Successful Full-Thickness Skin Regeneration Using Epidermal Stem Cells in Traumatic and Complex Wounds: Initial Experience

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Abstract
Skin grafts generated from cultured autologous epidermal stem cells may have potential advantages when compared to traditional skin grafting. In this report, we will share our initial experience with a new technique for the treatment of difficult cutaneous wounds. Eight patients with traumatic or complex wounds underwent full-thickness skin harvesting and processing of epidermal stem cells, followed by the application of our novel management protocol. The patients were at high risk for non-healing and/or severe scar formation due to large traumatic de-gloving crush injuries, wounds from necrotizing fasciitis, or chronic wounds from osteomyelitis. We examined the percent graft success, recipient to donor size ratios, the median time to epithelialization, and two-point sensory discrimination. An international scale (The Patient and Observer Scar Assessment Scale - POSAS) was used to evaluate wound cosmesis and included parameters such as pain, pruritus, vascularity, pigmentation, and thickness of the healing wound. In total, 10 out of 11 wounds had 100% survival of the graft, and one patient had an 80% graft take. The largest wound was 1600 cm² and all wounds were harvested from small-donor sites, which were closed primarily. The mean wound to donor ratio was >25:1. Most wounds were fully epithelialized within 30 days. Neurologically, four out of six patients studied exhibited two-point discrimination similar to the adjacent native uninjured skin. The majority of patients reported their wounds to have limited pain or pruritus, and similar pigmentation to adjacent skin.

Introduction
Major advancements in skin grafting did not occur until the 19th century with the advent of general anesthesia. The limitations of the commonly utilized split-thickness skin grafts are well described: wounds often develop severe scarring, hyperpigmentation, and/or hyperalgesia 

Stem cells have been shown to proliferate, differentiate, and survive when cultured, and may revolutionize the treatment of disease 

Skin grafts generated from cultured autologous epidermal stem cells may have potential advantages when compared to traditional skin grafting. In this report, we will share our initial experience with a new technique for the treatment of difficult cutaneous wounds. Eight patients with traumatic or complex wounds underwent full-thickness skin harvesting and processing of epidermal stem cells, followed by the application of our novel management protocol. The patients were at high risk for non-healing and/or severe scar formation due to large traumatic de-gloving crush injuries, wounds from necrotizing fasciitis, or chronic wounds from osteomyelitis. We examined the percent graft success, recipient to donor size ratios, the median time to epithelialization, and two-point sensory discrimination. An international scale (The Patient and Observer Scar Assessment Scale - POSAS) was used to evaluate wound cosmesis and included parameters such as pain, pruritus, vascularity, pigmentation, and thickness of the healing wound. In total, 10 out of 11 wounds had 100% survival of the graft, and one patient had an 80% graft take. The largest wound was 1600 cm² and all wounds were harvested from small-donor sites, which were closed primarily. The mean wound to donor ratio was >25:1. Most wounds were fully epithelialized within 30 days. Neurologically, four out of six patients studied exhibited two-point discrimination similar to the adjacent native uninjured skin. The majority of patients reported their wounds to have limited pain or pruritus, and similar pigmentation to adjacent skin.

Case Presentation
Eight patients presented to our institution with difficult to heal wounds. These included large traumatic wounds, debrided wounds from severe necrotizing fasciitis, and chronic wounds, which had failed prior skin grafting. Following our own novel protocol, a full-thickness segment of skin for harvest was removed from the thigh, groin, or abdomen. Care was taken to avoid cautery during graft harvest, so cellular morphology was not altered. The specimen was excised and placed in a sterile cup that contained a gentamicin antibiotic. The size of the graft was typically a small fraction of the size of the wound. The specimen donor site was then irrigated, and hemostasis was obtained prior to being closed primarily. The donor sites are all listed as one dimensional because they were closed primarily.

After processing of the donor specimen at a bioengineering laboratory (Polarity TE, Salt Lake City, USA), the graft material was returned as a tissue paste in a sterile package. The recipient wound bed, at this point, had...
a clean bed of healthy granulation tissue. During the deployment, the graft material was spread on the wound bed as a paste similar in consistency to peanut butter. The wound was then covered by a thin sheet of silicone that was meshed in a 1.5:1 fashion using a Skin Graft Mesher (Zimmer® Surgical, Dover, USA) and placed directly over the graft material. The silicone sheet was secured by staples to the wound edge and then covered by a fibrin glue (Evicel™, Somerville, USA). Lastly, a white Granulofoam™ dressing (KCI, San Antonio, USA) was applied with an Ioban™ drape (3M™, St. Paul, USA) to secure the dressing. Negative pressure therapy was initiated and set to -75 mmHg. This dressing was then removed and reapplied every 5-7 days for three weeks. The dressing was then changed to a regular non-adherent material in between the wound and the overlying gauze bandage. Successful graft maturation was noted by the presence of dermal islands (see Figure 1). These were portions of healthy granulation that subsequently epithelialized and formed functional and glandular tissue (see Figure 2). The final graft resembled healthy skin by general appearance, pigmentation, and sensation. Additional images are provided in appendices.
The tissue was evaluated postoperatively in several ways. The Patient and Observer Scar Assessment Scale (POSAS) was used to evaluate the cosmesis and functionality of the wound. This assessment scale has been shown to have inter-observer reliability and is widely used amongst dermatologists and plastic surgeons worldwide [7]. It measures scar quality metrics, including pigmentation, pliability, thickness, and similarity to adjacent skin architecture. It is also unique in that it combines the patient’s viewpoint of the wound with the observer’s assessment. In addition to the POSAS evaluation, we assessed the grafts for two-point discrimination and time to epithelialization.

The average patient age was 46 years (see Tables 1-2). All patients were at high risk for non-healing and severe scar formation. Nearly all of the wounds were acute and treated during their initial hospitalization. In total, 10 out of 11 patients had 100% graft survival. The single patient who had only an 80% graft survival had been extensively and repeated debrided for Fournier’s gangrene of the perineum (Table 1, patient 7), and likely failed 100% grafting because of a flare-up of pyoderma gangrenosum. The largest wound was over 1600 cm$^2$ and all wounds were harvested from a donor site, which was closed primarily. The wound to donor ratio exceeded 25:1 on most patients. The average donor site scar was seven centimeters in length. All donor...
site scars were healed without pain or deformity. The average time to epithelialization of the graft recipient wound beds was 30 days. Neurologically, more than 50% of the patients exhibited two-point discrimination, which was similar to the adjacent native skin. Overall, patients reported the grafted wounds to be low in pain, pruritus and had similar pigmentation to adjacent skin.

| Patient | Type/location                  | Wound/donor ratio | Two-point discrimination | Overall scar score | Percent take |
|---------|--------------------------------|-------------------|--------------------------|--------------------|--------------|
| 1       | Fournier's gangrene            | 28:1              | Yes                      | 100%               | 100%         |
| 2       | LUE traumatic degloving        | 37:1              | Yes                      | 70%                | 100%         |
| 3       | RUE traumatic degloving        | 13:1              | Yes                      | 80%                | 100%         |
| 4       | LLE traumatic degloving        | 75:1              | Yes                      | 50%                | 100%         |
| 5       | Chronic osteomyelitis          | 10:1              | No                       | 100%               | 100%         |
| 6       | Sacral ulcer and B/L heel      | 30:1              | No                       | 100%               | 100%         |
| 7       | Fournier's gangrene            | 18:1              | Pending                  | Pending            | 80%          |
| 8       | RUE traumatic degloving        | 22:1              | Pending                  |                     |              |

**TABLE 1: Wound characteristics**

LUE - left upper extremity; RUE - right upper extremity; LLE - left lower extremity; B/L - bilateral
TABLE 2: Demographics

| Patient | Type/location | Time to epithelization | Size of the wound | Harvest site | Size of donor site | Risk factors for poor wound healing |
|---------|----------------|------------------------|-------------------|-------------|-------------------|-------------------------------------|
| 1: 48-year-old female | Necrotizing fasciitis - bilateral groin and perineum | 3 weeks | Left groin: 20x8cm; right groin: 4x3cm | Right lateral thigh | 6cm | DM |
| 2: 56-year-old female | Traumatic degloving - left upper extremity | 4 weeks | 20x15cm | Left lateral thigh | 8cm | none |
| 3: 37-year-old male | Traumatic degloving - right upper extremity | 8 weeks | 8x5cm, 15x5cm | Right lateral thigh | 8cm | none |
| 4: 48-year-old female | Traumatic degloving - left lower extremity x2 | 4 weeks | 50x20cm (thigh); 30x20cm (calf) | Right medial thigh, right groin | 12cm (right thigh), 9cm (right groin) | none |
| 5: 37-year-old male | Chronic osteomyelitis - right lower extremity | 4 weeks | 7x6cm | Lower abdomen | 4cm | PVD, DM, RSD |
| 6: 52-year-old male | Sacral ulcer and bilateral heel | 4 weeks | Bilateral heel: 8x4cm; sacrum: 10x16cm | Lower abdomen | 7cm | DM |
| 7: 65-year-old male | Fournier’s gangrene - perineum | 8 weeks | 6x12cm | Lower abdomen | 4cm | PVD, DM, pyoderma |
| 8: 27-year-old male | Right upper extremity degloving | 4 weeks | 8x20cm | Right groin | 7cm | none |

DM - diabetes mellitus; PVD - peripheral vascular disease; RSD - reflex sympathetic dystrophy

Discussion

The main findings of our study were: 1. epidermal stem cell skin grafts had a nearly 100% survival despite their use in highly complicated wounds; 2. the cosmetic appearance and functionality (including sensation) of the graft site was similar to that of adjacent native skin.

Split thickness skin grafts are commonly employed for coverage of various types of granulating wound beds, but have a number of limitations. These grafts are absent of normal sensation, and also lack the durability, appearance (i.e., pigmentation), and functionality of native skin [1, 3]. This is an inherent limitation of wound biology. Re-epithelization is limited by competing and opposing forces of fibroblasts during the proliferative phase of wound healing and is most apparent in large or deep wounds. This process promotes scar formation and secondary wound contracture without the functionality of normal skin [8]. Published literature has shown that high-risk patients with compromised wound biology have a high rate of failure (nearly 30%) with even the most advanced skin substitutes [6, 9]. On a cellular level, both chronic and complex wounds have dysregulated growth factors, cellular activity, and the persistence of a poorly differentiated state of keratinocytes [8].

An increased understanding of the nature of stem cells, molecular signaling, and tissue engineering in the past two decades has made the clinical application possible (see Figure 3) [5]. Anatomically, the reservoir for epithelial stem cells lies in the outer root sheath in the proximal bulge of a hair follicle. Cell lineage studies performed by Ito and colleagues in 2004 showed that these pluripotent bulge cells and their progeny have the ability to proliferate and undergo terminal differentiation to form the native full-thickness architecture. This includes the hair shaft, melanin-producing cells, and glandular cells [10]. The same investigator was able to show, in animal studies, that these isolated bulge cells rapidly divide and migrate in the setting of the acute wound-healing response [11]. Radio-labeled leucine-rich repeat-containing G-protein coupled receptor, a transmembrane protein marker for stem cells, has been extensively studied and
found throughout epithelial surfaces, specifically in the follicular bulge. In animal studies, these isolated cells were shown to have the ability to proliferate, migrate, and differentiate into native hair follicles [12].

A recent pediatric case report using the same skin construct from our investigation supported this cellular phenomenon. In this case, a biopsy of a child who had received the stem cell-derived graft five months prior demonstrated a normal distribution of melanocytes and structurally normal hair follicles. On further cell staining, this biopsy demonstrated a fully developed stratified epidermis with organized peripheral vasculature [2]. The ability for these pluripotent stem cells to differentiate has been studied extensively and is the result of precise signals from their microenvironment. This gives these stem cells the capacity to recognize, regenerate, and replace damaged or dying cells in their vicinity [5].

Our case series included only very complex wounds in a complicated patient population (Table 2). We focused on high risk, sometimes previously grossly infected, large wounds that traditional split-thickness skin grafting would have likely failed, and in two of our patients had failed. We feel that our excellent graft survival, along with the cosmesis and functionality of the grafts, would not have been possible without this epidermal stem cell-derived product. With regards to safety, the graft is autologous, so there does not appear to be a risk of immunogenic reactions.

There are a number of limitations to our investigation. The subjectivity of the scar assessment and time to epithelialization were apparent. The wounds were examined on follow up appointments that were spread apart by at least a week after discharge, limiting our analysis to periodic observations. The percent graft survival was somewhat subjective as well because it reflects a visual estimation of how well the graft took throughout the wound. This was not a major limitation because almost all the wounds had 100% graft survival. Our study is retrospective and limited by the size of the study population and, as a result, warrants further study. Additionally, there is no long-term data because our patients have only recently received grafting; however, this is the first report of its kind, evaluating a series of patients with complex wounds receiving successful grafting from epidermal stem cells. Future studies would include looking at tissue specimens and performing a skin biopsy of the recipient wounds beds and comparing them to adjacent native skin.

Conclusions
We describe the index case series utilizing a novel tissue based, autologous, homologous epidermal stem cell derived product in complex surgical wounds. These wounds on average were very large and likely not amenable to traditional skin grafting. Despite requiring only a small full thickness donor skin specimen, more than 90% of the patients had a 100% graft survival covering large and complicated wound surfaces, and many experienced normal sensation at the healed graft site. Furthermore, our clinicians felt that cosmesis was far superior to the results seen with split thickness skin grafts. This is the first case series in the literature describing the use of epidermal stem cells.

Appendices
FIGURE 4: Traumatic degloving injury referenced in Figure 1
FIGURE 5: Large wound after multiple debridements from necrotizing fasciitis

FIGURE 6: Previous patient several months after deployment of stem
FIGURE 7: Traumatic degloving of right upper extremity (shown after multiple debridements)

FIGURE 8: Previous patient shown weeks after deployment of stem cell skin construct

**Additional Information**

**Disclosures**

**Human subjects:** Consent was obtained by all participants in this study. Hackensack University Medical Center Institutional Review Board issued approval PRO2019-0622. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.
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