Distinct Patterns of Hair Graft Survival After Transplantation Into 2 Nonhealing Ulcers: Is Location Everything?

Majid Alam, PhD,*†‡ Jerry Cooley, MD,§ Magdalena Plotczyk, MSc,‖ María Soledad Martínez-Martín, MD,¶ Ander Izeta, PhD,# Ralf Paus, MD,**†† and Francisco Jimenez, MD*†‡‡

BACKGROUND Studies highlighting the role of hair follicles (HFs) in wound healing have raised the challenge of bringing this knowledge to clinical applications. A successful translation is the transplantation of scalp HFs into chronic wounds to promote healing.

OBJECTIVE To characterize scar formation and hair growth in nonhealing ulcers after transplantation.

PATIENTS AND METHODS Nonhealing ulcers were treated with hair transplantation to promote wound healing. Hair follicles were harvested from the patient's scalp and inserted into the wound bed. Wound repair and hair growth were assessed clinically. Further analyses were performed in situ, using biopsies from the central and peripheral scar.

RESULTS Rapid wound closure and differences of scar quality and hair growth between the central and peripheral wound areas were observed: the periphery healed with no hair shaft survival and an almost scarless appearance, the center healed with a fibrotic scar, with some hair shaft growth. In situ analyses revealed differences in dermal remodeling and collagen formation between central and peripheral scar areas.

CONCLUSION Besides confirming the effectiveness of this therapy to promote wound healing in human skin, location-dependent disparities in scar quality and hair growth raise the intriguing question whether they are due to clinically important differences in mechanical forces and/or wound microenvironments between ulcer center and periphery.

The authors have indicated no significant interest with commercial supporters.

The hair follicle (HF) is an integral part of the healing process in cutaneous wounds, with the migration of epithelial and dermal HF cells contributing substantially to wound re-epithelialization and repair.[1–4] It is believed that this is based, at least in part, on the angiogenesis- and reinnervation-promoting properties of the HF,[5,6] the release of potent wound healing–promoting growth factors such as TGFß1 and VEGF by HFs, and the endowment of HFs with multiple different progenitor cell populations[7,8] that, in principle, are capable of regenerating all major skin compartments.

As an attempt to translate this well-known, but still underappreciated role of HFs as wound healing

*Mediteknia Skin and Hair Laboratory, Las Palmas de Gran Canaria, Spain; †Universidad Fernando Pessoa Canarias, Las Palmas de Gran Canaria, Spain; ‡Monasterium Laboratory, Münster, Germany; §Carolina Dermatology Hair Center, Pineville, North Carolina; ‖Department of Bioengineering, Imperial College London, London, United Kingdom; ¶Department of Morphology, Universidad Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; #Biodonostia Health Research Institute, Donostia University Hospital, San Sebastian, Spain; **Centre for Dermatology Research, University of Manchester, and NIHR Manchester Biomedical Research Centre, Manchester, United Kingdom; ††Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida; ‡‡Grupo de Patología Médica, Instituto Universitario Investigación Biosanitaria, Universidad Las Palmas Gran Canaria, Las Palmas, Spain

ISSN: 1076-0512 • Dermatol Surg 2019;00:1–9 • DOI: 10.1097/DSS.0000000000001748

promoters, several clinical reports have shown the utility of autologous HF grafts in stimulating the healing of chronic ulcers or other nonhealing wounds.[7–11] Some of these reports refer to patients with deep and extensive burns or surgical wounds successfully treated with the combination of an artificial dermis coverage that provides an immediate coverage of the wound and subsequent formation of a neodermis, followed by autologous hair follicle grafting, which was shown to significantly accelerate overall wound healing and re-epithelialization.[9–11] Other clinical studies show effectiveness of transplanting exclusively hair follicle grafts in chronic venous leg ulcers[12–15] and in surgical or traumatic wounds.[16] In these cases, the hair follicle grafts were harvested using skin punches from the patient scalp as the donor source.[17,18]

It is interesting to note that in all wound healing studies where scalp hair grafts were used, the hair shafts grew in far lower quantity than would be expected in a normal hair transplant procedure performed in a patient with androgenetic alopecia.[4,12,13,16] However, none of the previous studies have addressed the hair growth pattern distribution generated on the scar once the wounds were healed. In the 2 cases reported here, we highlight the strikingly distinct hair growth pattern seen in the central and peripheral zones of the wound, and note that, this correlates with differences in the quality of scar formation. Although limited only to clinical observations in just 2 male patients at different extremes of the age spectrum, we feel that these consistently observed phenomena raise biologically fascinating and clinically highly relevant questions as to the potential underlying mechanisms. This motivates us to share these case reports with the field so as to stimulate further research that may answer these intriguing open questions.

Materials and Methods

Standards and Ethics Compliance

Helsinki Declaration principles, relevant Spanish legislation, and Good Clinical Practice regulations were followed. Informed, written patient consent was given before punch biopsies were taken.

Patients

An 18-year-old male patient came for a second opinion regarding a traumatic ulcer located on the inner aspect of the left arm that had not improved with conventional therapy, that is, a variety of dressings and local antibiotics, after 3 months. The ulcer was due to a thermal burn caused by contact with the exhaust pipe of a motorcycle in a traffic accident. On physical examination, the ulcer was circular in shape, 2.7 cm in diameter and covered with a boggy red granulation tissue (Figure 1A).

Given the patient's lack of response to standard therapy and the coauthor's (F.J.) previous positive experience using hair transplant autografts in nonhealing ulcers,[12] we offered the patient this therapeutic option. In short, after shaving and administering local anesthesia in a 4×4-cm area of the patient's occipital scalp, 100 follicular unit (FU) grafts were harvested using a 1-mm punch graft (serrated hybrid trumpet punch; Devroye Instruments, Brussels, Belgium) attached to a motorized device (Waw FUE system, Devroye Instruments). This harvesting technique, known as FU excision, is identical to the one normally used in hair transplantation for androgenetic alopecia.[19] These hair grafts were immersed in physiologic saline until their immediate insertion in the recipient wound. The wound bed of the ulcer was locally anesthetized with lidocaine 1% and 1:100,000 adrenaline, cleaned, curetted, and all FU grafts were then inserted into the wound bed using hair graft implanters (Lion Hair Implanter; Hansbiomed, Seoul, Korea). Grafts were evenly distributed in the wound area, which measured 5.72 cm^2, at an average density of 17 grafts per cm^2 (Figure 1B). The procedure lasted approximately 45 minutes, and the patient was discharged with an occlusive wound dressing for 48 hours.

An 83-year-old man was referred for evaluation of a nonhealing wound of iatrogenic origin located on his right upper arm. Three months prior, a malignant

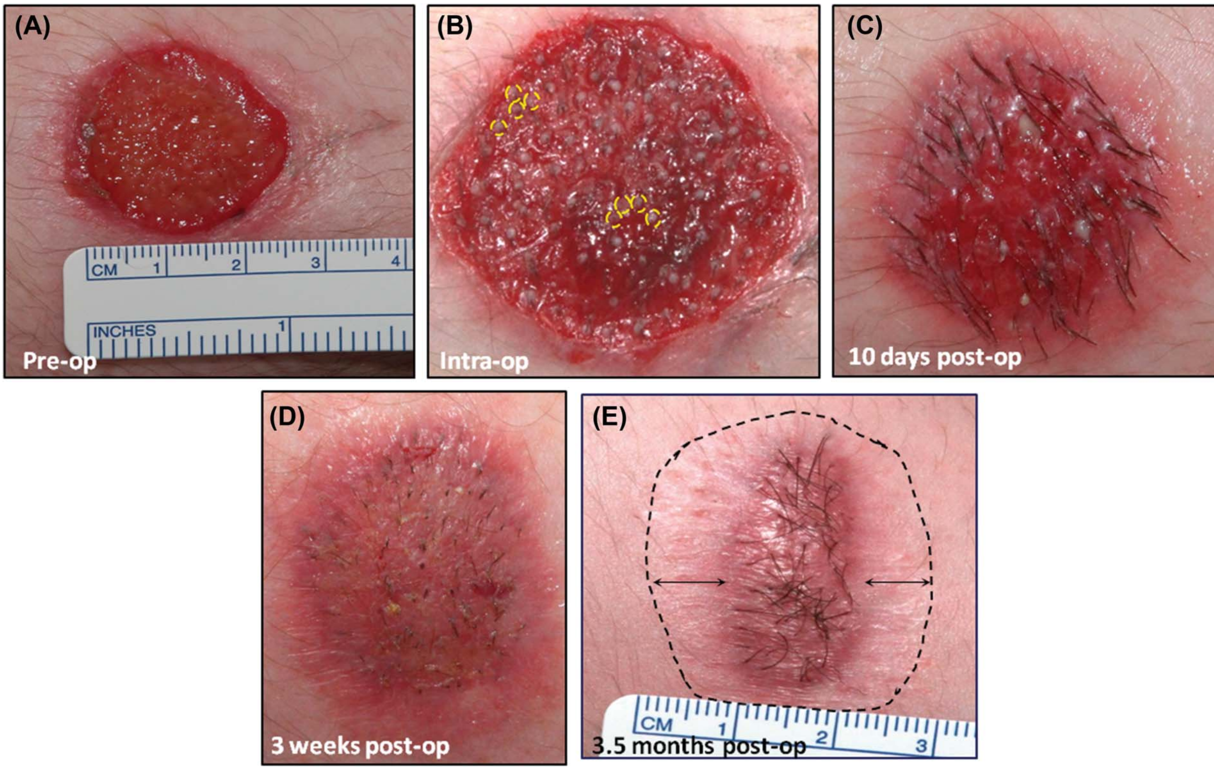

Figure 1. Distinct pattern of hair shaft growth in a nonhealing wound treated with FU grafts. A wound approximately 2.7 cm in diameter caused by a thermal burn after an accident (A). Hundred follicular unit (FU) punch grafts (from the scalp) containing terminal hair follicles were transplanted into the wound bed of the ulcer (epidermis of FU grafts demarcated by dotted yellow circles at the center and periphery of the wound bed (B). After 10 days, rapid healing of the ulcer was observed with increased wound closure and reduction in inflammation (C). After 3 weeks, the wound had completely healed over with no granulation tissue remaining (D). Three and a half months after transplantation, the original wound had shrunk in size (demarcated by dotted lines) and an unusual pattern of hair growth was observed (E). No hair shaft growth was observed at the periphery (double arrow heads), in comparison with the central area, which contained growing hair shafts and was visibly more fibrotic.

melanoma was excised followed by primary closure. Before excising the tumor, the referring primary care provider had inadvertently injected approximately 40 cc of 10% buffered formalin into the skin, mistaking it for tumescent anesthetic. Recognizing the error immediately, the tumescent anesthetic was injected into the skin in an attempt to dilute the formalin, and the surgery was performed in standard fashion including a 2-layered sutured closure. However, 2 weeks after the surgery, when the surface sutures were removed and over the subsequent days, the wound dehisced completely, and necrosis was noted along the wound edges. Wound care consisted of regular debridement of necrotic skin followed by saline-soaked gauze and compression dressings. Wound healing occurred very slowly, and at 6 weeks, the diameter of the wound did not seem to have reduced (Figure 2A).

The patient was referred to one of the coauthors (J.C.), a dermatologist specialized in the wound healing process. After discussing the potential benefit of a hair transplant–promoting wound healing, the patient gave consent for this therapy. At the time of hair transplantation, the ulcer measured 9 × 4.5 cm (~40.5 cm^2). Using the strip-harvesting hair transplant method, a total of 405 FUs grafts were implanted into the wound bed at an average density of 10 grafts per cm^2 (Figure 2B). In this patient, in an attempt to further stimulate wound healing, the surgeon lightly coated the FU grafts with a concentrated suspension of ACell MicroMatrix (ACell, Columbia, MD), a medical product routinely used for both acute and chronic wound treatment.[20,21] In addition, after the procedure, the patient was instructed to frequently spray a saline-based solution containing liposomal ATP (Energy Delivery

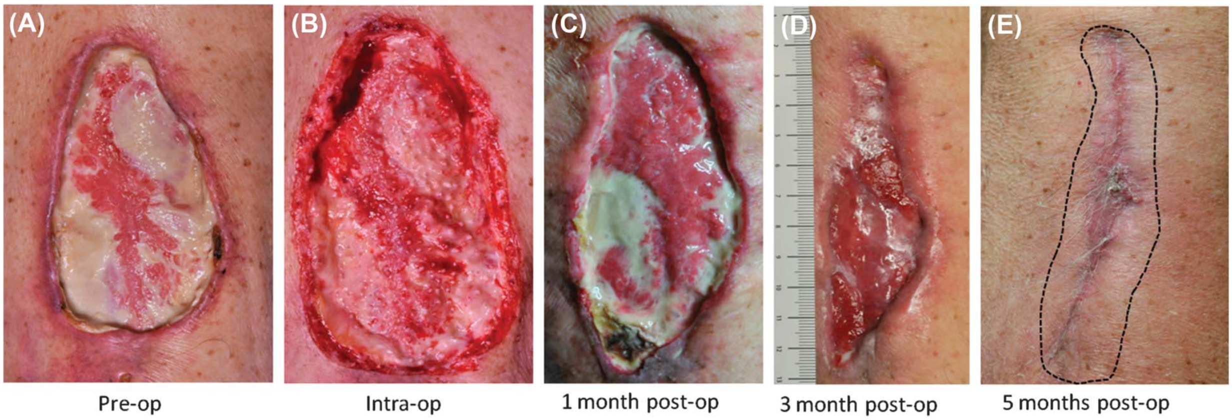

Figure 2. Distinct pattern of hair growth of a nonhealing surgical wound treated with follicular unit grafts. An iatrogenic wound after accidental injection of formalin (A). Four hundred follicular unit grafts from the scalp were evenly transplanted into the wound bed (B). After 1 month, rapid healing of the wound can be seen (C). After 3 months, the wound shrunk further in size (D), and 5 months post-op (E), the wound had completely healed and shrunk in size (dotted line), and hair growth can only be seen in the center of the scar. Scar tissue at the periphery is almost indistinguishable from normal skin, whereas the center is more fibrotic.

Solutions, Jeffersonville, IN) into the wound as an adjuvant therapy.

Histochemistry and Immunofluorescence Microscopy

Given the striking differences observed at 7 months after operation in scar quality and hair growth pattern between the central and peripheral zones of the healed ulcer, the scar of patient 1 was biopsied after written informed consent. Two 3-mm punch biopsies were obtained, one from the central fibrotic hairy zone and another from the nonhairy peripheral zone for further histological and histochemical analyses.

Paraffin-embedded skin samples were sectioned at 7 μm for hematoxylin and eosin (H&E) and Masson trichrome staining to examine general morphology and collagen structure (Collagen I, III, and IV) between the 2 well-defined scar areas.

For immunohistology, paraffin-embedded skin samples were deparaffinized using Histo-Clear (National Diagnostics, HS-200) and rehydrated after 3 washes: 100% ethanol, 70% ethanol, and water. For antigen retrieval, a drop of proteinase K (Abcam, ab64220) was placed on top of each section and incubated for 7 minutes at room temperature (RT). Sections were then fixed with 4% paraformaldehyde (PFA) for 5 minutes at RT, blocked with 5% goat serum (Vector Laboratories, Burlingame, CA) for 30 minutes at RT, and incubated overnight at 4°C with primary antibodies; 1:200 anti-COLIV (Abcam, ab6311) and 1:200 anti-COLIII (Abcam, abcam7778). Secondary antibody anti-mouse and anti-rabbit Alexa Fluor 546 (Life Technologies, A21207 and A21203) were used at 1:500 for 1 hour at RT in the dark. Sections were mounted using Vectashield mounting media and a cover slide.

Second Harmonic Generation and Fluorescence Imaging

For COLI imaging, we used second harmonic generation (SHG) microscopy, which offers deeper tissue penetration, improved resolution, and without the need for labeling, as compared to standard linear optical techniques. In the process, 2 photons interact with a nonlinear material, such as COLI fibers, and form a new photon of twice the energy, which is then detected as an SHG signal. Fluorescence imaging was performed on an upright confocal microscope (Leica SP5) coupled to a Ti:Sapphire laser (Mai Tai, Newport Spectra-Physics), a water immersion 25X objective (IRAPO, NA 0.95, Leica) and a photomultiplier tube detector. The excitation was accomplished at wavelength 820 nm obtained at 400 to 420 nm for the SHG signal to image collagen I fibers, and at 430 to 480 nm for the multiphoton signal (DAPI). Through sequential scanning, separated excitation of 594 nm and emission 500 to 580 nm were used for fluorescence imaging of COLIV and COLIII. High-resolution images (1,024 × 1,024 pixels) with 12-bit pixel depth were obtained with a view field of 620 × 620 μm.

Regarding image processing and statistical analysis, 5 images of randomly selected areas in the dermis of the central and peripheral scars were taken for each staining and analyzed using an image processing package, Fiji (NIH, Bethesda, MD). AngioTool software was used as a Fiji plugin for the quantitative analysis of angiogenesis, focusing on the assessment of vessel percentage area.[22] The average pixel density was measured using an in-built Analyse Fiji tool. All statistical analyses were performed using GraphPad 5 (La Jolla, CA).

Results

Hair Follicle Scalp Grafts Promote Ulcer Healing

Irrespective of the major difference in age, skin ulcer healing was induced by autologous scalp hair transplantation in both patients. In the first clinical case, very rapid healing was seen 10 days after transplantation, with a notable reduction in inflammation and partial re-epithelialization (Figure 1C). After 3 weeks, the ulcer was considered by the surgeon to be completely re-epithelialized and healed (Figure 1D). In the second case, rapid healing was also observed after transplantation, and after 1 month, a reduction in ulcer size, wound contraction, and granulation tissue was observed (Figure 2C). After 3 months, the ulcer had further reduced in size (Figure 2D), and after 6 months post-op, the wound was considered completely healed (Figure 2E).

The Postulcer Scar Displays Two Well-Defined Zones With Strikingly Distinct Hair Growth Patterns

Interestingly, when Patient 1 returned for a clinical assessment after 3.5 months, we noted that the scar presented 2 clearly defined zones: the central part, more red, fibrotic, and covered with hair shafts; and the peripheral zone, devoid of hairs and more atrophic, blending with the surrounding normal skin. At that time, of the 100 transplanted FUs (approximately 250 hairs), 67 FUs containing a total of 82 hairs were counted. This clinical appearance persisted until 7 months post-op, when the biopsies were obtained.

In Patient 2, again, hair shaft growth was exclusively noted only in the center of the scar while the periphery was absent of transplanted hairs (Figure 2E). After the transplantation of 400 grafts, only 75 growing hair shafts were counted at 11 months post-op.

Central and Peripheral Scar Areas Display Distinct Dermal Morphology and Collagen Composition

Biopsy and histology of the scar of Patient 1 revealed striking differences in the dermal morphology and collagen composition between the central (Figure 3A–D) and peripheral zones (Figure 3E–H). As expected from the clinical phenotype, the central scar zone was more fibrotic (Figure 3A–D), with thicker and more prominent collagen bundles (Figure 3C–D) compared with the scar periphery (Figure 3E–H). In addition, terminal HFs could be seen growing in the central scar area (Figure 3I).

Analysis of the predominant collagen types was consistent with fibrosis in the central area: excessive accumulation of fibrillar collagen Type I (Figure 4), but also a higher expression of collagen Type III (Figure 5).[23,24] COLIII is one of the dominant collagens alongside COLI during wound repair and plays a role in fibrillogenesis,[25] which further consolidates the difference in fibrotic scar tissue formation between the 2 areas. Moreover, quantification of collagen Type IV expression, which labeled the basal membrane of blood vessels, revealed a 35.5% higher vessel area in the central scar compared with the peripheral. The increased COLIV expression in the central area would be typical of early wound healing phases before vascular remodeling.[26,27]

Discussion

Follicular unit transplantation for wound healing applications is a relatively recent clinical development.[12–14] As further experience is gained in using this

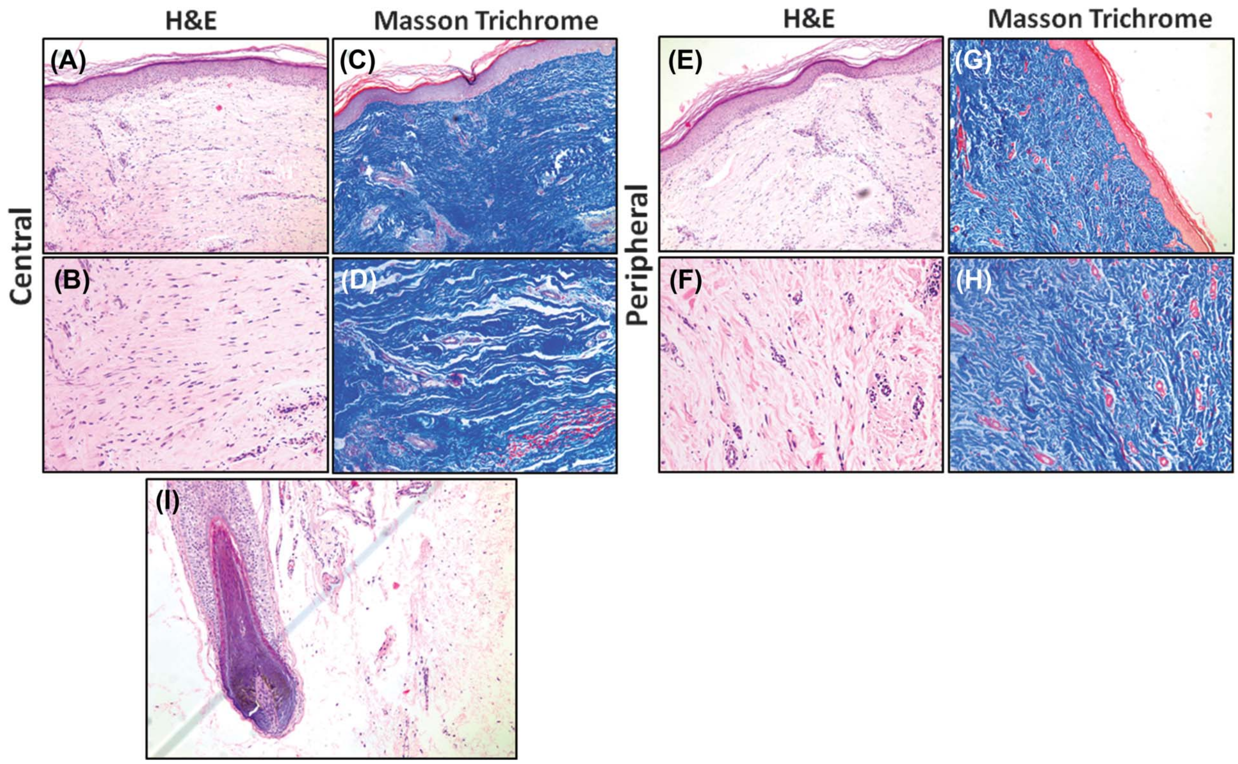

Figure 3. Differential dermal remodeling of the central and peripheral scar areas after follicular transplantation. H&E and Masson Trichrome staining revealed a difference in dermal remodeling in the central scar (A–D) in comparison with the peripheral scar (E–H). The central scar is more fibrotic (B) and contains visibly thicker collagen bundles (D), whereas the peripheral scar is less fibrotic (F) and collagen seems to be more fragmented (H). A transplanted anagen VI hair follicle remains in the central scar (I).

hair transplantation technique for treating ulcers and difficult-to-heal wounds, outcome measurements and clinical indications are becoming better defined. The current, independently observed and documented cases reported here once more underscore the utility of transplanting autologous hair follicle grafts for promoting the healing of chronic wounds. More importantly, however, our 2 cases also revealed a striking, location-dependent difference in the hair regrowth pattern between the central (hair-bearing) and peripheral (non–hair-bearing) zones of the postulcer scar. Although further observations of this nature are required before one knows how reproducible and robust this phenomenon is, our findings already raise biologically intriguing and clinically relevant questions.

In theory, the observed phenomenon might have reflected differences in wound healing–associated HF neogenesis.[28–30] However, the latter has never been conclusively demonstrated to occur in adult human skin and can therefore be ignored as an explanation here. On this basis, we hypothesize that these differences in hair growth and scarring patterns could be due to one or more of the following 3 reasons:

Different Inflammatory/Proteolytic/Wound Healing Signaling Milieu

In a chronically stressed tissue environment such as a nonhealing wound with its excess of reactive oxygen species and accumulation of toxins, coupled with hypoxia and a profoundly proinflammatory and proteolytic signaling milieu,[26,27] the peripheral and central portions of the wound likely show a very different inflammatory/proteolytic/wound healing signaling milieu. This may favor the transplanted HFs in the periphery to participate primarily in the more immediately pressing task of wound repair rather than in the less vital routine task of hair shaft formation (as previously proposed by Jahoda[31]). This would also explain the lower rate of transplanted hair shaft

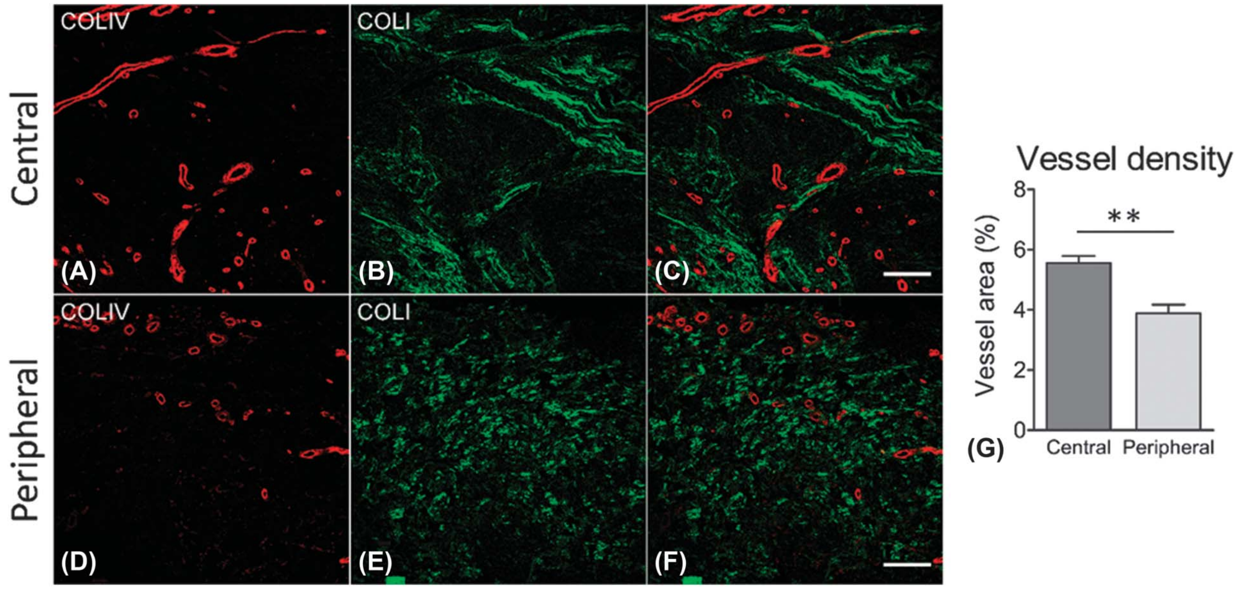

Figure 4. COLIV and COLI expression revealed a significant reduction in vessel density and structural differences of collagen in the peripheral scar area. COLI (green) second harmonic generation (SHG) imaging and COLIV (red) immunofluorescence in (A–C) central and (D–F) peripheral areas of the scar dermis (scale bars = 100 μm). Quantification of vessel density based on percentage vessel area after COLVI staining revealed a significant reduction in the peripheral scar area compared with the central. (G) Data shown as mean ± SEM (n = 5 microscopic fields); unpaired Student's t-test; $^{**}p \leq .01$.

regrowth seen (<50%) in comparison with what would be expected if the same number of hair grafts were transplanted in healthy skin (80%–100%).

Differences in Tension

Tissue tension differs markedly in the wound center from that in the wound margins, and this difference in wound tension creates a distinct Wnt signaling environment.[32] This may underlie the observed differences in regenerative versus scar-prone environments (namely growth of hair shafts and fibrotic skin)—possibly in a similar manner to what occurs in wound-induced HF neogenesis in rodent skin (WIHN), where HFs can regenerate de novo in the center of large mouse wounds as a result of Wnt signaling.[33,34]

Hair Follicle Stem Cell Exhaustion

Given that wound healing typically begins at the wound periphery and that this involves, at least in the later stages of wound healing, an activation and participation of HF-associated stem cells,[7,35] the transplantation of HFs is likely to impact on the normal healing process.[4,12] Therefore, HFs transplanted into the periphery of a chronic wound may receive distinct signals that provoke HFSC exhaustion as a reflection of maximal stem cell activation and recruitment to optimally promote wound healing.

Interestingly, the different hair distribution patterns in the central versus peripheral zones also correlated with a clear difference in the quality of the scar: the latter was more fibrotic in the center and almost scarless (nonvisible scar) in the periphery. Fibrosis may be seen as the result of a dysregulation in the coordination of normal tissue repair, in which mechanical cues and the extracellular matrix have a dominant instructive role in signaling for undue persistence of macrophages and myofibroblasts.[36] In mouse development, different fibroblast subpopulations are responsible for the switch from fetal scarless healing to the scar-prone healing of adult skin wounds.[37–39] Recent studies in human skin also highlight the existence of a (possibly profibrotic) dermal fibroblast subpopulation, characterized by the expression of SFRP2 and CD26 (DPP4).[40,41]

Therefore, it is tempting to speculate that HF transplantation, which provides a fresh source of hair follicle–associated dermal stem/progenitor cells[42] to the wound bed, may somehow induce such a switch in fibroblast subpopulations. Why this would happen at

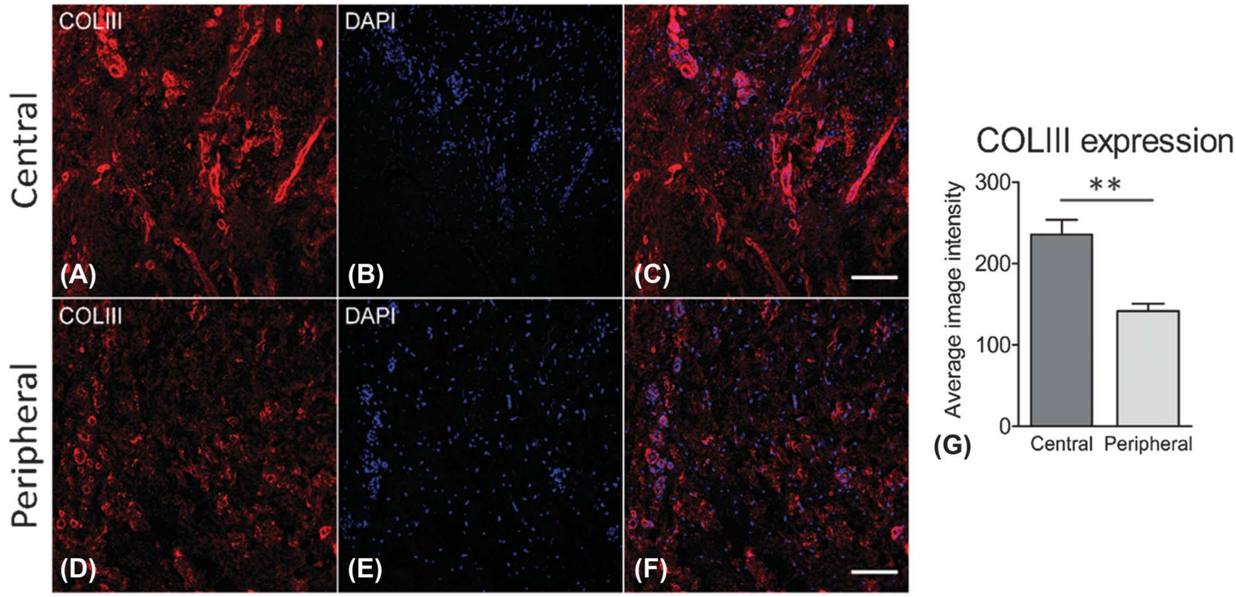

Figure 5. Significant reduction of COLIII expression in the dermis of the peripheral scar area. Immunofluorescence staining of COLIII in the dermis of central (A–C) and peripheral (D–F) scar area. Quantification of average pixel intensity across images revealed a significant reduction in the peripheral scar (G). Data shown as mean ± SEM (n = 5 microscopic fields analyzed); unpaired Student's t-test; $**p \leq .01$, scale bars = 100 μm.

the wound periphery but not at the wound center is currently unclear. However, as mentioned before, extracellular matrix composition, mechanical/tissue tension cues, and hypoxia differ between these biologically very distinct wound areas and may explain the different outcomes. On the basis of these considerations, we would like to suggest a novel concept of "plastic fibrosis," which can be conceptualized as a late granulation tissue/early fibrosis stage that may be specific for the very unique characteristics of the central hair-bearing area of the healed wound.

In summary, these 2 cases from 2 independent clinics and physicians reaffirm the clinical effectiveness of the still underutilized technique of hair follicle transplantation into nonhealing wounds to promote ulcer healing. Most importantly, the reported cases support the theory that the hair shaft regrowth process must be dictated by differences in the local microenvironment of the recipient zone, and that the functional differences in central versus peripheral healing ulcers are of much larger clinical relevance than we may have recognized so far. Once the molecular and cellular mechanisms that underlie the striking spatial dependence of hair regrowth and fibrosis patterns in distinct zones of healing ulcers reported here have become revealed, these may be directly targeted pharmacologically so as to render the future management of nonhealing wounds more efficient.

Acknowledgments The authors thank Prof. Juan Jose Cabrera-Galvan from the University of Las Palmas de Gran Canaria for help with histologic analyses and the Facility for Imaging by Light Microscopy (FILM) at Imperial College London for their support in obtaining SHG images.

References

1. Takeo M, Lee W, Ito M. Wound healing and skin regeneration. Cold Spring Harb Perspect Med 2015;5:a023267.
2. Ansell DM, Kloepper JE, Thomason HA, Paus R, et al. Exploring the "hair growth-wound healing connection": anagen phase promotes wound re-epithelialization. J Invest Dermatol 2011;131:518–28.
3. Garcin CL, Ansell DM. The battle of the bulge: re-evaluating hair follicle stem cells in wound repair. Exp Dermatol 2017;26:101–4.
4. Jimenez F, Poblet E, Izeta A. Reflections on how wound healing-promoting effects of the hair follicle can be translated into clinical practice. Exp Dermatol 2015;24:91–4.
5. Mecklenburg L, Tobin DJ, Müller-Röver S, Handjiski B, et al. Active hair growth (anagen) is associated with angiogenesis. J Invest Dermatol 2000;114:909–16.
6. Botchkarev VA, Botchkareva NV, Peters EM, Paus R. Epithelial growth control by neurotrophins: leads and lessons from the hair follicle. Prog Brain Res 2004;146:493–513.

7. Lau K, Paus R, Tiede S, Day P, et al. Exploring the role of stem cells in cutaneous wound healing. Exp Dermatol 2009;18:921–33.

8. Paus R. Migrating melanocyte stem cells: masters of disaster? Nat Med 2013;19:818–9.

9. Narushima M, Mihara M, Yamamoto Y, Iida T, et al. Hair transplantation for reconstruction of scalp defects using artificial dermis. Dermatol Surg 2011;37:1348–50.

10. Navsaria HA, Ojeh NO, Moiemen N, Griffiths MA, et al. Reepithelialization of a full-thickness burn from stem cells of hair follicles micrografted into a tissue-engineered dermal template (Integra). Plast Reconstr Surg 2004;113:978–81.

11. Zakine G, Mimoun M, Pham J, Chouat M. Reepithelialization from stem cells of hair follicles of dermal graft of the scalp in acute treatment of third-degree burns: first clinical and histologic study. Plast Reconstr Surg 2012;130:42–50e.

12. Martínez ML, Escario E, Poblet E, Sánchez D, et al. Hair follicle-containing punch grafts accelerate chronic ulcer healing: a randomized controlled trial. J Am Acad Dermatol 2016;75:1007–14.

13. Jiménez F, Garde C, Poblet E, Jimeno B, et al. A pilot clinical study of hair grafting in chronic leg ulcers. Wound Repair Regen 2012;20:806–14.

14. Fox JD, Baquerizo-Nole KL, Van Driessche F, Yim E, et al. Optimizing skin grafting using hair-derived skin grafts: the healing potential of hair follicle pluripotent stem cells. Wounds 2016;28:109–11.

15. Budamakuntla L, Loganathan E, Sarvajnamurthy SA, Nataraj HV. Follicular unit grafting in chronic nonhealing leg ulcers: a clinical study. J Cutan Aesthet Surg 2017;10:200–6.

16. Yang Z, Liu J, Zhu N, Qi F. Comparison between hair follicles and split-thickness skin grafts in cutaneous wound repair. Int J Clin Exp Med 2015;8:15822–7.

17. Jimenez F, Martínez ML, Escario E, Izeta A. The hair follicle as a wound healing promoter and its application in clinical practice. In: Turksen K, editors. *Wound Healing*. Hoboken: John Wiley & Sons, Inc; 2018; pp. 149–65.

18. Martínez Martínez ML, Escario Travesedo E, Jiménez Acosta F. Healing of recalcitrant chronic venous leg ulcers by punch grafts harvested from the scalp. Dermatol Surg 2015;41:1085–7.

19. Jiménez-Acosta F, Ponce-Rodríguez I. Follicular unit extraction for hair transplantation: an update. Actas Dermosifiliogr 2017;108:532–7.

20. Polanco TO, Xylas J, Lantis JC. Extracellular matrices (ECM) for tissue repair. Surg Technol Int 2016;28:43–57.

21. Turner NJ, Badylak SF. The use of biologic scaffolds in the treatment of chronic nonhealing wounds. Adv Wound Care 2015;4:490–500.

22. Zudaire E, Gambardella L, Kurcz C, Vermeren S. A computational tool for quantitative analysis of vascular networks. PLoS One 2011;6:e27385.

23. Klingberg F, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol 2013;229:298–309.

24. Karsdal MA, Nielsen SH, Leeming DJ, Langholm LL, et al. The good and the bad collagens of fibrosis—their role in signaling and organ function. Adv Drug Deliv Rev 2017;121:43–56.

25. Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care 2015;4:119–36.

26. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 2014;6:265sr6.

27. Eming SA, Tomic-Canic M. Updates in wound healing: mechanisms and translation. Exp Dermatol 2017;26:97–8.

28. Ito M, Yang Z, Andl T, Cui C, et al. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature 2007;447:316–20.

29. Gay D, Kwon O, Zhang Z, Spata M, et al. Fgf9 from dermal γδ T cells induces hair follicle neogenesis after wounding. Nat Med 2013;19:916–23.

30. Nelson AM, Loy DE, Lawson JA, Katseff AS, et al. Prostaglandin D2 inhibits wound-induced hair follicle neogenesis through the receptor, Gpr44. J Invest Dermatol 2013;133:881–9.

31. Jahoda CA, Reynolds AJ. Hair follicle dermal sheath cells: unsung participants in wound healing. Lancet Lond Engl 2001;358:1445–8.

32. Harn HI, Ogawa R, Hsu CK, Hughes MW, et al. The tension biology of wound healing. Exp Dermatol 2017 [epub ahead of print].

33. Plikus MV, Guerrero-Juarez CF, Ito M, Li YR, et al. Regeneration of fat cells from myofibroblasts during wound healing. Science 2017;355:748–52.

34. Horsley V, Watt F. Repeal and replace: adipocyte regeneration in wound repair. Cell Stem Cell 2017;20:424–6.

35. Gonzales KAU, Fuchs E. Skin and its regenerative powers: an alliance between stem cells and their niche. Dev Cel 2017;43:387–401.

36. Pakshir P, Hinz B. The big five in fibrosis: macrophages, myofibroblasts, matrix, mechanics, and miscommunication. Matrix Biol 2018;68-69:81–93.

37. Driskell RR, Lichtenberger BM, Hoste E, Kretzschmar K, et al. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature 2013;504:277–81.

38. Rinkevich Y, Walmsley GG, Hu MS, Maan ZN, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science 2015;348:aaa2151.

39. Jiang D, Correa-Gallegos D, Christ S, Stefanska A, et al. Two succeeding fibroblastic lineages drive dermal development and the transition from regeneration to scarring. Nat Cel Biol 2018;20:422–31.

40. Tabib T, Morse C, Wang T, Chen W, et al. SFRP2/DPP4 and FMO1/LSP1 define major fibroblast populations in human skin. J Invest Dermatol 2018;138:802–10.

41. Philippeos C, Telerman SB, Oulès B, Pisco AO, et al. Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. J Invest Dermatol 2018;138:811–25.

42. Agabalyan NA, Rosin NL, Rahmani W, Biernaskie J. Hair follicle dermal stem cells and skin-derived precursor cells: exciting tools for endogenous and exogenous therapies. Exp Dermatol 2017;26:505–9.

Address correspondence and reprint requests to: Francisco Jimenez, MD, Clínica Mediteknia, Av. Alcalde Ramírez Bethencourt 20, Las Palmas de Gran Canaria 35004, Spain, or e-mail: fjimenez@mediteknia.com