Received: 25 January 2019 | Revised: 11 February 2019 | Accepted: 19 February 2019

DOI: 10.1111/iwj.13109

IWJ WILEY

ORIGINAL ARTICLE

In vivo expansion and regeneration of full-thickness functional skin with an autologous homologous skin construct: Clinical proof of concept for chronic wound healing

Mark S. Granick[1] | Nicholas W. Baetz[2] | Pratima Labroo[2] | Stephen Milner[2] | William W. Li[3] | Nikolai A. Sopko[2]

[1]Division of Plastic Surgery, Department of Surgery, Rutgers New Jersey Medical School, Newark, New Jersey

[2]Department of Research and Development, PolarityTE, Inc., Salt Lake City, Utah

[3]The Angiogenesis Foundation, Cambridge, Massachusetts

Correspondence
Mark S. Granick, MD, Division of Plastic Surgery, Department of Surgery, Rutgers New Jersey Medical School, 140 Bergen Street Room E1620, Newark, NJ 07103.
Email: mgranickmd@rutgers.edu

A new cell-tissue technology uses a patient's skin to create an in vivo expanding and self-organising full-thickness skin autograft derived from potent cutaneous appendages. This autologous homologous skin construct (AHSC) is manufactured from a small full-thickness skin harvest obtained from an uninjured area of the patient. All the harvested tissue is incorporated into the AHSC including the endogenous regenerative cellular populations responsible for skin maintenance and repair, which are activated during the manufacturing process. Without any exogenous supplementation or culturing, the AHSC is swiftly returned to the patient's wound bed, where it expands and closes the defect from the inside out with full-thickness fully functional skin. AHSC was applied to a greater than two-year old large (200 cm^2) chronic wound refractory to multiple failed split-thickness skin grafts. Complete epithelial coverage was achieved in 8 weeks, and complete wound coverage with full-thickness functional skin occurred in 12 weeks. At 6-month follow-up, the wound remained covered with full-thickness skin, grossly equivalent to surrounding native skin qualitatively and quantitatively equivalent across multiple functions and characteristics, including sensation, hair follicle morphology, bio-impedance and composition, pigment regeneration, and gland production.

KEYWORDS

appendage development, autologous homologous skin construct (AHSC), functional skin, hair regeneration, regenerated full-thickness skin, split-thickness skin graft

1 | INTRODUCTION

Wound healing has traditionally been defined through four distinct physiological phases; haemostasis, inflammation, proliferation, and tissue remodelling. Each phase involves complex and interdependent signalling and coordination of diverse cellular populations including inflammatory, endothelial, stromal, and progenitor, or stem cells. Notable cell populations present in skin include cells expressing leucine-rich repeat-containing G protein-coupled receptor 6 (Lgr6), CD34, and keratin 15 that reside within dermal appendages including the follicular bulge, sebaceous glands, and inter-follicular epidermis.[1–3]

In large skin defects, many cell populations are lost, and the remaining stem cells along the wound's periphery are unable to adequately regenerate lost tissues. Failure to achieve early and appropriate wound coverage can result in refractory non-healed wounds. As the population ages and comorbidities such as diabetes become more prevalent, chronic wounds are expected to increase and already account for 3% of total health care expenditure in developed countries with an excess of $5 and $50 billion spent annually in the United Kingdom and United States, respectively.[4–7]

While full-thickness skin grafts and split-thickness skin grafts (STSGs) can achieve early autologous wound coverage,

both have measurable rates of failure approaching 30%, especially in the setting of chronic wound treatment.[8] Graft failure can result from numerous factors including systemic comorbidities, the inability of the wound bed to support the metabolic demand of intact tissue, traumatic detachment of nascent vasculature, and physical shearing.[8,9] Commercially available skin substitutes can replicate the hierarchical morphology of skin, but they do not replace functional appendages, such as follicular and glandular structures, and they too are vulnerable to loss from shear stress leading on the graft (Table 1). A novel, commercially available, cell-tissue therapy, derived from a patient's own skin can expand and regenerate appendage-bearing skin and be used to heal chronic wounds. This autologous, homologous skin construct (AHSC) treatment is created from a small full-thickness skin harvest (epidermis, dermis, and hypodermis) taken from an unaffected healthy area, which is shipped to a biomanufacturing facility. The AHSC is manufactured without ex vivo expansion and swiftly returned to the provider. A single application of AHSC is applied to the properly debrided chronic wound and covered by a standard wound dressing. Once engrafted, the AHSC self-propagates and expands into full-thickness skin that contains all the critical components for native tissue function including dermal appendages, such as hair follicles and sweat glands.

Here, we present this technique and report the first outcome of AHSC-treated tissue compared with native skin and STSG in a chronic lower extremity wound, which had repeatedly failed the clinical standard of care (autograft) and other advanced wound care skin substitutes.

Key Messages

- The growing clinical and financial burden of chronic non-healing wounds mandates effective wound coverage options that result in robust permanent skin.
- Skin grafts are one standard of care but they require specialised surgeons, create a large and painful donor site defect, and can fail because of shear stress on the graft.
- An autologous homologous skin construct (AHSC) can be derived from a patient's own skin. Innate regenerative cellular populations from a small full-thickness healthy skin harvest are fractionated and activated during manufacturing. The product expands within the wound bed using the natural healing environment created by the patient's body to heal the wound from the inside out.
- Compared with native uninjured skin, neo-regenerated skin from the AHSC was found to be equivalent across multiple functions and characteristics, including sensation, hair follicle composition, pigment regeneration, and gland production.

2 | METHODS

Patient authorisation and consent were obtained for use of all photographs, images, and figures contained within this manuscript, in accordance with institutional policies. Following production and application of a single application of AHSC, the patient was followed for 6 months and assessed for AHSC safety, efficacy, donor site morbidity, graft take, time to wound closure, pigmentation, hair follicle development, and sweat and sebaceous gland production, sensation, contracture, and pliability.

2.1 | Preparation of AHSC therapy

A full-thickness harvest is taken from an unaffected area of the patient such as the groin or thigh, and the site is closed primarily. The tissue is shipped in normal saline at 4°C to an Food and Drug Administration–regulated biomedical manufacturing facility (PolarityTE, Salt Lake City, Utah). All the tissue is processed into AHSC (SkinTE; PolarityTE), which involves processing of tissue to improve the surface area to volume ratio and activation of the endogenous regenerative cellular populations akin to the activation that occurs when native skin is injured. The AHSC can be returned to the clinical site the same day depending on the location, or up to 11 days following tissue harvest with the goal of providing the patient with their own AHSC as expeditiously as possible. The wound bed is sharply debrided and the AHSC is spread evenly across the wound bed analogous to distributing a skin graft. It is dressed with a non-absorbent, non-adherent dressing such as silicone with regular dressing changes until mature epithelialisation in a manner consistent with STSG dressing.

2.2 | Wound Healing and Functional Tissue Assessment

Pre-treatment cutaneous defect and post-treatment wound healing were documented with high-resolution digital single lens reflex (DSLR) photography (Cannon, Melville, New York). Pain was subjectively rated by the patient. Baseline static two-point discrimination was performed on AHSC-treated areas, native skin, and STSG-treated areas as previously described taking the average of 5 random locations for each group.[10] Bioimpedance analysis (RJL Systems, Clinton Township, Michigan) was performed using the two-electrode method as previously described on AHSC-treated areas, native skin, and STSG-treated areas to assess water content, oil content, and pliability. Briefly, electrodes are placed 4 cm apart in the region of skin to be tested with time-varying sinusoidal 1 V applied in a 200 Ω resistance circuit with voltage drop measured with current passage less than 10 μA.[11–14] Differences between means were measured using two-way analysis of variance with a Tukeys post-hoc test with a P-value of <0.05 considered as significant.

2.3 | Tissue architecture and compositional analysis

Molecular composition of skin and hair follicles from AHSC-treated areas and native skin was analysed using Raman

TABLE 1 Table of AHSC and advanced wound care products

Treatment	Tissue source	Viable tissue	Regenerative stem cell niche	Complete skin architecture
AHSC	Autologous full-thickness skin	Yes	Yes	Yes
Split-thickness skin graft	Autologous partial-thickness skin	Yes	No	No
Animal collagen scaffolds	Xenograft collagen	No	No	No
Acellular dermal matrix	Allogeneic acellular dermis	No	No	No
Amnio-placental membranes	Allogeneic human amnion	Yes/No	No	No
Cultured keratinocytes on matrices	Allogeneic neonatal foreskin	Yes	No	No
Allograft (cadaveric) skin	Allogeneic human skin	Yes	No	No
Cultured epithelial autograft (CEA)	Cultured epidermal cells+ mouse cells (xenograft)	Yes	No	No

spectroscopy (ThermoFisher Scientific, Madison, Wisconsin). Hair follicles removed from uninjured skin and AHSC-treated skin were whole mount imaged with a digital compound microscope (Zeiss V16, Oberkochen, Germany), a confocal microscope (Leica TPS SP8, Wetzlar, Germany) following labelling with wheat germ agglutinin and phalloidin (ThermoFisher Scientific), an environmental scanning electron microscope (ESEM, Zeiss EVO LS10, Oberkochen, Germany), and a second harmonic multi-photon microscope (Leica SP, Wetzlar, Germany).

3 | CLINICAL CASE

A 31-year-old previously healthy African American male suffered polytraumatic injuries from a motorcycle accident 24 months earlier. His acute traumatic injuries included wounds in both lower extremities, with a large traumatic soft tissue avulsion covering the majority of the anterior RLE resulting in bone exposure and a large avulsion flap that later failed and resulted in complete necrosis. The flap was debrided and a STSG was placed 1-month post-injury. The STSG failed with complete graft loss likely because of the inadequacy of the wound bed to support the graft. Over the subsequent 2 years, the wound chronically failed to heal despite application of multiple advanced wound care products, skin substitutes, and additional STSG efforts. The patient initially presented for AHSC therapy with a pretibial defect >200 cm^2 in size with drainage, exposed bone and reported absent or impaired light touch sensation, and increased pain throughout wound and peri-wound bed surfaces (Figure 1). The patient elected to undergo AHSC treatment to avoid another STSG.

Two days prior to the AHSC application procedure, a small (6 cm long), elliptical full-thickness skin harvest was performed in clinic by a sterile technique from the patient's right groin for the creation of AHSC. At 48-hours following harvest, the wound bed was prepared using direct contact low frequency ultrasonic debridement (SonicOne; Misonix, Farmingdale, New York) followed by topical application of the AHSC product into the full-thickness wound bed (200 cm^2). AHSC was spread evenly applied across the entire wound surface and the treated wound was covered with an occlusive, non-adherent, non-absorbent silicone dressing similar to a skin graft, and wrapped with multi-layer compression dressings. The patient was discharged home with instructions to return for weekly follow-up for 8 weeks followed by monthly follow-up for a total of 6 months.

4 | RESULTS

4.1 | Wound healing and donor site

AHSC had complete (100%) graft take and resulted in complete epithelial coverage within 8 weeks and full-thickness functional skin coverage within 12 weeks (Figure 2). At the last follow-up at 6 months post-application of AHSC, the wound remained completely closed and covered with full-thickness functional skin, which was grossly equivalent to the surrounding native

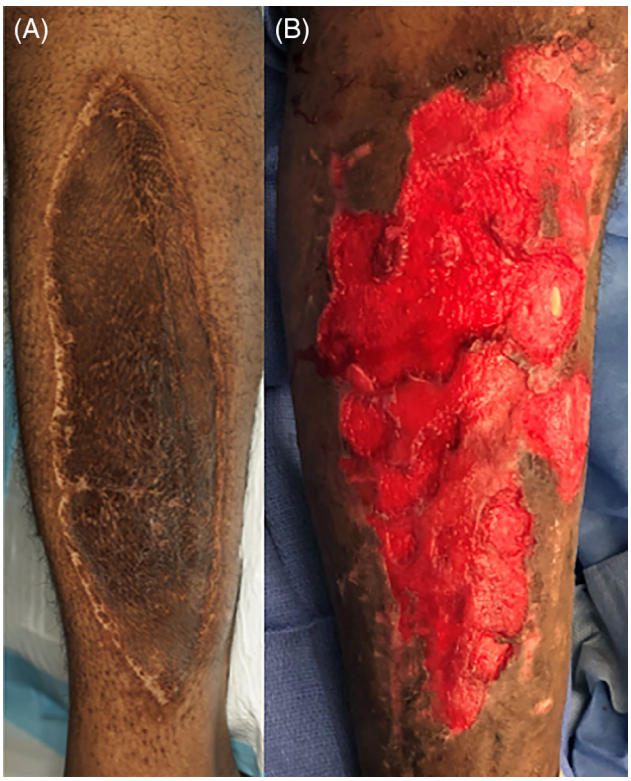

FIGURE 1 A, Image of left lower extremity fasciotomy wound resurfaced with a split-thickness skin graft 30 months previously. B, Image of debrided chronic right lower extremity wound 24 months following injury with granulation tissue and exposed tibia

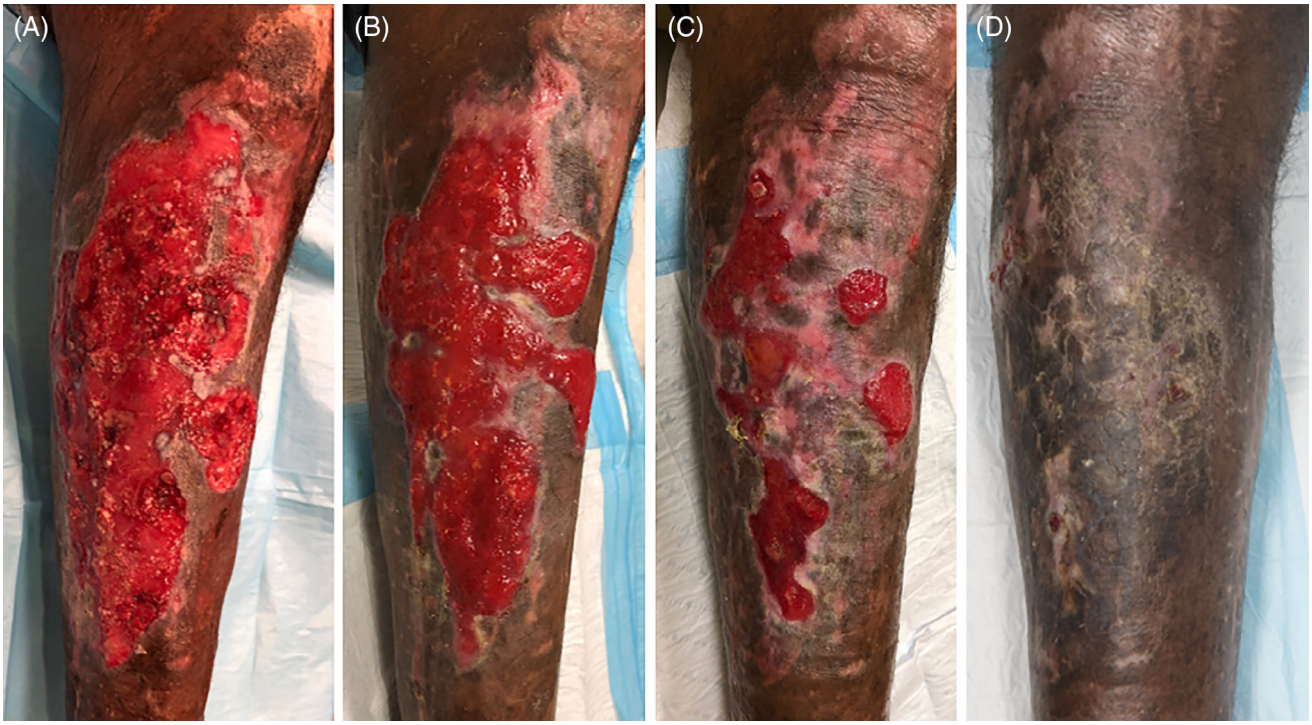

FIGURE 2 Progressive healing of right anterior lower extremity wound following autologous homologous skin construct (AHSC) application with propagation of melanocyte pigmentation and regeneration of full-thickness skin at A, 1 week; B, 4 weeks; C, 8 weeks; D, 6 months

skin. Serial examination of the wound following application of AHSC demonstrated regeneration of full-thickness skin tissue and associated cutaneous appendages (hair follicles, sweat/oil glands). In addition to wound closure by epithelialisation, serial follow-up assessments showed progressive restoration of tissue volume, melanin pigment deposition, and improvement of gross cutaneous sensation. The patient subjectively reported decreased pain and improved functional and aesthetic outcome compared with the contralateral STSG, which he reported continuously believed dry and required moisturisation.

4.2 | Functional and compositional analysis

Digital single-lens reflex photography demonstrated focal expansion of AHSC within the full-thickness wound bed, with progressive melanocyte pigmentation and full-thickness skin regeneration throughout the wound, with minimal contracture (Figure 2). Static 2-point discrimination demonstrated no difference between AHSC and native skin ($P = 0.076$). In contrast, the healed STSG placed on the contralateral extremity demonstrated a reduction in sensation relative to native skin

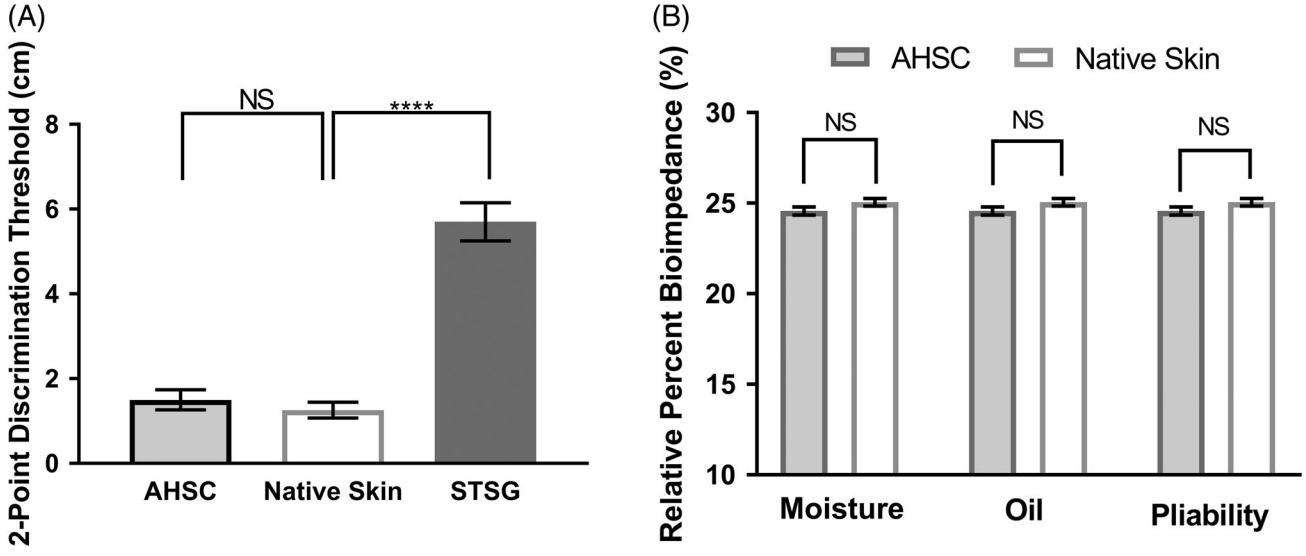

FIGURE 3 A, Static 2-point discrimination test results comparing right lower extremity autologous homologous skin construct (AHSC) (14 weeks post-operative), left lower extremity native skin, and left lower extremity STSG (30 months post-operative). B, Relative bio-impedance expressed as a percent (%) comparing right lower extremity AHSC (14 weeks post-operative), left lower extremity native skin, and healed left lower extremity split-thickness skin graft (STSG) (30 months post-operative). NS, non-significant). *P-value <0.05; ****P-value <0.0001

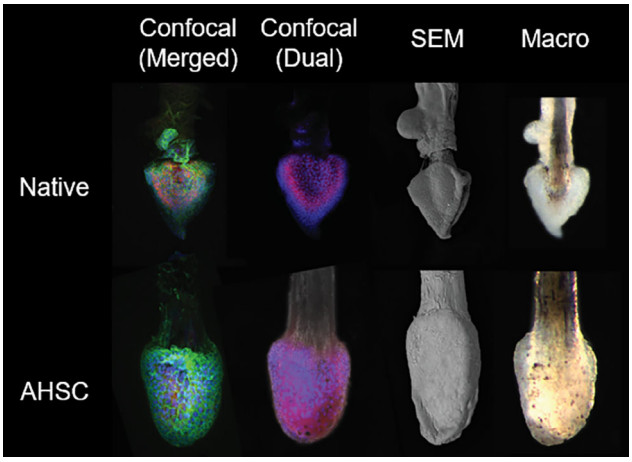

FIGURE 4 Comparative multi-modality imaging of hair follicles (HFs) harvested from either native skin or autologous homologous skin construct (AHSC) at 14-week post-application. Correlative fluorescent probe imaging was conducted using confocal microscopy to determine relative quantity, and colocalisation of structures including nuclei (blue), F-actin (red) and collagen (green) analytes. Environmental scanning electron microscopy (ESEM) and dark field stereoscopic imaging was conducted to determine the relative surface/subsurface microanatomy of the structures

and AHSC ($P < 0.0001$) (Figure 3). Bio-impedance analysis of AHSC-regenerated skin relative to native skin showed no significant difference in moisture, oil, or pliability ($P = 0.25$), whereas the healed STSG of the LLE had significantly reduced features along all three parameters ($P < 0.05$) (Figure 3). Hair follicles that were regenerated from AHSC demonstrated normal cellular and structural architecture, with complex hierarchical dermal papilla morphology, and properly oriented keratinised hair shaft growth similar to that of native hair follicles by compound microscopy, fluorescent confocal microscopy, second-harmonic multiphoton microscopy, and ESEM (Figure 4). Notably, the complex cellular architecture of the follicular bulge where regenerative cellular populations are located was completely recapitulated with AHSC treatment. Additionally, Raman spectroscopy demonstrated no significant difference in molecular composition of AHSC-regenerated and native hair follicles (Figure 5).

5 | DISCUSSION

True successful healing of chronic wounds requires complete epithelialisation with the regeneration and replacement of normal skin end organs and function. Advanced skin substitutes and STSGs are limited by their inability to fully recapitulate the cellular physiology of skin. AHSC uses autologous cells derived from intact skin that can activate in situ the complex coordination of epidermal and dermal cellular populations, extracellular matrix, and repair pathways required for successful healing.[15] These differences improve the quality of complete healing beyond epithelialisation, and represent a desired state for clinical wound care.

The quest to develop a fully recapitulative topical healing modality has been pursued for years. Chronic wounds are characterised by multiple impaired physiological processes that perturb normal healing, including ischaemia, inflammation, infection, reduced levels of growth factors, proteinase imbalance, and cellular senescence as well as local factors such as foreign bodies and tissue insult.[15] Impairment of cellular activity and efficacy has been attributed to decreased mitogenic response of wound fibroblasts to growth factors and the persistence of a hyperproliferative but less differentiated state of keratinocytes.[15,16]

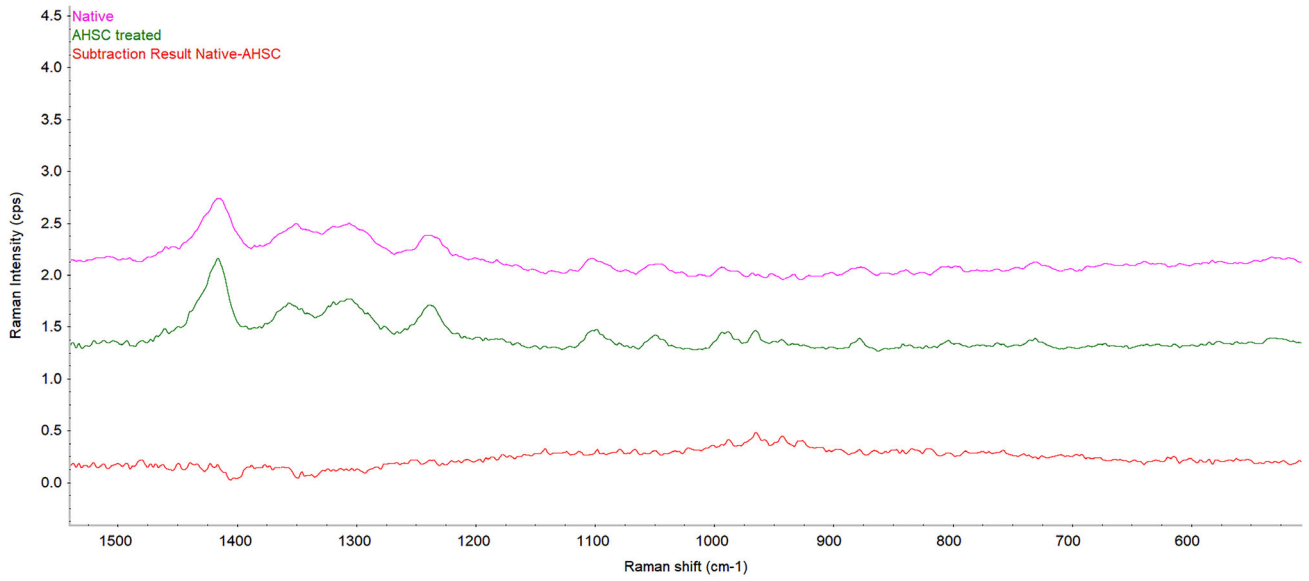

FIGURE 5 Comparative Raman spectroscopic fingerprinting of hair follicles (HFs) harvested from (A) autologous homologous skin construct (AHSC)-regenerated tissues and (B) native skin to determine the relative molecular composition, quantity, structure, and energy state of the specimens shifted to show their respective signal peaks. C, Direct comparison of the spectra by subtraction creates a flat line demonstrating minimal differences in the molecular fingerprint of the tissues

Within this setting of compromised wound biology, conventional therapies and advanced skin substitutes commonly fail with reported failure rates approaching 30% in clinical trials with no specific skin replacement therapy demonstrating clinical superiority over another in a Cochrane Database Systematic Review.[17,18] Similarly, skin grafts with their relatively large tissue mass and metabolic demands have reduced graft take in the setting of ischaemia and poor tissue perfusion found in chronic wounds.[9] The AHSC technology was developed to survive austere tissue environments and in vitro, in vivo, and emerging clinical data support these capabilities. The entirety of the harvested tissue is used during the manufacturing of AHSC, so it contains all necessary structural and cellular elements, including the endogenous stem cell populations. The manufacturing of AHSC activates these cells, with the segmentation of the skin tissue. The processing of AHSC improves the surface area to volume ratio of reimplanted tissue, which aids cell survival via plasmatic imbibition, the passive diffusion of oxygen, nutrients, and metabolite, until inosculation and blood vessel formation can take place. Because AHSC is swiftly returned to the patient and the cells are not cultured in vitro, they expand physiologically in the wound bed using the body's endogenous wound repair support pathways, in contrast to cells produced in tissue culture, which can alter gene expression and cell behaviour.[19]

In the clinical case presented in this report, the functional capability of the resulting AHSC-regenerated tissue was similar to that of uninjured native skin and a healed STSG on the contralateral across all parameters tested, including digital single-lens reflex photography, microscopic imaging, sensory examination, and bio-impedance analysis. In contrast, STSG-treated areas were found to be significantly different from native skin (Table 1). Patient-reported outcomes demonstrated a strong preference by the patient for treatment with AHSC compared with STSG, for parameters of pain, function, and cosmesis.

We have thus presented a clinical proof of concept that a patient-derived autologous cell-tissue therapy, AHSC, can achieve regenerative healing in a chronic wound, complete with replacement of skin end organs. Remarkably, the successful healing occurred in the setting of multiple failed prior STSG and use of xenograph skin substitute through a single delivery of AHSC. Repeated AHSC applications were not required. The achievement of complete wound closure by AHSC with full-thickness skin regeneration with functional appendages (hair, glands, and light touch sensation) is unique and has not been previously reported. We point out that this patient was a young otherwise healthy patient without the comorbidities frequently encountered in patients with chronic wounds such as with diabetic lower extremity wounds, venous stasis ulcers, and arterial ulcers. Therefore, further translational and clinical research of AHSC is warranted to explore its full potential for successful, high quality wound healing under more challenging host environments.

CONFLICT OF INTEREST

M.S.G. is a clinical advisor to PolarityTE.

REFERENCES

1. Yi R. Concise review: mechanisms of quiescent hair follicle stem cell regulation. *Stem Cells*. 2017;35(12):2323-2330.
2. Snippert HJ, Haegebarth A, Kasper M, et al. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science*. 2010;327(5971):1385-1389.
3. Lough DM, Yang M, Blum A, et al. Transplantation of the LGR6+ epithelial stem cell into full-thickness cutaneous wounds results in enhanced healing, nascent hair follicle development, and augmentation of angiogenic analytes. *Plast Reconstr Surg*. 2014;133(3):579-590.
4. Järbrink K, Ni G, Sönnergren H, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. *Syst Rev*. 2017;6:15.
5. Nussbaum SR, Carter MJ, Fife CE, et al. An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. *Value Heal*. 2018;21(1):27-32.
6. Guest JF, Ayoub N, McIlwraith T, et al. Health economic burden that wounds impose on the National Health Service in the UK. *BMJ Open*. 2015;5(12):e009283.
7. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med*. 2017;376(24):2367-2375.
8. Reddy S, El-Haddawi F, Fancourt M, et al. The incidence and risk factors for lower limb skin graft failure. *Dermatol Res Pract*. 2014;2014:582080.
9. Buchanan PJ, Kung TA, Cederna PS. Evidence-based medicine: Wound closure. *Plast Reconstr Surg*. 2014;134(6):1391-1404.
10. Dellon AL, Mackinnon SE, Crosby PMD. Reliability of two-point discrimination measurements. *J Hand Surg Am*. 1987;12:693-696.
11. Kekonen A, Bergelin M, Eriksson J-E, Vaalasti A, Ylänen H, Viik J. Bioimpedance measurement based evaluation of wound healing. *Physiol Meas*. 2017;38(7):1373-1383.
12. Davies L, Chappell P, Melvin T. Modelling the effect of hydration on skin conductivity. *Skin Res Technol*. 2017;23(3):363-368.
13. Uchiyama T, Ishigame S, Niitsuma J, Aikawa Y, Ohta Y. Multi-frequency bioelectrical impedance analysis of skin rubor with two-electrode technique. *J Tissue Viability*. 2008;17(4):110-114.
14. Martinsen ØG, Grimnes S, Kirkhus T, Mørkrid L, Aas K. Increased moisture content in children's atopic skin. *Eur J Pediatr Dermatol*. 2007;17(1):17-20.
15. Harding KG, Morris HL, Patel GK. Science, medicine and the future: healing chronic wounds. *BMJ*. 2002;324(7330):160-163.
16. Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. *Adv Skin Wound Care*. 2012;25(7):304-314.
17. Santema TB, Poyck PPC, Ubbink DT. Skin grafting and tissue replacement for treating foot ulcers in people with diabetes. *Cochrane Database Syst Rev*. 2016;2:CD011255.
18. Tettelbach W, Cazzell S, Reyzelman AM, Sigal F, Caporusso JM, Agnew PS. A confirmatory study on the efficacy of dehydrated human amnion/-chorion membrane dHACM allograft in the management of diabetic foot ulcers: A prospective, multicentre, randomised, controlled study of 110 patients from 14 wound clinics. *Int Wound J*. 2019;16(1):19-29.
19. Januszyk M, Rennert RC, Sorkin M, et al. Evaluating the effect of cell culture on gene expression in primary tissue samples using microfluidic-based single cell transcriptional analysis. *Microarrays (Basel, Switzerland)*. 2015;4(4):540-550.

How to cite this article: Granick MS, Baetz NW, Labroo P, Milner S, Li WW, Sopko NA. 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*. 2019;16:841–846. https://doi.org/10.1111/iwj.13109