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Case report

Sprayed cultured autologous keratinocytes used alone or in combination with meshed autografts to accelerate wound closure in difficult-to-heal burns patients

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1. Introduction

Extensive deep burns present a significant challenge to the burns surgeon. The limited availability of skin donor sites in such patients means that the time to achieve complete skin cover is prolonged and consequently increases the risk of complications such as sepsis. Research has shown that prompt excision of burned tissue [1] and early wound cover [2,3] will lead to improved survival, more rapid recovery and reduction in scarring [4].

Skin graft expansion is an essential tool in major burns. However, as expansion ratios increase so do the difficulties in handling the skin graft. Furthermore, higher expansion ratios result in larger areas of exposed, unhealed tissue (interstices) which are then dependent on epithelial migration from the meshed skin graft margins to close the wound. Lattice meshed grafts [5] have become the mainstay in burns treatment, but at expansion ratios of greater than 1:4 they become difficult to handle, have unpredictable take rates with increased hypertrophic scarring [6] as well as leading to increased contracture of the wound area [7,8]. It has also been demonstrated that the actual expansion may be significantly less than expected [9–11].

'Meek' meshing of skin graft is particularly useful in situations where higher expansion ratios are required. The Meek micrografting technique was initially described in 1958 [12] and although its subsequent use was eclipsed by the use of lattice meshed skin, recent revisions to the technique have led to an increase in its use [8,11,13–15]. Expansion ratios of 1:9 are easily achieved and this is combined with easier handling of the skin when compared with widely meshed lattice graft. Large areas can be covered with this technique with more accurate expansion, but the time to complete healing may still be prolonged because of the significant area between the islands of autograft tissue. It is also thought that 'take' rates are not as badly affected by microbial infections when compared with lattice grafts [8,10,13–15].

Cultured autologous keratinocytes, developed by Rheinwald and Green in the mid-70s [16] have been used extensively to help treat severe burns [17–19,21–23] and chronic ulcers [24]. Initially the cultured cells were applied to the wound bed as a mature sheet, and were able to form a

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neo-dermis even when grafted onto muscle facia [20]. However, long-term survival and stability were a problem in some instances, resulting in varying degrees of success [25–28], reviewed further in [29].

The unpredictability of successful 'take' rates may be due to the fact that the sheets can be very fragile and difficult to handle. It has been proposed that on their transport from the laboratory to the patient they could be prone to breaking up [30]. Histological analysis of CEA sheets has also been undertaken [31–33], in order to establish the possible cause of this fragility. These studies demonstrated delayed Rete ridge and anchoring fibril formation leading to a delayed maturation of the basement membrane. Further studies revealed an alteration in expression of integrins by which attachment of the CEA sheet is mediated [34–36]. The release of the epithelial sheet from the plastic culture flask by enzyme digestion may be responsible for the destruction or alteration in the integrins and basement membrane proteins [37] necessary for a successful attachment to the wound bed. The time delay between harvest and application may also be a factor [38].

We postulate that the condition of the cell sheet prior to removal from the plastic flask also influences its survival on the wound bed. It is important to have viable basal cells in the cell sheet as these are the cells which are going to attach and grow when placed on the wound bed. Within the cell culture flask the upper layers of the developing cell sheet will start to keratinise and provide a barrier between the liquid nutrients of the growth medium and the basal cells. This barrier may become more pronounced as the cell layers increase in number and as such, the thicker cell sheets, which may be easier to remove from the flask and easier to handle clinically, may have fewer viable basal cells and thus give rise to poorer take rates. It is proposed that these fully formed sheets contain, within the basal layer, increasing numbers of dying cells as well as reducing numbers of viable dividing cells as they become further isolated from the surface growth medium. This may be responsible for the unpredictability of take rates when compared with that of conventional split thickness skin grafts [20,26,28,39–41]. The successful use of immature, but difficult to handle sheets to re-epithelialise a wound pre-treated with the dermal substitute Integra [42] would confirm this hypothesis.

It is thought that by administering the cultured cells to the wound in a pre-confluent state may avoid some of the above problems [30]. There have been many different methods of delivery of these cells described (reviewed in [29,43]). We have used the sprayed method in our clinical practice which has been shown to have reasonable clinical success in the treatment of dermal burns at other centres [19,44,45].

In this article we report the use of sprayed, cultured autologous keratinocytes in combination with widely meshed autografts (both Meek and lattice techniques) and report here three cases with significant full thickness burns. The patients had 8% (elderly), 31% (non-healing) and 90% TBSA burns. We also report 2 patients treated with cultured cells alone; a 56% TBSA (non-healing) and a 15% TBSA female ballerina who wished to avoid unnecessary donor-site scars.

2. Methods

2.1. Cell culture

Skin biopsies were harvested from a non-burned area away from the site of injury, and at the same time as a scheduled procedure carried out under a general anaesthetic. In order to ensure sterility of the tissue, the harvest site was prepared by washing with 70% aqueous chlorhexidine gluconate (Hibiscrub, ICI), rinsed with sterile saline, washed with 5% povidine iodine and then left for a minimum of 5 min. The iodine was washed off with sterile saline, followed by a final wash with 70% alcohol then left to air dry. A thin split thickness skin graft (SSG) was harvested using a Zimmer dermatome (setting No. 4, equivalent to a thickness of 0.2 mm). The sample size varied with available donor site and extent of burn area to be treated, but usually between 3 and 8 cm^2. The tissue was wrapped in a sterile, saline-soaked gauze and transported to the laboratory in a sterile container.

In the laboratory, under sterile conditions, the skin sample was washed 3 times in Hanks Balanced Salt Solution (HBSS) (Invitrogen, UK). It was then submerged in a solution of 2% Dispase (Invitrogen, UK) and placed in an orbital shaking incubator for up to 1 h at 37 °C to release the epidermal layer. After peeling away the unwanted dermal layer, the epidermis was incubated in a 0.5% trypsin solution (Difco) for 30–60 min at 37 °C in order to generate a suspension of epithelial cells mainly from the basal side of the epithelial layers. The cell suspension was transferred to tissue culture flasks and grown in complete growth medium of (3:1) DMEM: F12; 20% heat inactivated foetal calf serum; hydrocortisone (0.4 mcg/ml); epidermal growth factor (10ng/ml) and 10^{-10}M cholera toxin final concentrations, with the addition of lethally γ-irradiated 3T3 feeder cells based on the original methods of Rheinwald and Green [16]. We routinely isolate about 2-4 $\times$ 10^6 epithelial cells from each 1 cm^2 of skin. The number of cultured epithelial cells harvested for each patient application varied depending on the length of time in culture as well as frequency of harvesting the cells for clinical application.

After repeated passaging and expansion, the cells were harvested from large numbers of flasks to give a cell suspension of 10^7 cells per ml in RPMI 1640 medium (Sigma). The cell suspension was then transported to the operating theatre and applied to the patient through a spray nozzle (insert V06222 Coster Aerosols Ltd) attached to a 5 ml hypodermic syringe and delivered at a density of approximately 5 $\times$ 10^5 cells per cm^2. Where cells were likely to be required for further applications at later dates, only some of the flasks were harvested and the rest maintained in culture.

The cells routinely completed ~6 population doublings over a 3-week period. For example, in Case 3 an initial yield of 18 $\times$ 10^6 cells from a skin biopsy of 8 cm^2 gave rise to a harvest of 415 $\times$ 10^6 cells from two thirds of the culture flasks after 20 days in culture. The remaining flasks were kept for further continued growth, allowing a second application 3 days later with 450 $\times$ 10^6 cells (total of 5.6 population doublings). Three vials of cells (3 $\times$ 10^6 each) were frozen for later revival and further clinical applications. The harvested cells were delivered to the patient in a 40 ml volume on day 20 and a 45 ml volume on day 23.

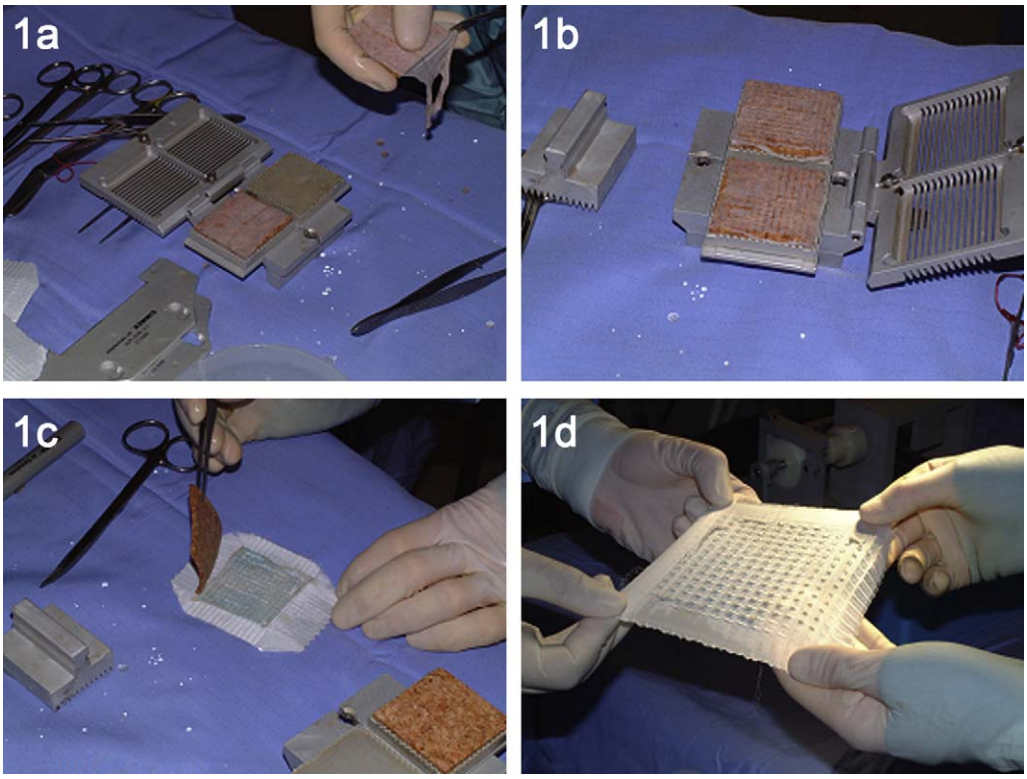

Fig. 1 – Meek meshing. (a): A piece or pieces of skin placed on top of a 42 mm × 42 mm cork board. (b): The skin meshed into 196 3 mm × 3 mm squares. (c): The meshed skin islands glued to a double-pleated, polyamide gauze. (d): The gauze un-pleated to expand the skin islands (the graft) to ratios ranging from 1:4 to 1:9.

2.2. *Application of cultured cells*

The burn wound was debrided using a Watson dermatome and haemostasis achieved using bipolar diathermy and adrenaline soaked gauze (1:500,000 in 0.9% normal saline). Where used, any remaining cryopreserved human allograft was debrided from the wound bed.

For the 3 patients requiring autografts, the subcutaneous tissues were infiltrated with a 1:500,000 solution of adrenaline in 0.9% normal saline and the skin lubricated with Aquagel. A Zimmer dermatome was used to harvest the skin grafts which were then meshed.

Lattice meshing was performed using a Brennan meshing device. In the cases described here the expansion ratios for lattice meshing used were 1:4 on patients 1 and 2. The meshed autografts were expanded, placed onto the prepared wound bed and stapled in position. Cultured cells were then sprayed evenly over the meshed autografts to provide cells within the interstices.

Meek meshing on patient 3: Harvested skin was placed on top of a 42 mm × 42 mm cork plate (Fig. 1a). This was then meshed into 196 3 mm × 3 mm squares (Fig. 1b) and glued to a double-pleated, polyamide gauze (Fig. 1c). The gauze was then un-pleated to expand the graft to ratios ranging from 1:4 to 1:9 (Fig. 1d). Cultured cells were sprayed evenly over the prepared wound bed (Fig. 4a) and the expanded meshed graft, still attached to the backing dressing, was then applied directly over the sprayed cells (Fig. 4b).

Patients 4 and 5 had cultured cells sprayed onto the prepared wound bed without the use of meshed autograft.

The grafted areas were covered with Tegapore dressing (secured with staples) over which standard tulle gras, gauze, gamgee and a crepe bandage were applied. The dressings were left intact for 3 days at which point all dressings except the Tegapore were changed and the wound area cleaned. Inspection of the skin graft was performed at this time through the Tegapore. The Tegapore was removed at day 7 and photographs taken at this and subsequent dressing changes. In the absence of cultured cells, the donor sites were dressed initially with Kaltostat (Convatec, BMS), tulle gras and absorbent dressings. Whenever possible, if there were remaining cultured cells, these were used to cover donor sites to accelerate their healing.

3. Histology

Minimal sized tissue biopsies were taken for cryosectioning and were prepared and cut by standard techniques. The 15-micron cut sections were visualised either by standard Haematoxylin and Eosin (H&E) staining for general morphology or standard immuno-histochemical staining with fluorescein-tagged antibodies to cytokeratin 14 for epithelial cell identification.

4. Patients

4.1. *Case 1: Lattice mesh + cultured cells on an elderly patient*

An 80-year-old woman sustained 8% full thickness flame burns circumferentially to both thighs after dropping a lit cigarette onto a polyester nightdress. She suffered from advanced secondary progressive multiple sclerosis and dementia. Exposure time was prolonged and subsequent first aid was delayed. On admission her burns were debrided and cryopreserved human allograft was applied.

At day 5 after burn a small area (~4 cm^2) of thin split-thickness skin was taken for cell culture as previously described.

At day 24 after burn she underwent lattice grafting with sprayed cultured cells to the right thigh as described above. The grafts were dressed with our standard dressing of Tegapore (3 M) and absorbent dressings. It was noted that the small donor site harvested for culture on day 5 had still not healed; therefore, cultured cells were sprayed over all donor sites to aid healing.

At 7 days after autograft application, the grafted areas were stable and epithelialisation of the wound had occurred, demonstrating excellent take of cells with good healing. It was noted that certain areas of the wounds were bleeding following removal of the primary dressing (Fig. 2a). It was uncertain whether the wound had not healed in these areas or whether any cells initially attached to the wound bed were being removed with the dressing. The dressing was, therefore, histologically assessed for the presence of keratinocytes. Cytokeratin 14 positive staining was observed above and below the mesh of the dressing (Fig. 2b) demonstrating the growth of the cells through the mesh, forming a continuous cell sheet above the dressing. Extra care to moisten the area was subsequently applied when removing primary dressings.

In order to determine the presence of epithelium rather than just dried wound exudate or granulation tissue, a 0.5 cm long elliptical biopsy was taken from a sprayed, and apparently healed, area for histological analysis with the cytokeratin 14 antibody. A section from an area close to the apex of the interstices reveals two areas of dermis from the autograft tissue with a continuation of epithelial cells within the gap (Fig. 2c). A section taken from the area of maximum gap in the mesh pattern demonstrates that even across the widest part, there is a continuation of epithelial cells (Fig. 2d).

At this time point, the combination procedure was repeated on the left thigh. The left calf area was treated with meshed autograft alone. A further 7 days later, the left thigh area had fully epithelialised (Fig. 2e). By this time point the meshed skin on the calf area had contracted but had still not fully epithelialised (Fig. 2f). There did not appear to be any contraction of the grafted area treated with cultured cells. Fig. 2i and j show the patient's legs before and after healing, respectively.

Haematoxylin and Eosin (H&E) stained 3 mm punch biopsy sections from each area of the left leg showed the presence of a thick epithelial layer beyond the autograft tissue from the thigh (Fig. 2 g), but a thinner outgrowth from the autograft tissue on the calf area which failed to cover the whole interstices (Fig. 2 h).

Subsequent dressings-changes showed good maturation of the grafts as well as donor site healing. The patient was transferred back to her referring hospital for further medical follow up prior to discharge home at day 41 after burn. The patient was reviewed by the outreach team weekly and was discharged from our care 3 weeks later.

4.2. *Case 2: Lattice mesh + cultured cells on non-healing wounds*

A 19-year-old man sustained a 31% self-inflicted burn to his face, trunk, upper thighs and both upper limbs. It was of mixed depth with at least 16% deep dermal burn.

On day 1, 16% of his burn was debrided and autograft applied. The remaining burned areas were treated with the antimicrobial agent Flammacerium (cerium nitrate-silver sulphadiazine) (Duphar–Solvay). On day 4 a further 4% was debrided and autografted. At subsequent dressing changes his grafts were found to have failed to take. On days 11 and 15 he was further debrided and the wounds covered with meshed cryopreserved allograft skin. By day 23 after burn most of his donor sites had failed to heal and much of the deep burn remained uncovered; at this stage a 3 cm^2 thin sample of skin was harvested for cell culture.

On day 44 post burn he underwent lattice grafting with sprayed cultured cells to the anterior trunk, right groin and both arms (Fig. 3a). The patient's left groin received only meshed autograft. The donor site on one arm also received a spray of cultured cells as it was adjacent to a treated area.

The cultured cells-treated areas were all found to have excellent graft take 1 week later. The interstices in areas treated with cultured cells had also filled in. There was improved, but not complete, closure of the groin area treated with just the meshed skin. This was earlier than normally expected for 1:4 lattice mesh used alone, and was virtually healed by day 10 post graft (Fig. 3b). It was noted that the donor site which had been sprayed with cells had also healed by day 7 while that on the other arm took longer (images not shown). The treated areas continued to heal without further problems.

There appeared to be some contraction of the left groin area treated with meshed skin alone when compared with the right groin area treated with cultured cells and meshed skin. Also, the meshed patterning was more prominent in the area treated with meshed skin alone, even by day 23 post graft (Fig. 3c and d).

4.3. *Case 3: Meek micrograft + cultured cells on extensive full-thickness burns*

A 26-year-old man was doused in petrol and set alight during an alleged assault. His injuries were estimated at 90%, mostly full thickness with some partial thickness areas. The lower third of his legs and part of his buttocks were the only areas available as donor sites. On the third day after burn, a 5 cm^2 skin sample was sent for tissue culture. Meanwhile, over the next 7 days the upper limbs, both thighs, the chest and the abdomen were excised to fascia and 1:2 meshed, cryopreserved, cadaver skin applied. Over the next 2 weeks the patient underwent repeated dressings changes with the antimicrobial cream Flammacerium.

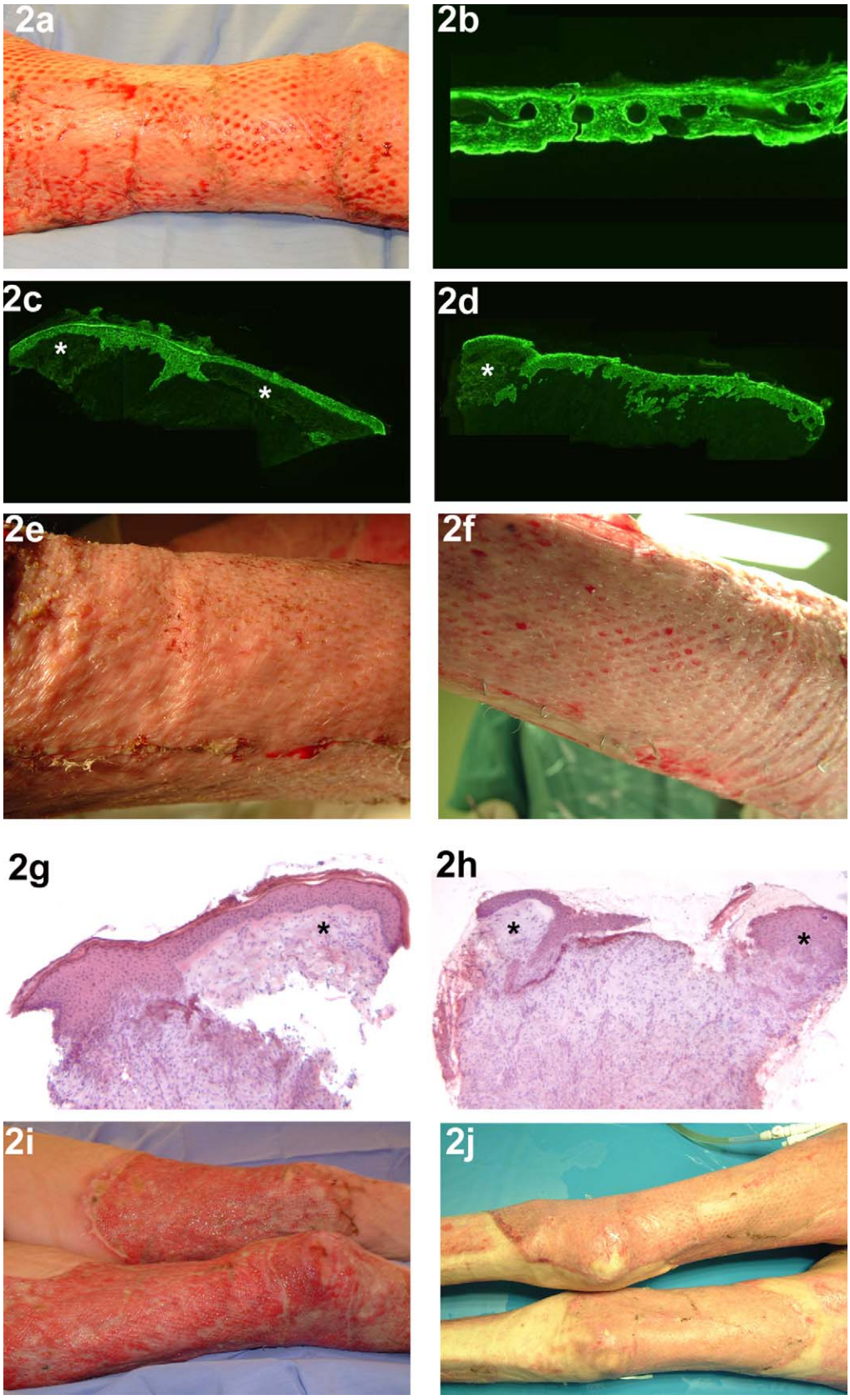

Fig. 2 – Patient 1—Lattice mesh plus cultured cells on an elderly patient. (a) Right leg, 7 days after grafting with meshed autograft and sprayed cultured keratinocytes, showing healed areas but some areas where cells have been removed with the dressing. (b) Histological section of the dressing removed after 7 days showing keratinocytes (stained positive with fluorescein tagged antibodies to cytokeratin 14) growing above and below the woven fibres of the dressing. (c and d)

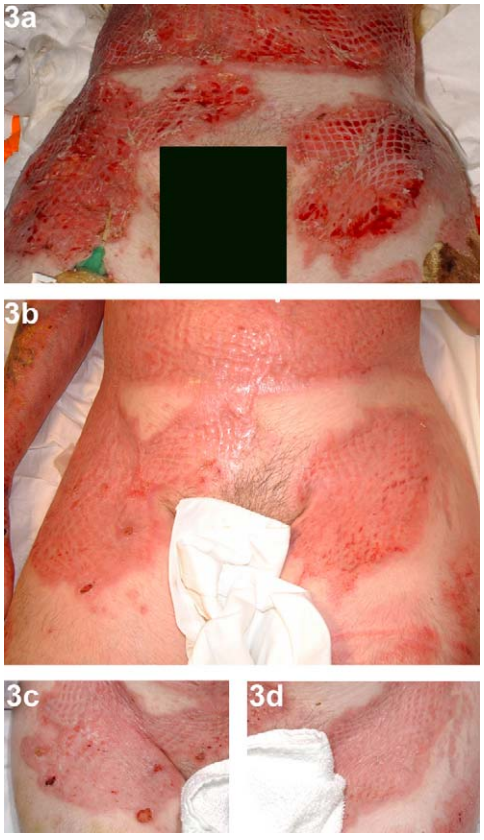

Fig. 3 – Patient 2—Lattice mesh plus cultured cells on a non-healing wound. (a) Mixed depth burns failed to heal 44 days after injury. Meshed autograft plus sprayed cultured cells applied to right groin and meshed autograft alone on the left groin. (b) Day 10 post graft shows both groin areas healing with complete closure of the right groin. (c and d) Day 23 post grafting showing both groin areas healed. There is a suggestion of greater contraction and a more prominent mesh pattern on the left side.

Following debridement of the cadaver skin, the sprayed cultured cells were applied (Fig. 4a) prior to the Meek meshed skin which was expanded 1:4 to the right upper limb and 1:9 to the chest (Fig. 4b).

Fig. 4c shows the right arm at 10 days post grafting and Fig. 4d shows a close-up view 3 weeks post grafting. Complete closure of the full thickness burn was achieved. Areas of epithelialisation can be seen between the Meek autografts even at day 10. This is too early to have grown out from the Meek autografts. It is more likely to be the cultured cells closing the gaps.

Staggered debridement of selected areas, balanced with the management of infection allowed time for the limited donor sites to heal and permitted re-cropping for further autograft.

On day 39 after burn, cultured cells and Meek autograft (used at 1:9) were applied to the chest. Day 43 saw the use of the same technique for closure of the abdomen but no cultured cells were used on this occasion. Approximately 3 weeks later the chest was healed whereas the abdomen still showed isolated islands of autograft with heavy granulation preventing epithelialisation (Fig. 4e). The abdomen was re-debrided and treated with further Meek meshed autograft combined, this time, with sprayed cells in order to achieve closure.

The patient continued to progress clinically and by day 142 had only 2% body surface area that was not yet skin-covered (Fig. 4f).

4.4. *Case 4: Cultured cells alone on non-healing wounds*

An 18-year-old male sustained a significant burn following a petrol bomb being thrown into his house. His burns were estimated to be 21%, mainly superficial but with deep dermal and full thickness areas to the lower abdomen and thighs.

By day 10 after burn the lower abdomen had failed to heal so a 3.5 cm^2 piece of skin was taken for skin culture. Sixteen days later his lower abdomen had still failed to heal (Fig. 5a). He underwent debridement of his abdominal burns and had the cultured cells sprayed onto this wound. On day 5 after application of the cultured cells the sprayed cultured cells had taken well across the whole area and the wound had healed completely by day 10 (Fig. 5b). This type of injury would normally be treated by excision and autograft when it failed to heal. The use of cultured cells helped these burn wounds to heal, avoiding the need for a skin graft.

4.5. *Case 5: Cultured cells alone to avoid scarring associated with donor sites*

A 19-year-old female ballet dancer from Belarus sustained burns to her right flank, thigh, buttock and both arms after accidentally falling into a bonfire. Her burns were assessed at 11% TBSA mainly partial thickness with deeper areas on the right forearm, flank and thigh.

On day 7 after burn her wounds were healing well except within the deeper burn areas mentioned above. In order to avoid harvesting donor skin to graft these areas a very small (<2 cm^2), thin skin sample was taken for culture. After just 7 days the cells were ready to spray back onto the patient's debrided wound. Fig. 6a shows the unhealed wound on her right flank prior to the application of cultured cells.

Histological sections of biopsies taken from the apex (2c) and the widest point (2d) of the interstices of the meshed autograft (*) from a healed area. Keratinocytes are stained for cytokeratin 14 and can be seen across the whole surface of the wound. (e and f) Left leg 7 days after grafting the thigh with meshed autograft and sprayed cells (2e) or the calf with meshed autograft alone (2f). The thigh has healed completely whereas there are still unhealed gaps in the meshed autograft on the calf. (g and h) Haematoxylin and Eosin stained biopsies taken from areas in 2e and f, respectively. The epithelium has clearly failed to extend far and close the gap between autograft tissue (*) in the absence of sprayed cells (2 h). (i and j) Patient's legs pre-graft (2i) and at discharge (2j).

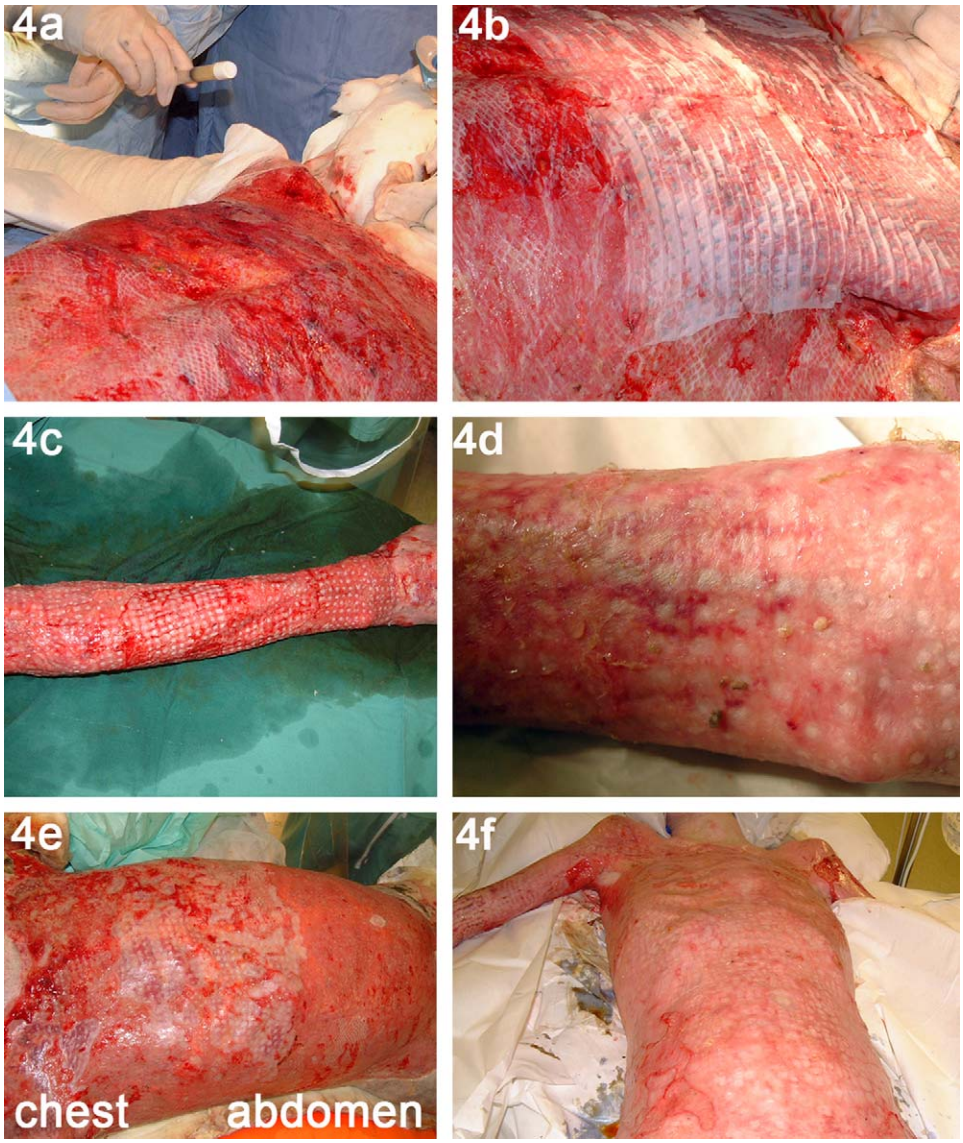

Fig. 4 – Patient 3—Meek micrografts plus cultured cells on a major burn. (a) Spray application of cultured keratinocytes onto the prepared wound bed. (b) Meek micrografts glued to the underside of the expanded dressing and applied over the sprayed cells. (c) Day 10 post grafting showing epithelialisation over most areas of the arm. (d) Day 24 post grafting showing a close-up of the arm demonstrating complete epithelialisation with micrografts still discernable. (e) Three weeks after grafting of chest (plus cultured cells) and abdomen (without cultured cells). The abdomen failed to epithelialise. Further debridement and reapplication of Meek micrografts, this time plus cultured cells, was necessary to achieve wound closure. (f) Three months post first grafts; all but 2% TBSA has healed.

Day 5 after sprayed-cells the wounds were re-inspected and the cultured cells had taken well. Her wounds continued to heal (Fig. 6b) and she travelled back to Belarus. She was seen there by one of our own Burns Surgeons 6 months after injury and her wounds had fully healed with minimal scarring (Fig. 6c).

5. Discussion

The ultimate aim for the treatment of any large burn is rapid cover of the entire wound with the patient's own skin. However, as burn wound size increases the amount of available donor site diminishes while the risks of sepsis increase. Sepsis increases the risk of graft failure thereby further challenging the burns surgeon.

It has been well documented that widely meshing skin allows greater coverage of the wound but still leaves a significant area of wound in the interstices to epithelialise. Expanded skin is difficult to handle and expansions of 1:6 and beyond are not readily possible with the lattice technique. The grafts are then subject to significant contracture and hypertrophic scarring. There are however indications in this work that the addition of sprayed cultured autologous keratinocytes may help to clinically reduce the contraction of meshed autografts (cases 1 and 2). These clinical observations have

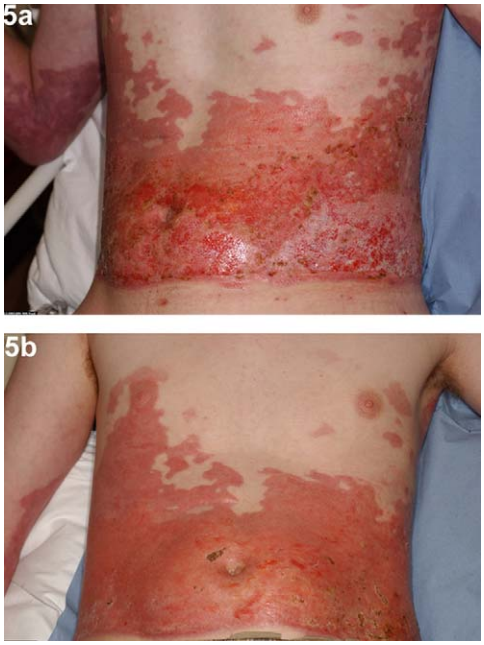

Fig. 5 – Patient 4—Cultured cells alone on non-healing wounds. (a) Day 27 post injury, the lower abdomen has failed to heal. The wounds were debrided and cultured cells sprayed onto the prepared wounds at this time point. (b) Day 10 post application of cultured cells; the wounds have closed.

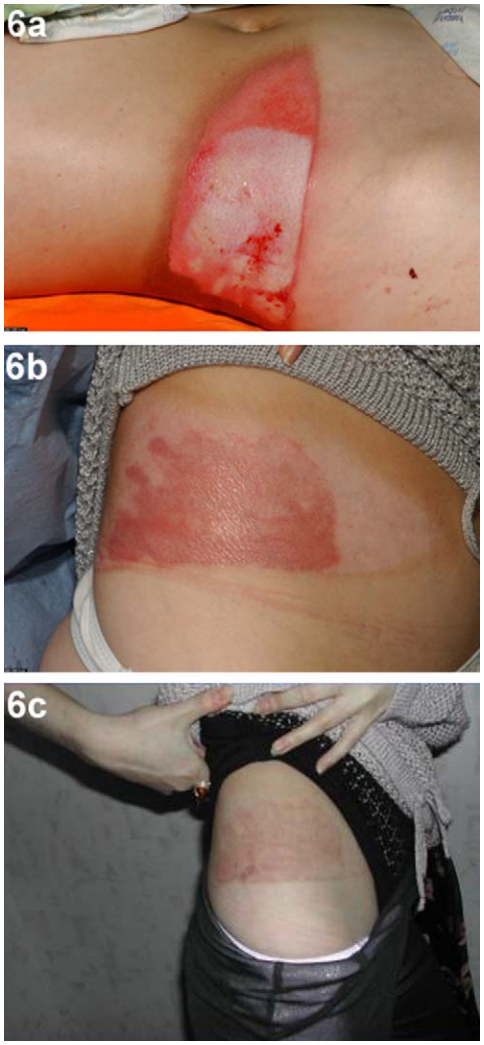

Fig. 6 – Patient 5—Use of cultured cells to avoid donor site scarring. (a) Day 14 post injury, cultured cells were sprayed onto the right flank wound. (b and c) Right flank healed with minimal scarring, day 38 (6b) and 6 months (6c) post injury.

subsequently been corroborated in our *in vivo* animal model [46]. The combination of lattice meshed graft and cultured epithelial sheet grafts has been reported with improved results as compared with either technique alone [23]. The healing times were, however, longer than we have reported here with the use of sprayed cells and in their work, no comment was made on contraction.

The versatility of sprayed cultured cells in a variety of settings has been demonstrated here. This case review report has demonstrated the benefits of cultured cell therapy, either alone, or in a combined approach to the skin coverage of large burns as well as difficult-to-treat cases.

Cultured autologous skin has been used in the treatment of large burns for many years and in many ways (reviewed in [9,29,45]). Disappointing results with sheets of cultured cells led to the use of sprayed cells in an attempt to increase epithelialisation of the burn wound. The cases we describe here are an attempt to fully utilise all the methods available to the burns surgeons to achieve their ultimate goal of closing burn wounds as rapidly as possible. The combination of the cultured cells and meshed autograft technique has merit in those cases where donor sites are limited; where initial treatment has failed; where difficulties are anticipated (e.g. in the elderly) and where donor sites are also failing to heal. The use of Meek micrografting allows expansion ratios of up to 9 times in a system that is relatively easy to learn and more importantly, easy to handle. Such expansion ratios would normally mean a prolonged time to full epithelialisation but the addition of cultured cells to the wound bed appears to significantly reduce this time. There is also merit in the use of cultured cells alone to help heal smaller areas, potentially avoiding the need to crop donor sites for autografting small, full thickness wounds (as in Case 5) with the associated donor-site scars. It may also prove useful in patients whose skin grafts have previously failed, as in Case 2. Equally, the benefit of spraying cultured cells onto donor sites to accelerate healing has also been observed by us and others [19,29,45].

The cultured cell therapy seemed to clinically reduce healing times when compared with the use of widely meshed skin alone. In Case 1, where the left leg was treated, with the thigh receiving both cells and lattice meshed skin graft, and the calf receiving skin graft alone. The thigh healed more rapidly and appeared to contract less. In Case 3 where Meek mesh was used, there was a significant difference between two areas of equal depth burn (full thickness). The healing time of the chest (with cells) was more rapid than that of the abdomen (no cells) with the abdomen having to be debrided and re-grafted after significant granulation tissue developed.

The literature does suggest that over-grafting with allograft in such expansion ratios is advised with Meek meshing but this still showed longer healing times than in our case [8,10,11,14,15]. There is also evidence that allograft can improve the take and outcome of grafted cultured epithelial sheets when used without meshed skin graft [21,22] but again healing times were longer than we have reported here.

In recent literature where overlay was not used in widely expanded Meek grafts, good results were achieved but the healing times were prolonged [13]. Reports on the use of cultured sheets and widely meshed Meek grafts have been reported with reasonable success and suggest that if used, allograft overlay is not required [11]. Healing times were again much slower than we experienced in our case in which sprayed cells were used. While direct comparison cannot be made between individual cases, as some of the methodologies differ; there is a suggestion that the addition of spraying cells as opposed to applying them as sheets may expedite healing in this setting.

The cell-therapy approach however does require a certain period of delay to allow time for the cell culture process, especially in cases where very large cell numbers are required to cover large burn areas. In such cases any patient who may require cultured autologous cells needs to be identified early and close liaison between the surgical team and the cell culture laboratory is essential to plan any procedures. Culture times will vary from laboratory to laboratory depending on the density of cells applied to the wound bed and the area to be treated. The high density of cells used on our patients, deposits cells onto the wound bed even when the supernatant runs off the surface, thus improving the potential for rapid wound closure.

Other laboratories are addressing the issue of time—where their aim is to deliver as many autologous epithelial cells as possible to the wound bed as rapidly as possible. CellSpray® products, provided by Clinical Cell Culture company (C3, Perth, Australia), use either cultured or non-cultured autologous keratinocytes. This technology is based on the possibility of harvesting subconfluent keratinocytes in their most active proliferating state followed by their application by spraying with further *in vivo* proliferation to confluency, and their differentiation to form an epithelium of typical structure [19,47]. Such an approach results in a reduced cell culture time and earlier wound coverage with viable activated keratinocytes [48]. Although this method of delivery to the wound bed at earlier stages post-wounding, such application is limited to partial-thickness and donor site wounds. Full-thickness wounds still require dermal pre-treatment to achieve functional permanent skin restoration [45]. The sprayed, non-cultured cells have been successfully applied to only relatively small wound areas but have the advantage that they can be prepared in the operating theatre from freshly harvested donor skin samples as required.

Another successful approach to earlier delivery of autologous epithelial cells (after 5–7 days in culture) to the wound has been as a sub-confluent layer of cells attached to a silicone support membrane with a specially formulated surface coating [49]. This product (MySkin™) is indicated for the treatment of neuropathic, pressure and diabetic foot ulcers, superficial burns and skin graft donor sites with reported positive clinical outcomes [50,51]. It can also be applied to full-thickness burns in combination with meshed skin grafts. The application of these sub-confluent keratinocytes following short-term culture allows a greater degree of flexibility in the coordination of cell propagation, harvesting and clinical application processes [43].

While the observational data we have reported here gives us hope for the future use and development of sprayed cultured autologous keratinocyte therapy, it is by no means conclusive. There have been several calls within the burns community for a higher level of research to be conducted to evaluate the efficacy of cultured epithelial cells in the treatment of burns [19,29,45]. A detailed retrospective audit of 84 patients over an 11-year period at one Centre demonstrates the great potential of this treatment [45]. A blind, prospective study of areas treated with a combination of meshed autograft plus cultured cells compared with matched areas treated with meshed autograft alone is underway at our Centre. It is hoped that the results of such a study will be able to answer the questions posed by the burns community as to the true efficacy of cultured cell therapy.

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REFERENCES

[1] Hendon DN, Barrow RE, Rutan RL, Rutan TC, Desai MH, Abston S. A Comparison of conservative versus early excision: therapies in severely burned patients. Ann Surg 1989;209:547–53.
[2] Burke JF, Bondoc CC, Quinby WC. Primary burn excision and immediate grafting: a method shortening illness. J Trauma 1974;14(5):389–95.
[3] Burke JF, Quinby Jr WC, Bondoc CC. Primary excision and prompt grafting as routine therapy for the treatment of thermal burns in children, 1976. Hand Clin 1990;6(2):305–17.
[4] Deitch EA, Wheelahan TM, Rose MP, Clothier J, Cotter J. Hypertrophic burn scars: analysis of variables. J Trauma 1983;23:895–8.
[5] Tanner JC, Vandeput J, Olley JF. The mesh skin graft. Plast Reconstr Surg 1964;34(3):287–92.
[6] Abdullah A, Blakely P, Hunt RBL, Philips L, Herndon DN, Robson MC. Visible scars and self-esteem in paediatric patients with bums. J Burn Care Rehabil 1994;15:164–8.
[7] Alexander JW, NacMillan BG, Law E, Kittur DS. Treatment of severe burns with widely meshed skin autograft and meshed skin allograft overlay. J Trauma 2008;21(6):433–7.
[8] Kreis RW, Mackie DP, Vloemans A-WFP, Hermans RP, Hoekstra MJ. Widely expanded postage stamp skin grafts

using a modified Meek technique in combination with an allograft overlay. Burns 1993;19(2):142–5.
[9] Peeters R, Hubens A. The mesh skin graft—true expansion rates. Burns 1988;14(3):239–40.
[10] Kreis RW, Mackie DP, Hermans RP, Vloemans AR. Expansion techniques for skin grafts: comparison between mesh and Meek island (sandwich-) grafts. Burns 1994;S39–42.
[11] Raff T, Hartmann B, Wagner H, Germann G. Experience with the modified Meek technique. Acta Chir Plast 1996;142(4):142–6.
[12] Meek CP. Successful Grafting using the Meek-Wall microdermatome. Am J Surg 1958;96(4):557–8.
[13] Hsieh C, Schuong JY, Huang WS, Huang TT. Five years experience of the modified Meek technique in the management of extensive burns. Burns 2008;34:350–4.
[14] Lari AR, Gang RK. Expansion technique for skin grafts (Meek technique) in the treatment of severely burned patients. Burns 2001;27(l):61–6.
[15] Zermani RGC, Zarabini A, Trivisonno A. Micrografting in the treatment of severely burned patients. Burns 1997;23(7/8):604–7.
[16] Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975;6(3):331–43.
[17] O'Connor NE, Mulliken JB, Banks-Schlegal S, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. Lancet 1981;1(8211):75–8.
[18] Dascome W, Britt LD, Jones GT, Futrell JW, Lind J. Cultured keratinocyte grafts: a review and clinical update. Curr Surg 1993;50:428–38.
[19] Chester DL, Balderson DS, Papini RP. A review of keratinocyte delivery to the wound bed. J Burn Care Rehabil 2004;25(3):266–75.
[20] Woodley DT, Briggaman RA, Herzog SR, Meyers AA, Peterson HD, O'Keefe EJ. Characterization of ''neo-dermis'' formation beneath cultured human epidermal autografts transplanted on muscle fascia. J Invest Dermatol 1990;95(1):20–6.
[21] Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. Lancet 1986;1(8490):1123–4.
[22] Cuono CB, Langdon R, Birchall N, Barttelbort S, McGuire J. Composite autologous-allogeneic skin replacement: development and clinical application. Plast Reconstr Surg 1987;80(4):626–37.
[23] Braye F, Oddou L, Bertin-Maghit M, Belgacem S, Damour O, Spitalier P, et al. Widely meshed autograft associated with cultured autologous epithelium for the treatment of major burns in children: report of 12 cases. Eur J Pediatr Surg 2000;10(1):35–40.
[24] Leigh IM, Navsaria H, Purkis PE, McKay I. Clinical practice and biological effects of keratinocyte grafting. Ann Acad Med Singapore 1991;20(4):549–55.
[25] Kangesu T, Navsaria HA, Manek S, Fryer PR, Leigh IM, Green CJ. Kerato-dermal grafts: the importance of dermis for the *in vivo* growth of cultured keratinocytes. Br J Plast Surg 1993;46(5):401–9.
[26] Phillips TJ, Bhawan J, Leigh IM, Baum HJ, Gilchrest BA. Cultured epidermal autografts and allografts: a study of differentiation and allograft survival. J Am Acad Dermatol 1990;23(2.1):189–98.
[27] Woodley DT, Peterson HD, Herzog SR, Stricklin GP, Burgeson RE, Briggaman RA, et al. Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstitution of anchoring fibrils. JAMA 1988;259(17):2566–71.
[28] Desai MH, Mlakar JM, McCauley RL. Lack of long-term durability of cultured keratinocyte burn-wound coverage: a case report. J Burn Care Rehabil 1991;12(6):540–5.
[29] Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autografts in the treatment of major burn injuries: a critical review of the literature. Burns 2006;32:395–401.
[30] Harris PA, Leigh IM, Navsaria HA. Pre-confluent keratinocyte grafting: the future for cultured skin replacements? Burns 1998;24(7):591–3.
[31] 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. A light, electron microscopic and immunohistochemical study. Lab Invest 1989;60(5):600–12.
[32] Carver N, Navsaria HA, Fryer P, Green CJ, Leigh IM. Restoration of basement membrane structure in pigs following keratinocyte autografting. Br J Plast Surg 1993;46(5):384–92.
[33] Putland M, Snelling CF, Macdonald I, Iron VA. Histologic comparison of cultured epithelial autograft and meshed expanded split-thickness skin graft. J Burn Care Rehabil 1995;16(6):627–40.
[34] Adams JC, Watt FM. Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes alpha 5 beta 1 integrin loss from the cell surface. Cell 1990;63(2):425–35.
[35] Hynes RO. Integrins: a family of cell surface receptors. Cell 1987;48(4):549–54.
[36] Marchisio PC, Bondanza S, Cremona O, Cancedda R, De LM. Polarized expression of integrin receptors (alpha 6 beta 4, alpha 2 beta 1, alpha 3 beta 1, and alpha v beta 5) and their relationship with the cytoskeleton and basement membrane matrix in cultured human keratinocytes. J Cell Biol 1991;112(4):761–73.
[37] Poumay Y, Roland IH, Leclercq-Smekens M, Leloup R. Basal detachment of the epidermis using dispase: tissue spatial organization and fate of integrin alpha 6 beta 4 and hemidesmosomes. J Invest Dermatol 1994;102(1):111–7.
[38] Siwy BK, Compton CC. Cultured epidermis: Indiana University Medical Center's clinical experience. J Burn Care Rehabil 1992;13:130–7.
[39] Lam PK, Chan ES, Liew CT, Yen RS, Lau HC, King WW. Dermal fibroblasts do not enhance the graft take rate of autologous, cultured keratinocyte suspension on full-thickness wounds in rats. Ann Plast Surg 2001;46(2):146–9.
[40] Leigh IM, McKay I, Carver N, Navsaria H, Green C. Skin equivalents and cultured skin: from petri dish to the patient. Wounds 1991;3:141–8.
[41] Hefton JM, Caldwell D, Biozes DG, Balin AK, Carter DM. Grafting of skin ulcers with cultured autologous epidermal cells. J Am Acad Dermatol 1986;(3):399–405.
[42] Pandya AN, Woodward B, Parkhouse N. The use of cultured autologous keratinocytes with integra in the resurfacing of acute burns. Plast Reconstr Surg 1998;102(3):825–8.
[43] MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007;445:874–80.
[44] Hartmann B, Ekkernkamp A, Johen C, Gerlach JC, Belfekruon C, Kuntscher MV. Sprayed cultured epithelial autografts for deep dermal burns of the face and neck. Ann Plast Surg 2007;58(l):70–3.
[45] Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: eleven years of clinical experience. Burns 2006;32:538–44.
[46] Reid MJ, Currie LJ, James SE, Sharpe JR. The effect of Integra®, cultured keratinocytes and split thickness skin graft on wound contraction. Wound Rep Regen 2007;15:889–96.
[47] Navarro FA, Stoner ML, Park CS, Huertas JC, Lee HB, Wood FM, et al. Sprayed keratinocyte suspensions accelerate

epidermal coverage in a porcine microwound model. J Burn Care Rehabil 2000;21:513–8.
[48] Atiyeh BS, Costagliola M. Cultured epithelial autograft (CEA) in burn treatment: three decades later. Burns 2007;33:405–13.
[49] Moustafa M, Simpson C, Glover M, Dawson RA, Tesfaye S, Creagh FM, et al. A new autologous keratinocyte dressing treatment for non-healing diabetic neuropathic foot ulcers. Diabet Med 2004;21:786–9.
[50] Zhu N, Warner RM, Simpson C, Glover M, Hernon CA, Kelly J, et al. Treatment of burns and chronic wounds using a new cell transfer dressing for delivery of autologous keratinocytes. Eur J Plast Surg 2005;28:319–30.
[51] Moustafa M, Bullock AJ, Creagh FM, Heller S, Jeffcoate W, Game F, et al. Randomized, controlled, single-blind study on use of autologous keratinocytes on a transfer dressing to treat nonhealing diabetic ulcers. Regen Med 2007;2:887–902.