
Hair follicle—containing punch grafts accelerate chronic ulcer healing: A randomized controlled trial



María-Luisa Martínez, MD,^a Eduardo Escario, MD,^b Enrique Poblet, MD,^c David Sánchez, MS,^d
Fernando-Francisco Buchón, PhD,^d Ander Izeta, BSc, PhD,^e and Francisco Jimenez, MD^f
Albacete, Murcia, Valencia, San Sebastian, and Gran Canaria, Spain

Background: A prominent role of hair follicle—derived cells in epidermal wound closure is now well established but clinical translation of basic research findings is scarce. Although skin punch grafts have been used as a therapeutic intervention to improve healing of chronic leg ulcers, they are normally harvested from nonhairy areas, thus not taking advantage of the reported role of the hair follicle as a wound-healing promoter.

Objective: We sought to substantiate the role of hair follicles in venous leg ulcer healing by transplanting hair follicle—containing versus nonhairy punch grafts.

Methods: This was a randomized controlled trial with intraindividual comparison of hair follicle scalp grafts and nonhairy skin grafts transplanted in parallel into 2 halves of the same ulcer.

Results: Ulcer healing measured as the average percentage reduction 18 weeks postintervention was significantly increased ($P = .002$) in the hair follicle group with a 75.15% (SD 23.03) ulcer area reduction compared with 33.07% (SD 46.17) in the control group (nonhairy grafts).

Limitations: Sample size was small ($n = 12$).

Conclusion: Autologous transplantation of terminal hair follicles by scalp punch grafts induces better healing than punch grafts harvested from nonhairy areas. Hair punch grafting is a minimally invasive surgical procedure that appears to be effective as a therapeutic tool for chronic venous leg ulcers. (J Am Acad Dermatol 2016;75:1007-14.)

Key words: follicular unit; hair follicle stem cells; hair transplantation; punch grafting; skin grafting; venous leg ulcer; wound healing.

Numerous studies emphasize the role of hair follicle—derived cells in promoting the wound-healing response.¹⁻¹¹ Bishop¹² pioneer experiments demonstrated that re-epithelialization started around the remaining hair follicles, and that when the skin was destroyed down to the reticular dermis, the granulation

tissue originated from the connective tissue surrounding the follicles. However, despite decades of clinician awareness of the importance of hair follicles in wound healing, relatively few attempts have been made to use hair follicles or hair follicle—derived cells as a therapeutic tool in clinical practice.¹³⁻²¹

From the Department of Dermatology, General Hospital of Villarrobledo, Albacete^a; Department of Dermatology, Hospital General Universitario de Albacete and Universidad de Castilla La Mancha^b; Department of Pathology, Hospital General Universitario Reina Sofía de Murcia and Universidad de Murcia^c; Department of Ingeniería Cartográfica, Geodesia y Fotogrametría, Universidad Politécnica de Valencia^d; Instituto Biodonostia, Hospital Universitario Donostia, San Sebastian^e; and Mediteknia Dermatology Clinic, Medical Pathology Group, University of Las Palmas de Gran Canaria.^f

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Reprint requests: Francisco Jimenez, MD, Mediteknia Dermatology Clinic, Av. Alcalde Ramírez Bethencourt, 20, Las Palmas de Gran Canaria 35004, Canary Islands, Spain. E-mail: fjimenez@mediteknia.com.

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A surgical procedure normally used to treat non-healing venous leg ulcers is the autologous skin transplantation (skin grafting) in all its variations, including pinch grafts, punch grafts, and split-thickness and full-thickness skin grafts.²²⁻²⁵ However, none of these surgical methods were specifically aimed to include intact terminal hair follicles. In particular, punch graft harvesting has traditionally been performed using the thigh or buttocks²⁶⁻²⁸ where the skin graft is preferentially composed of epidermal and dermal tissue with an absence of terminal hair follicles.

A pilot study showed that hair scalp punch grafts transplanted into the wound bed of chronic leg ulcers stimulated healing in the majority of patients.¹⁸ Recently, a retrospective 14-case series supported those observations,²¹ showing total re-epithelialization when nonhealing ulcers were covered by hair follicle units, although assessment was difficult because it was not randomized and lacked a control group.

Here, the results of a randomized controlled trial comparing the healing capacity of hair follicle scalp grafts and nonhairy skin grafts are reported. To compare whether a difference in healing capacity exists, both types of grafts were transplanted into the same ulcer, at the same density, and using the same punch size.

METHODS

Study design

This study was designed as an open, single-center, randomized controlled trial with intraindividual comparison. Each patient had 1 leg ulcer that was divided into 2 halves of as similar a size as possible. To avoid bias ulcers were divided vertically in 2 halves because the venous return flows upwards from bottom to top. The 2 halves were randomized for each wound into an experimental and control area by a methodologist blinded to study data (Fig 1). The random allocation sequence was concealed from the surgeon until intervention. The experimental half was transplanted with 2-mm punch grafts harvested from the scalp, and the control half with 2-mm punch grafts from abdominal skin with no visible hairs.

To fix the references for each half throughout the duration of the study, an ink dot was tattooed onto

the proximal and distal points of a line that divided the ulcer into 2 halves (Fig 2). The number of grafts to be transplanted per ulcer depended on its size. Because each ulcer was divided into 2 equal-sized halves, the same number of grafts was implanted in each half with a density of 5 grafts/cm².

Patient eligibility

In all, 17 patients with chronic leg ulcers of venous origin that had not responded to topical medications and standard ambulatory medical care (leg elevation, compression therapy, debridement of devitalized tissue, occlusive dressings) for a duration longer than 4 months were examined. Patients with ulcers of other, nonvenous causes; aged younger than 18 years or older than

95 years; with diagnosed coagulopathies; having hip or knee prosthesis on the same limb; having ulcers with signs of active infection; with contraindications for the hair transplant technique; who were incapacitated to participate in the study; or who did not sign the informed consent form were not considered for this study. Five patients were excluded and 12 patients were included in the study.

The diagnosis of venous insufficiency was based on clinical and physical examination (telangiectasia or reticular veins, varicose veins, edema, red-to-brown skin discoloration, lipodermatosclerosis). All patients had severe chronic venous disease according to the Venous Clinical Severity Score.²⁹ A Doppler study was performed to rule out associated arterial disease. Only patients in whom the arterial peripheral system was not affected were included in this study.

Description of the intervention

All interventions were performed in an outpatient setting by the same dermatologic surgeon (F. J.) experienced in hair transplantation. After local anesthesia (lidocaine 1% and epinephrine 1/100,000; B. Braun, Melsungen, Germany), hairy and nonhairy grafts were harvested using 2.00-mm biopsy punches (GlaxoSmithKline, Bad Oldesloe, Germany). A slit was made in the wound bed with a needle and the grafts were completely inserted with a fine-tipped forceps leaving only the epidermis at the surface level of the ulcer. After the intervention,

CAPSULE SUMMARY

- Hair follicles play a role in wound healing. Punch grafts induce healing of chronic venous leg ulcers.
- Hair follicle-containing punch grafts accelerate wound closure as compared with nonhairy grafts.
- Punch grafts from the scalp appear to accelerate wound healing and represent a therapeutic option for chronic venous leg ulcers.

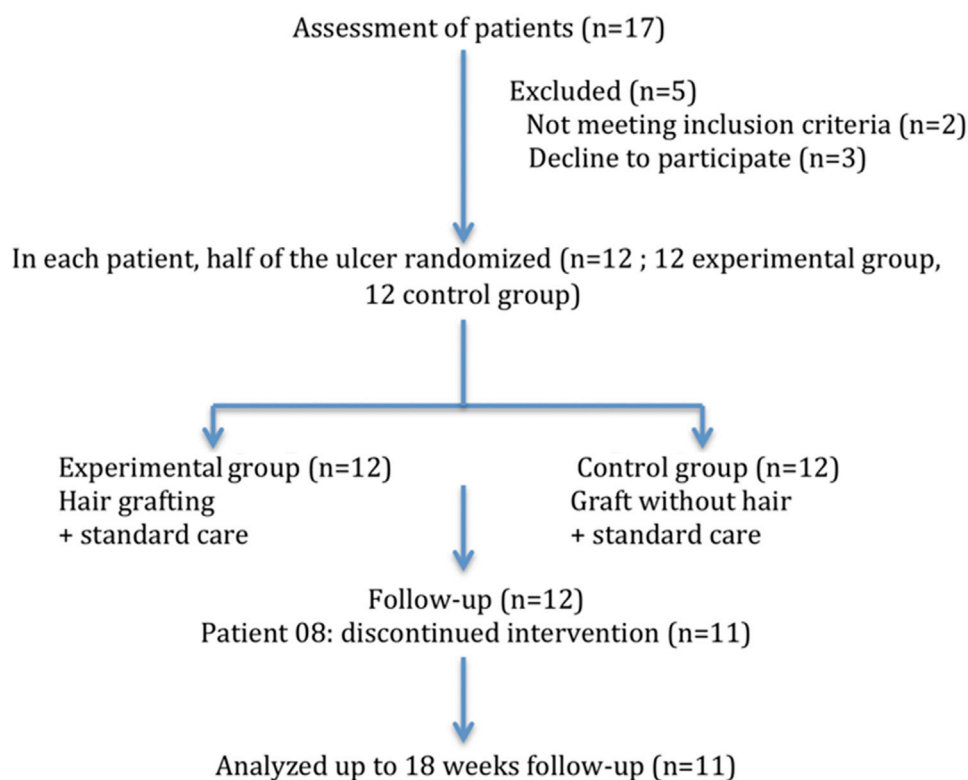


Fig 1. Trial design.

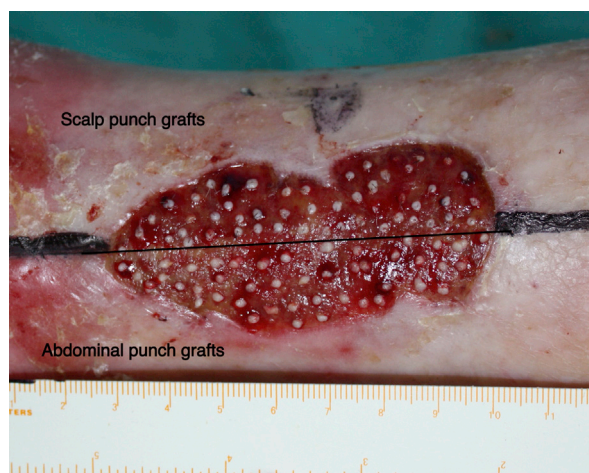


Fig 2. Black ink tattooed on the proximal and distal points of a line that divides the ulcer in 2 halves: experimental and control area.

the ulcer was covered with a nonadherent dressing (Mepitel, Mölnlycke, Goteborg, Sweden) and elastic bandage for 72 hours. Patients were instructed to maintain relative rest and reduce physical activity of the treated leg for the first 2 to 3 days.

Ulcer area measurements

The ulcers were photographed (digital IXUS 800 IS, Canon, Tokyo, Japan) alongside a millimeter ruler to assist in the calibration. Digital photographs were taken twice weekly for 18 weeks. Two dermatologists measured the areas of the ulcers independently. It was impossible to blind the fact that the dermatologists knew if the area being measured was the one that contained scalp hair punches or nonhairy punches because the hair shafts from the scalp punches were visible while doing the assessment. A virtual line that demarcated the external border of the ulcer was traced using software (Photoshop, CS6 extended, Adobe Systems Inc, San Jose, CA). Software (ImageJ; National Institutes of Health, Bethesda, MD)³⁰ was used to perform all area measurements.

Outcome measurement

The experimental and control areas were measured weekly until the end of the study. The primary outcome measurement was ulcer healing measured as the average percentage reduction in the experimental and control areas 18 weeks postintervention (end point of the study).

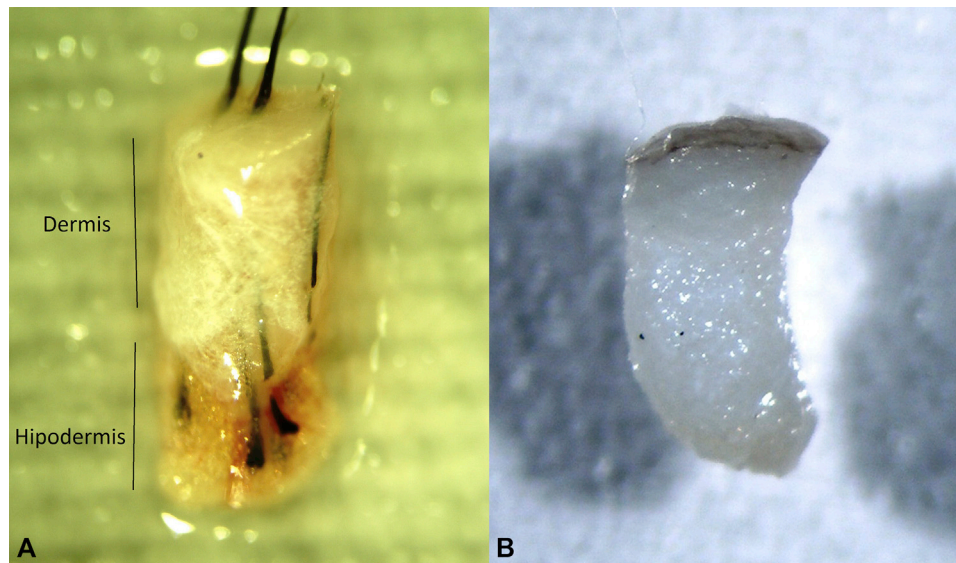


Fig 3. Harvested 2-mm punch grafts. **A**, From the scalp. Note the presence of several terminal hair follicles per graft that root deep into the subcutaneous tissue (depth 4.3 mm). **B**, From abdominal skin (depth 3.03 mm).

Secondary outcomes included the appearance of granulation tissue, wound border reactivation, and regular interviews to record symptoms such as pain.

Statistical methods

Intention-to-treat analyses were performed. The Student *t* test was used to compare the 2 treatment groups. When one group of data did not follow a normal distribution, medians were compared using the nonparametric Mann-Whitney test. A *P* value less than .05 was considered as significant and *P* less than .01 as very significant. Software was used (SPSS, Version 20 for Macintosh; IBM Corp, Armonk, NY).

Standards and ethics compliance

The standards of the Helsinki Declaration, all relevant Spanish legislation, and Good Clinical Practice regulations were followed. Approval from the regional and university ethics committee was obtained.

RESULTS

Patients had a mean age of 73 years; 33.3% were men and 66.7% were women. Average initial ulcer size was 23.34 cm². Average initial size for the experimental group was 11.35 cm² and 11.99 cm² for the control group. Average duration of the ulcers before surgery was 6 years.

Morphological differences of the punch grafts to be transplanted

Macroscopically, scalp punch grafts were composed of epidermis, dermis, terminal hair

follicles, and subcutaneous fat (Fig 3, A), whereas abdominal punch grafts were composed of epidermis, dermis, and small fragments of subcutaneous fat with no visible terminal hairs (Fig 3, B). Accordingly, the main difference between the 2 halves of the treated ulcers was that terminal hair follicle grafts were added to the experimental half and not to the control half.

Primary outcome

A significant 75.15% (SD 23.03) ulcer area reduction in the experimental group compared with 33.07% (SD 46.17) in the control group (*P* = .002) was found at end point (Figs 4 and 5).

At the end of the study, the mean reduction in total ulcer area (both halves taken together) was 55.69% (SD 29.19) (*P* < .001), and in absolute numbers 12.99 cm² (SD 14.29) (*P* = .013). Maximum reduction in total ulcer area was observed at week 18 in both the experimental and the control group.

Secondary outcomes

By comparing photographic images, improvement granulation tissue and wound border reactivation was seen in 100% of patients. In general, more granulation tissue was formed over the course of the study in the experimental than in the control half.

A numerical scale was used to assess the degree of pain (1 was no pain and 5 was worst pain imaginable). All patients reported that pain was reduced after the intervention both during wound care and daily life.

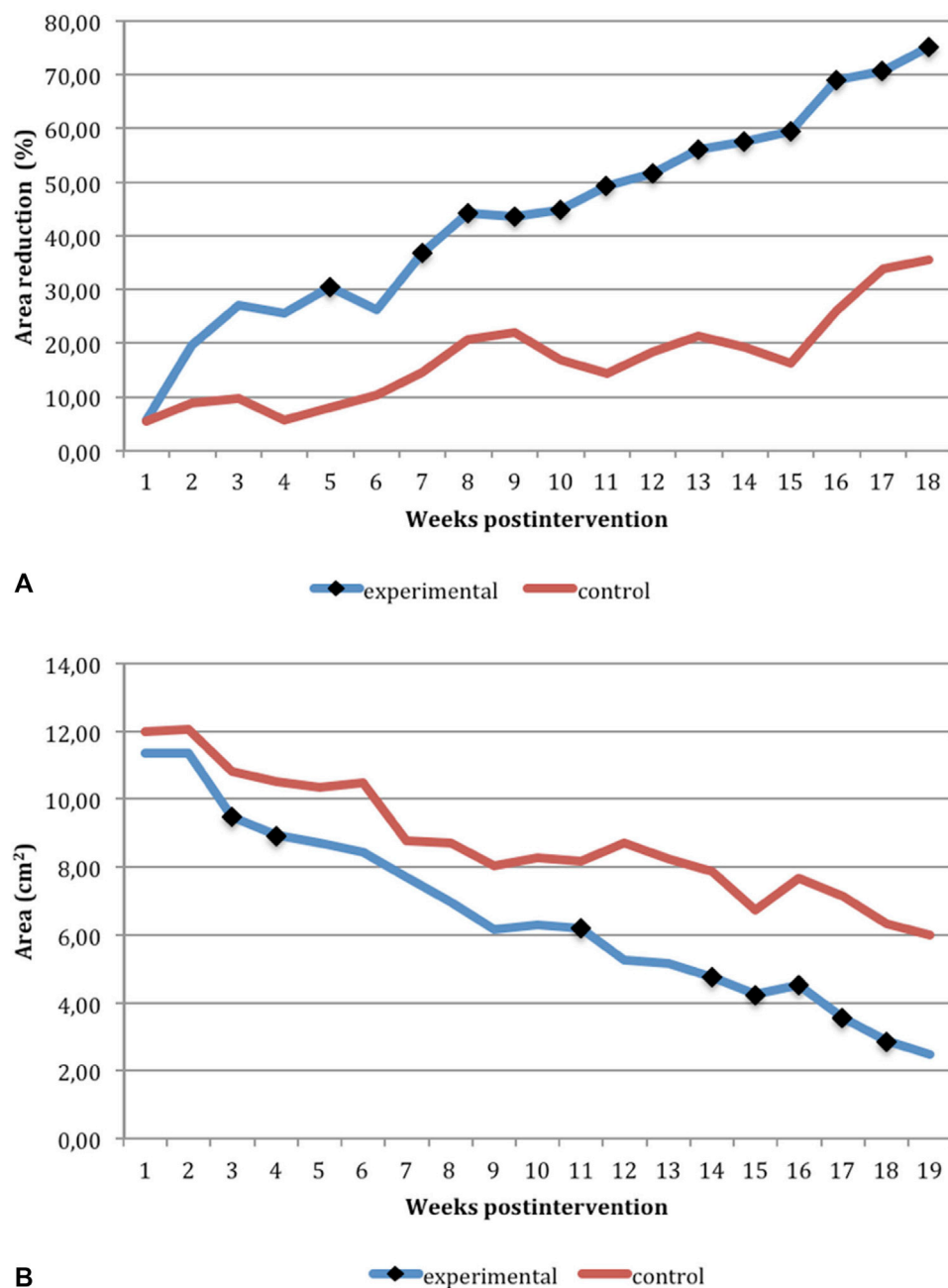


Fig 4. Evolution of experimental and control areas throughout the study. **A**, Average area reduction as a percent of baseline, 0-18 weeks postintervention. The rhombus indicates the weeks in which the difference was significant (weeks 5, 7, and 10, $P < .05$; weeks 8-9 and 11-18, $P < .01$). **B**, Expressed as mean reduction (cm^2) compared with the initial measurement, 0-18 weeks postintervention. The rhombus indicates the weeks in which the difference was significant, $P < .05$.

Complications and side effects

No major complication was reported during the study. One patient (patient 8) discontinued the study because of an intestinal perforation unrelated to the trial. Infectious complications occurred in 2 patients (patients 3 and 8), with resolution after antibiotic administration.

Hair shafts persisted in the ulcer an average of 8.75 weeks (SD 3.49) before falling out.

DISCUSSION

This study demonstrates that the half of the ulcer transplanted with scalp punch grafts experienced a



Fig 5. Digital photographs of patient 3 at weeks 0, 4, 8, 12, 15, and 18 postintervention. The line divides the ulcer in the experimental and control area. Scalp punch grafts transplanted into the upper half of the ulcer and abdominal skin grafts transplanted into the lower half.

statistically significant better outcome than the half transplanted with nonhairy grafts.

The fact that follicle-rich scalp punches improve wound healing reflects the clinical translation of the latest discoveries in hair follicle biology related to wound healing.¹⁻¹¹ Considering that the hair follicle is a well-known reservoir of several types of stem

cell,^{1,4,31,32} the transplantation of hair follicles in the wound bed provides a new supply of epithelial (bulge) and mesenchymal (dermal sheath) stem cells.

The observation of increased granulation tissue formation in the ulcer half transplanted with hair grafts could be attributed to the proliferation/migration of

the perifollicular dermal sheath and a hair follicle—promoted increase in vascularization and innervations of the wound bed.¹⁹ New evidence supports that upon wounding the perifollicular dermal sheath mesenchymal cells proliferate and differentiate into myofibroblasts, which migrate to the wound bed and participate in the dermal repair process.³³⁻³⁶

Another theoretical advantage of having the scalp as a source for skin harvesting is that the majority of scalp hair follicles (approximately 85%) are in anagen phase at any given time³⁷ and wounds heal faster when made on skin with hair follicles in anagen as opposed to telogen.⁶ Therefore, not only is the scalp ideal as a donor area because it heals faster,³⁸ but also a punch containing mainly anagen hairs would be ideal to boost the healing response in the recipient wound.

Regarding the manipulation of the grafts during implantation, their complete insertion into the wound bed enhances its interaction with the dermal tissue of the wound and increases the chance of graft take. An important difference between scalp and nonhair-bearing grafts is that although harvested with the same punch size (2 mm), the scalp punches had more tissue volume because the roots of the terminal hairs were located at a depth of 4 to 5 mm and included more subcutaneous tissue around the follicles. Consequently, the graft implantation is technically easier because the stiffness of the scalp graft facilitates its full insertion into the wound bed. In addition, the greater tissue volume of the scalp graft does not allow one to discount the possibility that other nonfollicular adult stem cells (eg, adipose-derived stem cells) could be responsible for the increased healing response.

One question often asked is what happens to the growth of hair shafts in the ulcer after the transplantation process. In hair transplantation, hairs are normally shed after 3 to 4 weeks, and 80% to 100% of the transplanted hairs grow back 3 to 4 months after transplantation.^{39,40} However, we have observed that the hairs transplanted in the ulcer grow in far less quantity than would be expected in a normal hair transplantation procedure, an observation also reported by Liu et al.²¹ Hair surgeons have long known that the recipient area has an influence on hair growth and it would appear that in the case of nonhealing wounds, the influence is a negative one.⁴¹ A possible explanation is that the microenvironment of an injured wound bed sends molecular cues to direct the transplanted follicles to provide cells for repairing the wound and not for hair shaft production. This idea, originally proposed by Jahoda and Reynolds,³³ argues that the cellular

machinery of the hair follicle has a choice between trying to regenerate a new follicle or participating in wound healing, depending on environmental factors.³ Although difficult to reach definitive conclusions because of the limited number of patients, it seems that this hypothesis holds true.

In conclusion, this study provides further evidence that autologous transplantation of scalp punch grafts is a minimally invasive procedure that appears to be effective as a therapeutic tool for chronic venous leg ulcers, and induces better healing than skin grafts harvested from nonhairy areas. The results support the notion that an important factor in relation to wound-healing stimulation is the presence of terminal hair follicles in the scalp punch graft. More studies need to be performed to investigate the utility of scalp punch grafts in other types of chronic ulcer conditions.

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