ABSTRACT

Rapid and effective closure of full-thickness burn wounds remains a limiting factor for survival after burns involving most of the total body surface area (TBSA). Hypothetically, engineered skin substitutes (ESS) consisting of autologous cultured keratinocytes and fibroblasts attached to collagen-based sponges may reduce requirements for donor skin, numbers of grafting procedures, and time of intensive care during hospitalization. To demonstrate feasibility for this approach, ESS were prepared from split-thickness skin biopsies collected after enrollment of 2 burn patients by Informed Consent into a study protocol approved by the local Institutional Review Board. Patient A was a 10 year-old male who sustained 94% TBSA burns, and patient B was a 2 year-old female who sustained 90% TBSA burns. The injuries were all full-thickness, and occurred in separate building fires in 2007. ESS and split-thickness skin autograft (AG) were applied in a matched-pair design with each patient serving as their own control. Data collection consisted of photographs, area measurements of donor skin and healed wounds after grafting. Data are expressed below as: A) % area closed at post-operative day (POD) 14, B) %TBSA closed at POD 28, and C) ratio of closed to donor areas at POD 28. Patient A received 12 applications of ESS over 4 months, and patient B received 6 applications of ESS over 3 months. Average % area closed (dry epithelium) at POD 14 was 72.4% for ESS and 96.9% for AG. Frequency of partial regrafting was higher for ESS than for AG. Average %TBSA closed at POD 28 was 51.4% for ESS, and 40.6% for AG. The average ratio of closed wound area to donor skin area at POD 28 was 125.5 for ESS, compared to 4.0 for AG. ESS which was healed at POD 28 did not blister or ulcerate subsequently. Patients wore pressure garments over all treated areas. Pigmentation of areas treated with ESS was deficient, but pliability of healed skin was acceptable. These results demonstrate that ESS reduce requirements for donor skin harvesting for grafting of excised, full-thickness burns involving most of the TBSA. Availability of ESS for treatment of extensive, deep burns may reduce time to wound closure, morbidity and mortality in this patient population.

1. INTRODUCTION

Rapid and effective closure of full-thickness burn wounds remains a limiting factor for survival after burns involving most of the total body surface area (TBSA). Hypothetically, engineered skin substitutes (ESS) consisting of autologous cultured keratinocytes and fibroblasts attached to collagen-based sponges may reduce requirements for donor skin, numbers of grafting procedures, and time of intensive care during hospitalization. Preclinical studies have shown that ESS (previously referred to as cultured skin substitutes, CSS) form partial epidermal barrier and basement membrane in vitro (Boyce et al. 2002b), and express angiogenic factors, including but not limited to Vascular Endothelial Growth Factor, basic Fibroblast Growth Factor and Transforming Growth Factor β-1 (Supp et al. 2000; LePoole and Boyce 1999). After grafting to full-thickness wounds in athymic mice, ESS containing epidermal melanocytes restore skin pigmentation (Swope et al. 2006), or containing microvascular endothelial cells form human vascular analogs (Supp et al. 2002). Previous clinical studies with ESS have demonstrated a reduction in requirements for harvesting of donor skin autograft in burns greater than 50% TBSA (Boyce et al. 2002a; Boyce et al. 2006), grafting of excised giant congenital melanocytic nevus (Passaretti et al. 2004), and chronic wounds (Boyce et al. 1995a). Although several alternatives for treatment of extensive, deep burns have been reported (MacNeil 2007; Supp and Boyce 2005), closure of very large TBSA burns remains challenging during acute hospitalization, and can result in long-term morbidity from scars. In this study, autologous ESS were compared with split-thickness, meshed skin autograft treatment of two pediatric patients with burns of 90% TBSA or greater, and evaluated quantitatively for formation of scar, and qualitatively for engraftment at post-operative day (POD) 14, for ratio of closed wound to donor skin areas at POD 28, and for % TBSA closed with ESS or AG.
2. METHODS

To demonstrate feasibility for this approach, ESS were prepared from split-thickness skin biopsies collected after enrollment of 2 burn patients by Informed Consent into a study protocol approved by the local Institutional Review Board. Patient A was a 10 year-old male who sustained 94% TBSA burns, and patient B was a 2 year-old female who sustained 90% TBSA burns. The injuries were all full-thickness, and occurred in separate building fires in 2007. ESS were prepared from autologous keratinocytes and fibroblasts which were isolated from split-thickness skin, cultured, and cryopreserved for later use (Figure 1). Cells were combined with collagen-based sponges, and incubated at the air-liquid interface to promote formation of epidermal barrier (Figure 2). ESS and split-thickness skin autograft (AG) were applied in a matched-pair design with each patient serving as their own control (Figure 3). The first application of ESS was compared to AG for all end points, and subsequent applications of ESS were added to the first and quantify device efficacy. Data collection consisted of photographs, area measurements of donor skin and healed wounds at post operative days (POD) 14 and 28 after grafting, and healed tissue biopsies as available. Data are expressed below as mean values for these two subjects for: A) % area closed at post-operative day (POD) 14, B) %TBSA closed at POD 28, and C) ratio of closed to donor areas at POD 28. Due to the small sample size, no statistical analyses were performed.

Prior to treatment with ESS, wounds were excised, and grafted with either meshed, allograft skin or Integra Dermal Regeneration Template. Two-stage grafting was performed in which the allograft or silicone layer of Integra was removed, and wounds were treated overnight at two-hour intervals with alternating irrigations of 5% Sulfamylon solution and double antibiotic solution (200 U/mL polymyxin B and 40 μg/mL neomycin) (Warden et al. 1982). The following morning, the dressings were removed in the operating room, hemostasis was obtained with electrocautery and compression. Autograft skin was harvested at a thickness of 0.010-0.012 inches thickness, and meshed and expanded 1:2. ESS were applied with a dressing of N-Terface, and AG was applied directly to the prepared wounds. Grafts were stapled to the wounds, dressed with fine-meshed gauze and bulky gauze with perforated red rubber catheters and secured either with a Spandex stent or with elastic wrap bandages. Sites were irrigated for five days with a formulation of non-cytotoxic antimicrobial agents (Boyce et al. 2006) at a dosage of 1mL/cm2 three times per day. Dressings were changed on POD 2. On POD 5, wet dressings were discontinued, and all dressings and staples were removed. Open areas of ESS were dressed with a topical ointment consisting of equal parts Neosporin, Bactroban and Nystatin on Adaptic. Open areas of AG were dressed with a topical cream consisting of equal parts Silver sulfadiazine, Bacitracin and Nystatin on Adaptic. Keratinized areas of ESS were treated with moisturizing cream (i.e., Eucerin) beginning at POD 11, and moisturizing lotion (i.e., Curel) beginning at POD 15.

3. RESULTS

Patient A received 12 applications of ESS over 4 months, and patient B received 7 applications of ESS over 3 months. Average % engraftment (dry epithelium) at POD 14 was 72.4% for ESS and 96.9% for AG (Figure 4A). Partial regrafting was performed in 8 of 12 ESS sites (66%) for Patient A, and 4 of 7 ESS sites (57%) for Patient B. The average ratio of closed wound area to donor skin area at POD 28 was 125.5 for ESS, compared to 4.0 for AG (Figure 4B). Average %TBSA closed at POD 28 was 51.4% for ESS, and 40.6% for AG (Figure 4C). Physical therapy was resumed beginning at POD 7, and ESS which was healed at POD 28 did not blister or ulcerate subsequently. Patients wore pressure garments over all treated areas. Pigmentation of areas treated with ESS was deficient, but pliability of healed skin was acceptable. Figure 5 shows image of Patient A at the time of hospital discharge. 187 days after the first treatment with autologous engineered skin.

CONCLUSIONS

These results demonstrate that ESS reduce requirements for donor skin harvesting for grafting of excised, full-thickness burns involving most of the TBSA. Survival of these two patients after treatment with ESS is consistent with previous findings that autologous engineered skin is associated with reduced harvesting of donor skin autograft (Boyce et al. 2006), and decreased mortality in matched patient populations (Armour et al. 2007). Availability of ESS for treatment of extensive, deep burns may reduce time to wound closure, morbidity and mortality in this patient population.

ACKNOWLEDGEMENTS

This study was supported by Shriners Hospitals for Children. The authors acknowledge the technical skills and expertise of Christopher Lloyd, Elizabeth Maier, Rachel Zimmerman, Jill Pruszka, John Besse, and Deanna Leslie for delivery of this investigative device to assist the burn care team with the recovery of these patients.
Figure 1. Diagram of the process for fabrication of engineered skin substitutes. A biopsy of split-thickness skin is harvested from an uninjured site, epidermal keratinocytes and dermal fibroblasts are isolated, the cells are cultured to very large populations, harvested as cell suspensions, inoculated onto collagen-based sponges, incubated in contact with air to stimulated epidermal keratinization, and grafted to excised, full-thickness wounds. The entire process requires approximately 4 weeks.

Figure 2. Microscopic and macroscopic anatomies of engineered skin substitutes (ESS). A) Populations of dermal fibroblasts cover the surface of the biopolymer, and are also distributed into its interior to form the dermal substitute (d). Epidermal keratinocytes (e) attach to the fibroblasts, form partial basement membrane, stratify, and form partial epidermal barrier before grafting. The engineered skin is avascular and has a total thickness of approximately 0.3 mm. Scale bar = 0.1 mm. B) Macroscopic anatomy of ESS shows a uniform construct approximately 30cm² which can be handled readily by a surgeon. Scale in cm.
Figure 3. Surgical application of engineered skin substitutes (ESS). Left panel) ESS were applied as meshed, non-expanded sheets, stapled to wounds and irrigated for 5 days with non-cytotoxic antimicrobial agents (Boyce et al. 1995b). Right panel) Grafting of the anterior torso of Patient A with ESS, and split-thickness skin autograft (AG). Integra Dermal Regeneration Template (Integra) was grafted previously. Scale in cm.

Figure 4. Quantitative comparisons of engineered skin substitutes (ESS) and split-thickness skin autograft (AG). A) Engraftment at post-operative day (POD14) 14 was 72.4% for ESS, and 96.9% for AG. B) The ratios of closed wounds to biopsy area were 125.5 for ESS and 4.0 for AG. This value represents a reduction of donor skin requirements by treatment with ESS. C) Mean total body surface area (TBSA) closed with ESS was 51.4%, and with AG was 40.6%.
Figure 5. Patient A, healed ESS and AG at POD 187. Top panels) Torso and arms. Bottom panels) Legs. Wounds close rapidly because of epidermal keratinization \textit{in vitro}, and do not blister because of basement membrane formation. Pigmentation of most areas treated with ESS was deficient, but pliability of healed skin was acceptable. The patient wore pressure garments over all treated areas. Application as non-expanded sheets reduces granulation tissue and scar to generate a relatively smooth surface. Scale in cm.
REFERENCES


