this "reconstituted skin" into athymic mice. Both human papillary dermis in which the basement membrane zone has been retained and human reticular dermis that has been repopulated with human dermal fibroblasts are good substrates for keratinocyte attachment, stratification, growth, and differentiation. Both of these dermal preparations can be lyophilized and stored at room temperature without losing their ability to support keratinocyte growth. In contrast, human papillary dermis that has been treated with trypsin lacks laminin and collagen type IV in the BMZ and supports keratinocyte attachment and differentiation less well. *J Invest Dermatol 97:843–848, 1991*

Third-degree burns by definition destroy both epidermis and dermis, necessitating skin replacement. Usually, uninjured areas serve as donor sites for split-thickness skin grafts. In extensive burn injuries (> 50% TBSA), autologous grafts from patient donor sites are inadequate to cover all burned areas. Repeated harvesting of healed donor sites is limited by the ability of these sites to regenerate. A functional and permanent skin replacement is needed in these patients. In 1975, Rheinwald and Green described a technique for growing human keratinocytes as stratified cultures [1]. In 1979, Green et al suggested the use of these cultures as grafts [2]. Cultured epidermal sheets were introduced in 1981 for the therapy of burns [3] and have been used in burn therapy since then. Grafting of epidermal sheets on fat, fascia, or granulation tissue, however, does not provide an immediate dermal replacement and results in problems such as wound contraction, incomplete reconstituion of the basement membrane zone [4], and grafting fragility [5]. A "neodermis" is formed, however, within a few years [6]. In order to replace the dermal component, Bell et al [7], Burke et al [8], and recently Hansbrough et al [9] have studied composites consisting of collagen-based membranes associated with thin autografts or cultured keratinocytes.

Manuscript received November 27, 1990; accepted for publication July 10, 1991.

This work was supported in part by N.I.H. grants AR-13929 and T32 AR07422 and by a fellowship from the Deutsche Forschungsgemeinschaft, Kr 10422/2-1.

This work was presented in part at the Society of Investigative Dermatology, Washington, DC, May 1990.

Reprint requests to: Dr. Niels Krejci, Department of Dermatology, Stanford University, Edwards Building R-144, Stanford, CA 94305.

Abbreviations:

ALI: air-liquid interface
DED: de-epidermized dermis
DMEM: Dulbecco's modified Eagle's medium
PBS: phosphate-buffered saline
SCD: second-cut dermis (reticular dermis)
TBSA: total body surface area

In 1986 a technique was described in which burned skin was replaced with cadaver skin. Following escharectomy of the burned skin, the wound was resurfaced with viable cryopreserved cadaver skin. After 4–6 weeks, the allogeneic epidermal component was mechanically removed and replaced with cultured stratified autologous keratinocyte sheets. This approach resulted in a skin with complete reconstitution of the basement membrane zone [10,11].

The present study was undertaken to determine whether skin could be reconstituted in vitro from allogeneic acellular dermis and autologous keratinocytes and fibroblasts. We investigated the ability of various dermal substrates to support epidermal growth, differentiation, and dermal-epidermal integration and the subsequent in vivo behavior after transplantation into athymic mice.

## MATERIALS AND METHODS

**Dermal Preparations** Split-thickness skin 0.4 mm thick was harvested with a dermatome from either the thigh or back of a cadaver and devitalized by three freeze-thaw cycles and subsequent exposure to 5000 rad gamma irradiation delivered over a 12-min period. The skin was then soaked at 37°C in PBS containing penicillin (50 u/ml), streptomycin (50 μg/ml), and amphotericin B (1.25 μg/ml) for 7–14 d, after which the epidermis was mechanically removed leaving "de-epidermized dermis" (DED) as originally described by Prunieras [12]. The DED was soaked in PBS at 37°C for an additional 3 weeks until it became acellular as assessed by light microscopy. In order to degrade components of the basement membrane, DED was incubated in trypsin 1 : 250 (GIBCO) at 37°C for 5 h and then transferred to culture medium containing 10% calf serum to inactivate the trypsin. Reticular dermis of 0.6-mm thickness was harvested by recutting a previously harvested area. It was devitalized as above and subsequently soaked for 5 weeks in PBS at 37°C. This acellular, reticular dermis is referred to as second-cut dermis (SCD). Viable reticular dermis was prepared by cutting skin obtained from mammoplasty to a thickness of 1 mm using a drum dermatome. A second cut at 0.4 mm from the epidermal surface yielded reticular dermis of 0.6-mm thickness. Small amounts of blood on the dermis were removed by rinsing the dermis in PBS; it was then placed in culture medium.

0022-202X/91/$03.50 

**Explant Assay** In order to measure the ability of various dermal preparations to support epithelial growth, we used the method of Freeman et al [13] and stained the preparations with hematoxylin. Neonatal foreskins were trimmed free of subcutaneous fat and cut into 1 $mm^2$ explants. Explants were placed on dermal preparations and cultured for 2 weeks at the air-liquid interface (ALI) in keratinocyte culture medium (see below). From five to eight explants were grown on each dermal substrate. Each experiment was carried out in duplicate (lyophilized DED and trypsinized DED) or triplicate (normal DED and SCD). Explants were fixed in 10% buffered formalin. Epidermal outgrowth from the explant was measured after staining with aqueous hematoxylin. Specimens were submerged for 20 seconds in hematoxylin (0.68% W/V Shandon) and washed in tap water. This treatment stains dermis, but not epidermal outgrowth, which appears as a white area on a purple stained dermal background. Specimens were photographed at fixed magnification. The images were projected and traced on paper. The area of keratinocyte outgrowth (less the area of the central explant) was calculated by gravimetric planimetry. Dermal-epidermal integration was evaluated by lifting the outgrowing epidermis with forceps. Attachment was considered strong if the epidermis tore before lifting from the dermis. Attachment was considered poor if the explant and its epidermal outgrowth could be lifted easily from the dermis as a sheet.

**Keratinocyte and Fibroblast Culture and Seeding** Fibroblasts from neonatal foreskin dermis were cultured in DMEM with 10% bovine calf serum and antibiotics. Fibroblasts ($2.5 \times 10^6$ cells) in second to fourth passage were seeded in a circular area of 2.5 $cm^2$ on SCD and cultured submerged for 3 d before seeding with keratinocytes. Neonatal foreskin keratinocytes were cultured in a variation of the method of Wu and Rheinwald [14] using a 3T3 feeder layer and a 3:1 mixture of DMEM and Ham's F12 supplemented with 10% fetal calf serum, adenine ($1.8 \times 10^{-4}$ M), hydrocortisone (4 $\mu$g/ml), insulin (5 u/ml), triiodothyronine ($2 \times 10^{-9}$ M), cholera toxin ($1 \times 10^{-10}$M), EGF (1 ng/ml), transferrin (5 $\mu$g/ml), penicillin (50 $\mu$g/ml), streptomycin (50 $\mu$g/ml), and amphotericin B (1 $\mu$g/ml). Keratinocytes ($9.5 \times 10^5$ cells) from subconfluent secondary cultures were confined to a 0.95-$cm^2$ circular area of dermis. Each condition was represented by two or three circles. If keratinocytes were seeded on SCD that had been pre-seeded with fibroblasts, the keratinocytes were placed in the same location as the fibroblasts. The specimens were cultured for 1 week submerged and for 1 week on a plastic grid at the air-liquid interface (ALI), as described by Prunieras [15].

**Grafting** Reconstituted skin from normal DED (n = 2), lyophilized DED (n = 2), and SCD (n = 2) were grafted. Athymic mice (balb/c, nu/nu, NIH) were anesthetized by intraperitoneal injection of ketamine and xylazene (Rompun) and a 2-cm longitudinal incision was made through skin and panniculus carnosus on the lateral side of the thorax. Reconstituted skin was placed dermis down on the chest wall, covered with a sterile 0.007-inch-thick silicone sheet (Silastic, Dow Corning, Midland, MI) and the overlying skin incision was closed. The subpannicular grafts were removed and examined after 1-3 weeks.

**Immunostaining** Specimens for immunostaining were fixed in acetone for 4-6 h and directly embedded in paraffin. Four-micrometer sections were placed on poly-L-lysine-coated slides, baked at 56°C for 1 h, and deparaffinized with xylene and acetone. Polyclonal rabbit antibody against laminin was purchased from Telios, San Diego, CA. Polyclonal rabbit antibody against collagen type IV was purchased from Heyl, Berlin, Germany. Fluorescein-conjugated goat-anti-rabbit antibody was purchased from Accurate, Westbury, NY. Peroxidase staining was performed with the Vectastain ABC Kit from Vector, Burlingame, CA.

**Freeze-Drying** Dermal specimens were cut into pieces of 6-8 $cm^2$ and placed into 60-mm culture dishes that were closed and loosely taped. The specimens were frozen by floating the dishes on liquid nitrogen and then lyophilized, after which they were packaged and stored at room temperature. Specimens were rehydrated by soaking at 37°C for 2 h in 2-5 ml of distilled water. Specimens were then transferred to culture medium for 16 h at 37°C before cells were applied.

**Application of Extracellular Matrix Components** Fibronectin was purchased from Sigma, St. Louis, MO and collagen type IV was purchased from Heyl, Berlin, Germany. Dermal specimens were transferred to a sterile plastic grid and placed in a laminar flow hood for 30 min. Fibronectin or collagen type IV (0.2 $\mu g/cm^2$) was applied in a volume of 50 $\mu$l and spread over the dermis with a sterile scalpel. The dermis was allowed to air dry for 5-10 min and the dish was filled with enough culture medium to touch the lower surface of the dermis.

**Transmission Electron Microscopy** Specimens were placed in Karnovsky's fixative for 24 h at 4°C and processed by LifeCell Corporation, Woodland, TX, following the general procedure detailed previously [16].

## RESULTS

**Hematoxylin Surface Staining** Keratinocytes cultured on dermis from either explants or keratinocyte cell suspensions could be visualized and measured as white, unstained areas on a purple stained background. Phase-contrast microscopic evaluation of a hematoxylin-stained, transparent collagen gel demonstrated that a monolayer of keratinocytes was sufficient to prevent dye penetration into the underlying collagen; the unstained area corresponded to the area covered by outgrowing keratinocytes. Hematoxylin staining of specimens did not interfere with subsequent histologic evaluation.

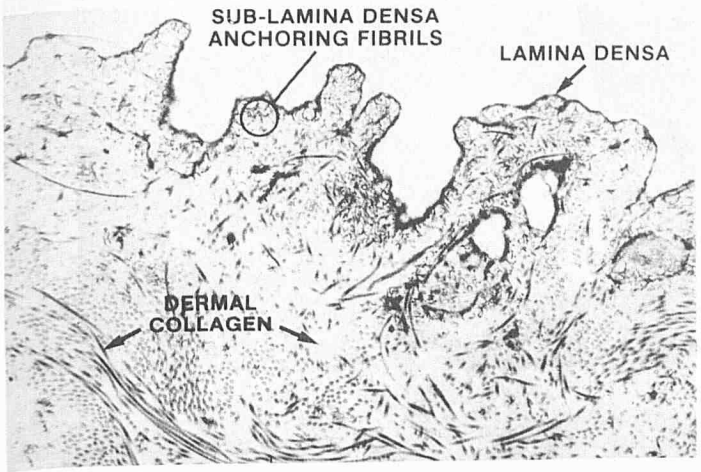
**De-Epidermized Dermis** Soaking de-epidermized dermis in PBS at 37°C after killing the resident cells resulted in loss of basophilic nuclear staining. Nuclei of fibroblasts were not detectable after 2 weeks and endothelial nuclei were not detectable after 4 weeks. After this time the dermis was "acellular" by light microscopy. Transmission electron microscopy revealed a lamina densa and anchoring fibrils (Fig 1*a*). DED was used either immediately or was freeze-dried and then stored at room temperature. Lyophilized de-epidermized, acellular dermis that had been rehydrated after being stored at room temperature for 3 months showed no gross changes and was identical histologically to fresh DED. Both laminin and collagen type IV could be demonstrated in both conditions by immunofluorescence (Fig 1*b,c*). Functional behavior such as support of keratinocyte growth, differentiation, attachment, and engraftment in athymic mice was identical for both.

Epidermal outgrowth from explants placed on DED covered an average area of 17.4 $mm^2$ at 2 weeks on fresh DED and an area of 15.5 $mm^2$ on freeze-dried DED (Fig 2). The outgrowing epidermal apron was flat, one to three cell layers thick, and tightly adherent to the dermis (Fig 3*a*). Keratinocytes also epibolized the explant (Fig 3*b*). Keratinocytes that were applied to the DED as a cell suspension attached within 16 h. Cultures submerged for 1 week and kept for an additional week at the air-liquid interface produced a five to 15 cell-layer-thick, stratified epidermis. Basal, spinous, and granular layers, and stratum corneum were identifiable (Fig 3*c*). Meshing of this reconstituted skin was possible without detachment of the epidermis from the dermis.

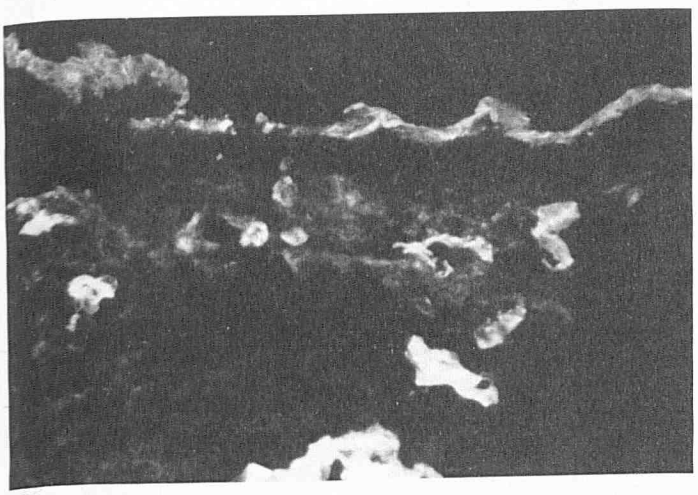
Subpannicular grafting of reconstituted skin to the chest wall resulted in engraftment within 2 weeks. The epidermis after 2 weeks appeared white and shiny and was not attached to the overlying silicone sheet. The formerly acellular dermis was repopulated by fibroblasts and blood vessels, presumed to be of host origin. The epidermal component was histologically viable and differentiated (Fig 3*d*).

**De-Epidermized Dermis after Trypsin Treatment** Freshly prepared DED was subjected to digestion by trypsin for 5 h at 37°C. Following such treatment collagen type IV and laminin could not be identified by immunofluorescence.

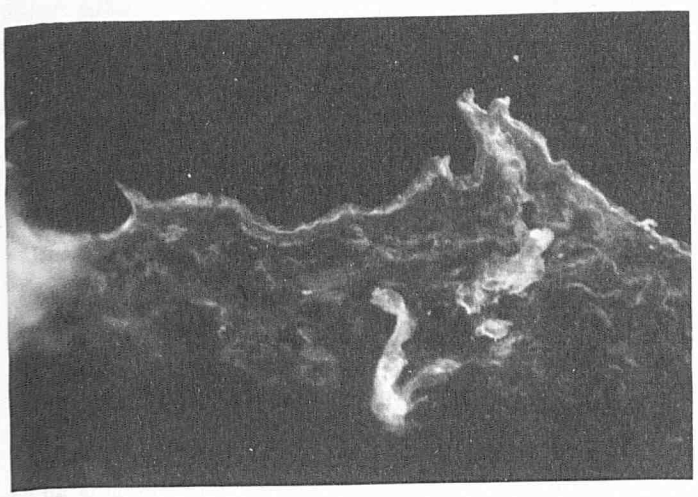
Outgrowth area from explants on trypsin-treated DED averaged




a



b



c

**Figure 1.** Preservation of basement membrane zone components during preparation of DED. *a*) TEM of fresh, non lyophilized DED. (Magnification × 13,700); *b*) IIF for collagen type IV in DED that has been lyophilized and rehydrated; *c*) IIF for laminin in DED that has been lyophilized and rehydrated DED.

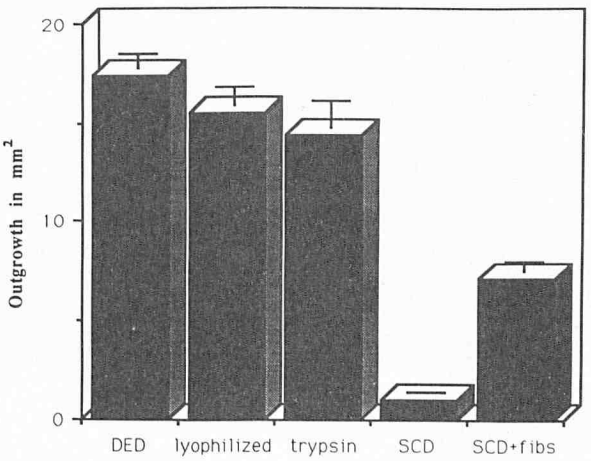



**Figure 2.** Comparison of the outgrowth from foreskin explants on various dermal preparations. Outgrowth is expressed as $mm^2$ of keratinocyte outgrowth minus the area of the original explant.

14.4 $mm^2$ (Fig 2), and these explants usually did not epibolize. The dermal-epidermal integration of the outgrowing epidermis was poor; the explant with its outgrowing margin of keratinocytes could be lifted easily with forceps (Fig 3*e*) and the epidermis frequently detached partially from the dermis during histologic processing (Fig 3*f,g*). Keratinocytes that were first seeded on trypsinized DED as a cell suspension and then cultured for 1 week submerged and for a second week at the ALI produced a 3–8 cell-layer–thick epidermis. There was less differentiation compared to epidermis on PBS-split DED (Fig 3*g*). Attempts to mesh the reconstituted skin resulted in detachment of epidermis from dermis.

**Human Reticular Second-cut Dermis (SCD)** There was very limited or no epidermal outgrowth (< 1 $mm^2$, Fig 2) from explants placed on reticular, acellular dermis (Fig 4*a*). Suspended keratinocytes seeded on reticular acellular dermis did not attach and spread, but formed small islands of rounded cells (Fig 4*b*). Conditioning the surface with either fibronectin or collagen type IV did not enhance keratinocyte attachment or growth. Seeding cultured dermal fibroblasts on SCD resulted in both colonization of the surface as well as invasion of the acellular dermis. Skin explants placed on fibroblast-conditioned SCD grew out an average of 7.6 $mm^2$ in 2 weeks (Fig 4*c*). Suspended keratinocytes seeded on such dermal preparations formed a 4–10 cell-layer–thick epidermis that stratified and cornified with frequent suprabasalar mitoses (Fig 4*d*). Dermal-epidermal integration was sufficient to allow meshing without detachment of the epidermis (Fig 4*e*). Staining for collagen type IV 2 weeks after keratinocyte seeding was negative.

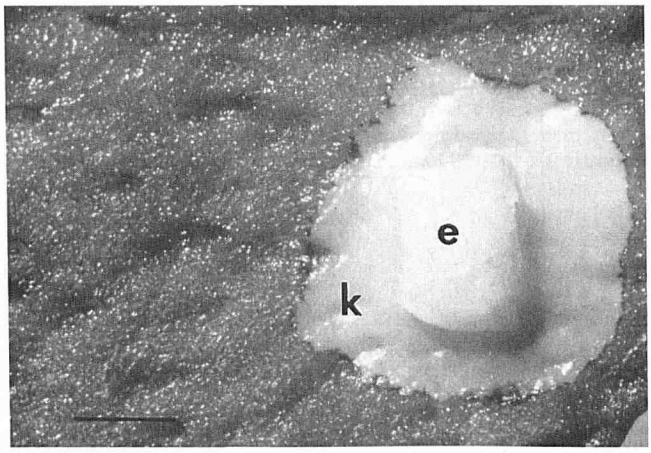
When grafted into athymic mice this reconstituted skin was populated by blood vessels at day 25. The overlying epidermis was viable and differentiated. The dermo-epidermal junction stained positively for collagen type IV (Fig 4*f*).

No differences in fibroblast ingrowth or keratinocyte growth could be detected when freshly prepared SCD was compared with lyophilized and rehydrated SCD.

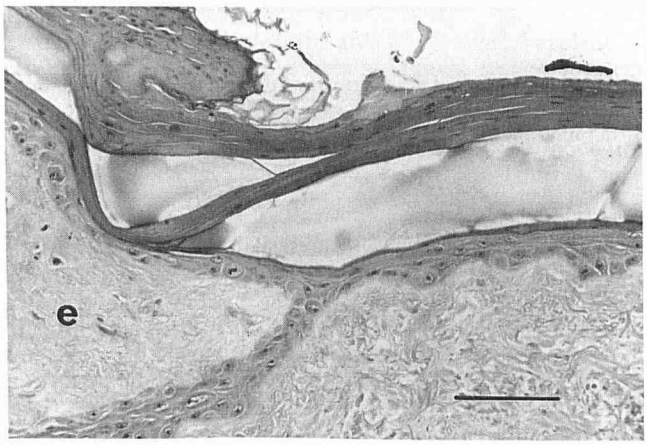
Fresh, viable reticular dermis did not support keratinocyte growth from either explants or keratinocyte suspensions.

## DISCUSSION

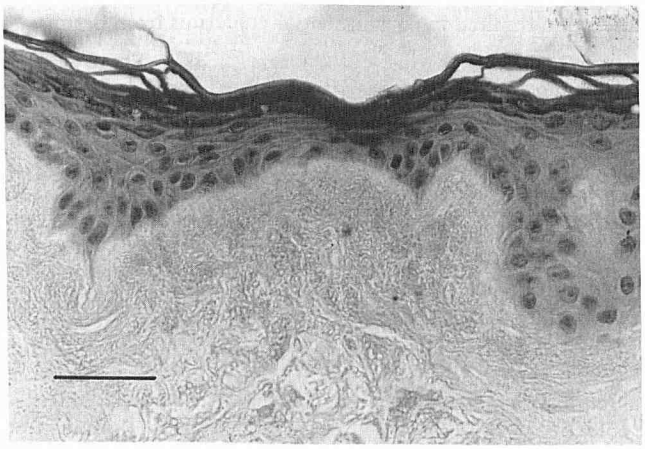
The integration of dermis and cultured keratinocytes in vitro provides a potential source of "skin" for grafting. Providing a dermal component as opposed to grafting cultured epidermal sheets on muscle fascia enhances stability of the resulting skin, and reduces scarring and contraction [11,16]. The greater stability of epidermis engrafted on a dermal bed as compared to muscle fascia correlates with development of anchoring fibrils that are present 3 months



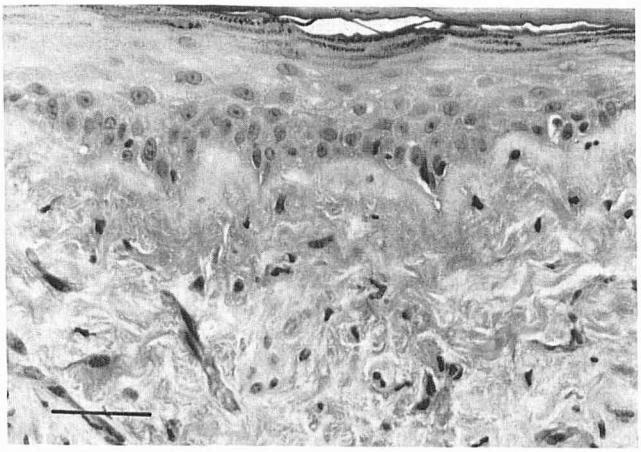

a



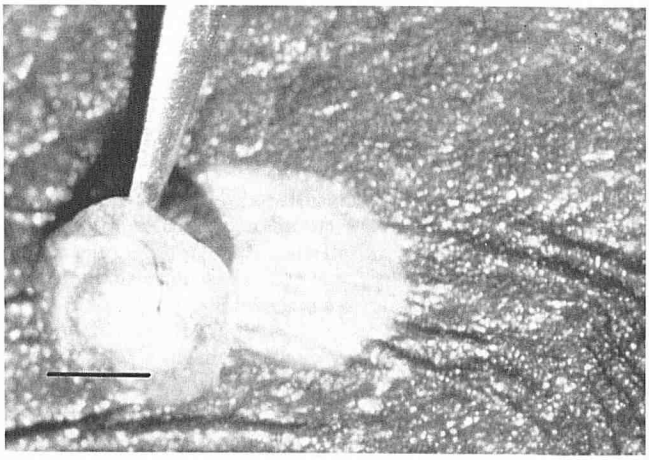

b



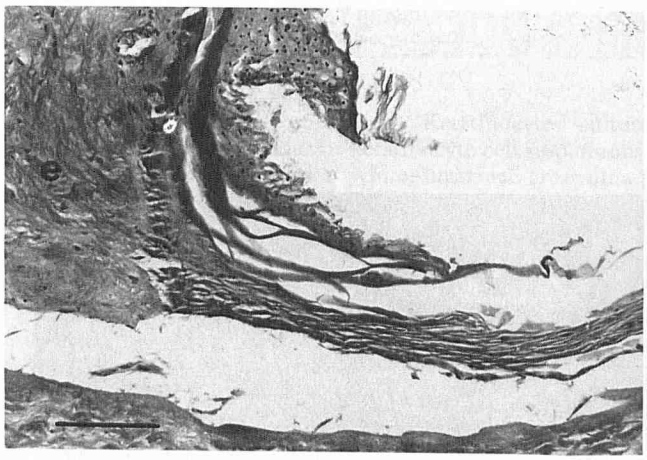
c



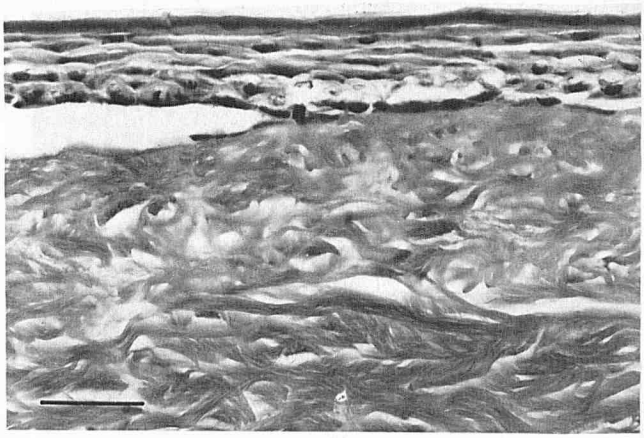
d



e

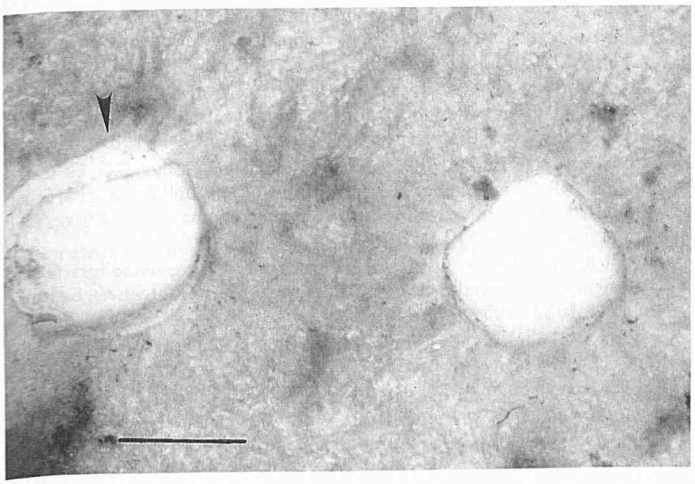



f

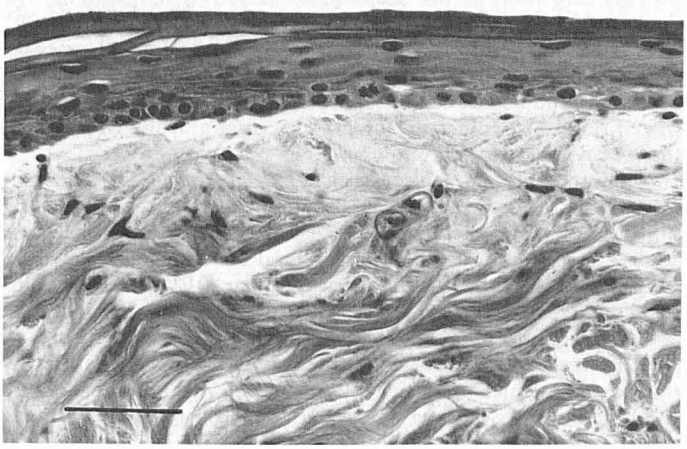


g

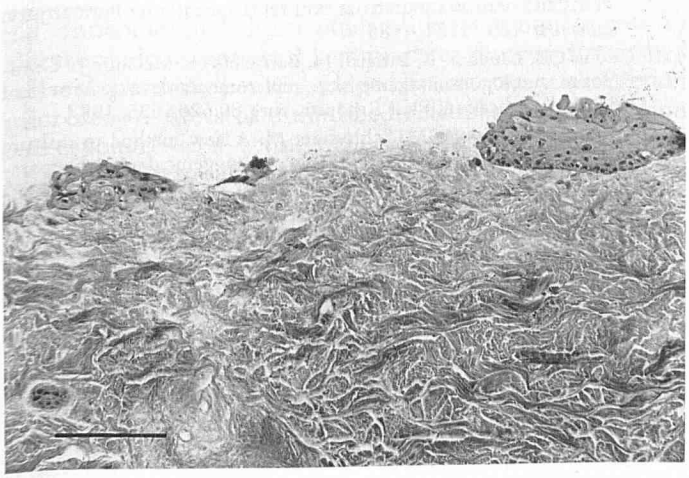
**Figure 3.** Behavior of keratinocytes and explants on untreated DED (*a–d*) and on trypsin-treated DED (*e–g*). Fig *a,b,c,e,f,* and *g* show specimens after 2 weeks in culture at the air liquid interface. Figure 3*d* shows a specimen after 2 weeks in culture and 2 weeks of engraftment in an athymic mouse. *a*) Foreskin explant (*e*) on DED showing outgrowth of keratinocytes (*k*). Hematoxylin stain. (*Bar*, 1 mm.) *b*) Foreskin explant (*e*) and its outgrowing keratinocytes on de-epidermized dermis. Vertical section. H&E. (*Bar*, 80 μm.) *c*) Reconstituted skin composed of DED and epidermis that developed from a suspension of keratinocytes that had been seeded 2 weeks before fixation. Vertical section, H&E. (*Bar*, 50 μm.) *d*) Reconstituted skin after 2 weeks in vitro culture as in Fig 3*c* and subpannicular engraftment in an athymic mouse for 2 weeks. Vertical section, H&E. (*Bar*, 50 μm.) *e*) Foreskin explant and outgrowing keratinocytes on trypsin-treated DED. The explant and its outgrowth are easily lifted by forceps from the dermis leaving an unstained area. Hematoxylin stain. (*Bar*, 1 mm.) *f*) Foreskin explant (*e*) and its outgrowing keratinocytes on trypsin-treated DED. Note poor attachment. Vertical section, H&E. (*Bar*, 130 μm.) *g*) Reconstituted skin, composed of DED that has been treated with trypsin, and epidermis derived from a keratinocyte suspension. H&E. (*Bar*, 50 μm.)



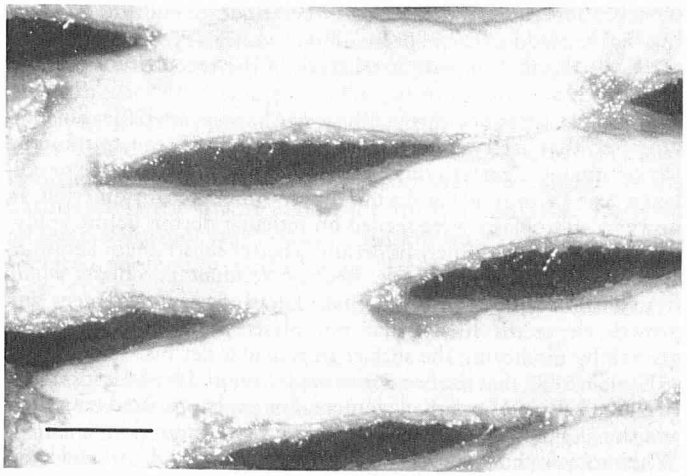
a



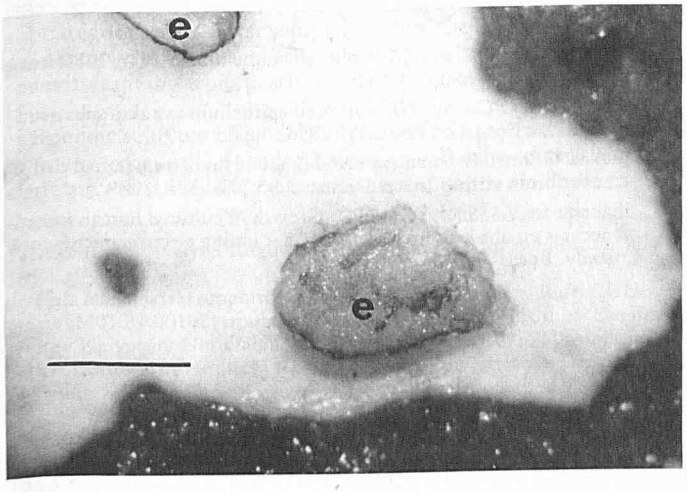
d



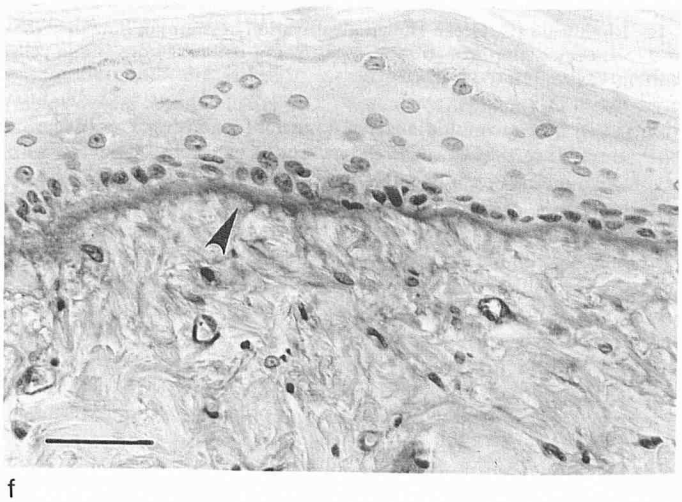
b



e




c



f

**Figure 4.** Behavior of keratinocytes on untreated (*a–b*) reticular second-cut dermis (SCD) and on SCD that had been pre-treated with fibroblasts (*c–f*). Figures *a–e* show specimens at 2 weeks of culture at the air-liquid interface. Figure 4*f* shows a specimen after culture for 2 weeks in vitro and 3 weeks of engraftment in an athymic mouse. *a*) Foreskin explant without (right) or with minimal epidermal outgrowth (left; *arrow*) on SCD. Hematoxylin stain. (*Bar*, 1 mm.) *b*) Keratinocytes that had been seeded on SCD as a cell suspension. Vertical section, H&E. (*Bar*, 160 μm.) *c*) Foreskin explants (*e*) and outgrowing keratinocytes on SCD that had been pretreated with fibroblasts. Hemaxtoxylin stain. (*Bar*, 1 mm.) *d*) Reconstituted skin composed of SCD that had been pre-treated with fibroblasts, then seeded with a suspension of keratinocytes. Vertical section, H&E. (*Bar*, 50 μm.) *e*) Reconstituted skin from fibroblast-treated SCD as in Fig 4*d* after meshing 1 : 1.5. The unstained white surface of the meshed skin demonstrates attached epidermis that excludes hematoxylin from the dark stained dermis (*Bar*, 1 mm.) *f*) Demonstration of type IV collagen (*arrow*) by immunperoxidase in reconstituted skin consisting of fibroblast-conditioned SCD seeded with a suspension of keratinocytes as in Fig 4*d*. The skin was then engrafted into an athymic mouse for 2 weeks. Hematoxylin counter stain. (*Bar*, 50 μm.)

post grafting in the dermal-epidermal junction [16] but are absent or decreased in the epidermal-muscular junction [4].

Split-thickness skin engrafts reliably on most sites, whereas epidermal sheets are reported to take between 30% [17] and 80–90% [18]. If wound bed-dermis integration is more easily accomplished than wound bed-epidermis integration, then grafting of reconstituted skin is preferable.

Air-liquid interface cultures of keratinocytes on DED as pioneered by Prunieras [15] are the best in vitro system for producing highly differentiated epidermis. We confirmed and extended their studies in order to establish the potential for clinical applications. Our experiments demonstrate that acellular DED can be lyophilized and conveniently stored at room temperature and retain its ability to support keratinocyte growth after rehydration. Further, the attachment of reconstituted epidermis on DED is strong enough to allow subsequent meshing. This is clinically relevant because meshing increases the area and reduces the probability of graft failure due to the occurrence of sub-graft seromas and hematomas.

Epidermal differentiation on native and trypsinized human DED has been reported by others to be identical (19). We, however, observed superior differentiation of keratinocyte cultures on DED that has retained a basement membrane zone. Tryptic digestion of the BMZ resulted in poor attachment of the reconstituted epidermis.

Our observations confirmed those of Shakespeare [20] who demonstrated that acellular reticular dermis is an inadequate substrate for keratinocyte growth in vitro. Application of fibronectin or collagen type IV to reticular dermis did not improve this function. If, however, fibroblasts were seeded on reticular dermis before applying keratinocytes, the dermis became a better substrate for keratinocyte attachment and growth. Because resident fibroblasts *within* fresh viable SCD did not facilitate keratinocyte attachment and growth, it seems likely that fibroblasts promote keratinocyte growth by modifying the surface of reticular dermis.

Human SCD that has been preserved through lyophilization may find use in clinical burn management. It is easily procured with little additional effort at the time of routine post-mortem skin donation. When rehydrated it could be repopulated with fibroblasts and keratinocytes of the intended recipient and grafted.

## REFERENCES


1. Rheinwald JG, Green H: Serial cultivation of strains of human keratinocytes: the formation of keratinizing colonies from single cells. Cell 6:331–344, 1975
2. Green H, Kehinde O, Thomas J: Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci USA 76:5665–8, 1979
3. O'Connor NE, Mulliken JB, Banks-Schlegel S, Kehide O, Green H: Grafting of burns with cultured epithelium prepared from autologous epidermal cells. Lancet I:75–78, 1981
4. Woodley D, Peterson HD, Herzog SR, Stricklin GP, Burgeson RE, Briggamam RA, et al: Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstitution of anchoring fibrils. JAMA 259:2566–2571, 1988
5. Herzog SR, Meyer A, Woodley D, Peterson HD: Wound coverage with cultured autologous keratinocytes: use after burn wound excision; including biopsy followup. J Trauma 28:195–198, 1988
6. Compton CC, Gill JM, Bradford DA, Regauer S, Gallico GG, O'Connor NE: Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting. Lab Invest 60:600–612, 1989
7. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T: Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science 211:1052–1054, 1981
8. Burke JF, Yannas IV, Quinby WC, Bondoc CC, Jung WK: Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg 194:413–428, 1981
9. Hansbrough JF, Boyce ST, Matthew LC, Foreman TJ: Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. JAMA 262:2125–2130, 1989
10. Cuono C, Langdon R, McGuire J: Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. Lancet I:1123–1124, 1986
11. Cuono CB, Langdon R, Birchall N, Barttelbort S, McGuire J: Composite autologous-allogenic skin replacement: development and clinical application. Plast Reconstr. Surg 80:626–635, 1987
12. Prunieras M, Regnier M, Schlotterer M: A new method to culture human epidermal cells on allogenic or xenogenic dermis: preparation of recombined grafts. Ann Chir Plast 24:357–362, 1979
13. Freeman AE, Igel HJ, Herrman BJ, Kleinfeld KL: Growth and characterization of human skin epithelial cell cultures. In Vitro Cell Devel Biol 12:352–362, 1976
14. Wu YJ, Parker LM, Binder NE, Beckett MA, Sinard JH, Griffiths C, et al: The mesothelial keratins: a new family of cytoskeletal proteins identified in cultured mesothelial cells and nonkeratinizing epithelia. Cell 31:693–703, 1982
15. Prunieras M, Regnier M, Woodley DT: Methods for cultivation of keratinocytes with an air-liquid interface. J Invest Dermatol 81:28S–33S, 1983
16. Langdon RC, Cuono CB, Birchall N, Madri JA, Kuklinska E, McGuire J, et al: Reconstitution of structure and cell function in human skin grafts derived from cryopreserved allogenic dermis and autologous cultured keratinocytes. J Invest Dermatol 91:478–485, 1988
17. Eldad A, Burt A, Clarke JA: Cultured epithelium as a skin substitute. Burns 13:173–180, 1987
18. Gallico GG, O'Connor NE: Cultured epithelium as a skin substitute. Clin Plast Surg 12:149–157, 1985
19. Guo M, Grinnell F: Basement membrane and human epidermal differentiation in vitro. J Invest Dermatol 93:372–378, 1989
20. Shakespeare VA, Shakespeare PG: Growth of cultured human keratinocytes on fibrous dermal collagen: a scanning electron microscopy study. Burns 13:343–348, 1987