Comparison of Healing Parameters in Porcine Full-Thickness Wounds Transplanted with Skin Micrografts, Split-Thickness Skin Grafts, and Cultured Keratinocytes

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BACKGROUND: Transplantation of skin micrografts (MGs), split-thickness skin grafts (STSGs), or cultured autologous keratinocytes (CKs) enhances the healing of large full-thickness wounds. This study compares these methods in a porcine wound model, investigating the utility of micrograft transplantation in skin restoration.

STUDY DESIGN: Full-thickness wounds were created on Yorkshire pigs and assigned to one of the following treatment groups: MGs, STSGs, CKs, wet nontransplanted, or dry nontransplanted. Dry wounds were covered with gauze and the other groups' wounds were enclosed in a polyurethane chamber containing saline. Biopsies were collected 6, 12, and 18 days after wounding. Quantitative and qualitative wound healing parameters including macroscopic scar appearance, wound contraction, neoepidermal maturation, rete ridge formation, granulation tissue thickness and width, and scar tissue formation were studied.

RESULTS: Transplanted wounds scored lower on the Vancouver Scar Scale compared with nontransplanted wounds, indicating a better healing outcome. All transplanted wounds exhibited significantly lower contraction compared with nontransplanted wounds. Wounds transplanted with either MGs, STSGs, or CKs showed a significant increase in re-epithelialization compared with nontransplanted wounds. Wounds transplanted with MGs or STSGs exhibited improved epidermal healing compared with nongrafted wounds. Furthermore, transplantation with STSGs or MGs led to less scar tissue formation compared with the nontransplanted wounds. No significant impact on scar formation was observed after transplantation of CKs.

CONCLUSIONS: Qualitative and quantitative measurements collected from full-thickness porcine wounds show that transplantation of MGs improve wound healing parameters and is comparable to treatment with STSGs. (J Am Coll Surg 2011;213:728–735. © 2011 by the American College of Surgeons)

Full-thickness wounds caused by major burns or trauma expose the body to physical, mechanical, thermal, and microbial assaults.[1-3] In addition, extensive loss of skin leads to increased water loss, impaired thermoregulation, and decreased local immune defense. Rapid wound closure is a major factor in successful recovery and is usually achieved by debridement and skin grafting.[4-6]

In an attempt to accelerate wound healing, cultured epithelial autografts (CEAs) can be transplanted to full-thickness wounds.[7-9] The method enables a large expansion ratio but suffers from a number of drawbacks.[10-12] The CEA is fragile, devoid of a dermal component with basement membrane, and susceptible to mechanical shear.[13,14] Furthermore, the required time and cost of CEA preparation limit their indications, and the delayed wound coverage exposes the patient to risk of infection and sepsis.

The most widely used skin graft in the treatment of full-thickness wounds is split-thickness skin grafts (STSGs). Transplantation with STSGs diminishes excessive extracellular matrix deposition and wound contraction compared with nontrans-

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Abbreviations and Acronyms

CEA	= cultured epithelial autograft
CK	= cultured autologous keratinocyte
MG	= micrograft
STSG	= split-thickness skin graft

planted full-thickness wounds.[15,16] However, due to the restricted availability of donor sites and limited expansion ratios obtained with current surgical practice, early wound coverage cannot always be achieved.

In order to increase the expansion ratio of STSGs, Meek,[17] in 1958, introduced a method of mechanically dividing skin into small pieces, allowing up to 10-fold skin expansion. Using this technique, the skin pieces need to be placed with the dermal side down in order to survive and proliferate.[18] This makes the technique cumbersome and time consuming, resulting in limited use of the methodology among surgeons.[19] In an attempt to overcome these hurdles, our laboratory has previously shown that autologous minced skin grafting accelerates re-epithelialization of fluid-treated skin wounds.[20] The controlled mincing of skin can generate smaller micrografts of uniform size and shape (Fig. 1). Our data show that the orientation of skin micrografts (MGs) is irrelevant (dermis up or down) when placed in a wet or moist environment. Furthermore, MGs transplanted in a 1:100 ratio have been shown to proliferate and provide new epithelium to wounds independent of graft orientation (unpublished data). The technique enables early wound coverage of large full-thickness wounds and provides an alternative to current treatment options.

Several studies have compared healing outcomes after transplantation with STSGs and CEAs. We have previously shown that transplantation with MGs accelerates re-epithelialization and delays contraction.[20] In this study, we used qualitative and quantitative parameters to study healing of full-thickness wounds after transplantation with MGs, STSGs, or cultured keratinocytes (CKs). To better assess the quality of healing after transplantation with autologous skin grafts to full-thickness wounds, multiple morphometric healing parameters were studied. The macroscopic appearance of a healed wound can be assessed using scar assessment scales. The Vancouver Scar Scale, developed by Sullivan in 1990, is a widely used scale that scores pigmentation (0 to 2 points), vascularity (0 to 3 points), pliability (0 to 5 points), and scar height (0 to 3 points), with a total possible score between 0 points (unwounded skin) and 13 points (hypertrophic scar) (Table 1).[21] This study sought to test the hypothesis that MGs improve the healing of full-thickness wounds using a porcine model. We hypothesized that transplantation with MGs can improve healing parameters as well as currently available techniques.

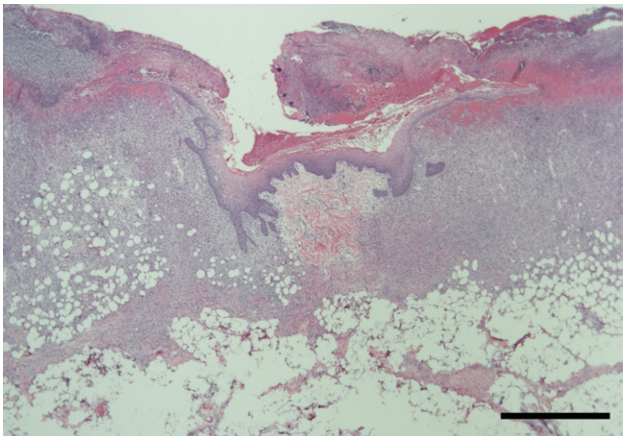

Figure 1. Morphology of skin micrografts. Hematoxylin-eosin staining of a micrograft (MG) 6 days after transplantation to a full-thickness porcine wound. The MGs consist of both epidermis and dermis, separated by an intact basement membrane. Scale bar equals 1 mm.

METHODS

Animals

Four female Yorkshire pigs (Parson's Farm) weighing approximately 50 kg at arrival were allowed to acclimatize for 1 week before the start of any experiments. All animal procedures were approved by the Harvard Medical Area Standing Committee on Animals and conformed with regulations related to animal use and other federal statutes.

Skin graft collection

Anesthesia was induced with intramuscular administration of 4.4 mg/kg tiletamine and zolazepam (Telazol; Fort Dodge Veterinaria) and 2.5 mg/kg xylazine (Xyla-Ject; Phoenix). General anesthesia was maintained with 1% to 3% isoflurane (Novaplus, Hospira) and oxygen via endotracheal intubation. Oxygen saturation and heart rate were measured with pulse oximeter ear sensors, and respiratory rate and rectal temperature were monitored throughout the procedure.

The skin was thoroughly disinfected with successive applications of 10% povidone-iodine scrub (Betatide; Purdue Products LP) and 70% isopropanol (Aaron Industries). An STSG of 0.35 mm (0.014 inch) was harvested from the dorsal neck region with a pneumatic Zimmer dermatome (Zimmer Inc) and the donor site was dressed with petrolatum gauze (Medline Industries). After the procedure, pigs were transferred back to the pen and monitored during recovery from anesthesia. A transdermal patch releasing 25 μg fentanyl per hour for 72 hours (Duralgesic, Janssen) was given for pain management during surgical recovery.

Table 1. Macroscopic Wound Healing Was Quantified Using the Vancouver Scar Scale

Parameter	Score
Pigmentation	
Normal	0
Hypopigmentation	1
Hyperpigmentation	2
Vascularity	
Normal	0
Pink	1
Red	2
Purple	3
Pliability	
Normal	0
Supple	1
Yielding	2
Firm	3
Ropes	4
Contracture	5
Height	
Flat	0
<2 mm	1
2–5 mm	2
> 5 mm	3

(Modified from: Sullivan T, Smith J, Kermode J, et al. Rating the burn scar. J Burn Care Rehabil 1990;11:256–260, with permission.)

Porcine cell culture

Porcine keratinocytes were isolated from split-thickness skin as previously described.[22] Briefly, skin samples were treated with 2.5 U/mL dispase overnight (GIBCO, Invitrogen) and the epidermis was mechanically separated from the underlying dermis. Separated epidermal sheets were then treated with 0.025% trypsin and 0.01% ethylenediaminetetra-acetic acid (GIBCO). After centrifugation for 7 minutes at 180*g* (Beckman CRP centrifuge) the supernatant was removed and the pellet was resuspended in keratinocyte medium. The keratinocytes were plated on collagen-1 coated cultured dishes (Becton Dickinson,). Keratinocytes were grown in EpiLife medium (GIBCO) supplemented with bovine insulin (5 μg/mL), hydrocortisone (0.5 μg/mL) human recombinant epidermal growth factor (0.1 μg/mL), 0.4% bovine pituitary extract, 65 μM calcium chloride (GIBCO), and 8% sterile filtrated fetal bovine serum (Hyclone). Subconfluent cells were washed with phosphate buffered saline and detached by treatment with 0.025% trypsin and 0.01% ethylenediaminetetra-acetic acid (GIBCO). Cells from passages 1 to 3 were used for the experiments.

Wound model and surgical procedure

To minimize the effect of wound location on healing outcomes, experimental groups were placed in a randomized fashion. The wounds were spaced a minimum of 4 cm apart, in 4 columns parallel to the dorsal midline. Thirty squares measuring 1.5 × 1.5 cm were outlined and the wound margins were traced using a tattoo gun and black ink (Special Electronic Tattoo Marker, Spaulding Enterprises). Full-thickness excisional wounds were created down to the fascial plane along the tattooed lines. An STSG was harvested from the dorsal neck region as described above.

Experimental groups

Wounds were assigned to 1 of 5 treatment groups (MGs, STSGs, CKs, wet nontransplanted, dry nontransplanted) with 6 wounds per group per pig. Using a handheld mincing device consisting of 24 parallel rotating cutting discs 0.8 mm apart, the STSG was minced with 2 perpendicular cuts, creating skin MGs measuring 0.8 mm × 0.8 mm. The MGs were then evenly applied in a 1:10 ratio to the wound bed. No attention was paid to the orientation of the MGs (dermal side up or down). In the second treatment group, wounds were transplanted with an STSG sutured in place with 4 5−0 Vicryl sutures (Ethicon Inc) in the corners of the graft and wound. In the third treatment group, autologous CKs were transplanted to the wound at a density of 220,000 cells per wound.

Wounds transplanted with MGs, STSGs, or CKs, as well as saline-treated nontransplanted controls (wet nontransplanted), were enclosed in flexible polyurethane chambers (Corium International). Dry dressing–treated nontransplanted wounds (dry nontransplanted) were covered with sterile gauze.

Wounds were biopsied with a 0.5-cm margin of surrounding unwounded tissue at 6, 12, and 18 days after wounding. Tissue samples were fixed in 4% neutral buffered formalin (Sigma-Aldrich), embedded in paraffin, and sectioned for staining with either hematoxylin-eosin or Masson's trichrome.

Gross wound examination

Before being biopsied, wounds were photographed on days 0, 6, 12, and 18 to assess autograft take, wound contraction, granulation tissue formation, infection, and scaring. On day 18 wounds were assessed for height, pliability, vascularity, and pigmentation using the Vancouver Scar Scale (Table 1).

Wound contraction

Wound contraction was determined by digitalized planimetry of the tattooed margins. The area of each wound was measured using Scion Image Software (Scion Corp) and expressed as a percentage of its original size on day 0.

Table 2. Transplantation of Skin Micrografts, Split-Thickness Skin Grafts, or Cultured Keratinocytes Leads to Better Macroscopic Healing Outcomes Compared with Nontransplanted Wounds

	Vancouver Scar Scale				
Treatment	Pigmentation	Vascularity	Pliability	Height	Total score
MG	0 ± 0	0.75 ± 0.5	0.75 ± 0.5	0 ± 0	1.5 ± 0.6
STSG	0 ± 0	1.5 ± 0.6	1.5 ± 0.6	1.0 ± 0.8	4.0 ± 0.8
CK	0 ± 0	1.5 ± 0.6	2.3 ± 0.5	1.3 ± 0.5	5.0 ± 0.8
Wet	0 ± 0	2.0 ± 0	4.5 ± 0.6	2.0 ± 0	8.6 ± 0.6
Dry	0 ± 0	2.3 ± 0.5	5.0 ± 0	2.8 ± 0.5	10.0 ± 0.8
Unwounded skin	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Grafted wounds had a better score on the Vancouver Scare Scale compared with nongrafted wounds.
A low score indicates less scar formation. Data are means ± SD for 8 samples.
CK, cultured keratinocyte; Dry, dry nontransplanted; MG, micrograft; STSG, split-thickness skin graft; Wet, wet nontransplanted.

Computerized morphometric wound analysis

Hematoxylin-eosin stained tissue sections were observed by light microscopy by 4 experienced, blinded observers. For morphometric analysis, slides were examined using an Eclipse E400 light microscope, and images captured using a DS-Fi1 camera (Nikon Corporation). Quantitative measurements were performed using NIS-Elements D3.0 digital image analysis software (Nikon Corporation).

Re-epithelialization was defined as the sum of the new epithelium divided by the original wound length indicated by the tattoo. Epidermal thickness was measured in 5 representative areas of neoepidermis for each wound cross-section. The number of rete formations per millimeter of neoepithelium was counted under the microscope from 5 standardized locations in each wound after 18 days of healing. A previously described 4-class numeric system was used to assess neoepidermal maturity:[23] Class I, little or no epithelial layer; Class II, 1 to 3 cell layers deep covered with little or no stratification; Class III, normal basal layer and stratification of 3 to 7 cell layers; and Class IV, basal layer, hyperproliferation, downward projections in neodermis.

Slides stained with Masson's trichrome were observed under a light microscope by 4 experienced and blinded observers. Granulation tissue thickness was measured from the surface to the wound bed on day 6. On day 18 after wounding, scar tissue width was measured and expressed in micrometers.

Statistical analysis

All statistical calculations were performed using GraphPad Prism (GraphPad).

Data are presented as mean ± SD. For statistical comparisons at a set time point, a nonparametric Kruskal-Wallis test with a Dunn's post-test was used. All parameters tracked over time were analyzed using a 2-way ANOVA. A p value < 0.05 was considered statistically significant.

RESULTS

Macroscopic wound healing assessment

All transplanted wounds scored better on the Vancouver Scar Scale compared with nontransplanted wounds after 18 days of healing (MGs, 1.5 ± 0.6; STSGs, 4 ± 0.8; CKs, 5 ± 0.8; wet nontransplanted, 8.6 ± 0.6; dry nontransplanted, 10 ± 0.8; unwounded skin, 0 ± 0) (Table 2).

Wound contraction

All transplanted wounds exhibited significantly lower contraction compared with nontransplanted wounds (Fig. 2). On day 6 after wounding, contraction of wet nontransplanted and dry nontransplanted was significantly higher

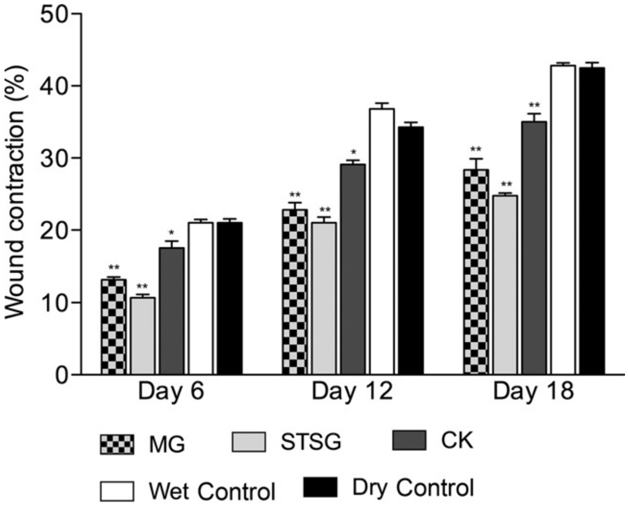

Figure 2. Transplantation with skin micrografts or split-thickness skin grafts reduced wound contraction. All transplanted wounds exhibited significantly lower wound contraction compared with dry nontransplanted wounds. Values are means ± SD for 8 samples. * indicates $p < 0.05$, ** indicates $p < 0.01$. CK, cultured keratinocyte; Dry, dry nontransplanted; MG, micrograft; STSG, split-thickness skin graft; Wet, wet nontransplanted.

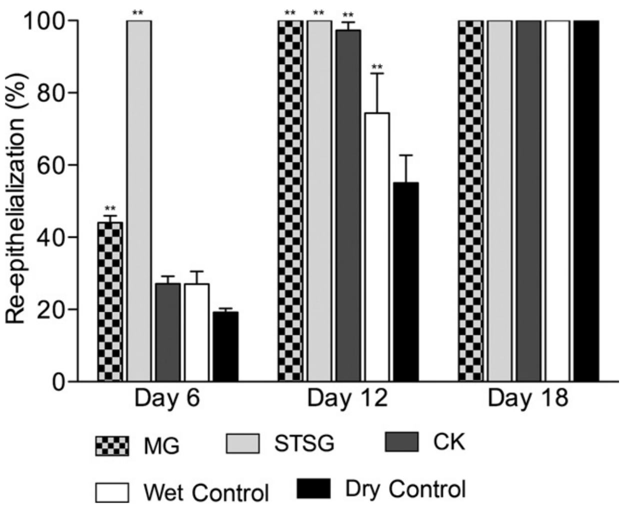

Figure 3. Transplantation with skin micrografts, split-thickness skin grafts, or cultured keratinocytes increased the rate of re-epithelialization. Re-epithelialization expressed as present on resurfaced full-thickness porcine wounds after 6, 12, and 18 days of healing. Wounds transplanted with STSGs were fully covered at all time points. MG transplantation enhanced re-epithelialization compared with wounds transplanted with CKs or untransplanted wounds. All wounds were fully re-epithelialized 18 days after wounding. Values are means ± SD for 8 samples. ** $p < 0.01$. CK, cultured keratinocyte; Dry, dry nontransplanted; MG, micrograft; STSG, split-thickness skin graft; Wet, wet nontransplanted.

(21.0% ± 0.9% and 21.0% ± 1.0%, respectively, $p < 0.0001$) than in wounds transplanted with MGs (13.2% ± 0.8%, $p < 0.01$), STSGs (10.7% ± 1.0%, $p < 0.01$), or CKs (17.5% ± 1.0%, $p < 0.05$).

Wound re-epithelialization

Wounds transplanted with STSGs were fully covered at all time points. Wounds transplanted with either MGs or CKs showed a significant increase in re-epithelialization compared with nontransplanted wounds (MGs, 44%, CKs, 27%, wet nontransplanted, 27%, dry nontransplanted, 19% after 6 days, and MGs, 100%; CKs, 97%; wet nontransplanted, 74%; dry nontransplanted, 55 % after 12 days, $p < 0.01$). All wounds were fully re-epithelialized on day 18 after wounding (Fig. 3).

Epidermal morphology

Epidermal maturation was assessed after 18 days of healing. Neoepidermis was thicker in all transplanted groups compared with nontransplanted groups, but only wounds transplanted with MGs reached a statistically significant increase in epidermal thickness, with a p value < 0.05 (Fig. 4A). Wounds transplanted with MGs or STSGs exhibited significantly greater rete ridge formation compared with nontransplanted wounds ($p < 0.05$); CKs did not reach statistical significance compared with nontransplanted wounds (Fig. 4B).

To detect morphologic differences in the healed epidermis, a 4-tier semiquantitative grading system was used.[23] Nontransplanted wounds showed a lower grade of epidermal maturity compared with transplanted wounds. After 18 days of healing, wet nontransplanted and dry nontransplanted wounds were covered with a basal layer of epithelium with little stratification (Class II). Wounds transplanted with CKs displayed epidermal stratification (Class

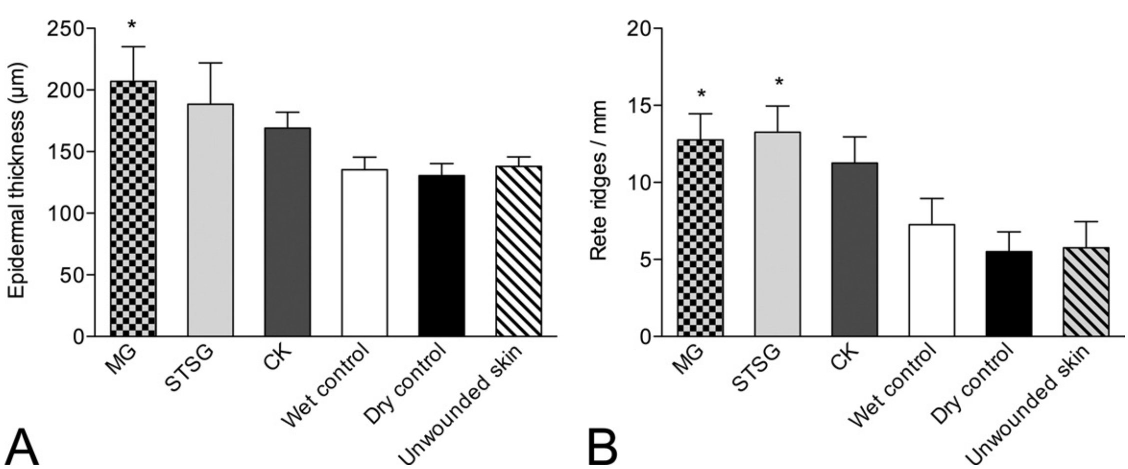

Figure 4. Quantitative morphometric analysis of epidermal maturation. Epidermal thickness and rete ridge formation was measured in 5 representative areas of neoepidermis after 18 days of healing. (A) Epidermal thickness was increased in wounds transplanted with MGs. * $p < 0.05$. (B) The number of rete ridges of each wound was counted and expressed as the number of rete ridges per millimeter of cross-section of neoepidermis. Wounds transplanted with MGs or STSGs exhibited significantly greater rete ridge formation. Values are means ± SD for 8 samples, * $p < 0.05$. CK, cultured keratinocyte; Dry, dry nontransplanted; MG, micrograft; STSG, split-thickness skin graft; Wet, wet nontransplanted.

III); transplantation with STSGs or MGs resulted in an epithelial coverage with an increased degree of stratification (Class IV).

Granulation tissue thickness and scar width

Granulation tissue thickness was measured in the central areas of the wound beds (Fig. 5A). Granulation tissue thickness was significantly increased ($p < 0.05$) in wounds transplanted with MGs (148 ± 9.4 μm) or STSGs (144 ± 10.8 μm) compared with nontransplanted wounds (wet nontransplanted, 97.7 ± 6.4 μm; dry nontransplanted, 99 ± 9.7 μm). In comparison, wounds transplanted with CKs did not show a statistically significant increase (103 ± 11.3 μm). MGs, STSGs, and CKs exhibited a decreased scar tissue width ($p < 0.05$) compared with nontransplanted wounds (Fig. 5B).

DISCUSSION

Transplantation with MGs to full-thickness wounds has been shown to accelerate re-epithelialization and delay contraction, and we have previously shown that MGs can be transplanted in a 1:100 ratio to full-thickness wounds, with complete re-epithelialization in 14 days.[20] In this study, we investigated the effect of MG, STSG, or CK transplantation on several scar and wound healing parameters including macroscopic scar appearance, wound contraction, neoepidermal maturation, rete ridge formation, granulation tissue thickness and width, and scar tissue formation. We showed that transplantation of MGs to full-thickness porcine wounds treated in a wet environment improves the healing outcome compared with nontransplanted wounds. Additionally, transplantation with MGs yields results comparable to treatment using STSGs. Both MGs and STSGs demonstrated enhanced healing outcomes compared with wounds transplanted with CKs.

Increased rate of re-epithelialization is one of the indicators of enhanced wound repair. Although the rate of epithelialization is an essential feature of the healed wound, additional parameters need to be taken into account to better evaluate the outcome of wound healing. This study specifically evaluated both the epithelial and dermal healing parameters after transplantation with MGs, STSGs, or CKs. As expected, grafted wounds had a better Vancouver Scar Scale score than nongrafted wounds (Table 2). The difference in macroscopic appearance between transplanted and nontransplanted wounds was significant despite the fact that Yorkshire pigs typically heal without hypertrophic scar formation. Even though the wounds did not form raised hypertrophic scars in this experimental design, there was clearly visible cutaneous scar tissue (Table 2). Due to the many similarities, porcine skin has been used as a model for human wound healing studies and skin grafting procedures.[24] In both porcine and human skin, the relative thickness of the epidermis and dermis is similar and the porcine wound healing model closely approximates the normal process of healing in humans.[25,26]

In this study, epidermal thickness and rete ridge formation were measured 18 days after wounding. Increased epidermal thickness was observed in wounds transplanted with MGs as compared with other treatment groups. Wounds transplanted with MGs or STSGs exhibited significantly greater rete ridge formation, which reflects an

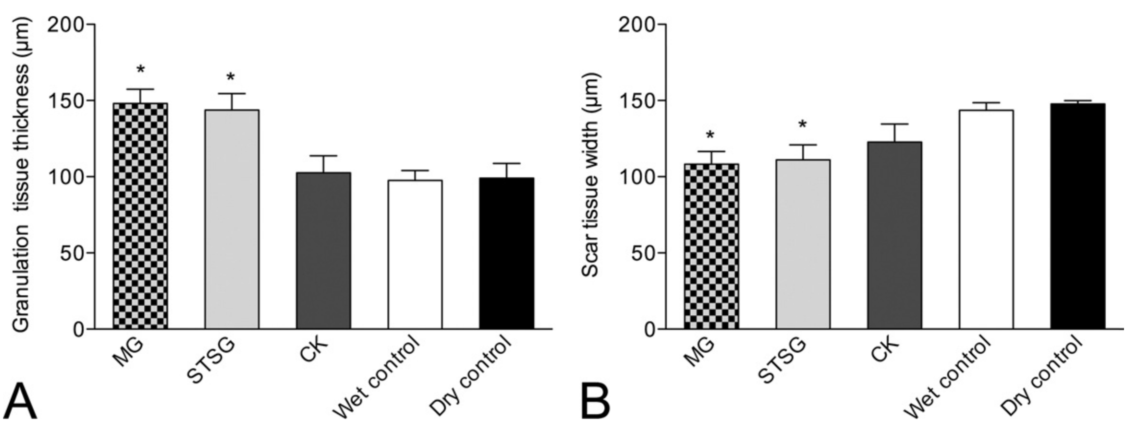

Figure 5. The effect on granulation tissue thickness and scar width after transplantation with skin micrografts, split-thickness skin grafts, or cultured keratinocytes. (A) Wounds transplanted with MGs or STSGs had an increased granulation tissue thickness compared with untransplanted wounds. Transplantation with CKs did not affect the granulation thickness. (B) Scar tissue width was measured 18 days after wounding. All transplanted wounds exhibited a significantly decreased scar tissue width compared with nontransplanted wounds. Values are means ± SD for 8 samples, * $p < 0.05$. CK, cultured keratinocyte; Dry, dry nontransplanted; MG, micrograft; STSG, split-thickness skin graft; Wet, wet nontransplanted.

improved basement membrane function due to greater epidermal-dermal surface area. In addition, morphologic factors such as the degree of epidermal stratification and differentiation support the notion that transplantation with MGs improves epidermal restoration. Taken together, these findings indicate improved epidermal healing after transplantation of MGs to full-thickness wounds, and these findings correlate with our earlier findings that minced skin grafting accelerates re-epithelialization of skin wounds treated in a wet environment.

In the early phase of wound healing, the wound bed consists of a loose granulation tissue sparsely populated with fibroblasts and inflammatory cells. As wound healing progresses, the loose granulation tissue forms a more mature neodermis, containing higher amounts of collagen type I. Granulation tissue thickness is a reliable assessment tool that portrays dermal events during wound healing. Transplantation with MGs or STSGs significantly increases granulation thickness; wounds transplanted with CKs did not show a statistically significant improvement. In this study, all transplanted wounds exhibited a decreased scar tissue formation compared with nontransplanted wounds.

There are several plausible reasons why transplantation with MGs or STSGs improves wound healing parameters to a greater extent than CKs. It has been demonstrated that keratinocytes in co-cultures with fibroblasts downregulate the synthesis of profibrotic factors and extracellular matrix components and upregulate the synthesis of matrix-degrading enzymes.[27-30] Upon transplantation to a full-thickness wound, MGs and STSGs contain both epidermis and dermis separated by an intact basement membrane. In MGs, the keratinocytes and fibroblasts are surrounded by a supportive microenvironment, increasing the regenerative capacity of the cells when transplanted (Fig. 1). One can speculate that the presence of these supporting structures facilitates keratinocyte migration and promotes wound healing.

CONCLUSIONS

Qualitative and quantitative measurements collected from the full-thickness porcine wound model show that transplantation of MGs improves wound healing parameters comparable to treatment with STSGs. In contrast, wounds transplanted with CKs exhibited a less favorable healing outcome. The MG methodology is a promising new technique for treatment of large full-thickness wounds. In addition, MGs can be useful in the study of keratinocyte-fibroblast-matrix interactions.

Author Contributions

Study conception and design: Kiwanuka, Hackl, Philip, Caterson, Junker, Eriksson
Acquisition of data: Kiwanuka, Hackl, Philip
Analysis and interpretation of data: Kiwanuka, Hackl, Philip, Caterson, Junker, Eriksson
Drafting of manuscript: Kiwanuka, Hackl, Philip, Junker
Critical revision: Kiwanuka, Caterson, Junker, Eriksson

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