A PROSPECTIVE, MULTICENTER, PILOT TRIAL OF A NOVEL HOMOLOGOUS SKIN CONSTRUCT ON DEEP PARTIAL-THICKNESS AND FULL-THICKNESS BURNS

UNE ÉTUDE PILOTE PROSPECTIVE ET MULTICENTRIQUE SUR L'UTILISATION D'UN NOUVEL HYBRIDE CUTANÉ HOMOLOGUE SUR LES BRÛLURES PROFONDES

Feldman M.J.,[1]✉ McLawhorn M.M.,[2] Han J.,[1] Marcu A.C.,[1] Burkey S.E.,[2] Travis T.E.,[2,3,4] Johnson L.S.[2,3,4]

[1]Division of Plastic and Reconstructive Surgery, Virginia Commonwealth University, Richmond, Virginia, USA
[2]Firefighters' Burn and Surgical Research Laboratory, MedStar Health Research Institute, Washington DC, USA
[3]The Burn Center, MedStar Washington Hospital Center, Washington, DC, USA
[4]Department of Surgery, Georgetown School of Medicine, Washington DC, USA

SUMMARY. Split-thickness skin grafting (STSG) is the standard of care for treating deep burns. They often contract, have unpredictable cosmetic outcomes, lack dermal appendages, and result in painful, conspicuous donor sites. An autologous homologous skin construct (AHSC) has been shown to produce full-thickness skin architecture. This study examined the safety profile, engraftment, and quality of healing of a pilot group of AHSC-treated burn wounds. Following IRB approval and informed consent, patients with deep-partial/full-thickness burns requiring grafting underwent side-by-side treatment with AHSC and STSG. A 2 cm^2 full-thickness harvest was processed into AHSC at an FDA-registered facility, returned within 48 hours, and applied to a 4 cm^2 area alongside a STSG. AHSC donor site was closed primarily. Wounds were evaluated for healing with digital photography and investigator assessments for 90 days. All adverse events (AEs) were recorded. Eight patients with average 13.3% TBSA [range 2-58%] burn wounds were treated: 5 Caucasian and 3 African American with an average body mass index (BMI) of 26.8. Injury was due to predominantly flame burn, with additional injury from grease, scald, contact, friction and flash. Mean time between injury and AHSC treatment was 11 days [range 5-35 days]. All patients had adequate engraftment and complete epithelialization by the end of the study. Patients required one application of AHSC and no other additional surgical procedures at the application sites. The most common AEs for STSG-treated wounds included hypertrophic scarring and pruritus. One non-infected AHSC harvest site experienced a dehiscence. There were no other AEs related to AHSC treatment. AHSC treatment is feasible in deep partial and full-thickness burn wounds warranting additional investigation.

Keywords: autologous homologous skin construct, deep partial-thickness burns, full-thickness burns, split-thickness skin grafting

***RÉSUMÉ**. La greffe dermo- épidermique (GDE) est le traitement de référence des brûlures profondes. La zone traitée est sujette aux brides, n'a pas d'appendices dermiques, a un aspect esthétique aléatoire et le site donneur est indéniablement douloureux. Un hybride cutané autologue- homologue (HCAH) a montré être architecturalement proche de la peau. Cette étude a pour but d'évaluer l'innocuité, la qualité de prise et la qualité cicatricielle obtenues sur un groupe pilote de brûlés profonds. Après autorisation des tutelles et consentement éclairé, les patients, nécessitant une greffe ont reçu, côte à côte, une GDE et un HCAH. Ce dernier est préparé à partir d'un prélèvement de 2 cm^2 de peau totale (auto- fermant), en 48 h, dans une structure approuvée par la FDA. On obtient une structure de 4 cm^2, installée à côté d'une GDE. Les brûlures ont été évaluées cliniquement et photographiées pendant 90 j. Tous les événements indésirables (EI) ont été répertoriés. Huit patients brûlés sur 13,3 % (2-58) de SCT ont été inclus. Il s'agissait de 5 blancs et 3 noirs* (je dois traduire même ceci, qui me semble foncièrement non éthique- NDRLF) *ayant un IMC de 26,8. Les brûlures étaient liées à un flamme mais aussi à de la graisse, par ébouillantement, contact, flash ou dermabrasion. Le délai moyen de mise en place de l'HCAH était de 11 jours (5-53). L'intégration de la greffe a été bonne et tous les patients étaient cicatrisés à la fin de l'étude, sans nécessité de nouvelle greffe. Les EI les plus fréquents observés sur les zones GDE étaient des cicatrices hypertrophiques et un prurit. Une zone HCAH s'est désunie (hors infection), seul EI observé dans ce groupe. L'HCAH semble utilisables sur les brûlures profondes et doit être étudié plus avant.*

***Mots-clés :** hybride cutané autologue- homologue, brûlure profonde, greffe cutanée autologous*

✉ Corresponding author: Michael J. Feldman, Department of Surgery, Division of Plastic and Reconstructive Surgery, Evans-Haynes Burn Center, Critical Care Hospital 8th Floor, 1213 East Clay ST, Richmond, VA 23298, USA. Tel.: +1 804-828-3033; fax: +1 804-828-3045; email: michael.feldman@vcuhealth.org
Manuscript: submitted 25/05/2020, accepted 26/08/2020

Introduction

Globally, an estimated 300,000 fatalities have been attributed annually to burns and fires.[1] In 2016, there were 486,000 burn injuries in the United States alone that required medical treatment.[2] Their societal and individual impact is huge. Burns may result in a painful recovery and leave patients vulnerable to infections, sepsis, circulatory and renal failure, and scarring with contractures and disfigurement. In addition, there is a high healthcare burden, with the median total cost per patient in high-income countries estimated at $88,218.[3,4]

Split-thickness skin grafts (STSG) are the standard of care for the resurfacing of excised burn wounds.[5] However, for patients with extensive burns, limited donor site area leads to challenging clinical scenarios.[3,6,7] For these patients, cellular and tissue-based products have emerged as a viable treatment alternative given their ability to provide acceptable wound coverage and healing, reduce scarring and other complications.[3,5] However, none of the alternative forms of skin substitutes can result in functionally polarized autologous skin. Polarity, which maintains the correct orientation of the cells, including the orientation of epithelial cells between the basal lamina and apical surface, is essential in driving stratification, maintaining cell adhesion, quiescence, and progenitor lineage developments, and enables generation of skin appendages.[8]

An autologous homologous skin construct (AHSC) has been developed to treat cutaneous defects.[9-16] It is created from a small full-thickness healthy skin harvest, which is sent to an FDA-registered manufacturing facility. The AHSC is manufactured in a physiological media void of enzymes or growth factors and retains the endogenous extracellular and cellular tissues important for native skin repair.[14,16] The AHSC is optimized for sustenance from passive diffusion by improving the surface area to volume ratio, which is important for AHSC engraftment. It is not cultured *ex-vivo* or preserved, rather it is returned expeditiously to the provider in a syringe usually within 48 hours of harvest. It is returned to the provider in a syringe and is spread evenly across the wound bed where the native wound environment supports the AHSC, which engrafts within the wound and facilitates wound closure.[13,14] Prior pilot studies demonstrate its ability to close chronic lower extremity wounds that were previously refractory to multiple split-thickness skin grafts. The ability of AHSC to close deep partial and full-thickness burn wounds with a single treatment was evaluated in this open-label, single-arm feasibility study.

Material and methods

Study design and population

This study was a prospective, multicenter, single-arm open-label pilot trial designed to evaluate the use of AHSC on deep partial and full-thickness burn wounds and to help design future studies using this novel therapy. The study took place from January 22, 2018 to May 22, 2019 at two burn centers in the United States. The Western Institutional Review Board, Inc. (Puyallup, WA) and the Advarra Institutional Review Board (Columbia, MD) approved the study protocol. This study adheres to the Declaration of Helsinki, the Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6, and is in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals.

Adult patients (aged 18 to 75 years old) with deep partial-thickness and full-thickness burns requiring autografting as determined by the treating provider, with a total burn surface area (TBSA) of at least 1% were recruited for this study. Burn wounds resulting from chemical or electrical injury were excluded. The complete inclusion and exclusion criteria are found in *Table I*. During the screening visits, the patients underwent a complete physical examination, had vital signs and clinical and wound history recorded, and standard of care laboratory tests were performed. Upon enrollment, the provider selected an anticipated study wound site, which was a single, contiguous wound area measuring at least 1% TBSA.

The primary endpoint was AHSC engraftment assessed by the provider. The loss of AHSC on follow-up and/or the need for additional AHSC or autografting of the treated site was captured. Primary

Table I - Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
• Adult (18-75 years old) patient with deep-partial or full-thickness burn wounds requiring surgical grafting of an area measuring at least 1% total surface burn area • Women of childbearing potential with a negative urine or serum pregnancy test at screening • Was willing and able to comply with the study procedures and follow-up • Gave informed consent, or if appropriate, the patient's legally authorized representative provided informed consent	• Had known or suspected active localized infection of the study site that, in the opinion of the investigator, increased the risk of graft failure • Had a vascular disorder that, in the opinion of the investigator, increased the risk of graft failure • Electrical burns and burns on genitalia and digits • Participated in another clinical trial within 30 days of enrollment involving another investigational product • Subjects with uncontrolled diabetes (HbA1C > 10%) • Had active immune deficiency • Was unstable with pulmonary compromise • Used cytotoxic agents with application of AHSC • Used chlorhexidine gluconate on study site

AHSC = autologous homologous skin construct

safety endpoint was AHSC treatment-related adverse events. AHSC harvest site closure, STSG donor site closure, and the time (in days) from burn injury and from graft harvest to application were recorded. Adverse events were defined as any untoward medical occurrence that happened to the patient over the time-period beginning with the harvest procedure and ending at the 90-day study visit. These included graft-specific events including graft failure, systemic events including cardiac, pulmonary, and gastrointestinal events, and any serious adverse events (SAEs). All events were reviewed for causality in relationship to the protocol.

A sample size of 8 was calculated to achieve 81% power to detect a mean of paired differences of 25.0 with a known standard deviation (SD) of 25.0 and with a significance level (alpha) of 0.05 using a 2-sided paired z-test.

Graft harvest and application procedures and postoperative follow-up

A treating provider performed all graft harvest and application procedures at the burn centers. Using sterile technique and local anesthesia, a 2 cm x 1 cm elliptical full-thickness skin harvest was excised from a healthy, non-injured area of skin of the groin in 4 patients, thigh in 2 patients, abdomen in 1 patient, and gluteal crease in 1 patient. Harvest sites were sutured closed. The skin sample was placed in crystalloid solution with gentamicin if not contraindicated due to allergy and shipped overnight to an FDA–registered biomedical manufacturing facility (PolarityTE MD Inc., Salt Lake City, UT) to manufacture the AHSC (SkinTE™). The AHSC was returned to the clinic within 48 hours of tissue harvest and applied to the wound bed within 4 days of the harvesting procedure.

Burn wounds were sharply debrided per standard of care. Allografting and xenografting were used to prepare the wound bed per provider's discretion. On the day of the application procedure, a split-thickness skin graft was excised from a skin graft donor site in standard fashion. The split-thickness skin graft was placed over the majority of the study wound site, leaving a 2 x 2 cm area for the application of the AHSC. The AHSC was then spread evenly across the designated area and covered with a non-absorbent, non-adherent silicone dressing, which was secured in place with either staples or suture. The silicone was the primary dressing for the AHSC-treated area for 2 weeks. Thereafter, non-adherent dressing was used similar to the STSG-treated areas.

Investigators evaluated patients on days 5, 14, 30 and 90 following treatment. Five subjects were also evaluated on day 60 following an amendment to the schedule of events. At each visit, patient data were collected and photography was performed. Any AEs that had occurred were recorded. For each time point when possible, a provider assessed graft take, wound closure, harvest site closure, and wound infection. Patients were free to withdraw from the study at any time. An investigator could also terminate their participation if any clinical AE, laboratory abnormality, or other medical condition or situation occurred such that their continued participation would not be in their best interest.

Data collection and statistical analysis

All data collected by the study investigators were deidentified and stored in a HIPAA compliant manner. An independent statistician (Strategic Solutions, Inc, Bozeman, MT) analyzed the study data using Excel. An intent-to-treat analysis, based on all patients enrolled into the trial, was carried out for all endpoints. All analysis was descriptive with means and standard deviations (SD) calculated for continuous parameters and frequencies and percentages for categories.

Results

Ten patients were screened, of whom 2 were screen failures, leaving 8 patients enrolled. The mean age was 45.8+/-16 years; five (62.5%) were men, 5 (62.5%) were White, and 3 (37.5%) were African American. Mean BMI was 26.8 +/- 5.1: 2 patients were overweight, and 2 were obese. Burn etiologies were heterogeneous with half caused by open flames or flash burns and involved varied anatomical locations of the body. The mean TBSA was 13.3% +/- 19% (range: 2%-58%; *Table II*).

Table II - Burn characteristics for each patient

Patient No.	TBSA (%)	Burn Injury	Body Areas Burned (Severity of Burn)	Study Area
1	8	Hot grease	Bilateral upper extremities (partial-thickness) and left thigh (full-thickness)	Left thigh
2	2	Friction burn	Left hand, left forearm, left shoulder, left knee, face, and buttocks (partial-thickness)	Left shoulder
3	58	Flash burn	Bilateral lower and upper extremities back and abdomen (partial-thickness/full-thickness)	Left thigh
4	11	Flame	Neck, face, bilateral thighs and buttocks (deep partial-thickness); right forearm and shoulder (full-thickness)	Right thigh
5	2.5	Flame	Left hand and arm (partial-thickness); right hand, arm, and knee (full-thickness)	Right forearm
6	4	Contact	Bilateral buttocks and thighs (full-thickness)	Right gluteal
7	15	Flash burn	Bilateral lower and upper extremities; back; abdomen	Right chest
8	6	Scald	Bilateral lower extremities (partial and full-thickness)	Left foot

TBSA = total burned surface area

Over the course of treatment, patients underwent a total of 28 surgeries related to their burn care: 6 of the areas that received AHSC were allografted at an operation prior to their autografting. All 8 patients required only 1 AHSC harvest procedure to obtain the necessary cells; when possible this was included in a previous operation, taking advantage of systemic anesthesia and analgesia. The mean time from burn injury to application of AHSC was 10.8 days (SD: 9.9; range: 5-35 days). The mean time from the harvest procedure to AHSC application was 4.4 days (SD: 1.9; range: 2-8 days). AHSC and split-thickness skin grafts were applied side-by-side to a single burn wound in all 8 patients. One patient was lost to follow-up after his first visit (No. 3 – Day 5). The remaining patients had AHSC engraftment and complete wound closure by their last follow-up visit. Patients required only one application of AHSC to achieve wound closure: no additional procedures involving the AHSC-treated site were needed. A total of forty-five adverse events were documented. The majority of these occurred in 2 patients who had severe injuries on presentation resulting in systemic physiologic derangements: one patient arrived in profound hemorrhagic shock from poly-trauma, and the other had a greater than 50% TBSA burn. Additionally, one serious adverse event deemed unrelated to study participation was noted in a patient who was involved in a motor vehicle collision and sustained a chest wall hematoma during the study follow up period. The average number of AEs per patient was 6; the average number of AEs per percent TBSA was 0.8. The most commonly reported adverse events were pruritis (4/8), a spectrum of stress disorders (4/8) and the development of hypertrophic scar (3/8). There were no AEs at the application site of the product, though non-AHSC autograft loss was specifically noted in one patient in multiple areas in the setting of sepsis and fungal infection. While this patient required repeat autografting of multiple areas, the AHSC treatment site specifically did not need repeat autografting. There was one AE at the AHSC harvest site secondary to a dehiscence (technical error) requiring secondary closure at the time of the patient's definitive grafting procedure (patient No. 1).

Discussion

Autologous skin grafting remains the gold standard for the resurfacing of deep-partial and full-thickness burn wounds.[17] However, there are inherent limitations. An STSG fails to capture the deeper dermal appendages and cellular entities. Lack of sebaceous glands renders the graft dry and brittle, a situation worsened by meshing because the meshed skin interstices heal by secondary intention with epithelialization over scar resulting in a 'fish-net' appearance. There is also the tendency for STSGs to undergo contraction, which can distort surrounding normal tissue and cause limited pliability and range of motion. In addition, donor site morbidity may include painful healing with long-term pigmentary changes.[18,19] Furthermore, donor sites themselves can only be harvested a limited number of times due to wound healing and the quality of the newly healed skin at the site. Some of these limitations are obviated by use of full-thickness skin grafting with primary closure of donor sites, however the use of such grafts is not feasible for the treatment of large burn wounds. Therefore, there is interest in finding new autografting options for the treatment of burn wounds.

The goal of this pilot study was to assess the feasibility of AHSC to engraft within and close deep-partial and full-thickness burn wounds. This first reported study of AHSC in burn wounds demonstrated that AHSC was able to successfully engraft and resulted in wound closure with one application.[14] It is important to note that this study was specifically designed not to address large area expansion, but rather to keep harvested and grafted areas similar in size. The approximately 2 cm^2 area elliptical harvest was more than sufficient to treat a 4 cm^2 wound area. The full-thickness harvest site is closed primarily and avoids large painful donor sites that can occur following STSG harvesting. Additionally, the use of full-thickness skin to source AHSC allows for the retention of the endogenous regenerative cellular populations, which may aid in wound closure and the quality of the resultant skin.[9,14,16]

AHSC treated areas were visually distinct from STSG treated areas with absence of the 'fishnet' appearance (*Fig. 1*), however the size of the ASHC

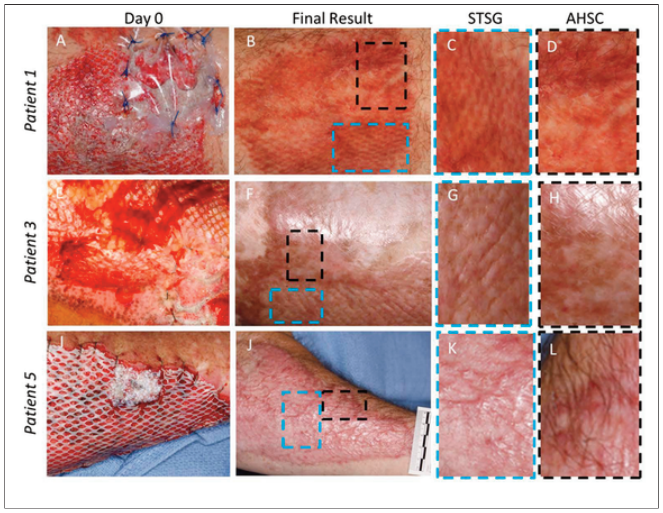

Fig. 1 - AHSC and STSG treated areas in representative patients (1, 3, 5)

treated area and relatively short follow-up precluded the ability for more qualitative comparisons. However, reduction of scarring with limited contraction has been reported in a case report.[14] Additionally, it would be difficult to make the distinction between pruritis in STSG and AHSC in view of the juxtaposition of the areas and the small graft size. We have, however, reported significant reduction in itching in AHSC treated areas compared to conventional STSG in a 45-year-old female with a 75% TBSA burn.[20]

The adverse events noted fall generally into two categories. The first are those experienced by burn patients relative to the severity of injury and duration of care, including arrythmias and infections, nutritional deficiencies and ileus, anemia and post-anesthesia complications. Unsurprising to burn practitioners, these were limited to the period of time the patients were acutely hospitalized. The second are those complications associated with the burn injury and healing process and involve themes such as psychologic injury, cutaneous sensory changes, decreases in skin pliability, the development of hypertrophic scar, and function-related range of motion changes. These represent the opportunities for burn practitioners to improve burn wound healing, and the potential promise of AHSC is largely in this second arena.

This feasibility pilot study has several important limitations. A small sample size, while sufficient for statistical comparisons, makes it difficult to draw generalizations for the many practical challenges seen at a busy burn center. The next in a series of trials using AHSC should include a focus on expansion to at least 100 cm^2 to address some of the questions raised by the small treatment area in this study, including the ability to biopsy the AHSC site to histologically demonstrate the reconstructed skin architecture. While this may make some blinded assessments less robust due to the obvious appearance of meshed STSG, it is balanced by the opportunity to make several additional assessments over a larger area, including pliability, hair growth, and sensory differences. Finally, as with any study evaluating scar formation, a longer follow-up time to evaluate scar maturation and a robust plan to minimize patient loss to follow up are needed.

Conclusions

AHSC was successfully able to close small areas of deep-partial and full-thickness burn wounds with a single application with no treatment site related adverse events. These data suggest AHSC-treatment of burn wounds is feasible and warrants further investigation in larger prospective studies.

BIBLIOGRAPHY

1 Peck MD: Epidemiology of burns throughout the world. Part I: Distribution and risk factors. Burns, 37(7): 1087-1100, 2011.
2 American Burn Association: Burn Incidence Fact Sheet. Accessed April 7 2020. https://ameriburn.org/who-we-are/media/burn-incidence-fact-sheet/.
3 Shpichka A, Butnaru D, Bezrukov EA et al.: Skin tissue regeneration for burn injury. Stem Cell Res Ther, 10(1): 1-16, 2019.
4 Hop MJ, Polinder S, van der Vlies CH, Middelkoop E, van Baar ME: Costs of burn care: a systematic review. Wound Repair Regen, 22(4): 436-450, 2014.
5 Rowan MP, Cancio LC, Elster EA et al.: Burn wound healing and treatment: review and advancements. Crit Care, 19(1): 1-12, 2015.
6 Atiyeh BS, Costagliola M: Cultured epithelial autograft (CEA) in burn treatment: three decades later. Burns, 33(4): 405-413, 2007.
7 Wood FM, Kolybaba ML, Allen P: The use of cultured epithelial autograft in the treatment of major burn injuries: a critical review of the literature. Burns, 32(4): 395-401, 2006.
8 Muroyama A, Lechler T: Polarity and stratification of the epidermis. Semin Cell Dev Biol, 23(8): 890-896, 2012.
9 Isbester K, Wee C, Boas SS, Sopko N, Kumar A: Regeneration

of autologous, functional, full thickness skin with minimal donor site contribution using autologous homologous skin construct. Plast Surg Case Stud, 6, 2020, doi:10.1177/2513826x19898810.
10 Johnson ON, Nelson M, Estabrooke I, Sopko N, Swanson EW: Successful treatment of war zone traumatic lower extremity wound with exposed tendons using an autologous homologous skin construct. Cureus, 12(5), 2020, doi:10.7759/cureus.7952
11 Armstrong DG, Orgill DP, Galiano RD, Glat PM et al.: Open-label venous leg ulcer pilot study using a novel autolologous homologous skin construct. Plast Reconstr Surg Glob Open, 8(7): e2972, 2020, doi:10.1097/GOX.0000000000002972
12 Armstrong DG, Orgill DP, Galiano R et al.: Complete wound closure following a single topical application of a novel autologous homologous skin construct: first evaluation in an open-label, single-arm feasibility study in diabetic foot ulcers. Int Wound J, 2020, doi:10.1111/iwj.13404
13 Mundinger GS, Armstrong DG, Smith D et al.: Autologous homologous skin constructs allow safe closure of cutaneous wounds: a retrospective, non-controlled, multi-centered case series. Plast Reconstr Surg Glob Open, 8(5): e2840, 2020.
14 Patterson CW, Stark M, Sharma S, Mundinger GS: Regeneration and expansion of autologous full-thickness skin through a self-propagating autologous skin graft technology. Clin Case Reports, 7(12): 2449-2455, 2019.
15 Murphy GA, Woelfel SL, Armstrong DG: What to put on (and what to take off) a wound: treating a chronic neuropathic ulcer with an autologous homologous skin construct, offloading and common sense. Oxford Med Case Reports, 2020(8): 259-263. 2020
16 Granick MS, Baetz NW, Labroo P, Milner S et al.: In vivo expansion and regeneration of full-thickness functional skin with an autologous homologous skin construct: clinical proof of concept for chronic wound healing. Int Wound J, 16(3): 841-846, 2019.
17 Simman R, Phavixay L: Split-thickness skin grafts remain the gold standard for the closure of large acute and chronic wounds. J Am Col Certif Wound Spec, 3(3): 55-59, 2011.
18 Rudolph R: The effect of skin graft preparation on wound contraction - PubMed. https://pubmed.ncbi.nlm.nih.gov/1105840/. Accessed August 21, 2020.
19 Davis SL, Shibasaki M, Low DA et al.: Impaired cutaneous vasodilation and sweating in grafted skin during whole-body heating. J Burn Care Res, 28(3): 427-434, 2007.
20 Smith DJ: Coverage of bilateral lower extremities with fully-functional, full-thickness skin following treatment with a novel autologous homologous skin construct. In: American Burn Association, 2019.

Acknowledgments. The authors would like to thank Dr. Marissa Carter (Strategic Solutions, Inc, Bozeman, MT) for her statistical analysis of this study and Kristen Eckert (Strategic Solutions, Inc) for her assistance in writing and editing the manuscript. The authors would also like to thank Christina Asare, Dr. Robert Ball, and Dr. Gaurav Garg for their clinical and administrative support to complete this research work.

Funding and role of funder: This study was funded by PolarityTE (Salt Lake City, UT), which provided the AHSC product and support in completing this study. The authors had access to all of the data, analysis, and writing the manuscript.