Pixel Grafting: An Evolution of Mincing for Transplantation of Full-Thickness Wounds

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Background: Split-thickness skin grafting is the gold standard for treatment of major skin loss. This technique is limited by donor-site availability in large burn injuries. With micrografting, a technique where split-thickness skin graft is minced into 0.8 × 0.8-mm pieces, the authors have demonstrated an expansion ratio of 1:100 and healing comparable to that achieved with split-thickness skin grafting.
Methods: In this study, the authors explore the regenerative potential of a skin graft by cutting split-thickness skin grafts to pixel size (0.3 × 0.3 mm) grafts. Wound healing was studied in full-thickness wounds in a porcine model by creating an incubator-like microenvironment using polyurethane wound chambers. Multiple wound healing parameters were used to study the outcome of pixel grafting and compare it to micrografting and nontransplanted wounds.
Results: The authors' results show that 0.3 × 0.3-mm pixel grafts remain viable and contribute to skin regeneration. The pixel graft–transplanted wounds demonstrated a faster reepithelialization rate, decreased wound contraction, and increased mechanical stability compared with nontransplanted wounds. The reepithelialization rates of the wounds were significantly increased with pixel grafting at day 6 after wounding compared with micrografting. Among the other wound healing parameters, there were no significant differences between wounds transplanted with pixel grafts and micrografts.
Conclusions: Pixel grafting technique would address the most commonly encountered limitations of the split-thickness skin graft with the possibility of an even larger expansion ratio than micrografting. This technique is simple and fast and can be conducted in the operating room or in the clinic. (*Plast. Reconstr. Surg.* 137: 92e, 2016.)

Full-thickness skin loss from major trauma or burn will require surgical reconstruction unless the area is very small.[1–3] The most common method of reconstruction is split-thickness skin grafting.[4,5] Split-thickness skin grafting provides epidermal regeneration and minimizes wound contraction compared with healing in nontransplanted full-thickness wounds.[6,7] In a very large burn that is accompanied by a limited donor site for split-thickness skin graft harvest, the skin graft is meshed and expanded up to sixfold (possibly ninefold), but the donor sites may still not suffice.[8,9] However, increased expansion ratios result in a fishnet appearance of the healed skin.[10–12]

In vitro expansion of skin using cultured epithelial autografts is useful but has three major drawbacks.[13] First, the process of harvesting and expanding the skin takes at least 2 weeks.[13–16] Second, the cultured epithelium is very fragile and has no dermal component.[17–21] The fragile cultured epithelial autografts are extremely susceptible to mechanical shear, which results in poor graft take. The delay between harvest and graft application will subject the patient to significant potential for invasive infection and prolonged negative metabolic balance. Third, it is

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an extremely expensive technique, which further limits its use.[22]

To address the limitation of split-thickness skin grafting, Meek described a technique for mincing a split-thickness skin graft into small pieces, allowing 10-fold expansion.[8] Meek's method never gained widespread clinical application, in part because the skin graft pieces needed to be placed with the dermal side down to ensure survival.[23] Moreover, the device for mincing the skin grafts was expensive and the method was labor intensive.

We have described a simple technique for creating 0.8 × 0.8-mm skin micrografts from an autologous split-thickness skin graft using a handheld mincing device. The transplanted micrografts regenerated epidermis and dermis in full-thickness porcine wounds in healthy and diabetic pigs.[24] The wounds were treated in a wet environment using a polyurethane wound chamber that has been tested in previous experiments.[25,26] Similar results have been achieved using moist dressings and with negative-pressure therapy.[27,28] In the moist or wet environment, orientation of the micrografts is unimportant, and they will survive with the dermal side up or down.[24–27] Using this technique, a previous study demonstrated expansion of the skin graft of 100-fold, with complete healing in 14 days.[26]

In the present study, we hypothesized that smaller grafts would increase the regenerative potential of the graft by creating many more pieces of the same original skin graft. These grafts can be compared to the pixels on a computer screen, and we have chosen to call them "pixel grafts."

In this study, 0.3 × 0.3-mm pixel grafts were compared to 0.8 × 0.8-mm micrografts taken from the same size piece of skin and grafted to the same size full-thickness wounds. The objective of this study was to investigate the feasibility of pixel grafting in healing of full-thickness wounds.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the Harvard Medical Area Standing Committee on Animals. Four female Yorkshire pigs (Parsons Farm, Hadley, Mass.) weighing 50 to 60 kg were used for this study. Pigs were allowed to acclimatize for 72 hours before the experiments. Anesthesia was induced with intramuscular administration of 4.4 mg/kg tiletamine and zolazepam (Telazol; Fort Dodge Veterinaria, Fort Dodge, Iowa) and 2.5 mg/kg xylazine (Xyla-Ject; Phoenix Pharmaceutical, Inc., St. Joseph, Mo.) according to protocol. General anesthesia was maintained with 1% to 3% isoflurane (Novaplus; Hospira, Inc., Lake Forest, Ill.) and oxygen by means of endotracheal intubation. Oxygen saturation and heart rate were routinely measured intraoperatively with pulse oximeter ear sensors. In addition, respiratory rate and rectal temperature were monitored throughout the procedure. After the procedure, pigs were transferred back to the pen and monitored during recovery from anesthesia. A transdermal patch releasing 25 μg of fentanyl per hour for 72 hours (Duragesic; Janssen Pharmaceuticals, Inc., Titusville, N.J.) was given for pain management during surgical recovery, and buprenorphine 0.005 mg/kg was administered intramuscularly immediately after the end of the procedure.

Wound Creation

After marking 2.5 × 2.5-cm wounds in two parallel paraspinal stripes on the dorsum of the pig, the outlines were tattooed with black ink using an electric tattoo marker (Spaulding & Rogers Mfg., Inc., Voorheesville, N.Y.). Full-thickness wounds down to the panniculus carnosus were excised. Wounds were separated by at least 4 cm of unwounded skin.

Skin Collection

The skin was prepared thoroughly with successive applications of 10% povidone-iodine scrub (Betadine; Purdue Products LP, Stamford, Conn.) and 70% isopropanol (Aaron Industries, Clinton, S.C.). A split-thickness skin graft of 0.30-mm (0.012-inch) thickness was harvested from the buttock region with a pneumatic Zimmer dermatome (Zimmer, Inc., Warsaw, Ind.) and the donor site was covered with gauze dressings (Medline Industries, Inc., Mundelein, Ill.).

Mincing of Pixel Grafts and Micrografts from Split-Thickness Skin Grafts

Split-thickness skin grafts were washed twice in the Dulbecco's Modified Eagle Medium (Sigma-Aldrich, St. Louis, Mo.) before mincing and transplantation. The mincing device consists of 24 parallel rotating cutting disks 0.8 mm apart (Xpansion Micrografting System; Wright Medical Technology, Inc., Memphis, Tenn.) (Fig. 1). Using this device, the graft was cut twice, in perpendicular directions, and micrografts measuring 0.8 × 0.8 × 0.30 mm were obtained.

For pixel grafts, split-thickness skin grafts were minced 10 times; five times in each perpendicular

Fig. 1. The mincing device. The skin graft is cut twice, in perpendicular directions, and micrografts measuring 0.8 × 0.8 × 0.30 mm are obtained. For pixel grafts, split-thickness skin grafts are minced 10 times; five times in each perpendicular direction.

direction. Preparation of micrografts or pixel grafts took approximately 5 to 10 minutes once the skin was harvested. The size of the pixel grafts was confirmed under a microscope in preliminary studies (unpublished data). Using this technique, an average size of 0.3 × 0.3 × 0.3 mm was obtained.

Transplantation of Micrografts and Pixel Grafts

Because the aim of this study was to demonstrate the feasibility of pixel grafts, both pixel grafts and micrografts were transplanted and spread evenly over the wound bed without regard to the orientation, with a 1:2 expansion ratio. A polyurethane wound chamber (Design Standards Corp., Charlestown, N.H.) was applied to cover each wound. The micrografts and the pixel grafts were allowed to adhere for 30 minutes before 5 ml of keratinocyte serum-free medium (Thermo Fisher Scientific, Waltham, Mass.) (containing human keratinocyte growth supplement, penicillin, and streptomycin) was added to the wound chamber through an injectable port.

Experimental Groups

The full-thickness wounds created on the dorsum of the pig were divided into four groups. The experimental group location was randomized to minimize the effect of wound location on healing. The first group underwent transplantation with pixel grafts, and the second wound group underwent transplantation with micrografts. The remaining two wound groups served as control groups. The first control group, the wet control, was covered with the polyurethane wound chamber and injected with keratinocyte medium. The second control group, the dry control, was covered with gauze and tape.

Biopsy was performed on wounds with a 0.5-cm margin of surrounding unwounded tissue at 6, 10, and 28 days after wounding. Tissue samples were fixed in 4% neutral buffered formalin (Sigma-Aldrich), embedded in paraffin, and sectioned for staining with hematoxylin and eosin.

Wound Contraction

Wound surface was measured by calculating the area within the tattooed margins from macroscopic wound photographs using ImageJ software (National Institutes of Health, Bethesda, Md.).[29] The area of each wound was measured and expressed as a percentage of its original size on day 0.

Computerized Morphometric Wound Analysis

Hematoxylin and eosin–stained tissue sections were examined with light microscopy by three experienced, blinded observers. For morphometric analysis, slides were examined using an Eclipse E400 light microscope, and images were captured using a DS-Fi1 camera (both from Nikon Corp., Tokyo, Japan). Quantitative measurements were performed using NIS-Elements D3.0 digital image analysis software (Nikon). Reepithelialization was expressed as a percentage of reepithelialized wound surface area compared with the original wound surface area. Epidermal thickness was measured in five representative areas of neoepidermis for each wound cross-section. The number of rete formations per millimeter of neoepithelium was counted under the microscope from five standardized locations in each wound after 28 days of healing.

Statistical Analysis

Statistical comparisons were performed using GraphPad Prism 6.0 (Graph Pad Software, Inc.,

La Jolla, Calif.). All of the tracked parameters were analyzed using a two-way analysis of variance test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Histologic Evaluation

Hematoxylin and eosin–stained slides of pixel graft–transplanted wounds demonstrated that the pixel grafts were completely incorporated into the wound and migrated to the epithelial surface by day 6 after wound creation (Fig. 2, *above*). Higher magnification demonstrated that the epidermis of the pixel grafts was already incorporated into the neoepidermis by day 6 (Fig. 2, *below*). This was different from the micrografting technique, as previous studies have shown that the transplanted micrografts are approximately midway between the wound bed and the surface by day 6 and become incorporated at the wound surface by day 10 after wound creation.[23] With the pixel graft technique, reepithelialization was complete by day 10, and the wound had well-matured neoepidermis and neodermis by day 28.

Wound Reepithelialization

Both pixel graft– and micrograft-transplanted wounds had significantly increased reepithelialization compared with the wet and dry control wounds at day 6 after wounding (pixel grafts, 77 percent; micrografts, 55 percent; wet control, 12 percent; dry control, 5 percent; $p < 0.01$) (Fig. 3, *left*) and at day 10 (pixel grafts, 100 percent; micrografts, 100 percent; wet control, 62 percent; dry control, 55 percent; $p < 0.01$) (Fig. 3, *right*). Wounds transplanted with pixel grafts demonstrated a significant increase in reepithelialization at day 6 compared with micrograft-transplanted wounds ($p < 0.05$). By day 10, both the pixel graft– and micrograft-transplanted wounds were completely reepithelialized. Wounds in all groups including both control groups had completely reepithelialized by day 28.

Wound Contraction

Wounds transplanted with pixel grafts or micrografts had decreased contraction compared with the nontransplanted control wounds. Twenty-eight days after wound creation, both the pixel graft– and micrograft-transplanted wounds had significantly higher percentages of original wound surface area compared with the nontransplanted wounds (pixel grafts, 62 percent; micrografts, 60 percent; wet control, 51 percent; dry control, 48 percent; $p < 0.05$) (Fig. 4). Thus, the wound contraction in the micrograft- and the pixel graft–transplanted groups was significantly less than in the nontransplanted groups.

Epidermal Morphology

Epidermal maturation was assessed after 28 days of wound healing. Neoepidermis thickness was significantly increased in both the pixel

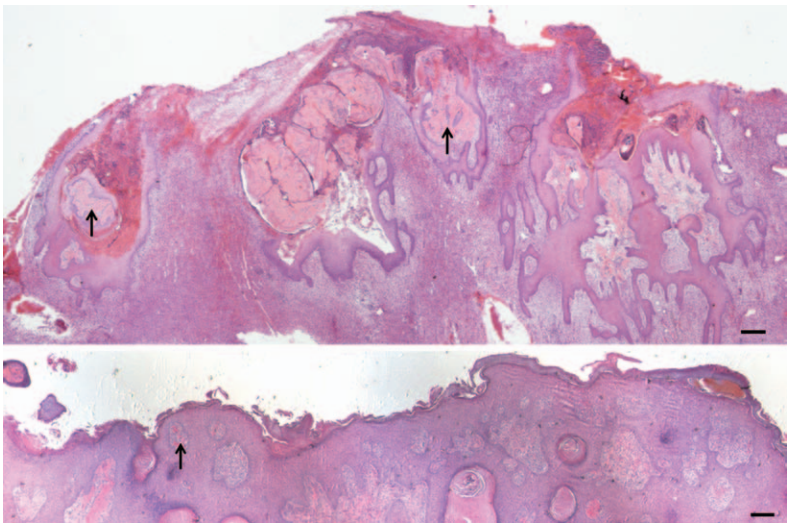

Fig. 2. Histologic images of pixel graft–transplanted wounds. (*Above*) Migration of the pixel grafts (*arrow*) to the wound surface and contribution to wound healing by day 6 (*above*) and day 10 (*below*). Wound edges are marked with the *arrow* in all slides. *Scale bar* = 300 μm.

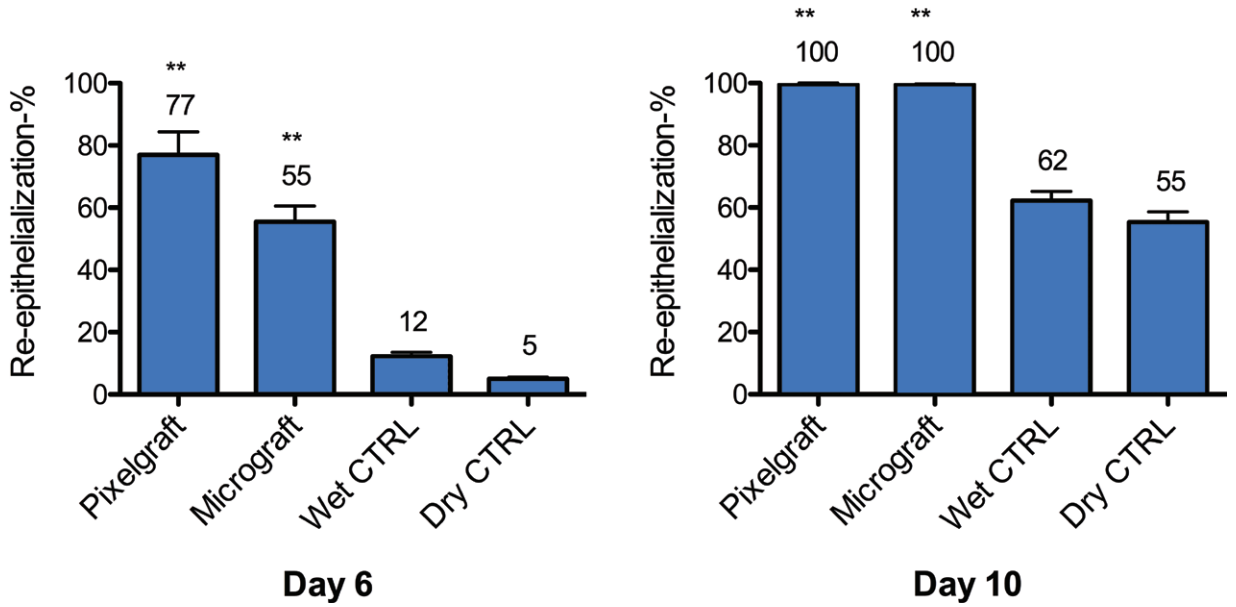

Fig. 3. Comparison of the reepithelialization of the full-thickness wounds in pixel grafts, micrografts, wet control, and dry control groups at 6 days (*left*) and 10 days (*right*) after wound creation (n = at least 8 for each group). **$p < 0.01$.

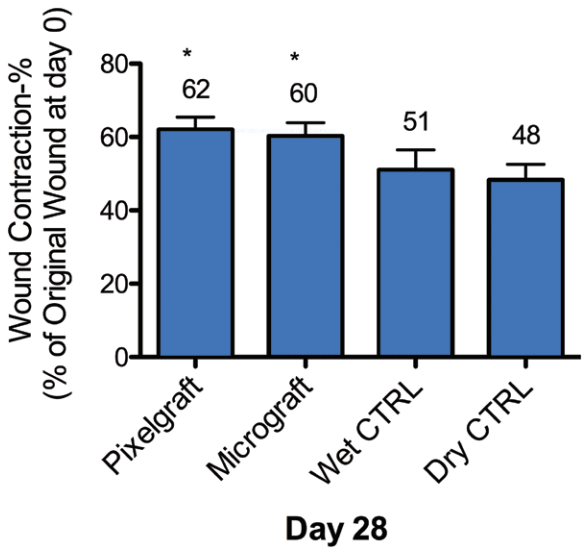

Fig. 4. Comparison of wound contraction of the full-thickness wounds in pixel graft, micrograft, wet control, and dry control groups at 28 days after wound creation (n = at least 8 for each group). *CTRL*, control. *$p < 0.05$.

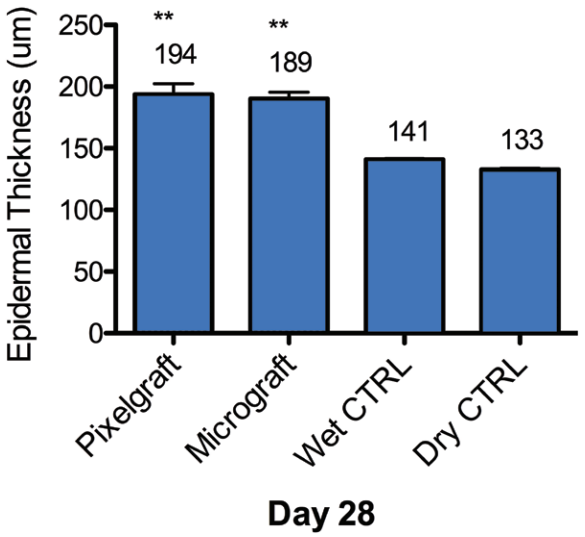

Fig. 5. Comparison of neoepidermis thickness of the healed full-thickness wounds in pixel graft, micrograft, wet control, and dry control groups 28 days after wound creation (n = at least 8 for each group). *CTRL*, control. **$p < 0.01$.

graft– and micrograft-transplanted wounds compared with the control wounds when measured 28 days after wound creation (pixel grafts,186 μm; micrografts, 188 μm; wet control, 140 μm; dry control, 130 μm; $p < 0.01$) (Fig. 5).

Rete Ridges per Millimeter

The number of rete ridges per linear millimeter has often been used as an indicator of the strength of the dermal-epidermal junction.[30] Both the pixel graft– and micrograft-transplanted wounds had a significantly increased number of rete ridges per millimeter compared with control wounds (pixel grafts, 9.8; micrografts, 9.0; wet control, 5.8; dry control, 5.5) (Fig. 6).

DISCUSSION

A 90 percent total body surface area burn illustrates the problem facing the patient and the

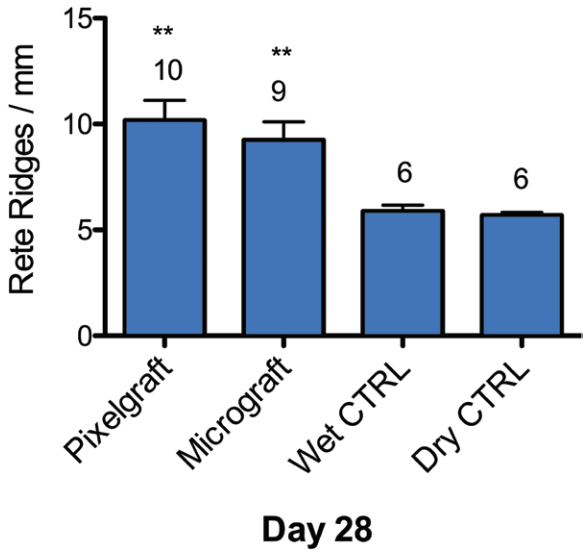

Fig. 6. Comparison of number of rete ridges per linear millimeter in the healed full-thickness wounds in pixel graft, micrograft, wet control, and dry control groups 28 days after wound creation (n = at least 8 for each group). *CTRL*, control. **$p < 0.01$.

burn surgeon. Only 10 percent of the total body surface area is available as skin graft donor sites, and sometimes this area includes face, hands, and feet, which are not suitable donor sites. Between the choices of immediate expansion of the skin graft 100-fold and the expansion by in vitro culture over 2 weeks or more, both micrografting and pixel grafting present as better alternatives to in vitro culture.

The surface area of an 0.8 × 0.8-mm micrograft is 0.64 mm^2. A 0.3 × 0.3-mm pixel graft has a surface area of 0.09 mm^2. This means that from the same piece of skin graft, one can produce seven times more pixel grafts than micrografts. If for instance a skin graft is expanded 100-fold with either methodology, the micrografts, in the optimal geometry, will be placed 5.5 mm from each other and the pixel grafts will be placed 2.3 mm from each other. This means that each pixel graft will need to regenerate skin over less than half that distance of the micrograft. It is quite possible or even likely that grafts smaller than pixel grafts will create an even greater regenerative advantage. However, there must be a point where the trauma from cutting a very small graft outweighs the benefit of the larger number of grafts. Svensjö et al. compared the regenerative potential of micrografts to that of nonexpanded and expanded suspensions of single cells.[31] They found that the micrografts had greater regenerative potential than the noncultured cells and almost the same regenerative potential as the cultured cells. In their experiments, the micrografts and the uncultured or cultured cells had each regenerated from skin grafts of the same size.

Mincing technique has been described for articular cartilage to create a larger surface area for cartilage expansion and has also been used for reimplantation of the parathyroids.[32,33] In previous studies, we have shown that transplantation of micrografts in a 1:100 expansion ratio results in complete epithelialization of both healthy and diabetic full-thickness wounds within 14 days after wounding.[25] In another study published by Kiwanuka et al., transplantation of micrografts in a full-thickness porcine wound model was found to improve wound healing parameters such as macroscopic scar appearance, wound contraction, neoepidermis maturation, and rete ridge formation compared with nontransplanted wounds and was comparable to treatment with split-thickness skin grafting.[34] The micrografting technique with a moist dressing was used to treat a large burn wound of an Iraqi civilian. This was especially impressive given the extremely high mortality rates of patients with greater than 50 percent total body surface area full-thickness injury receiving treatment outside of a nonspecialized burn center.[28]

The goal of this study was to explore the feasibility of pixel grafts in full-thickness wound healing. We therefore used a 1:2 expansion ratio for our study instead of a larger expansion. To make an objective comparison, the transplanted micrografts in the study also had the same expansion ratio. Both the pixel grafts and the micrografts were taken from the same size piece of skin and grafted to the same size full-thickness wounds.

The most interesting finding of our study was the significantly increased reepithelialization rate with pixel grafts compared with micrografts. The increased number of grafts with pixel grafting technique results in an increased number of islands of regeneration, which facilitates faster reepithelialization. The observation that almost all pixel grafts were at the wound surface by day 6 after wound creation can also contribute to faster reepithelialization. In contrast, the majority of the micrografts were only midway between the wound bed and the surface by day 6. Unsurprisingly, the reepithelialization rates were similar for both pixel grafts and micrografts by day 10 once all of the grafts were at the surface. Finally, because both micrografts and pixel grafts would survive by diffusion rather than by neovascularization, the probability and duration of survival of pixel grafts is higher than micrografts because of decreased diffusion distance for nutrients.

At day 28, the transplanted group had significantly decreased contraction compared with the control groups. Although contraction has some role in the transplanted wounds, the healing is primarily through reepithelialization from the grafts.

The epidermal thickness is a marker for skin integrity and was measured in all of the wounds after 28 days of wound healing.[35,36] The epidermal thickness was significantly increased in the transplanted wounds compared with the control wounds, indicating superior skin integrity in the transplanted wounds.

The strength of the dermal-epidermal junction is related to the number of rete ridges. An increased number of rete ridges suggests improved strength because of enhanced epidermal-dermal surface area. In our study, both the pixel graft– and micrograft-transplanted groups had a significantly increased number of rete ridges per linear millimeter than the control wounds. The higher number of rete ridges suggests that the regenerated skin possesses increased mechanical stability because of greater surface area for anchoring protein attachments.[37]

Cultured epithelial autografts have provided permanent epithelial healing for a large number of burn patients. Particularly in burns larger than 60 percent total body surface area, this is a very useful technique, and many patients have survived burns they would not have been able to survive without the cultured epithelial autografts.[13–16] However, a preparation time of over 2 weeks and the lack of a dermal component make it very susceptible to trauma, and blisters frequently form.[17–21] Another disadvantage is that there have been a number of reports of the development of squamous cell cancer in the areas grafted with cultured epithelial autografts.[38,39]

Currently, a number of laboratories are attempting to transdifferentiate stem cells, especially adipose-derived stem cells, into skin epithelial cells.[40] Many of these experiments show promising results but have yet to produce epidermis in vivo.[40]

The present study clearly shows that the pixel grafts have a superior regenerative potential compared with the micrografts. As with micrografts, it would be possible to use pixel grafts at a 100-fold expansion ratio, and it might offer a more practical alternative to in vitro cell cultures in the treatment of large burns. It also appears that the surface appearance of the scar is better, although this has to be confirmed in larger wounds.

CONCLUSIONS

Pixel grafting technique provides a promising advancement in the field of wound healing. Qualitative and quantitative measurements from the full-thickness porcine wound model demonstrate that transplantation of pixel grafts improves wound healing parameters compared with nontransplanted wounds and has a significantly increased reepithelialization rate compared with micrografting technique. This would help in addressing the most commonly encountered limitations of split-thickness skin grafting with the possibility of a very large expansion ratio. Our study establishes the premise of pixel grafting technique and demonstrates its efficacy in full-thickness wound healing.

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