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GRAFTING ON NUDE MICE OF LIVING SKIN EQUIVALENTS PRODUCED USING HUMAN COLLAGENS

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Abstract

Autologous epidermal transplantation for human burn management is an example of a significant breakthrough in tissue engineering. However, the main drawback with this treatment remains the fragility of these grafts during and after surgery. A new human bilayered skin equivalent (hSE) was produced in our laboratory to overcome this problem. The aim of the present work was to study skin regeneration after hSE grafting onto nude mice. A comparative study was carried out over a period of 90 days, between anchored bovine skin equivalent, hSE and hSE+, the latter containing additional matrix components included at concentrations similar to those in human skin in vivo. The addition of a dermal layer to the epidermal sheet led to successful graft take, enhanced healing, and provided mechanical resistance to the grafts after transplantation. In situ analysis of the grafts showed good ultrastructural organization, including the deposition of a continuous basement membrane 1 week after surgery.

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Large scale epithelial cell production was first achieved in 1975 (1). Since then, the concept of cultured epithelial autografts for permanent burn wound coverage has become a therapeutic reality in North America (2-4). Although full terminal epithelial differentiation is obtained in vivo after grafting (5-8), some drawbacks to this technique have been identified. Indeed, epithelial sheet handling demands particular care from the first to the last steps of the surgical procedure, as well as after grafting. These sheets also lack suppleness, plasticity, and stress resistance, inherent to dermis in vivo. Moreover, complete skin regeneration does not occur before 2-5 years in adults after grafting, mostly because of slow reorganization of the dermis (4).

Therefore, the addition of a dermal counterpart to these epithelial sheets was highly desirable in order to obtain some valuable mechanical properties. Furthermore, since mesenchyme-epithelium interactions are involved in wound healing after grafting (9), the graft of dermoepidermal equivalents would very likely reduce the dermal organization delays, limit the scar tissue formation steps, and accelerate skin regeneration in situ.

Collagen gels were first used as a supportive matrix to grow cells (10) and were subsequently modified in order to obtain a three-dimensional, dermis-like structure seeded with human fibroblasts (11). A floating (nonanchored) skin equivalent (SE *) was reported in 1981 (12,13), but its use for wound coverage was limited since a severe contraction process greatly limits the final surface in culture. This problem was solved recently in our laboratory by the establishment of an SE peripheral anchorage method (14).

During the last decade, different skin substitutes have been proposed for human burn management (15-22). Most of these skin constructs involve the use of animal collagens or allogeneic human material, notably human cadaver dermis (23-27). Indeed, the rejection of foreign biological material by the host in vivo may lead to serious consequences (28,29). Moreover, obtaining such material on a regular basis and in sufficient quantity can be quite demanding. Contamination risk with microorganisms such as human immunodeficiency virus is a threat.

Thus, a different three-dimensional SE was produced with collagens and human living cells for its eventual use as permanent wound coverage on patients. Indeed, such clinical application would involve the use of autologous living epidermal cells and dermal fibroblasts, isolated from a small skin biopsy, amplified in culture, and seeded in SE grown in batches. Furthermore, additional extracellular matrix components were added to the dermal layer to assess their role in the postgrafting evolution of such SE.

In this article, we report the results of a comparative study, based on histological and postsurgical observations, following in vivo transplantation of SE prepared with bovine and human collagen. Graft take and evolution over time are described and compared.

MATERIALS AND METHODS

Preparation of anchored SE. Human keratinocytes were isolated using thermolysin before trypsin digestion of the epidermis, and cultured as described previously (30). Human dermal fibroblasts were isolated by collagenase digestion and then cultured (21).

The anchorage method of dermal equivalents (DE) that avoids collagen gel contraction, described previously (14), was adapted to facilitate SE manipulations. Briefly, a 5-mm-wide ring of unglued paper filter (Whatman, Maidstone, UK) was used as peripheral anchorage (31). All SE were prepared in 81-cm^2 bacteriological dishes (already containing the anchorage) (32).

Bovine type I collagen DE production has already been described (32), and is based on the initial method described by Bell et al. (11). DE were covered with 20 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin G, and 25 µg/ml gentamicin following collagen polymerization. The final concentrations of bovine type I collagen and fibroblast in DE were 2 mg/ml and 12,500 cells/ml, respectively.

For human collagen DE production, a stock solution of human type I and III collagen (63:37) (Imedex, Chaponost, France) was prepared by dissolving 6.26 mg/ml of the mixture in apyrogen water. A solution of 9.3 ml of Dulbecco's modified Eagle's medium 2.7 × (containing 40% fetal calf serum, 200 IU/ml penicillin G, and 25 µg/ml of gentamicin), 8 ml of the stock collagen solution, and 1 ml of elastin (1.336 mg/ml in 0.2 M Tris [pH 8.0]) was mixed with 1 ml of a second solution containing 0.062 mg of hyaluronic acid, 0.122 mg of chondroitin sulfate, 0.002 mg of heparan sulfate (Sigma) dissolved in apyrogen water, 0.016 ml of 4 N HCl, and 1 ml of a dermal fibroblast suspension (2.5×10^5 cells/ml). This mixture was quickly distributed in bacteriological petri dishes to obtain human DE containing additional dermal matrix components. Another formulation that did not contain elastin and the second solution (replaced by 1 ml of Tris 0.2 M and 1 ml of apyrogen water) was also prepared to produce human DE. The final concentrations of human type I and III collagens and fibroblasts in these DE were 2.9 mg/ml and 12,500 cells/ml, respectively.

Keratinocytes were plated on 4-day-old bovine DE, human DE, and human DE containing additional dermal matrix components at a concentration of 9×10^5 cells/cm^2. SE were cultured in Dulbecco-Vogt modification of Eagle medium with Ham's F12 containing serum and additives (32). The three types of skin substitutes (bovine skin equivalent [bSE], human skin equivalent [hSE], and human skin equivalent containing additional dermal matrix components [hSE+]) were grafted onto nude mice after being cultured for 15 to 18 days. Before grafting, the anchorage was removed and SE were mounted on a Vaseline gauze to facilitate manipulation.

Animals and surgical manipulations. An animal model was established in our laboratory (33), and subsequently was further improved (7). Briefly, athymic nu/nu CD-1 male adult mice (Charles River Laboratories, Lasalle, QC, Canada) were anesthetized with a solution of ketamine-xylasine. A 2-cm incision was made through the dorsal skin. The loose connective tissue under the panniculus carnosus was excised. A silicone Fusenig's chamber (5,34) (made in our laboratory) was implanted (Fig. 1) and the SE, covered by a sterile gauze, was deposited directly on the dorsal muscle of the mouse. The gauze was removed on day 7 after grafting to allow epidermal keratinization.

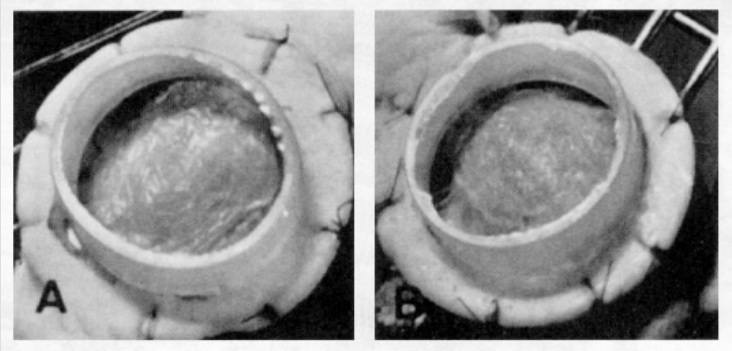

Figure 1. Human SE+ (A) and bSE (B) grafted onto the dorsal muscular bed of nude mice on the 7th day after transplantation (×1).

The graft take was assessed clinically on days 7, 14, and 21, and the standardized pictures produced were read with planimetric scales to obtain the percentage of take and contraction of each equivalent. One to four mice per group of SE tested were killed on the 4th, 14th, 21th, 28th, and 90th day after grafting for graft analysis. Each experiment was performed twice and gave similar results. Twenty-seven mice received grafts.

Histological and ultrastructural analyses. The grafts were excised, fixed in Bouin's solution, and embedded in paraffin. Four-micrometer-thick tissue sections were processed for standard hematoxylin, phloxine, and saffron staining. Some biopsies were also processed and mounted for electron microscope analyses, as described previously (7).

Indirect immunofluorescence staining. Rabbit polyclonal anti-type IV collagen and mouse monoclonal anti-HLA-A,B,C antibodies were kindly provided by Dr. Pascal Summer (Pasteur Institute, Lyon, France) and Dr. Raynald Roy (Laval University, Quebec, Canada), respectively. Rat monoclonal antilaminin antibody was bought from AMAC (BIO/CAN Scientific Inc., Mississauga, Ontario, Canada). Mouse monoclonal antiepithelial keratins AE3 and AE2 antibodies were purchased from ICN Immunobiologicals (Lisle, IL). Mouse monoclonal anti-human type I collagen antibody was bought from Biodesign (Kennebunk, ME). Mouse anti-human keratinocyte transglutaminase antibody was purchased from BTI (Stoughton, MA). Mouse monoclonal anti-human type VII collagen, FITC-conjugated goat anti-mouse IgG-IgM, sheep anti-rabbit IgG, and goat anti-rat IgG antibodies were purchased from Chemicon (Ingram-Bell, Montreal, Canada). Bullous pemphigoid human antigen anti-serum was kindly supplied by Dr. Ernest Heid's laboratory (Service de Dermatologie, Hôpital Civil de Strasbourg, France). FITC-conjugated protein A was purchased from Sigma. The histological immunostaining was performed on frozen tissue sections fixed with acetone.

Inflammatory response of C3H/HeN mice to bovine or human collagens. A volume of 100 µl of 2 mg/ml bovine type I (Sigma) or 2.9 mg/ml human types I and III collagen suspension was injected subcutaneously under sterile conditions into two independent groups of immunocompetent C3H/HeN mice. A control group was given injections with the same volume of glacial acetic acid diluted 1000 times with apyrogenic water. Mice skin biopsies were performed 14 days after injection in order to proceed with histological analysis.

RESULTS

In situ postgrafting analysis. The graft take was evaluated for each SE, as a percentage of the grafted surface. It was already possible to observe a good adhesion of all types of SE to the graft bed as soon as 7 days after their transplantation (hSE+ and bSE: Fig. 1, A and B, respectively; hSE: not shown). At this time, 99±1% graft take was observed with bSE (n=8) and hSE+ (n=5) after grafting. The hSE transplantation already led to 100±0% (n=4) graft take after the same period after surgery. From day 14 after grafting to the end of the experiment, 100% graft take was obtained for all types of transplanted SE. The development of a well-organized stratum corneum that progressively thickened in situ was seen in all cases. No sign of tissue necrosis was ever observed.

The contraction of all SE was also monitored at 7, 14, 21, and 28 days after grafting. Indeed, SE contraction was observed, increasing with time after grafting (Table 1). However, hSE and hSE+ showed lower contraction than bSE throughout the whole period evaluated after grafting (Table 1). On the 28th day after SE transplantation, 61±5% of the initial hSE surface area remained, compared with 48±4% for bSE (Table 1).

	Days postgrafting			
	7	14	21	28
bSE	65±3	58±4	56±11	48±4
hSE	77±3	68±11	66±2	61±5
hSE+	78±7	67±11	66±14	57±8

[a] Values are the mean of 2 or 4 mice ± SD (total of 27 mice grafted).

Table 1. Percentage of surface area after contraction of SE grafted on nude mice[a]

Histological analysis: Human epidermal production and grafting. Some SE from each tested group were analyzed to verify the histological integrity of the cultured tissues prior to transplantation (hSE+ and bSE: Fig. 2, A and B, respectively; hSE: not shown). As soon as 4 days after grafting, hSE epidermis was composed of 10-15 cell layers, including spinous and granular regions (Fig. 2C). In both hSE and hSE+, the basal cells had retained their cuboidal morphology and the formation of a stratum corneum was in progress. Similar observations were made on bSE (Fig. 2D). The human epidermis of all SE types progressively increased in thickness, leading to the establishment of stratified epidermal layers from 21 up to 90 days after grafting (hSE+: Fig. 3, A and B; bSE and hSE: not shown). Interestingly, the number of fibroblasts had decreased in the dermal layer of all types of SE (bSE, hSE, and hSE+) after 90 days (hSE+: Fig. 3B; bSE and hSE: not shown), compared with 21 days after grafting (hSE+: Fig. 3A; bSE and hSE: not shown).

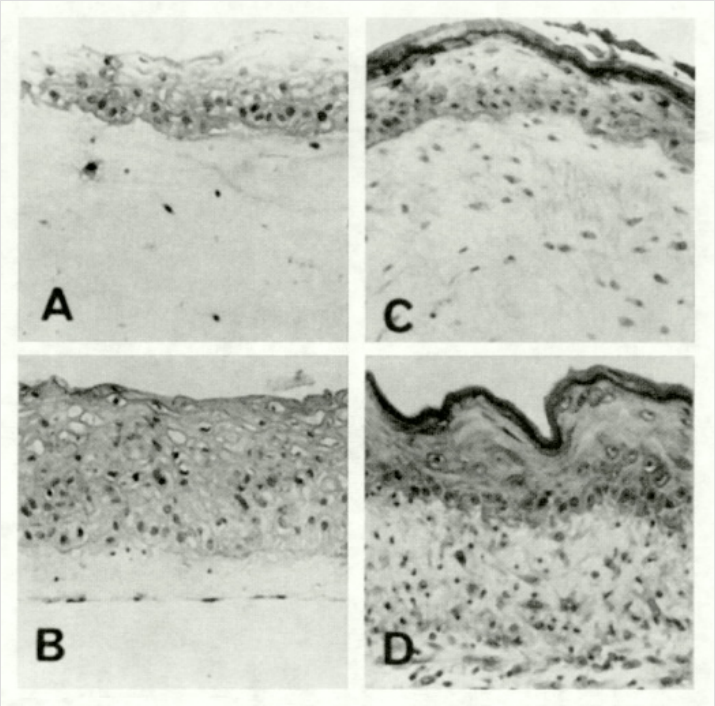

Figure 2. Histological analyses of cultured hSE+ (A) and bSE (B) before transplantation. Hematoxylin, phloxine, and saffron staining of hSE (C) and bSE (D) 4 days after transplantation (×130).

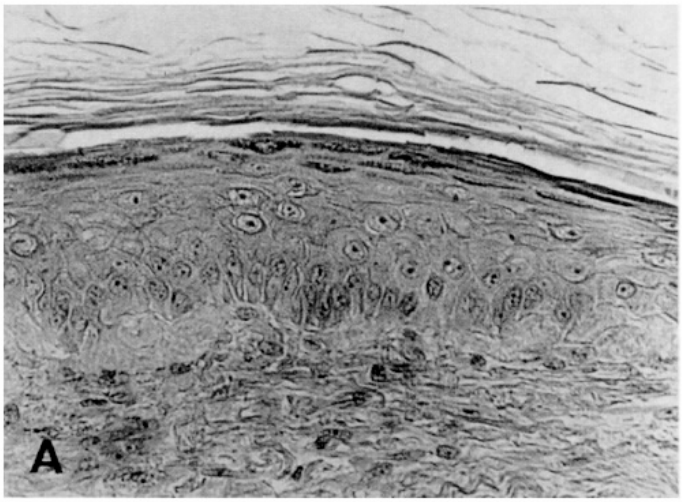

Figure 3. Hematoxylin, phloxine, and saffron staining of hSE+ 21 days (A) and 90 days (B) after transplantation (×340).

Viability of human skin cells over time after grafting. The human origin of the grafts was confirmed by immunofluorescence analyses with an anti-HLA-A,B,C antibody. Indeed, the positive labeling of all the human SE cells was maintained over time after grafting, for at least 90 days (hSE+: Fig. 4A; bSE: Fig. 4B; hSE: not shown).

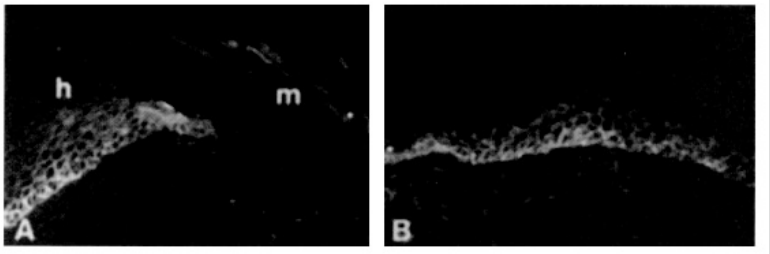

Figure 4. Immunofluorescent staining of hSE+ (A) and bSE (B) with mouse monoclonal anti-HLA-A,B,C antibody 90 days after grafting (×132). Note the junction of the human epidermis (h), labeled positively, to the mouse epidermis (m), not labeled.

Basement membrane development in situ. The deposition of various basement membrane constituents was evaluated by immunofluorescence tissue labeling over time after grafting. As soon as 4 days after all SE transplantation, a positive and continuous basement membrane labeling was observed with antilaminin (hSE+: Fig. 5A; bSE: Fig. 5B; hSE: not shown), anti-type IV collagen (hSE+: Fig. 5C; bSE: Fig. 5D; hSE: not shown), and bullous pemphigoid antigen antibodies (hSE+: Fig. 5E; bSE: Fig. 5F; hSE: not shown). Bullous pemphigoid antigens were also detected in the cytoplasm of the basal cells, 4 days after transplantation. The results obtained from hSE analyses were similar to the data observed with the two other groups of SE evaluated.

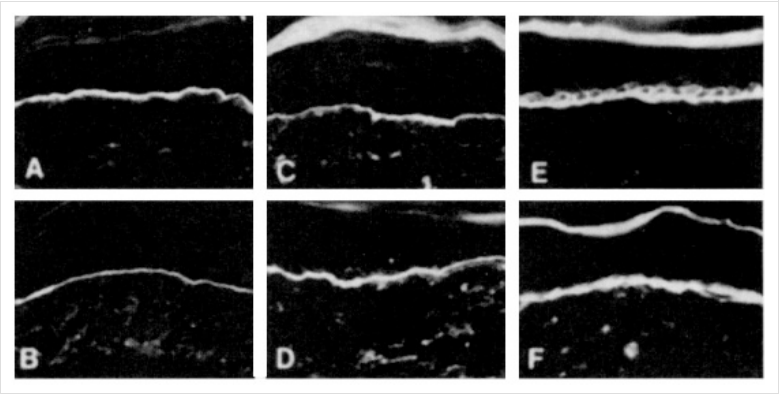

Figure 5. Immunofluorescent staining of hSE+ (A, C, E) and bSE (B, D, F) with rat monoclonal anti-laminin (A, B), rabbit polyclonal anti-human type IV collagen (C, D), and antiserum against bullous pemphigoid human antigens (E, F), 4 days after transplantation (×260).

Immunofluorescent staining of histological sections of bSE, hSE, and hSE+ revealed that the production of the basement membrane had been maintained by the human skin cells in situ, for at least 90 days after grafting. In all cases, laminin (hSE+: Fig. 6A), human type IV (hSE+: Fig. 6B), human type VII (hSE+: Fig. 6C) collagens, and bullous pemphigoid human antigens (hSE+: Fig. 6D) were positively labeled. The results obtained from bSE and hSE analyses were similar to the data observed with the hSE+ (data not shown).

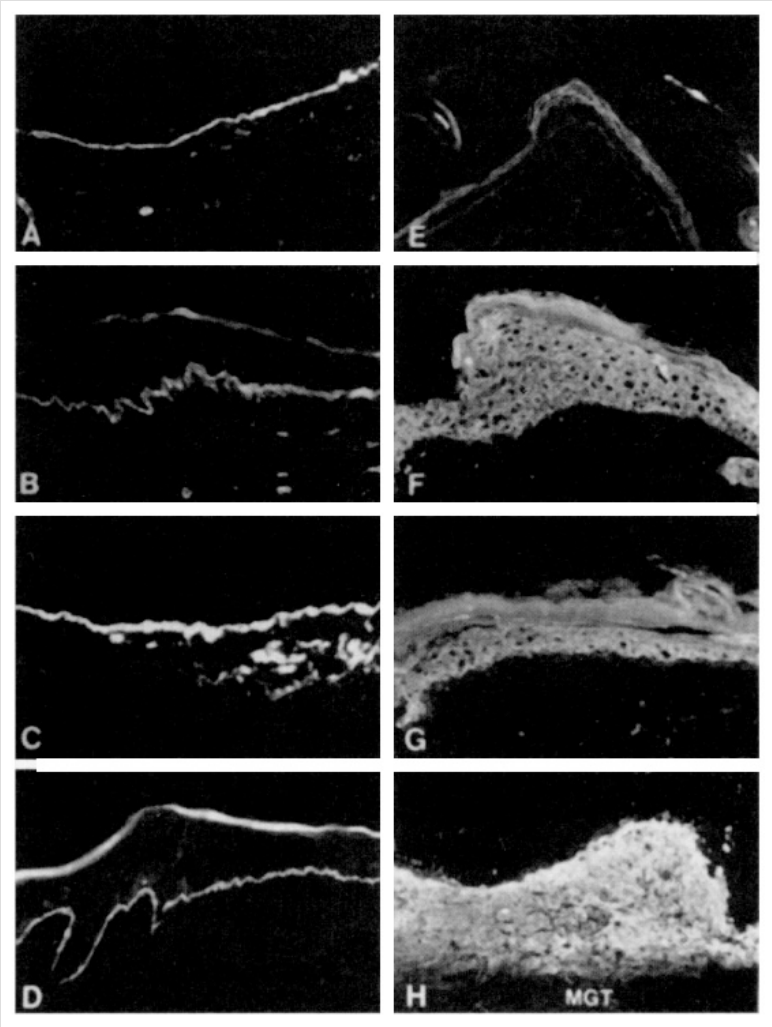

Figure 6. Immunofluorescent staining of hSE+ with rat monoclonal antilaminin (A), rabbit polyclonal anti-human type IV (B) and type VII (C) collagens, antiserum against bullous pemphigoid human antigens (D), anti-human transglutaminase (E), mouse monoclonal antikeratins AE3 (F) and AE2 (G), and rabbit polyclonal anti-human type I collagen (H), 90 days after grafting (×87) (MGT, mouse granulation tissue).

Transglutaminase detection. The detection of transglutaminase in the epidermal cell layers of the SE was evaluated by immunofluorescence staining of tissue sections. As shown in Figure 6E (hSE+), positive transglutaminase labeling was observed in the last suprabasal and the first stratum corneum epidermal cell layers, 90 days after grafting. Similar observations were done on bSE and hSE histological sections (data not shown). These results strongly suggest that the enzymatic cross-linking of the SE stratum corneum by transglutaminase is still going on in the human keratinocytes in situ.

SE epidermal cell differentiation and stratification. As expected from the histological observations, keratin labeling revealed that the human keratinocytes seeded on all SE groups can differentiate and adopt a stratified structural organization, for all time periods examined. The antikeratin antibody AE3 (hSE+: Fig. 6F) similarly labeled the whole epidermis of all SE groups (bSE and hSE: data not shown). The antikeratin antibody AE2, which reacts more specifically with keratins of the suprabasal epidermal layers, did not stain the basal epidermal cells of the grafts (hSE+: Fig. 6G). These observations are consistent with the differential pattern of keratin expression in keratinocytes in vivo.

Human type I collagen detection in the graft dermis. Human type I collagen was detected equally in all hSE and hSE+ dermal compartments, 21 days after grafting (data not shown). A similar immunolabeling signal was observed in the dermis of all bSE. At this point, it is important to mention that the anti-human type I collagen antibody cross-reacts with bovine type I collagen, but not with murine type I collagen.

Human type I collagen could still be detected in both hSE (not shown) and hSE+ (Fig. 6H) on day 90 after transplantation. However, human/bovine type I collagen staining led to a sparse distribution of weaker signals in all bSE histological sections grafted for the same time period.

Ultrastructural analyses of all SE revealed that some hemidesmosomes, lamina densa, and lamina lucida were present early after transplantation (hSE+: Fig. 7A; bSE and hSE: not shown), and all groups of grafts showed very good ultrastructural organization thereafter (hSE+: Fig. 7B; bSE and hSE: not shown).

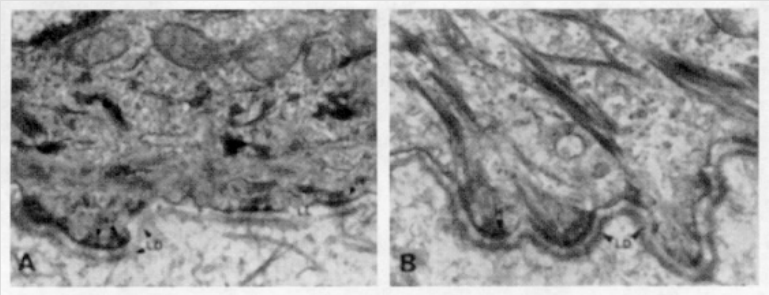

Figure 7. Photomicrographs of hSE+ thin sections analyzed under electron microscopy at 14 (A) and 90 days (B) after grafting (×34,200) (H, hemidesmosomes; LD, lamina densa; LL, lamina lucida).

Inflammatory response of C3H/HeN mice to bovine or human collagens. Polymorphonuclear leukocyte infiltration occurred at the site of the subcutaneous injection of bovine type I collagen after 14 days in immunocompetent mice (Fig. 8A). A slight inflammatory reaction was seen in response to the human collagen injection (Fig. 8B), but was never comparable to the immune cell migration observed with bovine collagen (Fig. 8A). Histological analysis of the injection sites did not reveal any inflammatory reaction in the control mice (data not shown).

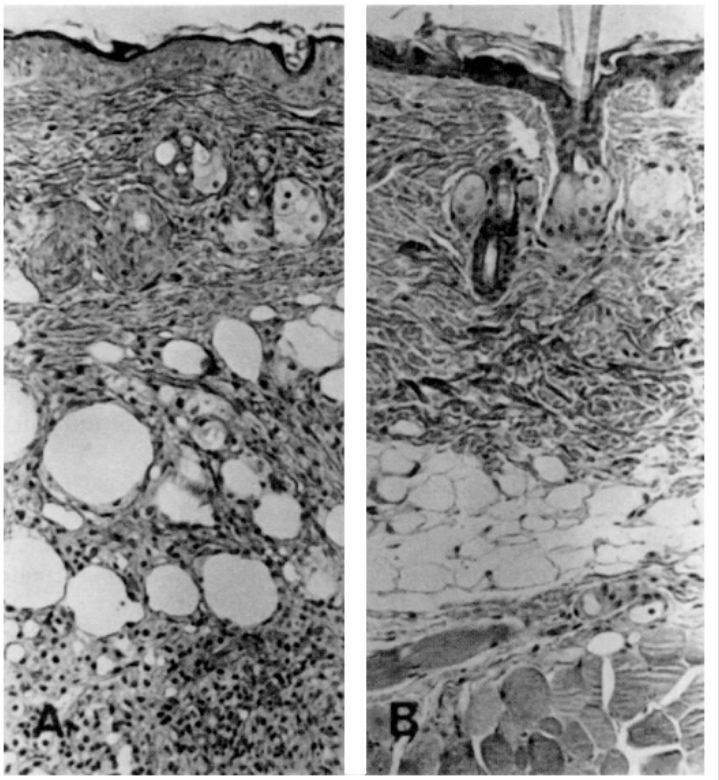

Figure 8. Hematoxylin, phloxine, and saffron staining of C3H/HeN mouse skin biopsy specimens taken at the site of subcutaneous injection of 200 µg (in 100 µl) of type I bovine collagen suspension (A) or 290 µg (in 100 µl) of type I + III human collagen (B), 14 days after the injections (×160).

DISCUSSION

The production of bioengineered human tissues has led to a fascinating diversity of medical applications, notably for permanent burn wound coverage (35). During the last 5 years, various skin substitutes have been introduced for extensive third-degree burn management, providing new approaches in reconstructive surgery (15-22). A choice between two therapeutic options or their combination may soon be possible: (1) temporary coverage of the burn sites with cadaver or animal skin (36,37), before autografting, or (2) permanent transplantation of various types of bioengineered SE (15-22). The second option may present practical and esthetic advantages but could entail some risks of microbial infections or long-term immunoreactions to the allogeneic components present in most of the bioengineered tissue grafts (28, 29, 38).

Collagens are frequently chemically modified or lyophilized so they can be used as specialized dressings, notably in skin substitutes (16-18, 39). These modifications often slow down its degradation after grafting in vivo (40). However, collagenic dermal substitutes should retain their permeability to allow the diffusion of nutriments or growth factors (39) and vascularization in vivo (41). Our living SE presents such advantages and can be produced with human collagens and autologous cells, which minimize the risks of inflammatory or immunological reactions in vivo. Moreover, the human living dermal and epidermal cells contribute to (1) initiate structural organization of the SE in culture (31) and (2) enhance permanent graft remodeling after transplantation (42). In our hSE+, we used bovine elastin, porcine chondroitin sulfate B, and bovine heparan sulfate in order to determine whether they improved graft take. These components will be introduced only in eventual patient autologous SE grafts when commercially available from a human source.

The contraction of cell-populated collagen lattice over time in culture has been well documented (11, 42-44). The contractile properties of fibroblasts lead to severe reduction of the initial surface of floating DE (11). Until now, the use of SE has been limited because of the contraction associated with maturation of the dermal component in vitro (19, 45). However, this drawback was obviated in culture by the peripheral anchorage method, established in our laboratory (14, 31). Our data showed that all types of living SE led to 100% graft take, even over the backbones of mice, a site particularly difficult to engraft in this animal model. The histological analyses of all SE groups (bSE, hSE, and hSE+) showed well-stratified human epidermal cell layers for the whole period studied (90 days) in situ after grafting. Moreover, immunofluorescence and ultrastructural studies of the grafts allowed the detection of a continuous basement membrane, containing several human components, such as laminin, bullous pemphigoid antigens, and types IV and VII collagen. In addition, human keratins and transglutaminase were also observed and expressed as expected, in respective epidermal cell layers on immunohistological SE sections, 90 days after transplantation. These results strongly suggest that the human epidermal cells, seeded in all groups of SE grafted, undergo a normal pattern of differentiation in situ and seem to retain functional activities for long periods of time after transplantation.

Immunohistological detection of human type I collagen in the dermal section of the hSE and hSE+ grafted for 90 days leads to two hypotheses. Either this human collagen was secreted by the human SE, or it was not completely degraded in situ after grafting. In the case of mice grafted with bSE, we could not determine whether the type I collagen detected was of human or bovine origin. Thus, the same two hypotheses still apply. However, on day 90 after transplantation, considerably less type I human (or bovine) collagen was observed on bSE dermal sections, compared with hSE and hSE+. This observation correlates with data obtained from in vitro studies which showed a higher amount of a 92-kDa gelatinase secreted in the culture supernatants of bSE, compared with hSE and hSE+ (32). Perhaps such an enzyme is also secreted by the human SE cells after grafting, and it may contribute to accelerating the degradation of the bSE dermal collagen matrix. Nevertheless, such results are very promising for the eventual use of living SE as autologous human wound coverage. Indeed, living SE contracted with time after transplantation; interestingly, however, our data suggest that the use of human collagen tends to reduce such contraction, compared with bovine collagen.

Our bilayered SE were readily transplanted in vivo in a single-step surgical procedure, under the best aseptic and physiological wound bed conditions. However, the situation may be different on extensively burned patients due to the dramatic extent of the cutaneous trauma and the size of each SE graft. We are conducting experiments to evaluate the feasibility of SE transplantation in a sequential approach. This dermal layer would first be grafted to close the wounds as soon as possible. This would be followed by the overlaying of epithelial sheets in a second step. The sequential transplantation of dermal and epithelial skin layers on nude mice will be compared with the single-step SE grafting. Covering of the wounds before epithelialization has already been applied in therapy for burned patients (23, 24, 42, 44). The presence of living fibroblasts in the dermal substitutes strongly enhances wound healing and vascularization compared with human acellular substitutes (42). The decreased number of fibroblasts in the dermal layer of SE 90 days after grafting suggests that a growth regulatory process may have occurred in vivo. Indeed, extracellular matrix secretion and reorganization, combined with the effects of several cytokines, may contribute to regulating growth of both fibroblasts and keratinocytes in situ.

The results of the present work show better graft take and histological evolution of anchored SE compared with cultured epidermal sheets transplanted on an animal model. Our data indicate that hSE can be used as a valuable therapeutic option for human burn management. This skin model can also be used for several biotechnological research applications. These new anchored SE have an adequate surface for clinical grafting and are readily amenable to large-scale production for autologous burn wound coverage by tissue-engineering methods.

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Footnotes

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REFERENCES

1. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975; 6: 331. [Context Link]

2. Green H, Kehinde O, Thomas J. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci USA 1979; 76: 5665. Bibliographic Links | [Context Link]

3. Gallico GG, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 1984; 331: 448. [Context Link]

4. Compton CC, Gill JM, Bradford DA, et al. Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting: a light, electron microscopic and immunohistochemical study. Lab Invest 1989; 60: 600. [Context Link]

5. Rouabhia M, Germain L, Bélanger F, Guignard R, Auger FA. Optimization of murine keratinocyte culture for the production of graftable epidermal sheets. J Dermatol 1992; 19: 325. Bibliographic Links | [Context Link]

6. Auger FA. The role of cultured autologous human epithelium in large burn wound treatment. Transplant Implant Today 1988; 5: 21. [Context Link]

7. Auger FA, Guignard R, López Valle CA, Germain L. Role and inocuity of Tisseel®, a tissue glue, in the grafting process and in vivo evolution of human cultured epidermis. Br J Plast Surg 1993; 46: 136. [Context Link]

8. Faure M, Mauduit G, Schmitt D, Kanitakis J, Demidem A, Thivolet D. Growth and differentiation of human epidermal cultures used as auto- and allografts in humans. Br J Dermatol 1987; 116: 161. Bibliographic Links | [Context Link]

9. Franzi AT, D'Anna F, Zicca A, Trabucchi E. Histological evaluation of human cultured epithelium before and after grafting. Burns 1992; 18 (suppl 1): S26. Bibliographic Links | [Context Link]

10. Ehrmann RL, Gey GO. The growth of cells on a transparent gel of reconstituted rat-tail collagen. J Natl Cancer Inst 1956; 16: 1375. Bibliographic Links | [Context Link]

11. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro.* Proc Natl Acad Sci USA 1979; 76: 1274. Bibliographic Links | [Context Link]

12. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T. Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. Science 1981; 211: 1052. [Context Link]

13. Bell E, Ehrlich HP, Sher S, et al. Development and use of a living skin equivalent. Plast Reconstr Surg 1981; 67: 386. Ovid Full Text | Bibliographic Links | [Context Link]

14. López Valle CA, Auger FA, Rompré P, Bouvard V, Germain L. Peripheral anchorage of dermal equivalent. Br J Dermatol 1992; 127: 365. [Context Link]

15. Hansbrough JF, Boyce ST, Cooper ML, Foreman TJ. Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. JAMA 1989; 262: 2125. [Context Link]

16. Boyce ST, Foreman TJ, English KB, et al. Skin wound closure in athymic mice with cultured human cells, biopolymers, and growth factors. Surgery 1991; 110: 866. Bibliographic Links | [Context Link]

17. Tinois E, Tiollier J, Gaucherand M, Dumas H, Tardy M, Thivolet J. *In vitro* and post-transplantation differentiation of human keratinocytes grown on the human type IV collagen film of a bilayered dermal substitute. Exp Cell Res 1991; 193; 310. [Context Link]

18. Contard P, Bartel RL, Jacobs L II, et al. Culturing keratinocytes and fibroblasts in a three-dimensional mesh results in epidermal differentiation and formation of a basal lamina-anchoring zone. J Invest Dermatol 1993; 100: 35. Bibliographic Links | [Context Link]

19. Rompré P, Auger FA, Germain L, et al. The influence of initial collagen and cellular concentrations on the final surface area of dermal and skin equivalents: a Box-Behnken analysis. In Vitro Cell Dev Biol 1990; 26: 983. Bibliographic Links | [Context Link]

20. Ponec M. Reconstruction of human epidermis on de-epidermized dermis: expression of differentiation-specific protein markers and lipid composition. Toxicol In Vitro 1991; 5: 597. Bibliographic Links | [Context Link]

21. Bouvard V, Germain L, Rompré P, Roy B, Auger FA. Influence of dermal equivalent maturation on a skin equivalent development. Biochem Cell Biol 1992; 70: 34. Bibliographic Links | [Context Link]

22. Hansbrough JE, Morgan J, Greenleaf G, Parikh M, Nolte C, Wilkins L. Evaluation of graftskin composite grafts on full-thickness wounds on athymic mice. J Burn Care Rehabil 1994; 15: 346. Ovid Full Text | Bibliographic Links | [Context Link]

23. Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. Lancet 1986; 1: 1123. Bibliographic Links | [Context Link]

24. Cuono CB, Langdon R, Birchall N, Barttelbort S, McGuire J. Composite autologous-allogeneic skin replacement: development and clinical application. Plast Reconstr Surg 1987; 80: 626. [Context Link]

25. Langdon RC, Cuono CB, Birchall N, et al. Reconstitution of structure and cell function in human skin grafts derived from cryopreserved allogeneic dermis and autologous cultured keratinocytes. J Invest Dermatol 1988; 91: 478. Bibliographic Links | [Context Link]

26. Young D, Langdon R, Kahn R, McGuire J, Cuono C. Analysis of the fate of allografted dermis using a DNA fingerprinting technique. Proc Am Burn Assoc 1989; 21: 71. [Context Link]

27. Compton CC, Hickerson W, Nadire K, Press W. Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting: a light, electron microscopic and immunohistochemical study. J Burn Care Rehabil 1993; 14: 653. [Context Link]

28. Chiocchia G, Boissier MC, Ronziere MC, Herbage D, Fournier C. T cell regulation of collagen-induced arthritis in mice. J Immunol 1990; 145: 519. [Context Link]

29. Hom JT, Bendele AM, Carlson DG. In vivo administration with IL-1 accelerates the development of collagen-induced arthritis in mice. J Immunol 1988; 141: 834. Bibliographic Links | [Context Link]

30. Germain L, Rouabhia M, Guignard R, Carrier L, Bouvard V, Auger FA. Improvement of human keratinocyte isolation and culture using thermolysin. Burns 1993; 19: 99. Bibliographic Links | [Context Link]

31. Michel M, Auger FA, Germain L. Anchored skin equivalent cultured *in vitro*: a new tool for percutaneous absorption studies. In Vitro Cell Dev Biol 1993; 29A: 834. [Context Link]

32. Auger FA, López Valle CA, Guignard R, et al. Skin equivalents produced with human collagens. In Vitro Cell Dev Biol 1995; 31: 432. [Context Link]

33. López Valle CA, Glaude P, Auger FA. Surgical model for *in vivo* evaluation of cultured epidermal sheets in mice. Plast Reconstr Surg 1992; 89: 139. Ovid Full Text | Bibliographic Links | [Context Link]

34. Worst PK, Valentine EA, Fusenig NE. Formation of epidermis after reimplantation of pure primary epidermal cell cultures from perinatal mouse skin. J Natl Cancer Inst 1974; 53: 1061. Bibliographic Links | [Context Link]

35. Langer R, Vacanti JP. Tissue engineering. Science 1993; 260: 920. Bibliographic Links | [Context Link]

36. Heck EL, Sergstrasser PR, Baxter CR. Composite skin graft: frozen dermal allograft support of the engraftment and expansion of autologous epidermis. J Trauma 1985; 25: 106. Ovid Full Text | Bibliographic Links | [Context Link]

37. Gallico GG III. Biologic skin substitutes. Clin Plast Surg 1990; 17: 519. Bibliographic Links | [Context Link]

38. Ellingsworth LR, De Lustro F, Brennan JE, Sawamura S, McPherson J. The human immune response to reconstituted bovine collagen. J Immunol 1986; 136: 877. Bibliographic Links | [Context Link]

39. Krejci NC, McGuire J. Treatment of burns with skin substitutes. J Dermatol Sci 1992; 4: 149. Bibliographic Links | [Context Link]

40. Koide M, Osaki K, Konishi J, Oyamada K, Katakura T, Takahashi A. A new type of biomaterial for artificial skin: dehydrothermally cross-linked composites of fibrillar and denatured collagens. J Biomed Mater Res 1993; 27: 79. Bibliographic Links | [Context Link]

41. Ross UH. *In vitro* production and subsequent transplantation of a living skin substitute in rat model. Eur Arch Otorhinolaryngol 1992; 249: 263. Bibliographic Links | [Context Link]

42. Demarchez M, Hartmann DJ, Regnier M, Asselineau D. The role of fibroblasts in dermal vascularization and remodeling of reconstructed human skin after transplantation onto the nude mouse. Transplantation 1992; 54: 317. Ovid Full Text | Bibliographic Links | [Context Link]

43. Gillery P, Maquart F-X, Le Corre Y, Kalis B, Borel J-P. Variability in the retraction of collagen lattices by scleroderma fibroblasts: relationship to protein synthesis and clinical data. Clin Exp Dermatol 1991; 16: 324. Bibliographic Links | [Context Link]

44. Nave M. Wound bed preparation: approaches to replacement of dermis. J Burn Care Rehabil 1992; 13: 147. Ovid Full Text | Bibliographic Links | [Context Link]

45. Germain L, Auger FA. Tissue engineered biomaterials: biological and mechanical characteristics. In: Wise DL, Trantolo DJ, Altobelli DE, Yaszemski MJ, Gresser JD, Schwartz ER, eds. Encyclopedic handbook of biomaterials and bioengineering. New York: Marcel Dekker, 1995: 699. [Context Link]

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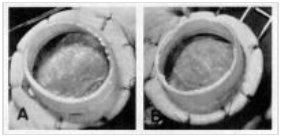

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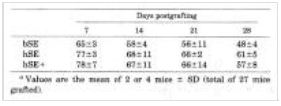

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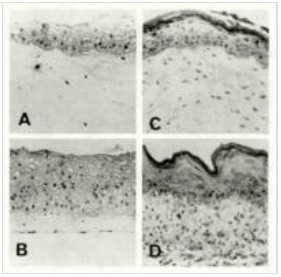

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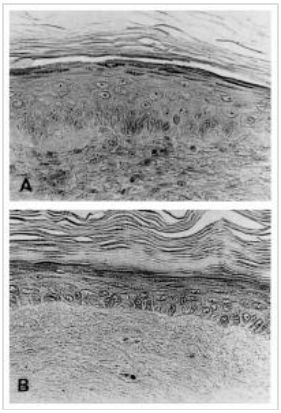

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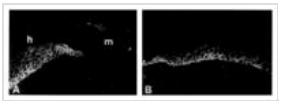

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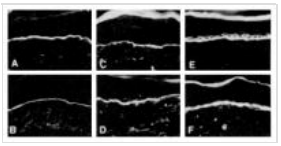

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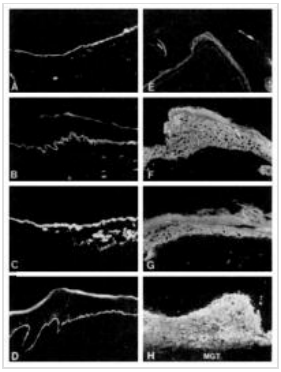

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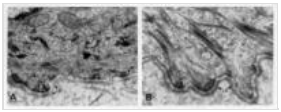

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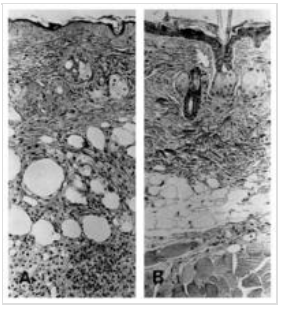

Figure 8

Retour au début