https://doi.org/10.15446/cr.v6n2.83815

TREATMENT WITH TYPE-I COLLAGEN SCAFFOLDS IN PATIENTS WITH VENOUS ULCERS. CASE REPORT

Keywords: Venous Ulcer; Collagen Type I; Regeneration.
Palabras clave: Úlcera venosa; Colágeno Tipo I; Regeneración.

Martha Isabel González-Duque
Julián Daniel Hernández-Martinez
Marta Raquel Fontanilla
Universidad Nacional de Colombia
- Bogotá Campus - Faculty of Science -
Department of Pharmacy - Tissue Engineering
Working Group - Bogotá D.C. Colombia.

Sofía Elizabeth Muñoz-Medina
Universidad Nacional de Colombia
- Bogotá Campus - Faculty of Medicine -
Health Technology and Policy Evaluation Group
- Bogotá D.C. - Colombia.

Corresponding author
Marta Raquel Fontanilla. Department of Pharmacy,
Faculty of Science,
Universidad Nacional de Colombia.
Email: mrfontanillad@unal.edu.co

Received: 01/12/2019 **Accepted:** 06/02/2020

RESUMEN

Introducción. La insuficiencia venosa crónica afecta alrededor del 5% de la población adulta en el mundo; una de sus mayores complicaciones son las úlceras en miembros inferiores, las cuales tienen una prevalencia mundial del 2%. Las úlceras afectan la calidad de vida de los pacientes e impactan al sistema de salud debido a los altos costos de atención que genera. El tratamiento de referencia es la terapia compresiva y la cura húmeda de las heridas, sin embargo estas intervenciones pueden no ser efectivas cuando las lesiones se complican.

Presentación del caso. Paciente femenina de 66 años con úlceras venosas en miembros inferiores acompañadas de fiebre y dolor local que no respondían a las terapias convencionales. La paciente fue tratada con un nuevo sustituto dérmico basado en una membrana acelular de colágeno tipo I que contribuye al cierre de la úlcera al estimular el remplazo del tejido lesionado por tejido similar al sano, con lo cual tuvo mejoría a las 16 semanas; después de 8 meses de terminado el tratamiento no se presentó recurrencia de las lesiones.

Conclusiones. La membrana acelular de colágeno tipo I desarrollada por el Grupo de Trabajo en Ingeniería de Tejidos del Departamento de Farmacia de la Universidad Nacional de Colombia es efectiva en el tratamiento de úlceras venosas en miembros inferiores y su bajo costo facilita el acceso de toda la población a terapias basadas en su aplicación.

ABSTRACT

Introduction: Chronic venous insufficiency affects about 5% of the global adult population. Venous leg ulcers are one of the most frequent complications of this pathology, with a global prevalence of 2%. This disease affects both the quality of life of patients and, due to the high cost of the treatment, the health system. Compressive therapy and moist wound healing have been the gold standard treatment. However, when complications occur, they may not be effective.

Case report: This is the case of a 66-year-old female patient with venous ulcers on her lower limbs and symptoms of fever and local pain that did not respond to conventional therapies. The patient was treated with a new dermal substitute made of an acellular type-I collagen membrane, which promotes the closure of the ulcer by stimulating the replacement of injured tissue with tissue similar to the healthy one. The condition of the patient improved at 16 weeks, and after 8 months of treatment there was no recurrence of the lesions.

Conclusions: Acellular type-I collagen membrane developed by the Tissue Engineering Working Group of the Department of Pharmacy of the Universidad Nacional de Colombia is effective in treating venous ulcers of the lower limbs. Its low cost facilitates the access of the whole population to therapies based on its application.

INTRODUCTION

Venous ulcers occur due to a venous valve insufficiency that causes venous hypertension, which generates hypervolemia (predominantly in the lower limbs) associated with lipodermatosclerosis and skin ulcers. In turn, the physiopathology of valve insufficiency is associated with an increase in the number of fibrin degradation products and pro-inflammatory

molecules in the dermis, leading to cell lysis and ulceration. (1)

The prevalence of chronic venous insufficiency ranges between 5% and 30% in the adult population, (2) and this condition is considered a public health problem due to high treatment costs and the negative impact it has on the quality of life of patients suffering from it. (3)

Available treatments for venous ulcers are based on compression measures, which improve venous return, and hydrocolloids that promote tissue repair. (4) However, these therapies often do not satisfy patients' needs, leading to therapeutic failure and recurrence. (5) To address this situation, the Tissue Engineering Working Group of the Department of Pharmacy of the Universidad Nacional de Colombia developed a sterile porous type-I collagen membrane of bovine origin approved by INVIMA through the Sanitary Registration Number No. 2017DM-0015999. (6) The membrane acts as a dermal substitute and promotes the replacement of injured tissue with healthy tissue similar to the demarcated borders of the wound. Besides, it has the advantage of being a low-cost and affordable product for any patient.

The present work reports the case of a patient who received the membrane designed at the Universidad Nacional de Colombia to treat two venous ulcers of her lower limbs with a satisfactory outcome.

CASE PRESENTATION

The following is the case of a 66-year-old female patient, mestizo, with basic secondary education, from the municipality of Soacha (Cundinamarca, Colombia), of low socioeconomic status, who was working as a caregiver for an elderly person. The patient attended a consultation due to having 2 ulcerated lesions in her lower limbs (the lower third of the right lower limb and the medial region in the malleolus of the left lower limb) for 12 months. The affected areas presented with erythema, local heat, ulceration and seropurulent secretion.

The patient reported the following history: personal: high blood pressure (in treatment with nifedipine 30 mg/12 hours orally) and venous insufficiency; surgical: hallux valgus correction, ligament reconstruction and second toe arthroplasty; gynecological-obstetric: menarche at age 15, 2 births, 0 abortions and menopause at age 58, and family: sibling diagnosed with high blood pressure. During the review of systems, cough and runny nose were observed. The patient was diagnosed with infected venous ulcers and was prescribed outpatient treatment with clindamycin (600 mg/day) and oral diclofenac (50 mg/12 hours). However, the symptoms did not improve.

After 2 weeks, she presented a 3-day episode of fever with intense pain in the lower limbs, predominantly on the right leg at the inner edge of the gastrocnemius muscle area, with limited walking and altered sleep pattern due to pain intensity. Therefore, she consulted the emergency department where she was evaluated by the general medicine service. On physical examination, the physician reported a medial ulcer in the malleolus (2cm x 2.5cm) of the lower right limb with well-defined borders, fibrin mesh, an area of necrosis, a perilesional edema and yellow discharge, and a satellite lesion (approximately 1cm deep) with fibrin mesh and irregular borders in the lower left limb.

Pedal pulses and ochre dermatitis were observed in both affected limbs. Considering the fever, the local pain, the functional limitation, and the characteristics of the injuries, the patient was admitted to the hospital for 20 days with a diagnosis of superinfected ulcers in the lower limbs and suspicion of osteomyelitis. The patient received antibiotic treatment with

trimethoprim/sulfamethoxazole (80+400mg in 5 mL vial, 2 vials intravenously every 12 hours) and clindamycin (600mg intravenously every 6 hours).

After osteomyelitis was ruled out, the woman was discharged with a confirmed diagnosis of superinfected venous ulcers and was prescribed with ciprofloxacin (500mg/12 hours for 7 days), 7 sessions of wound care at the wound clinic, and 5 sessions of rehabilitative physical therapy. In the wound clinic, the patient was treated with a medium stretch bandage for 2 months and then a hydrocolloid patch was applied for 1 month maintaining compression measures; however, no improvement was achieved with this treatment either. The woman consulted again due to the persistence of the lesions and yellow secretion; on that occasion, she was treated with erythromycin (500mg/12 hours for 10 days).

Given the persistence of the symptoms, the patient contacted the Tissue Engineering Working Group of the Universidad Nacional de Colombia with the aim of finding a solution to her medical problem. The group provided care and the doctor continued with the antibiotic treatment (which was on the fifth day at that point) until completing the 10-day cycle. He also recommended starting care with a dermal substitute based on porous type-I collagen scaffolding once the cycle was completed. The findings of the physical examination are presented in Table 1.

Table 1. Physical examination.

Assessment	Finding
Height	158cm
Weight	60kg
Body mass index	24
Blood pressure	140/70 mmHg
Heart rate	70 beats per minute
Respiratory rate	19 breaths per minute
Temperature	35.2°C
Head and neck	No alterations
Cardiorespiratory system	Well-ventilated lung fields without over-abundant noise and rhythmic heart sounds without murmurs
Abdomen	No alterations
Limbs	Left lower limb: ulcer of 1.5 cm^2 with necrotic edge and yellow secretion, fibrin background, erythema and perilesional skin. Right lower limb: ulcer of 7.5 cm^2 with indurated edge with yellow secretion and fibrin mesh.
Skin	Ochre-colored hyperpigmentation in lower limbs at middle third level and lesions described above.

Source: Own elaboration.

From the moment the ulcerous lesions ocurred and the patient was admitted to the hospital due to an infection of 20 days of evolution, 12 months had passed. She received 2 cycles of wound care, one for 2 months and another for 1 month, yet she developed a new superinfection, which

was treated for 10 days. Given the persistence of the ulcers, a new therapeutic management was considered, which began 10 days after the last superinfection took place. Data from the patient's laboratory tests at the beginning of treatment are shown in Table 2.

Table 2. Laboratory tests

Test	Results
Glycemia	104.7 mg/dL
Creatinine	0.81 mg/dL
Blood urea nitrogen	21.7 mg/dL
C-reactive protein	<6 mg/L
Erythrocyte sedimentation rate	12 mm
Hematocrit	44.5%
Hemoglobin	15.1 g/dL
Monocytes	5.3%
Neutrophils	72.9%
Platelets	488 x 103µL
Leucocytes	21.8%
Soft tissue ultrasound of the lower limbs	Inflammatory process of the soft tissues in the distal third of the legs with predominance in the right limb. No associated masses or focal lesions.
X-ray of the neck of the left foot	Osteopenia with no evidence of developmental or traumatic bone injury. Preserved joint relationships and evidence of soft tissue edema around the malleolus and calcaneal spur.
Arterial doppler ultrasound of the lower limbs	Study within normal limits for the patient's age. No evidence of hemodynamically significant arterial injury.
Lower limb venous Doppler	Valvular incompetence of the greater saphenous vein on both sides, including the saphenous arch, and incompetence of the left small saphenous vein.

Source: Own elaboration.

Diagnostic assessment

The patient was diagnosed with venous ulcers in the lower limbs and essential (primary) hypertension. Since she presented with superinfected ulcers, her prognosis was poor, and her condition did not improve after two conventional treatments, the management of her condition was considered to be challenging. One week after the antibiotic cycle established to control the infection was finished, a weekly wound care session using the acellular type-I dermal collagen substitute developed by the Universidad Nacional de Colombia was scheduled.

Therapeutic management

It should be noted that it was necessary to debride the ulcerated lesions. During the first four sessions, the ulcers were moistened with phosphate-buffered saline solution or autologous platelet-rich plasma to promote granulation. Then the acellular dermal substitute, adapted to fit the lesions, was placed in the bleeding bed. After the wound was covered with a vaseline gauze dressing and the dressing was secured with a hypoallergenic adhesive made of polyacrylate and polyester, a medium compression bandage was applied.

Follow-up and outcomes

The patient underwent 16 wound care sessions, achieving complete closure of both wounds. During the clinical follow-up, photographic records of the ulcers were taken to evaluate the characteristics of their evolution:

Figure 1 shows the evolution of the right lower limb ulcer: one week after the treatment with the acellular dermal membrane was started, it was possible to observe granulation tissue, defined borders, erythema, and absence of exudate

(Figure 1A). After 11 weeks of treatment, the injured area was completely epithelialized but there were still remnants of granulation tissue in the central region of the ulcer. At that point, the size of the ulcer had gone from 7.5cm^2 (initial size) to 0.56cm^2, that is, it had decreased by 91% (Figure 1B). At 16 weeks, complete epithelialization of the ulcerated lesion and mild hypopigmentation of the newly formed tissue was observed (Figure 1C). After 8 months of treatment, the appearance of the area indicated that the closure of the ulcer was maintained (Figure 1D).

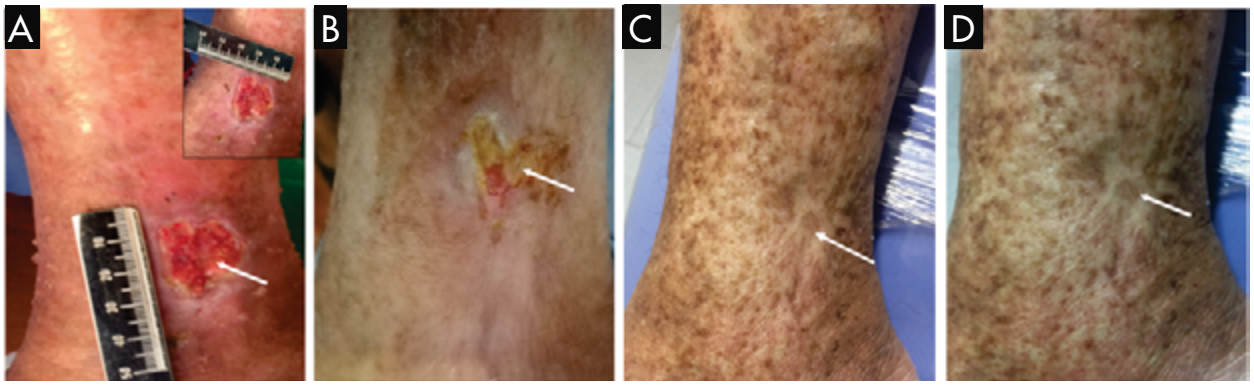

Figure 1. Clinical follow-up of right lower limb ulcers. A) 2 weeks of treatment; B) 11 weeks of treatment; C) 16 weeks of treatment; D) 8 months after treatment was completed.

Source: Own elaboration.

Figure 2 shows images of the evolution of the lower left limb: one week after the first session, an apparent decrease in depth was evident, as well as abundant granulation tissue, defined borders, absence of exudate and integrity of the perilesional area (Figure 2A). After 3 weeks of treatment, the size of the ulcer had gone from 1.5cm^2 (initial size) to 0.04cm^2, that is, it had decreased by 96% (Figure 2B). After 4 weeks, the wound was closed definitively (Figure 2C).

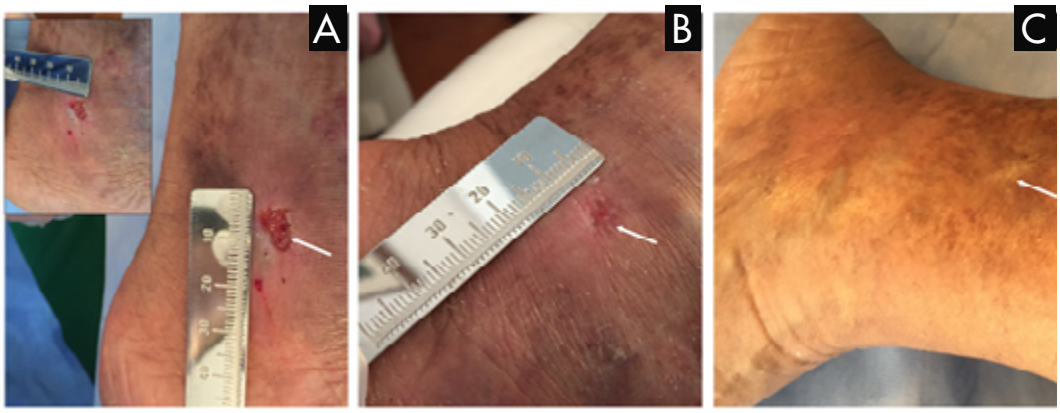

Figure 2. Clinical follow-up of ulcers in the lower left limb. A) 2 weeks of treatment; B) 3 weeks of treatment; C) 4 weeks of treatment.

Source: Own elaboration.

Throughout the follow-up, adherence to the treatment was complete: the patient attended all wound care sessions and always wore the compression bandage when she attended the control appointments. No adverse events associated with the intervention were reported. High compression stockings were indicated after ulcer closure.

DISCUSSION

Due to the length of the wound care sessions, venous ulcers in the lower limbs impact the cost of care and quality of life of people who suffer them. (7,8) These lesions can become chronic due to the inflammatory response, which is secondary to venous hypertension and caused by microcirculation damage. (9) Short- and long-term complications of venous ulcers are common due to local or systemic infections and the formation of new ulcers in the perilesional tissues. (10)

So far, there is no consensus on the most appropriate treatment for venous ulcers, and the devices used for wound care do not always promote closure of the lesion and regeneration of the skin. (11,12) Type-I collagen, the most abundant extracellular protein in the dermis, plays a key role in skin lesions as it guides cell migration, promotes the proliferation and differentiation of fibroblasts and keratinocytes, and sequesters and releases controlled growth factors that modulate wound closure and accelerate angiogenesis. (13)

The Tissue Engineering Working Group of the Universidad Nacional de Colombia developed some type-I membranes or acellular collagen scaffolds that have helped close connective tissue wounds and have favored the regeneration of skin and oral mucosa wounds in preclinical models. (13-16) To document their performance in cruciate areas, these membranes were used to treat two venous ulcers of different sizes in one patient. The closure time of the ulcers depended on the initial area of each lesion: the complete epithelization of the largest wound occurred at 16 weeks, while the smallest ulcer closed at 4 weeks. Overall, the data suggest that the product evaluated promotes the closure of the operated ulcers by stimulating the formation of tissue with characteristics similar to those of the surrounding healthy tissue, which, in turn, suggests it stimulates skin skin regeneration in the affected areas.

The main limitation of this case study is that it shows the data obtained during the treatment of two lower limb ulcers in the same patient; consequently, the results only suggest that debridement, weekly application of acellular dermal membranes and moderate compressive therapy promote the closure of lower limb venous ulcers and favor tissue regeneration over contracture repair. Therefore, in order to demonstrate the benefits of this therapeutic option, a clinical study of its safety and efficacy should be carried out with a representative sample that allows that allows drawing conclusions about its effects on the closure and healing of venous ulcers.

CONCLUSION

The application of acellular type-I collagen membrane in debrided and bleeding ulcers, together with compression measures and basic care, facilitated the closure of the lesions. The quality of the tissue formed and the non-recurrence of the ulcers after 8 months of treatment indicate that the product developed by the Tissue Engineering Working Group of the Department of Pharmacy at the Universidad Nacional de Colombia favors tissue regeneration and helps prevent scar contracture.

PATIENT'S PERSPECTIVE

After completing the treatment, the patient reported a subjective improvement of 80% in her

quality of life. At that time, the SF-36 quality of life questionnaire was administered and in the eight domains evaluated (vitality, physical functioning, pain, general health perception, physical role functioning, social role functioning, emotional role functioning and mental health) a score similar to that of patients without venous ulcer was obtained. (17,18) In addition, the patient stated that she could now perform activities that the ulcers had prevented her from doing, such as moving around without pain and bathing in the sea.

At the time of preparation of this case report, the woman was still wearing high-compression stockings and was assessed by the vascular surgery service for the treatment of venous insufficiency. The peripheral vascular surgeon who treated her indicated the need to perform a saphenectomy. Twenty months after completing the treatment with acellular type-I collagen scaffolds, the patient said she was satisfied since the venous ulcers had not reappeared.

INFORMED CONSENT

At the beginning of the treatment, the patient was informed of the possible adverse events that could be caused by the therapy. Likewise, all the details described in the report were explained to her and she was asked to sign to sign an informed consent form. The witness and the treating physician-investigator also signed it.

CONFLICT OF INTEREST

The Tissue Engineering Working Group declares a conflict of interest since it developed the new acellular type-I collagen dermal substitute used in this case.

FUNDING

Supplies for wound care were provided by the Tissue Engineering Working Group.

ACKNOWLEDGEMENTS

To Dr. Juan Bobadilla for his suggestions and comments.

REFERENCES

1. **Raffetto JD.** Pathophysiology of Chronic Venous Disease and Venous Ulcers. *Surg Clin North Am.* 2018;98(2):337-47. http://doi.org/gdcwqp.
2. **Eberhardt RT, Raffetto JD.** Chronic venous insufficiency. *Circulation.* 2014;130(4):333-46. http://doi.org/ds5p.
3. **Epstein D, Gohel M, Heatley F, Davies AH.** Cost-effectiveness of treatments for superficial venous reflux in patients with chronic venous ulceration. *BJS open.* 2018;2(4):203-12. http://doi.org/ds5q.
4. **Pinheiro L da S, Borges EL, Donoso MTV.** Uso de hidrocolóide e alginato de cálcio no tratamento de lesões cutâneas. *Rev Bras Enferm.* 2013;66(5):760-70. http://doi.org/ds5r.
5. **Lurie F, Bittar S, Kasper G.** Optimal Compression Therapy and Wound Care for Venous Ulcers. *Surg Clin North Am.* 2018;98(2):349-60. http://doi.org/gf2qzq.
6. **Suesca E, Dias AMA, Braga MEM, de Sousa HC, Fontanilla MR.** Multifactor analysis on the effect of collagen concentration, cross-linking and fiber/pore orientation on chemical, microstructural, mechanical and biological properties of collagen type I scaffolds. *Mater Sci Eng C Mater Biol Appl.* 2017;77:333-41. http://doi.org/gbjw97.
7. **Wittens C, Davies AH, Bækgaard N, Broholm R, Cavezzi A, Chastanet S, et al.** Editor's Choice - Management of Chronic Venous Disease : Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg.* 2015;49(6):678-737. http://doi.org/f3pgx8.
8. **Edwards H, Courtney M, Finlayson K, Shuter P, Lindsay E.** A randomised controlled trial of a community nursing intervention: improved quality of life

and healing for clients with chronic leg ulcers. *J Clin Nurs.* 2009;18(11):1541-9. http://doi.org/bnhp4f.

9. **Crawford JM, Lal BK, Durán WN, Pappas PJ.** Pathophysiology of venous ulceration. *J Vasc Surg Venous Lymphat Disord.* 2017;5(4):596-605. http://doi.org/ds5x.
10. **European Wound Management Association.** Position Document: Identifying criteria for wound infection. London: Medical Education Partnership LTD; 2005 [cited 2018 Oct 10]. Available from: https://bit.ly/35ckXz1.
11. **Andriessen A, Apelqvist J, Mosti G, Partsch H, Gonska C, Abel M.** Compression therapy for venous leg ulcers: risk factors for adverse events and complications, contraindications - a review of present guidelines. *J Eur Acad Dermatology Venereol.* 2017;31(9):1562-8. http://doi.org/gbv2c4.
12. **Evidence-based (S3) Guidelines for Diagnostics and Treatment of Venous Leg Ulcers.** *J Eur Acad Dermatology Venereol.* 2016;30(11):1843-75. *http://doi.org/ds5z.*
13. **Millán D, Jiménez RA, Nieto LE, Linero I, Laverde M, Fontanilla MR.** Preclinical evaluation of collagen type I scaffolds, including gelatin-collagen microparticles and loaded with a hydroglycolic Calendula officinalis extract in a lagomorph model of full-thickness skin wound. *Drug Deliv Transl Res.* 2016;6(1):57-66. http://doi.org/f8nnjf.
14. **Espinosa L, Sosnik A, Fontanilla MR.** Development and preclinical evaluation of acellular collagen scaffolding and autologous artificial connective tissue in the regeneration of oral mucosa wounds. *Tissue Eng Part A.* 2010;16(5):1667-79. http://doi.org/bsnnf2.
15. **Fontanilla MR, Espinosa LG.** In vitro and in vivo assessment of oral autologous artificial connective tissue characteristics that influence its performance as a graft. *Tissue Eng Part A.* 2012;18(17-18):1857-66. http://doi.org/f38qzr.
16. **Fontanilla MR, Casadiegos S, Bustos RH, Patarroyo MA.** Comparison of healing of full-thickness skin wounds grafted with multidirectional or unidirectional autologous artificial dermis: differential delivery of healing biomarkers. *Drug Deliv Transl Res.* 2018;8(5):1014-24. http://doi.org/ds52.
17. **Alonso J, Prieto L, Antó JM.** La versión española del SF-36 Health Survey (Cuestionario de Salud SF-36): un instrumento para la medida de los resultados clínicos. *Med Clin (Barc).* 1995;104(20):771-776.
18. **Dias TYAF, Costa IKF, Melo MDM, Torres SM da SGS de O, Maia EMC, Torres G de V, *et al.*** Quality of life assessment of patients with and without venous ulcer. *Rev Lat Am Enfermagem.* 2014;22(4):576-81. http://doi.org/ds53.