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Role and innocuity of Tisseel®, a tissue glue, in the grafting process and *in vivo* evolution of human cultured epidermis

F. A. Auger, R. Guignard, C. A. López Valle and L. Germain

Laboratoire de Recherche des Grands Brûlés, Saint-Sacrement Hospital, Quebec and Department of Surgery, Laval University, Faculty of Medicine, Sainte-Foy, Quebec, Canada

SUMMARY. **Cultured epidermal sheets are currently used for burn wound treatment but reported results on graft take are variable. This study was designed to evaluate the role and influence of Tisseel®, a fibrin glue, in the take of cultured human epidermal sheets in an athymic mouse model. On days 4, 10 and 21 post-grafting, histology, electron microscopy and immunofluorescence staining confirmed the presence of a human epithelium and the development of a basement membrane. Tisseel® was detectable on day 4 only, but overall treated and untreated grafts were similar. The use of Tisseel® enhanced the mechanical stability of these fragile grafts, increased the percentage of graft take, and its innocuity on the *in vivo* evolution of cultured epidermal sheets was demonstrated. For these reasons, we think that Tisseel® may be advantageous in a clinical setting.**

In burn management many methods have been devised for transient and permanent wound covering. One successful and clinically proven technique is the grafting of autologous cultured human epithelium (Gallico *et al.*, 1983; Auger, 1988; Donati *et al.*, 1992). Reported graft take varies from 0–100% with this technique (Woodley *et al.*, 1988; Hunyadi *et al.*, 1988; Desai *et al.*, 1991; Clugston *et al.*, 1991; Donati *et al.*, 1992). In some reports, these cultured sheets have displayed a poor level of attachment and vascularisation (Kumagai *et al.*, 1988).

Some clinical reports have appeared on the use of Tisseel® in the treatment of burn wounds with diverse skin grafts other than autologous cultured epidermis (Blümel *et al.*, 1986; Grabosch, 1986; Hettich, 1986). Tisseel® is a tissue glue containing fibrinogen, aprotinin solution, fibronectin, plasminogen, human albumin and factor XIII. When activated by thrombin and calcium, this product enhances adhesion of tissues and clotting. The addition of Tisseel® on a graft bed before skin application (mesh graft, split thickness skin and skin flap) leads to better graft take and more rapid revascularisation (Blümel *et al.*, 1986; Grabosch, 1986).

This report evaluates Tisseel® in the grafting of cultured epidermal sheets.

Materials and methods

Surgical procedures

Athymic nu/nu CD-1 male adult mice were anaesthetised with a solution of ketamine 10 mg/ml–xylasine 10 mg/ml (dosage 50 mg/kg intra-muscular). All work with these animals was carried out under a laminar flow hood as previously described (López-Valle *et al.*, 1992). A 2.5 cm incision was performed through the dorsal skin and panniculus carnosus and the loose connective tissue under the panniculus carnosus was excised in order to provide a muscular graft bed. A Fusenig's chamber (Worst *et al.*, 1974) was then implanted and cultured epidermal sheet was grafted on the muscular bed either directly or after Tisseel® application. Tisseel® (Immuno Canada Ltd, Windsor, Ontario) was sprayed using the Tissomat® system (Immuno). The glue solutions were reconstituted according to Immuno's recommendations.

Cultured epidermal sheets were produced as previously described (Green *et al.*, 1979; López Valle *et al.*, 1992). After graft deposition, the vaseline gauze was detached from the cultured epidermal sheet. The cap of the Fusenig's transplantation chamber was held in place by four cutaneous stitches.

After 5 days the chamber cap was removed to allow epidermal keratinisation. Since the viable graft could be readily identified, the percentage of graft take was established in the following manner: the ratio of the surface occupied by grafted cultured epidermis to the surface of Fusenig's chamber was measured on photographs taken on days 5, 10 and 21. Biopsies were performed 4, 10 and 21 days after grafting on 2 control and 2 Tisseel® treated mice. This experimental protocol was repeated twice and gave similar results. A total of 32 mice were grafted (16 with Tisseel®, 16 controls). An initial pilot study was done with 20 mice—10 with Tisseel® and 10 controls—to test for toxicity of Tisseel® to cultured grafts. The experimental groups reported here consisted of 12 mice—6 with Tisseel® and 6 controls.

Histology

Biopsies were fixed in Bouin's solution, paraffin embedded and processed for standard haematoxylin, phloxine and saffron staining.

Table The percentage of graft take of cultured human epidermal sheets grafted on mice muscular graft bed with or without previous application of Tisseel®

Days after grafting:	*5*	*10*	*21*
Tisseel® treated mice*	70% ± 3	70% ± 8	66% ± 1
Control mice**	52% ± 10	51% ± 9	46% ± 2

Values are the mean of 2 or 3 mice ± standard deviation (total of 12 mice grafted).
* Statistically different from control at 95% (Mann–Whitney test on all mice).
** Control mice were grafted with epidermal sheet without previous application of Tisseel®.

Immunofluorescence staining

Mouse monoclonal anti-HLA-A, B, C (Brodsky and Parham, 1982) and sheep polyclonal anti-type IV collagen (Morel-Maroger Striker *et al.*, 1984) courtesy of Dr Raynald Roy (Université Laval, Quebec, Canada) and Dr Paul D. Killen (University of Michigan, Michigan, USA), respectively. Rat monoclonal anti-laminin and FITC conjugates (goat anti-mouse, goat anti-rat, rabbit anti-sheep used at the dilution 1/100) were purchased from Chemicon (Ingram-Bell, Montreal, Canada). Mouse monoclonal anti-H_2-D.m47 came from Cederlane Laboratories (Hornby, Ontario, Canada) and monoclonal AE2 and AE3 anti-keratin polypeptides (Woodcock-Mitchell *et al.*, 1982; Sun *et al.*, 1983) from ICN Biochemical (Montreal, Quebec, Canada).

Antibody labelling was performed on frozen tissue sections fixed with acetone as previously described (Bouvard *et al.*, 1992).

Electron microscopy

Biopsies were immediately fixed in a solution of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.5 for 24 hours and post-fixed with 1% osmium tetroxide. Tissues were stained *en bloc* with 0.5% uranyl acetate for 1 hour at 4°C. After dehydration the samples were embedded in Epon 812. Contrasted sections (uranyl acetate and lead citrate) were observed with a Philips EM300 electron microscope.

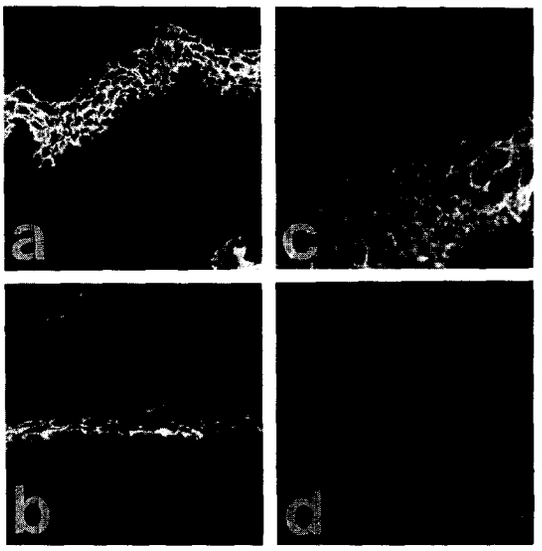

Fig. 2

Figure 2—Immunofluorescence microscopy of normal human skin (a) and of human cultured epithelium grafted on nude mice with previous Tisseel® application (b, day 10 post-transplantation; c and d, day 21) using mouse monoclonal anti-HLA-A, B, C (a–c) or anti-H_2D (d) antibodies. Anti-HLA-A, B, C antibody labelled cell membrane of all stratum (except stratum corneum). The positive staining with anti-HLA antibody (b, c) and negative staining with anti-H_2D antibody (d) confirm the human nature of grafted epidermis (E). The dotted line represents the epidermal–connective tissue junction (d). × 180.

Results

Cultured epidermal sheet grafting

The use of Fusenig's chambers in the present animal model allowed an excellent follow-up of transplanted

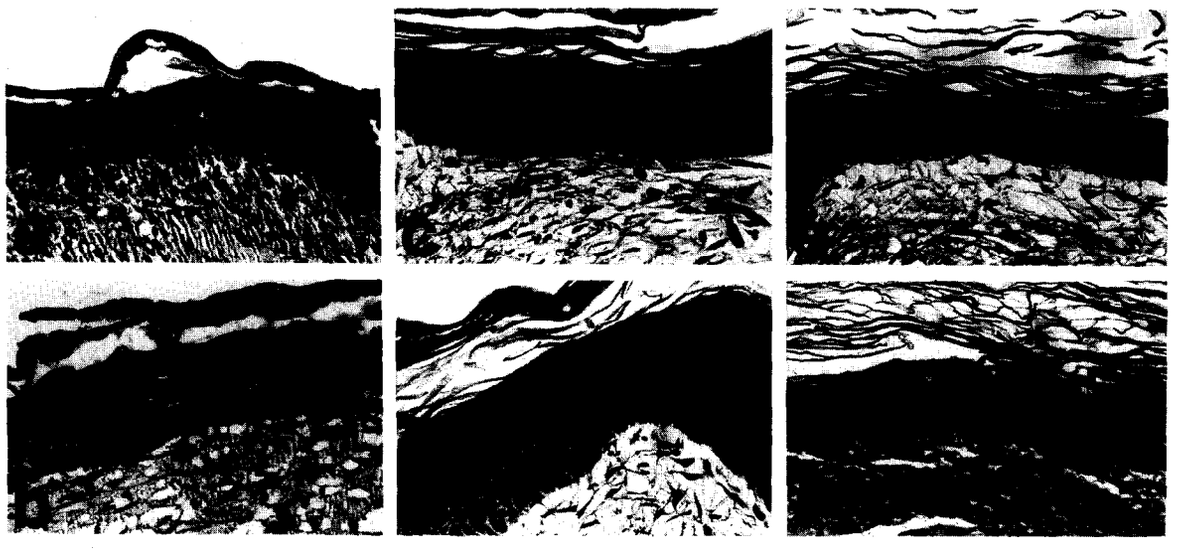

Fig. 1

Figure 1—Haematoxylin, phloxine and saffron staining of biopsies harvested 4 (a, b), 10 (c, d) and 21 (e, f) days after transplantation of human cultured epidermis grafted on muscular graft bed without (a, c, e) or with previous application of Tisseel® (b, d, f). Note the presence of the tissue glue at day 4 (b, arrow). × 180.

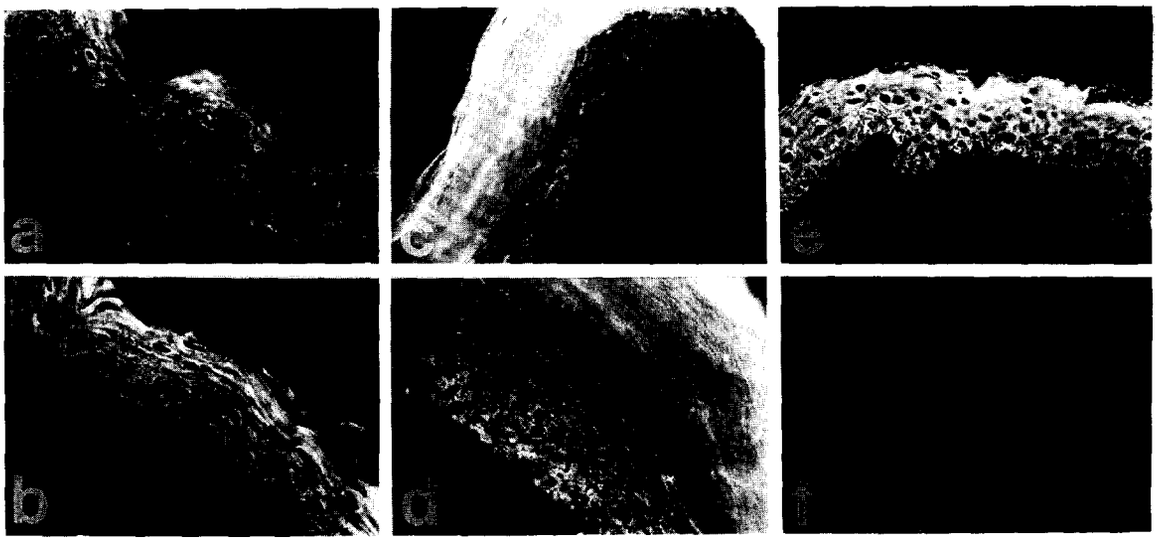

Fig. 3

Fig. 4

Figure 3—Immunofluorescence staining with mouse monoclonal anti-keratin antibody (AE3) of human cultured epithelium grafted on nude mice without Tisseel® (a, day 4 post-transplantation; c, day 21) or with previous Tisseel® application (b, day 4 post-transplantation; d, day 21). Positive control of normal human skin (e). Negative control (primary antibody omitted) (f). Note that AE3 labelled all epidermal cell layers (a–e). The dotted line represents the epidermal–connective tissue junction (f). E, epidermis. × 180. **Figure 4**—Immunofluorescence staining with mouse monoclonal anti-keratin antibody (AE2) of human cultured epithelium grafted on nude mice without Tisseel® (a) or with previous Tisseel application (b). Positive control of normal human skin (c). Note that AE2 labelled suprabasal layers (a–c). × 180.

cultured epidermal grafts. The addition of Tisseel® on the muscular surface before epidermal sheet deposition gave a better graft adherence. This characteristic of the glue was observed when the vaseline gauze was detached from the epidermal sheet immediately after deposition on the muscle. It was easier to free the gauze when Tisseel® was applied on the muscle. Furthermore, the graft take percentage was increased after application of Tisseel® (the Table shows the results of the 6 experimental mice and 6 controls).

Histological analysis

The histological appearance of the epidermis did not differ whether the sheets were transplanted directly on muscular tissue or on Tisseel®. On day 4 after grafting, keratinocytes within the epidermal sheet had begun to organise and stratify into five to eight cell layers (Fig. 1A, B). However, the tissue glue was observed under the epidermis 4 days after grafting (arrow, Fig. 1B) but by day 10 it had disappeared. On days 10 and 21, the epidermis was composed of several layers of keratinocytes at different stages of their normal terminal differentiation (Fig. 1C–F). On day 21, stratum corneum was thicker than at day 10 and the epidermis was stratified in a very satisfactory manner in both groups of mice (Fig. 1E, F).

Immunofluorescence analysis

The human nature of transplanted epidermis was determined by labelling the major histocompatibility complex (MHC) molecules class 1 (HLA-A, B, C) that are present on viable cells of human skin (Fig. 2A). The epidermis present on the biopsies was labelled by anti-HLA-A, B, C antibody (Fig. 2B, C) but not by the anti-H_2D antibody (mouse MHC molecules) (Fig. 2D), confirming its human nature.

Keratins in transplanted epidermis were detected using monoclonal antibodies AE3 and AE2 (Woodcock-Mitchell *et al.*, 1982). As described by Sun *et al.* (1983) in normal skin, AE3 stained all the layers of the epidermis (Fig. 3E). In transplanted cultured epidermis, normal patterns were observed in either Tisseel® treated or untreated mice (Fig. 3A–D). AE2 monoclonal antibody labelled the suprabasal layers of

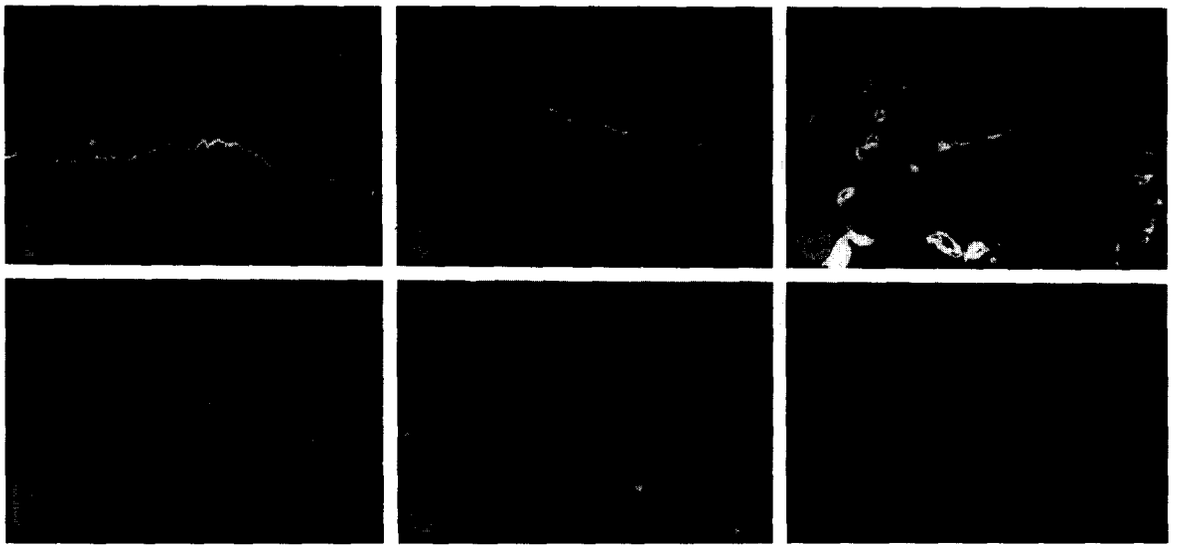

Fig. 5

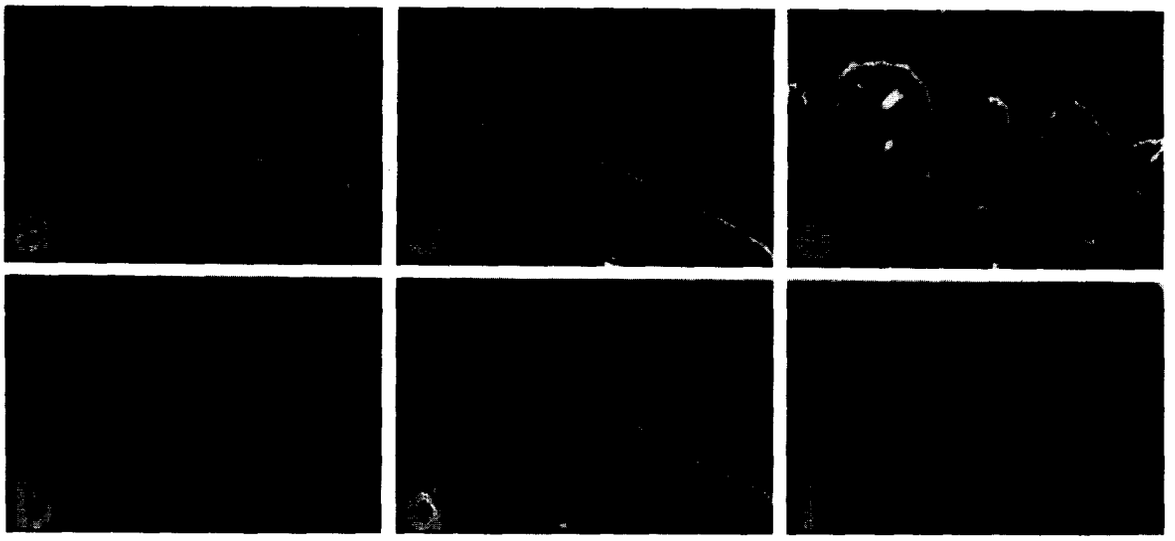

Fig. 6

Figure 5—Immunofluorescence staining with sheep polyclonal anti-human type IV collagen antibodies of human cultured epithelium grafted on nude mice without Tisseel® (a, day 4 post-transplantation; c, day 21) or with previous Tisseel® application (b, day 4 post-transplantation; d, day 21). Positive control of normal human skin (e). Negative control (primary antibody omitted) (f). Note the linear staining of the basement membrane. The fluorescence is slightly lower when Tisseel® was present at day 4 (b). E, epidermis. × 180. **Figure 6**—Immunofluorescence staining with rat monoclonal anti-laminin antibody of human cultured epithelium grafted on nude mice without Tisseel® (a, day 4 post-transplantation; c, day 21) or with previous Tisseel® application (b, day 4 post-transplantation; d, day 21). Positive control of normal human skin (e). Negative control (primary antibody omitted) (f). Note the linear staining of the basement membrane. The fluorescent line was thinner when Tisseel® was present at day 4 (b). E, epidermis. × 180.

keratinocytes in normal skin (Fig. 4C). On day 4 after transplantation, basal cells were distinguished from positive suprabasal cells in both groups of mice (Fig. 4A, B).

Progressive emergence of basement membrane macromolecules (type IV collagen and laminin) in transplanted cultured epidermis with and without Tisseel® was also studied. Before grafting, no basement membrane was present in cultured epidermal sheets released with Dispase (Boehringer Mannheim, Laval, Quebec, Canada). On day 4 after grafting, the labelling of type IV collagen was observed in basement membrane (Fig. 5A). In the presence of Tisseel® (Fig. 5B) the fluorescence of the basement membrane was slightly diminished compared to the control (Fig. 5A). However, on days 10 and 21 type IV collagen labelling was identical in both groups of mice (Fig. 5C, D) and its distribution was ubiquitous as in the dermal epidermal junction of human normal skin (Fig. 5E). The immunofluorescent pattern of laminin, a glycoprotein component of the basement membrane, was similar to the type IV collagen pattern (Fig. 6).

Ultrastructure of transplanted cultured human epidermis

The three regions of the basement membrane zone (plasma membrane, lamina lucida and lamina densa) were observed on day 4 in mice grafted without Tisseel® (Fig. 7A). The lamina densa was discon-

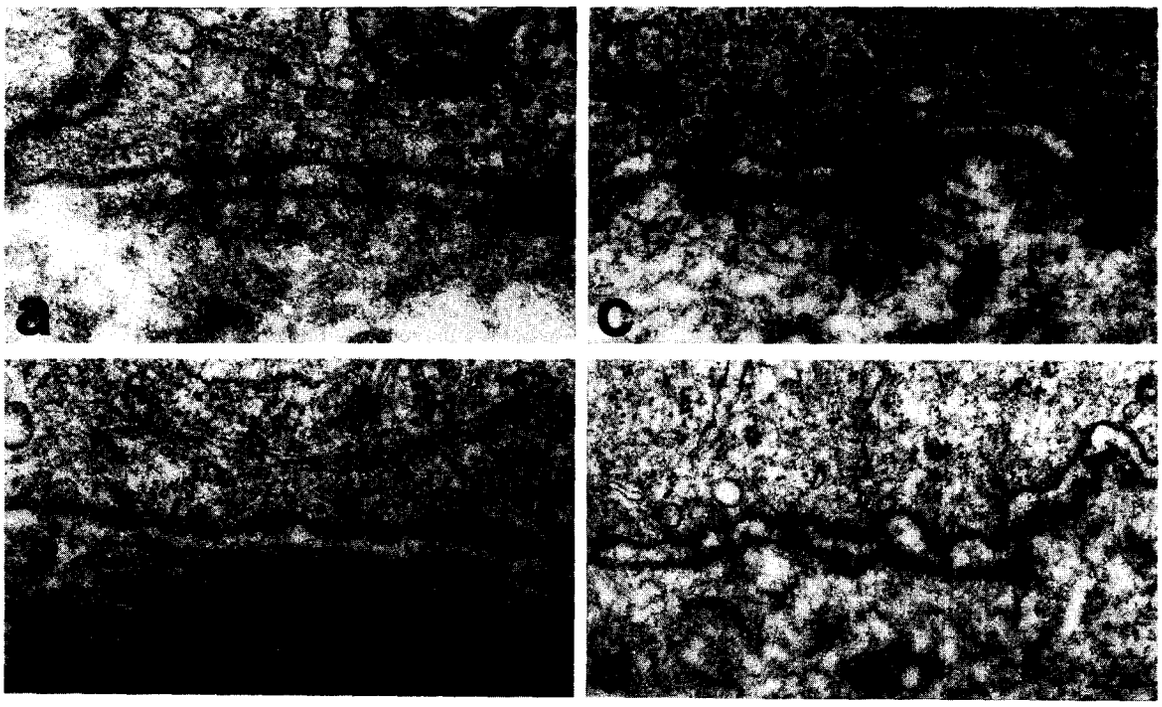

Fig. 7

Figure 7—Transmission electron microscopy of cultured human epidermal sheets grafted on nude mice without Tisseel® (a, day 4 post-transplantation; c, day 21) or with previous Tisseel® application (b, day 4 post-transplantation; d, day 21). The regions of the basement membrane zone, lamina lucida (LL) and lamina densa (LD) were already observed at day 4 post-transplantation in control group (a). Basal cell plasma membrane contained numerous electron dense plates characteristic of hemidesmosomes (HD). Note the presence of Tisseel® (T) at day 4 (b) which impedes the observation of lamina densa (LD). The ultrastructure of basement membrane is better defined 21 days post-transplantation (c, d). Bar represents 250 nm. ×29000.

tinuous and present mainly under hemidesmosomes. In mice treated with Tisseel®, the lamina densa of the basement membrane was difficult to distinguish because of the electron dense nature of the tissue glue (Fig. 7B). On day 10, Tisseel® had disappeared and the lamina densa was visualised under epidermal cells, although slight discontinuities were observed in both groups of mice. On day 21, the basement membrane components were well defined (Fig. 7C, D). The lamina densa was continuous. Hemidesmosomes and anchoring filaments were observed. The ultrastructural appearance of the transplanted tissue was similar in both groups of mice.

Discussion

The transplantation of autologous cultured epithelial sheets is an important therapeutic addition to the treatment of extensively burned patients (Gallico *et al.*, 1984; Donati *et al.*, 1992). However, these grafts are thin and fragile when first deposited on the burn wound. In some reports, cultured epidermal sheets do not attach and vascularise sufficiently well to close the wound routinely (Kumagai *et al.*, 1988). The possibility of membrane damage secondary to sheet separation from the culture flask surface (Merrick *et al.*, 1990) and abnormal anchoring fibril formation (Woodley *et al.*, 1988; Desai *et al.*, 1991) have been suggested. However, other investigators have described a time-dependent formation of anchoring filaments (Aihara, 1989; Compton *et al.*, 1989), implying that these phenomena are transient.

The reported percentage of take for cultured epidermal grafts varies from 0–100% (Hunyadi *et al.*, 1988; Herzog *et al.*, 1988; DeLuca *et al.*, 1989; Clugston *et al.*, 1991). Other factors that have to be considered in causation of this variability of clinical success include: insufficient graft immobilisation, lack of graft adherence caused by blood or serum collection, and the presence of numerous virulent bacteria on the wound bed. Furthermore, any shearing movement can also have an undesirable effect, presumably because it causes a disruption of the basal cell layer and basement membrane. Tisseel® should help with these problems.

The Fusenig's transplantation chamber physically separates the cultured human epidermal graft from the host mouse skin. This precaution was deemed to be quite important since we had previously noted a very significant graft size reduction after 10 days (López Valle *et al.*, 1992). The transplantation chamber ensured a sufficient graft size for the various biopsies without any mouse skin overlapping.

The main benefit of Tisseel® application is the better adherence of epidermal graft to the transplantation bed. In our animal model, even though the amount of graft movement was limited, we have seen at least a 20% increase in the percentage of graft take when Tisseel® was used (Table). In burn patients, grafts are strongly affected by any motion; the use of Tisseel® may allow better immobilisation of the grafted cultured epidermis and thereby increase the level of graft take.

Histological analysis revealed a normal cell strati-

fication with the expected keratin distribution whether Tisseel® had been applied or not. Thus Tisseel® did not impede the usual sequence of epidermal maturation. Moreover a basement membrane appeared along the junction of this epidermis and the newly formed connective tissue. It has been shown that prolonged graft stability and maintenance of a proliferating basal layer are dependent upon a complete basement membrane (Briggaman and Wheeler, 1975). Our immunofluorescent staining and electron microscope analyses suggested that the basement membrane was present on day 4 in both groups of mice and was very well defined on day 21 after grafting, as confirmed by other investigators (Stanley *et al.*, 1981; Demarchez *et al.*, 1985; Compton *et al.*, 1989).

The diminished fluorescence pattern for laminin and type IV collagen observed between the Tisseel® and control group at day 4 may be due to antigen masking by the fibrin tissue glue. Furthermore, the electron dense characteristics of Tisseel® prevent the electron microscopy from demonstrating the presence of a lamina densa. However, the positive labelling of type IV collagen which is localised in the lamina densa (Yaoita *et al.*, 1978) strongly suggests its presence. The absence of any difference in the immunofluorescence pattern and ultrastructural aspect of Tisseel® treated or untreated mice on days 10 and 21 is of particular significance. Combined with the disappearance of Tisseel® on these same days, these observations confirm that the transitory presence of Tisseel® has no deleterious effect on the ensuing sequence of basement membrane formation. We consider that these results may warrant further clinical evaluation of Tisseel® in the setting of cultured epidermal sheet transplantation.

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The Authors

François A. Auger, MD, Director and Associate Professor, Laboratoire de Recherche des Grands Brûlés/LOEX, Hôpital du Saint-Sacrement, 1050 Chemin Sainte-Foy, Québec (Québec), Canada G1S 4L8; and Département de Chirurgie, Université Laval, Faculté de Médecine, Sainte-Foy, Québec, Canada.

Rina Guignard, MSc, Research Assistant, Laboratoire de Recherche des Grands Brûlés/LOEX.

Carlos A. López Valle, MD, Resident, Laboratoire de Recherche des Grands Brûlés/LOEX and Département de Chirurgie, Université Laval.

Lucie Germain, PhD, Investigator and Associate Professor, Laboratoire de Recherche des Grands Brûlés/LOEX and Département de Chirurgie, Université Laval.

Requests for reprints to: Dr F. Auger at the Laboratoire de Recherche des Grands Brûlés/LOEX.

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