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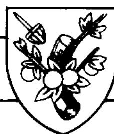

The effect of backing materials on keratinocyte autograft take

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SUMMARY. **A porcine model has been established to study keratinocyte autografts as a model of human keratinocyte grafting. Keratinocyte autografts were placed on 104 full thickness wounds in 13 pigs and backed with 3 dressings which varied in their ability to maintain an occlusive environment. Sixteen control wounds were ungrafted. No take was found using a backing of woven viscose and cotton gauze. Take was 20% at day 16 using a backing of woven viscose and paraffin gauze. Serial biopsies showed that keratinocytes frequently attached to the interstices of the viscose dressing and difficulty in detaching the viscose caused loss of epidermis. Hydrogel sheet backing made assessment at day 10 difficult because of wound hydration but dressing removal, enabling exudate evaporation, produced 22% take at day 13. The development of improved dressing techniques is certainly necessary for improved graft take.**

Extensive skin loss occurs in many clinical situations and is often managed by split thickness skin grafting. Advances in tissue culture now permit the growth of epidermal keratinocytes to produce sheets of cells which have been widely used as keratinocyte grafts on human wounds. However ethical problems limit clinical investigation and there is a need for a reliable animal model to evaluate graft structure and clinical behaviour.

The majority of workers have found that freshly excised wound beds provide the best take of keratinocyte autografts compared with chronic granulating wounds.[1–4] O'Connor, in the first report of keratinocyte grafting of burn wounds, used paraffin gauze as a backing material overlaid by cotton gauze and left in place for 6–10 days.[1] Since then the majority of workers have employed the same technique[5–9] whilst others have used synthetic mesh dressings which enable much easier graft mounting and handling[5,9–11] or sheet materials such as fibrin gel[6], collagen sheet,[5,6] glutaraldehyde treated porcine skin or amniotic membrane[5] and silastic sheet.[9]

Potential factors influencing graft take are legion and include the dressing, the wound bed, infection and the provision of a dermal component. We have established a model of keratinocyte grafting in pigs which allows controlled study of factors that may be relevant. The aims of the current study were: first, to quantify keratinocyte autograft take with three different dressings which varied in their ability to maintain an occlusive environment; secondly, to establish the duration of study of keratinocyte autograft epidermis possible in this model; and thirdly, to determine whether keratinocyte autograft application influenced wound healing as assessed by wound area measurement.

Materials and methods

For all procedures the pigs were deeply sedated using an intramuscular injection of ketamine and xylazine and anaesthesia was maintained by inhalation of 1–2% enflurane in oxygen and nitrous oxide.

Keratinocytes were cultured from split thickness skin obtained from the paravertebral region of the pig with a mechanical dermatome. Briefly, the skin was washed in EDTA, cut into strips and floated in 0.25% trypsin for 2–3 h at 37 °C to cleave it at the dermo-epidermal junction. The epidermis was then separated from the dermis using fine forceps and a single cell suspension prepared by filtration of the epidermal fragments through a fine wire mesh. The cells were inoculated into 75 cm^2 tissue culture flasks containing a feeder cell layer of lethally irradiated swiss 3T3 mouse fibroblasts and medium as previously described.[12] Confluent primary cultures were ready for grafting at 2 weeks and were detached from the plastic using the neutral protease dispase.

Eight 4 × 4 cm full thickness wounds (*i.e.* skin and fat including all epidermal appendages excised down

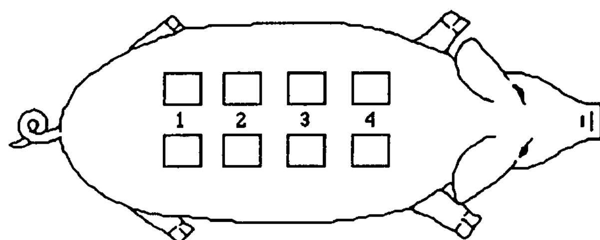

Fig. 1

Figure 1—Diagram of the eight 4 × 4 cm full thickness wounds excised to muscle fascia in bilateral lines of four on the paravertebral areas of each pig.

to muscle fascia) were created on each animal in bilateral lines of 4 on each paravertebral area (Fig. 1) and photographed with inclusion of a measurement scale before application of the keratinocyte autografts. The graft backing and immediate overlying dressings were secured under two tie-over sutures to normal skin beyond the wound margins and the trunk wrapped in circumferential elastic adhesive tape bandage.

On 4 pigs the keratinocyte autografts were dressed with the woven viscose graft backing (Tricotex^R, Smith & Nephew, UK) overlaid with a double layer of paraffin gauze and then cotton gauze. On another 2 pigs the grafts were similarly dressed with woven viscose and cotton gauze but without the intervening paraffin gauze. On a further 2 pigs the grafts were covered with a hydrogel sheet dressing (Vigilon^R, Seaton Health Care, UK) and then cotton gauze.

In the two groups employing viscose as the graft backing the cotton gauze was replaced at days 4, 7, 10 and 13. At day 16 the dressings including the backing material were removed and the wounds photographed. The wounds were then redressed with two layers of paraffin gauze and cotton gauze. They were again photographed at days 20 and 24.

In the group employing the hydrogel sheet as a graft backing the cotton gauze was changed on days 4 and 7. At day 10 the hydrogel sheet was removed and the wounds photographed. They were redressed with two layers of paraffin gauze and cotton gauze and photographed again at days 13, 16, 20 and 24.

A control group of a further 2 pigs had the same size and number of wounds created and photographed but keratinocyte autografts were not applied. The wounds were dressed with a double layer of paraffin gauze and cotton gauze maintained in position with tie-over sutures. The wounds were photographed and redressed at days 7, 10, 13, 16, 20 and 24.

The area of any visible keratinocyte autograft epidermis and the total area of each wound, bounded by the epidermis of the wound margin, was quantified by means of colour transparencies projected on to a digitising tablet. The areas were outlined with a stylus and quantified by means of a semi-automatic image analysis programme (Imagan 2, Kompira Ltd., Shotts, Strathclyde).

The results in each group were expressed as the number of wounds on which take was observed, the total absolute area of keratinocyte autograft epidermis generated, the area of the epidermis as a percentage of the total wound area at that time, and as a percentage of the original wound area. The areas of control and grafted wounds were expressed as a percentage of original wound area. The area of the keratinocyte autografted wounds was determined for those wounds on which epidermis was generated and those wounds on which it was not.

A further group of 5 pigs was used to provide biopsy material from days 2–24 for this and other studies. Biopsies were undertaken by wound excision without dressing removal after application of keratinocyte autografts backed with woven viscose and paraffin gauze. Sections of paraffin embedded tissue were stained with haematoxylin and eosin for light microscopy.

Results

The results are presented in Tables 1–5.

Keratinocyte autografts backed with woven viscose

No take was seen at any time point on any of the 16 wounds where the keratinocyte autografts were backed with viscose and simply dressed with cotton gauze.

Keratinocyte autografts backed with woven viscose and paraffin gauze

16 days after grafting 10 of 32 wounds had generated an epidermis from the keratinocyte autografts (Table 1, Fig. 2A). The absolute area of take was 4039 mm^2 (mean 126 mm^2/wound) and represented 20% of the total wound area at that time but only 9% of the original grafted area. At day 20, due to loss of 70% of the keratinocyte autograft epidermis (Fig. 2B), 6 wounds remained with a graft epidermis area totalling 930 mm^2 (mean 29 mm^2/wound) representing 9% of the wound area at that time but only 1.6% of the original grafted area. There was further loss of keratinocyte autograft epidermis by day 24 with 597 mm^2 remaining on 5 wounds (mean 25 mm^2/wound). One pig was lost from the study at day 20 (anaesthetic death). A further problem with the viscose dressing material was the ingrowth of granulation tissue where take had not occurred, causing trauma on dressing removal.

The take seen on 10 of the 32 wounds (31%) at day 16 ranged from 25–100% of wound area (mean 63.6%) (Table 2) with keratinocyte autograft epidermis continuous in parts with the epidermis of the wound edge. There was no take on any of the 8 wounds at position 1 (most cranial); 37% of the take was on 5 wounds at position 2; 35% on 3 wounds at position 3 and 28% on 2 wounds at position 4 (most caudal). 59% of the take at day 16 was on wounds on the left side of the animals and 41% on the right.

Keratinocyte autografts backed with hydrogel dressing

At 10 days after grafting there was no visible evidence of keratinocyte autograft epidermis on any of the 16 wounds (Fig. 4A). They remained wet under the hydrogel dressing. At day 13, however, islands of epidermis had appeared on 11 of the 16 wounds (Fig. 4B). None of these areas were continuous with the epidermis of the wound edge. Their total area was 2480 mm^2 (mean 155 mm^2/wound) representing 22% of the wound area at that time but only 9% of the original grafted area (Table 3). At day 16 there had been loss of epidermis from 4 wounds where it had adhered to the mesh of the paraffin gauze; the remaining area on 7 wounds was 1374 mm^2 (mean 86 mm^2/wound). The epidermis at day 16 had a thick top layer of keratin. There was no further loss of epidermis thereafter. However, islands of epidermis appeared on two wounds at day 20; in one case epidermis had been apparently lost at day 16 and in the other no visible epidermis had previously been present.

Table 1 Keratinocyte autograft take after removal of viscose and paraffin gauze backing at day 16 (32 wounds)

		Area of take (mm^2)			*% Take*	
Day	*No of wounds with take*	*Total*	*Mean*	*95% conf.*	*Mean*	*95% conf.*
16	10	4039	126	74	20	11.5
20	6	930	29	27.4	9	7.9
24*	5	597	25	20.9	13	9.7

* 24 wounds: one pig died under anaesthesia at day 20.

Table 2 Keratinocyte autograft take* after removal of viscose and paraffin gauze backing at day 16: Wound position and side

	Wound position				
	1	*2*	*3*	*4*	*Total*
Left: Pig A	–	242 (41)*	436 (61)	–	
B	–	–	508 (82)	617 (100)	
C	–	432 (59)	–	–	
D		139 (25)	–	–	2374
Right: Pig A	–	355 (65)	–	–	
B	–	–	470 (81)	–	
C	–	313 (46)	–	–	
D	–	–	–	517 (76)	1665
Total	–	1481 (37%)	1414 (35%)	1144 (28%)	4039

* mm^2 (% of wound area).

Table 3 Keratinocyte autograft take after removal of hydrogel backing at day 10 (16 wounds)

		Area of take (mm^2)			*% Take*	
Day	*No of wounds with take*	*Total*	*Mean*	*95% conf.*	*Mean*	*95% conf.*
10	–	–	–		–	
13	11	2480	155	80.4	22	10.6
16	7	1374	86	72.4	20	16.5
20	9	1357	85	51.6	32	19.7
24	8	1319	82	47.9	41	24.5

Table 4 Keratinocyte autograft take* at day 13 after removal of hydrogel backing: Wound position and side

	Wound position				
	1	*2*	*3*	*4*	*Total*
Left: Pig A	239 (32)*	–	269 (22)	103 9.4	–
B	224 (48)	292 (47)	82 (16)	–	1209
Right: Pig A	–	442 (52)	438 (55)	–	
B	162 (27)	153 (22)	76 (15)	–	1271
Total	625 (25%)	887 (36%)	865 (35%)	103 (4%)	2480

* mm^2 (% of wound area).

Table 5 Reduction in size* of control and keratinocyte autografted wounds

		Keratinocyte autografted wounds			
		Viscose Backing		*Hydrogel Backing*	
Day	*Control*	*Take*	*No Take*	*Take*	*No Take*
0	100*				
7	88.5 (3.6)				
10	60.3 (5.2)			–	57.8 (6.4)
13	38.0 (4.8)			42 (6.6)	43 (16.5)
16	32.7 (3.6)	36.1 (2.6)	32 (2.2)	27.6 (4.8)	29.6 (7.8)
20	14.8 (2.9)	16.8 (2.9)	15.5 (1.5)	15.3 (3.4)	16.1 (5.5)
24	10.8 (2.3)	10.8 (3.1)	10.6 (1.4)	11.4 (2.0)	9.5 (4.5)

* Percentage of original wound area (95% confidence limit).

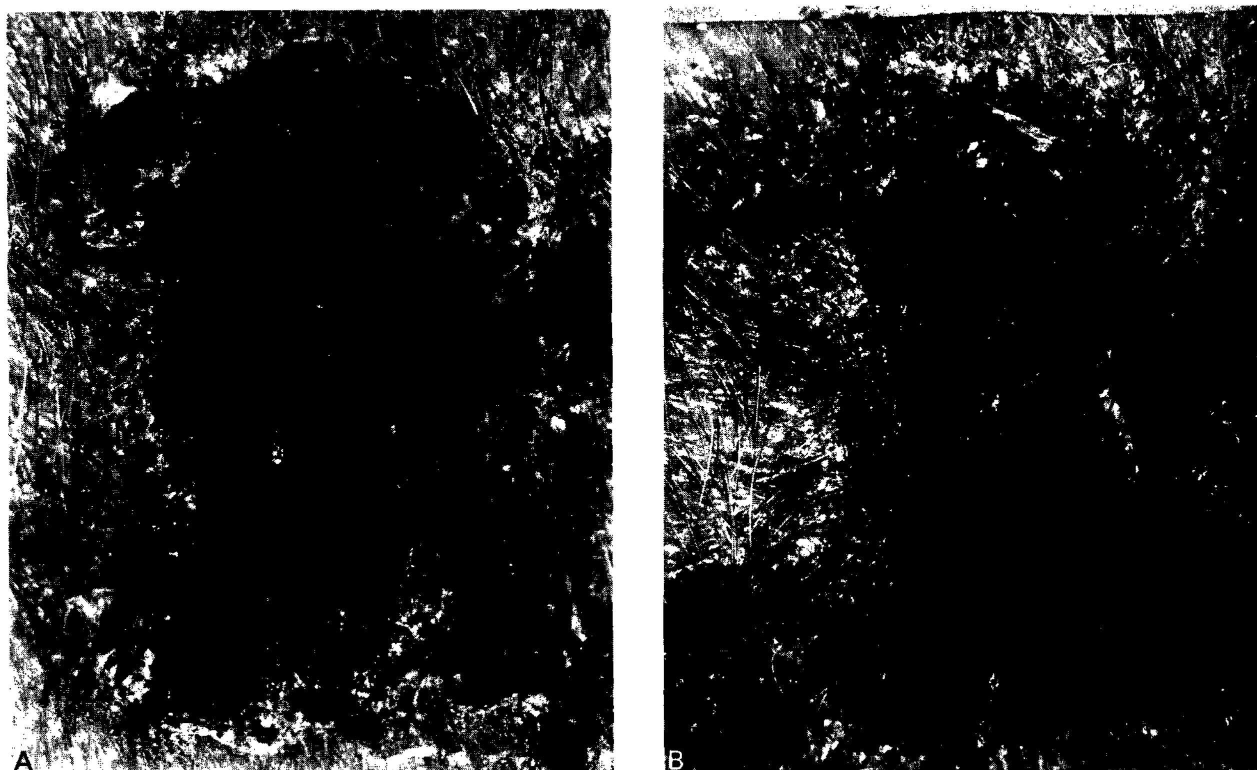

Fig. 2

Figure 2—(A) Keratinocyte autografted epidermis with a visible stratum corneum after removal of the viscose dressing at day 16. (B) Partial loss of keratinocyte autografted epidermis from the wound surface on wound redressing at day 20.

The take at day 13 seen on 11 of the 16 wounds (68 %) ranged from 9–55 % (mean 31.4 %) (Table 4). 25 % of the take was on 3 of the 4 wounds at position 1 (most cranial), 36 % on 3 wounds at position 2, 35 % on 4 wounds at position 3 and only 4 % on 1 wound at position 4 (most caudal). 49 % of the take was on wounds on the left side of the animals and 51 % on the right.

Wound area

The control wounds dressed only with paraffin gauze and cotton gauze healed mainly by wound contraction. At day 10 they were about 60 % of their original area (Table 5), 40 % at day 13 and 33 % at day 16 (equivalent to an area of 2.5 x 2.5 cm). Migration of the epidermal wound edge was not easily visible in the first 2 weeks but became more prominent after 16 days. It was easily distinguishable from the granulation tissue. No islands of epidermis were seen within the area of granulation tissue on these control ungrafted wounds.

The keratinocyte autografted wounds contracted in the same fashion as the control wounds. The 95 % confidence limits of the area of the keratinocyte autografted wounds fell within the 95 % confidence limits of the area of the control wounds at all times. This was irrespective of whether or not the keratinocyte autografted wounds had formed an epidermis.

By day 20 the wounds were only about 15 % of their original area and about 10 % by day 24 (equivalent to an area of 1.5 x 1.5 cm).

Histology

At day 2, light microscopy of the keratinocyte autograft epidermis showed the keratinocytes as a thin layer of flattened undifferentiated cells on the muscle of the wound bed. Wounds biopsied without dressing removal showed the keratinocyte sheet attached to, and proliferating into, the interstices of the viscose dressing. The keratinocyte sheet was on occasions found to be separated from the wound bed (Fig. 3). A stratified epidermis was formed by 7 days and a stratum corneum by 16 days.

Discussion

The backings of woven viscose with and without paraffin gauze and of hydrogel sheet provided environments of differing occlusion for the keratinocyte grafts. The viscose alone provided the least occlusion with exudate passing through to be absorbed by the cotton gauze and then evaporate. The addition of paraffin gauze would have maintained a semi-occlusive environment, at least in the early stage, with later loss of occlusion such that the dressing and areas of keratinocyte autograft epidermis were dry on dressing removal at day 16. The hydrogel was chosen to provide complete occlusion and also to try to prevent the adhesion of keratinocyte autograft epidermis to the viscose backing seen on microscopy of biopsied material and by the loss of clinically evident epidermis on dressing removal.

Fig. 3

Figure 3—Wound biopsied at day 4 without dressing removal showing the keratinocyte autograft sheet attached and corrugated to the shape of the overlying viscose backing material but separated from the wound bed (× 150, H + E).

Take was expressed as a percentage of the wound area present at the time of each dressing removal. This was less than the original wound area as a result of wound contraction. At day 16 the average wound size was equivalent to 2.5 × 2.5 cm and was sufficient for assessment of take. In addition, there was virtually no epidermal migration from the wound edge at this time so that any epidermis was clearly of keratinocyte autograft origin. Changes over time in take as a proportion of wound area are, however, confounded by wound contraction giving the impression of increasing wound cover. The absolute area of take is a better measure but beyond day 20 wound edge epidermis merged in parts with graft epidermis and assessment of take was therefore less accurate. The ungrafted control wounds were on separate animals in order that the maximum number of grafted wounds could be assessed in as few animals as possible with considerable economy of time in keratinocyte culture.

The complete absence of take using viscose without paraffin gauze indicates that cultured keratinocytes are unable to survive in this driest environment. The semi-occlusive environment provided by the addition of a double layer of paraffin gauze allowed keratinocyte survival and then development of an epidermis. Indeed, 100% take was found on one wound at day 16 and take was a mean of 68% on the 10 wounds (31%) which generated an epidermis. The lack of take on 22 (69%) of the wounds may be due to the failure of the dressing to maintain a consistent environment, or more probably to movement of the dressing adherent to the graft on the wound bed. The importance of movement of the dressings is suggested by the fairly rigid nature of the viscose, the histological finding of graft attached to the dressing but separated from the wound bed and the complete absence of take on wounds in position 1 nearest the shoulder girdle, where movement is greatest. Take in positions 2, 3 and 4 was similar; the hind limb did not encroach on the most caudal wound. The pattern of take under viscose and paraffin gauze with complete areas of autograft epidermis abutting in a straight line with the epidermis of the wound edge suggests that these areas of keratinocyte sheet had survived intact as opposed to epidermis being generated from islands of surviving graft.

The hydrogel sheet dressing provided a completely occlusive environment with the wounds remaining wet until the dressing was removed at day 10. This was initially viewed as failure, since no epidermis was seen in contrast to keratinocyte autografted wounds from which viscose and paraffin gauze was removed at day 10 in the group of 5 animals used to provide biopsy material. Since 11 of the 16 wounds had an epidermis at day 13, keratinocytes must have survived. Removal of the hydrogel dressing allowed the wound surface to dry, with subsequent generation of a clinically evident epidermis from the surviving keratinocytes. Epidermis formed on 68% of the wounds, greater than twice the proportion which generated an epidermis under viscose and paraffin gauze. However, the average area of take on these wounds was less than half the size of those on viscose and paraffin gauze dressed wounds. The appearance of islands of epidermis indicates that the keratinocyte sheet had not remained intact.

The mean area of take per wound at day 16 using viscose and paraffin gauze was similar to that at day 13 in the hydrogel dressed group. However, removal of the adherent viscose caused a more marked loss of epidermis than removal of paraffin gauze on the previously hydrogel dressed wounds.

The decrease in wound area, as measured in this study, gauged the combination of both wound contraction and epithelialisation. The control ungrafted wounds healed predominantly by wound contraction. The epidermal margin could be clearly defined but locating the position of the original wound edge, from which migration occurred, was highly subjective. Thus the contribution of epidermal migration to the reduction in wound size, although small, was difficult to determine. Keratinocyte autograft, however, did not alter the reduction in wound size compared with control wounds even when they generated an epidermis. As long ago as 1952 Billingham and Reynolds noted weakness of attachment of epidermis generated from a suspension of epidermal cells on full thickness wounds in rabbits.[13] Furthermore, in accordance with our results, the epidermis formed did not prevent wound contraction. In contrast, Eisenger claimed only

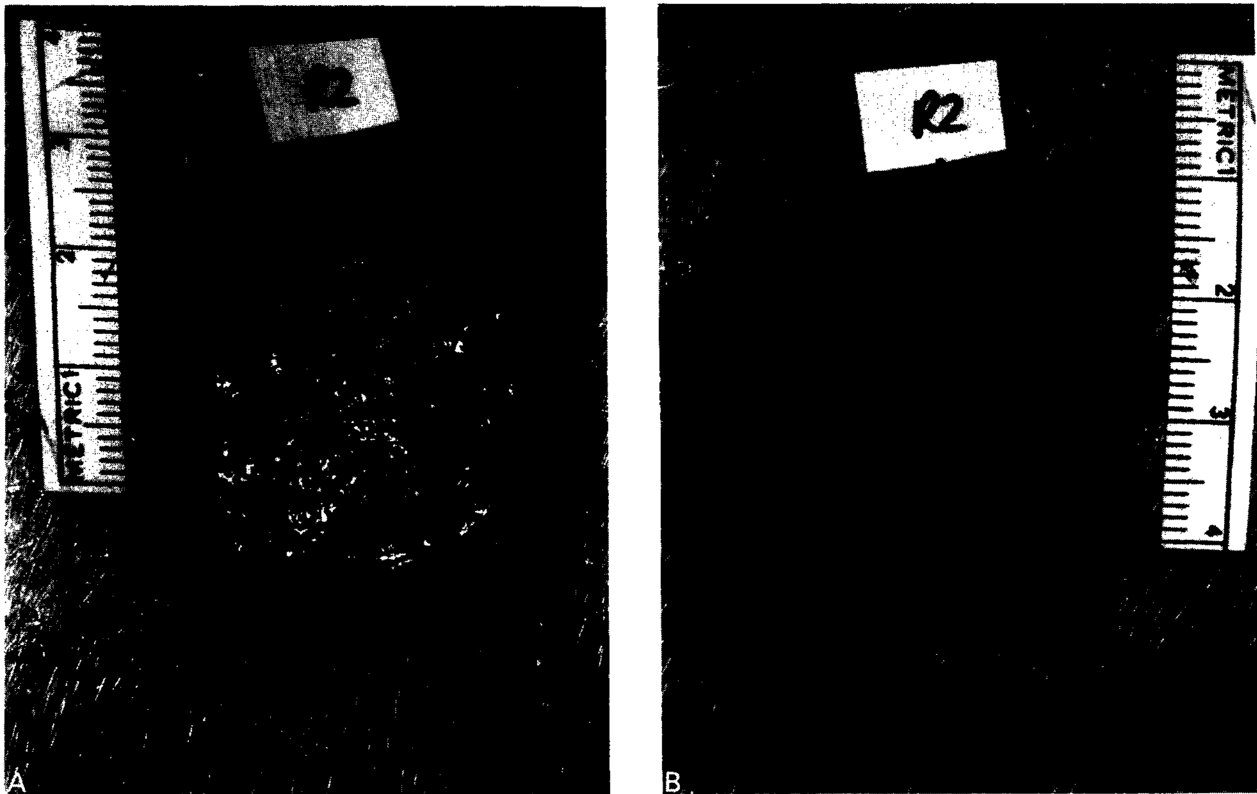

Fig. 4

Figure 4—(A) Hydrated wound after hydrogel dressing removal at day 10 without a clinically evident epidermis. (B) Development of an epidermis from the keratinocyte autograft at day 13.

25% reduction in wound area when keratinocyte grafts were applied to porcine wounds. However, the wounds were shallow, created with a dermatome, and were likely to have been covered by epidermis regenerating from remaining appendages.[14]

O'Connor, in the first report of keratinocyte grafting of burn wounds, used paraffin gauze overlaid by cotton gauze as a backing material left in place for 6–10 days.[1] Since then the majority of workers have employed the same technique.[5–9] Paraffin gauze provides a semi-occlusive environment in the early stages, allows the passage of exudate and later becomes non-occlusive. It appears to be a suitable dressing for keratinocyte survival and formation of an epidermis. Graft adherence to paraffin gauze has not been previously reported. Polyamide mesh (Surfsoft[R]) is structurally very similar to the viscose dressing used in this study and has been successfully used clinically[5,10] but it also adheres to the epidermis and suffers from granulation ingrowth where take does not occur, making it painful and traumatic to remove.[5] Gallico *et al.*,[9] however, found that this material retained exudate and did not observe any take with its use.

Sheet materials are an alternative to meshes. Those reported are plastic sheet,[15] fibrin gel,[6] collagen sheet,[5,6] glutaraldehyde treated porcine skin or amniotic membrane[5] and silastic sheet.[9] No take has been observed with these backing materials, which has been attributed to the retention of exudate. Our experience with hydrogel sheet seemed initially to be the same. However, an epidermis became evident after the wound exudate had evaporated. That grafted keratinocytes may remain alive for considerable time and show expansion only after presumed wound infection clears has previously been remarked upon[10,16] and meshed silastic sheet, preventing complete occlusion, has been found to be successful.[17] Hydrogel sheet was also used by Herzog in grafting of burn wounds but its qualities as a keratinocyte graft backing and the take produced compared to paraffin gauze were not recorded.[7]

In this study more than 70% of the area of transplanted keratinocyte autografts failed to survive. Graft structure may be a cause since in culture the cells lack a differentiated architecture and a protective stratum corneum. Infection has been cited as the main reason for graft failure. Although infection was not observed clinically in this study, it is likely that the wounds were colonised by commensal skin flora. However, quantitative bacteriology has previously not been correlated with poor keratinocyte autograft take.[5] In a recent study 20% of wounds with less than 10% take showed no bacterial growth and such wounds were also as frequently colonised as those with over 50% take.[11]

Failure of keratinocytes to survive could also result from lack of a favourable wound bed, with the epithelial cells isolated from their underlying dermal component. The best results have been seen on wounds where dermal elements are retained, but on such partial thickness wounds, take may be confused with healing from remaining appendageal epithelium. However,

good take has also been reported using cadaver allograft to provide the dermal component after removal of the allograft epidermis.[18, 19]

The results presented here illustrate the problems with currently available dressing materials. It appears that after grafting, occlusion is necessary for keratinocyte survival and attachment to the wound bed, but after this a semi-occlusive or dry environment is important for the formation of a differentiated epidermis. These conditions were met by removal of the hydrogel dressing at day 10 and by the drying out of the viscose and paraffin gauze dressing. This could be discerned from clinical observation even though the wound environment was not quantified. Further improvement in take may be possible by development of a dressing which is initially occlusive but later enables drying of the wound and which does not adhere to the graft.

The pig is a classical model of epidermal wound healing.[20] It lacks fur and has a large area of skin whose structure is similar to that of human skin.[21] It is thus a suitable model of human keratinocyte grafting and could be used to obtain a profile of the behaviour and fate of keratinocyte grafts. Using 4×4 cm wounds, the duration of study is limited to about 28 days because of wound contraction which was not prevented by the presence of keratinocyte autograft epidermis. Larger wounds may be used for longer periods of study. The structure of the graft-wound bed interface also warrants further investigation to ascertain the weakness responsible for loss of the keratinocyte autograft epidermis.

References

1. O'Connor NE, Mulliken JB, Banks-Schlegel S, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. *Lancet* 1981; **1**: 75.
2. Gallico III 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; **311**: 448.
3. Teepe RGC, Ponec M, Kreis RW, Hermans RP. Improved grafting method for the treatment of burns with autologous cultured human epithelium (letter). *Lancet* 1986; **1**: 385.
4. De Luca M, Albanese E, Bondanza S, Megna M, Ugozzoli L, Molina F, Cancedda R, Santi PL, Bormioli M, Stella M, Magliacani G. Multicentre experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. *Burns* 1989; **15**: 303.
5. Eldad A, Burt A, Clark JA, Gusterson B. Cultured epithelium as a skin substitute. *Burns* 1987; **13**: 173.
6. Kumagai N, Nishina H, Tanabe H, Hosaka T, Ishida H, Ogino Y. Clinical application of autologous cultured epithelia for the treatment of burn wounds and burn scars. *Plast Reconstr Surg* 1988; **82**: 99.
7. Herzog SR, Meyer A, Woodley D, Peterson HD. Wound coverage with cultured autologous keratinocytes: use after burn wound excision, including biopsy followup. *J Trauma* 1988; **28**: 195.
8. 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* 1989; **60**: 600.
9. Gallico III GG, O'Connor NE, Compton CC, Remensnyder JP, Kehinde O, Green H. Cultured epithelial autografts for giant congenital nevi. *Plast Reconstr Surg* 1989; **84**: 1.
10. Teepe RGC, Ponec M, Kempenaar B, Mauw B, Scheffer E. Clinical, histological and ultrastructural aspects of cultured epithelium. Proceedings of the International Symposium on the clinical use of cultured epithelium in surgery and dermatology. Leiden, The Netherlands, Medical and Scientific Conference Ltd., Birmingham, England. 1987: 36.
11. Blight A, Mountford EM, Cheshire IM, Clancy JMP, Levick PL. Treatment of full skin thickness burn injury using cultured epithelial grafts. *Burns* 1991; **17**: 495.
12. Leigh IM, Purkis PE, Navsaria HA, Philips TJ. Treatment of chronic venous ulcers with sheets of cultured allogeneic keratinocytes. *Br J Dermatol* 1987; **117**: 591.
13. Billingham RE, Reynolds J. Transplantation studies on sheets of pure epidermal epithelium and on epidermal cell suspensions. *Br J Plast Surg* 1952; **5**: 25.
14. Eisenger M. Regeneration of epidermis by cells grown in tissue culture. *J Am Acad Dermatol* 1985; **12**: 402.
15. Igel HJ, Freeman AE, Boeckman CR, Kleinfeld KL. A new method for covering large surface area wounds with autografts: II. Surgical application of tissue culture and expanded rabbit skin autografts. *Arch Surg* 1974; **108**: 724.
16. O'Connor NE, Gallico GG, Compton CC, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epithelial cells: II. Intermediate term results on three pediatric patients. In: Hunt TK, Heppenstall HBS, Pines E, *et al.* eds. Soft tissue and hard tissue repair: Biological and clinical aspects. New York: Praeger Publishers, 1984: 283.
17. Aubock J, Irschick E, Romani N, Kompatscher P, Hopfl R, Herold M, Schuler G, Bauer M, Huber C, Fritsch P. Rejection, after a slightly prolonged survival time of Langerhans cell-free allogenic cultured epidermis used for wound coverage in humans. *Transplantation* 1988; **45**: 730.
18. Heck EL, Bergstresser PR, Baxter CR. Composite skin grafts: frozen dermal allografts support the engraftment and expansion of autologous epidermis. *J Trauma* 1985; **25**: 106.
19. Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. *Lancet* 1986; **1**: 1123.
20. Winter GD. Epidermal regeneration studied in the domestic pig. In: Maiback HI, Rovee DT, eds. Epidermal wound healing. Chicago: Year Book Medical Publishers, 1972: 71.
21. Montagna W, Yun JS. The skin of the domestic pig. *J Invest Dermatol* 1964; **43**: 11.

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