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Autologous skin grafting in the treatment of severe scleroderma cutaneous ulcers: a case report

Sir, Systemic sclerosis (SSc) is a connective tissue disease characterized by collagen overproduction by altered fibroblasts and microvascular abnormalities, responsible for both visceral and skin involvement [1]. Vascular alterations lead to several typical manifestations of the disease, such as Raynaud's phenomenon and cutaneous ulcers. The latter are a frequent complication of SSc and are poorly responsive to the common pharmacological treatments. Vasodilator therapy with prostacyclin analogues has been reported to be effective in preventing and/or improving small cutaneous ulcers in some patients [2].

After the pioneering work of Rheinwald and Green in the mid-1970s [3], cultured keratinocyte grafting has become an alternative therapeutic approach to various ulcers (vascular, diabetic, post-traumatic) that are unresponsive to traditional treatments [4, 5]. The new class of semisynthetic biopolymers made from the benzyl ester of hyaluronan (Hyaff®; Fidia Advanced Biopolymers, Abano T., Italy) offers the advantage of good biointegration [6, 7]. The Hyalograft 3D™ device is composed of Hyaff fibres processed into a three-dimensional scaffold in which autologous fibroblasts find an ideal environment for adhesion, proliferation and the subsequent production of a dermal extracellular matrix [8–10].

The use of Hyalograft 3D therefore leads to a well-organized neo-dermis; its autologous nature guarantees complete biointregration. Moreover, Laserskin®, a microperforated membrane made from Hyaff biopolymer, was designed to allow the prompt grafting of actively proliferating keratinocytes, which constitute the neo-epidermis [8–11].

Here we describe the case of a patient with non-healing scleroderma skin ulcers who was usefully treated with this novel form of tissue-engineered skin autografting.

A 31-yr-old woman was first referred in 1988 to the Rheumatology Unit of the University of Pisa, where the diagnosis of SSc was made on the basis of typical clinico-serological manifestations [12, 13]. Since 1986 she had suffered from Raynaud's phenomenon, polyarthralgias, skin sclerosis of the hands, forearms and face, melanodermia and telangiectasias on the face, a typical scleroderma pattern on capillaroscopic examination, and the presence of anti-Scl70 in serum. During the following years she presented numerous episodes of ischaemic lesions on the fingertips, which were treated successfully with calcium channel blockers. In 1995 her skin ulcers worsened markedly, involving all fingers and toes; the skin lesions recovered completely after 5 months of combined therapy with calcium channel blockers and cyclosporin A. Three years later, there was severe relapse of the ischaemic lesions with autoamputation of two ungual phalanges of the left hand together with a large ulcer (5 cm diameter) at the right malleolus. The latter was unresponsive to different local and systemic therapies, including prostacyclin analogue (Iloprost), cyclosporin A, calcium channel blockers, steroids and cyclophosphamide. During 1999 the patient's general condition worsened progressively: besides skin lesions unresponsive to therapy, laboratory investigations revealed the presence of a severe anaemia (erythrocytes 2.13×10^6/ml; haemoglobin 5.5 g/dl). The anaemia recovered after 8 weeks of erythropoietin therapy (10 000 IU three times/week; Globuren; Dompè Biotech, Milan, Italy). The ulcer at the right malleolus became wider (Fig. 1), showing irregular margins, exposure of tibial tendons and superinfection by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and a new ulcer appeared at the left malleolus (Fig. 1). In November 1999, treatment with autologous skin grafting was decided upon. A 2.5 cm^2 skin biopsy from the patient's left thigh was taken and sent immediately to Fidia Advanced Biopolymers Laboratory (Padova, Italy) in sterile, serum-free medium (Dulbecco's Modified Eagle Medium). Autologous fibroblast were seeded into the non-woven scaffolding of Hyalograft 3D for 2 weeks. Inside this biomaterial fibroblasts deposit extracellular molecules, such as type III and IV collagens, fibronectin and laminin and produce a dermis-like tissue. Autologous keratinocytes were seeded onto the microperforated Laserskin membrane for 3 weeks. During this period, keratinocytes were able to proliferate and colonize both sides of the membrane, migrating through the holes, giving rise to a multistratified epithelial sheet

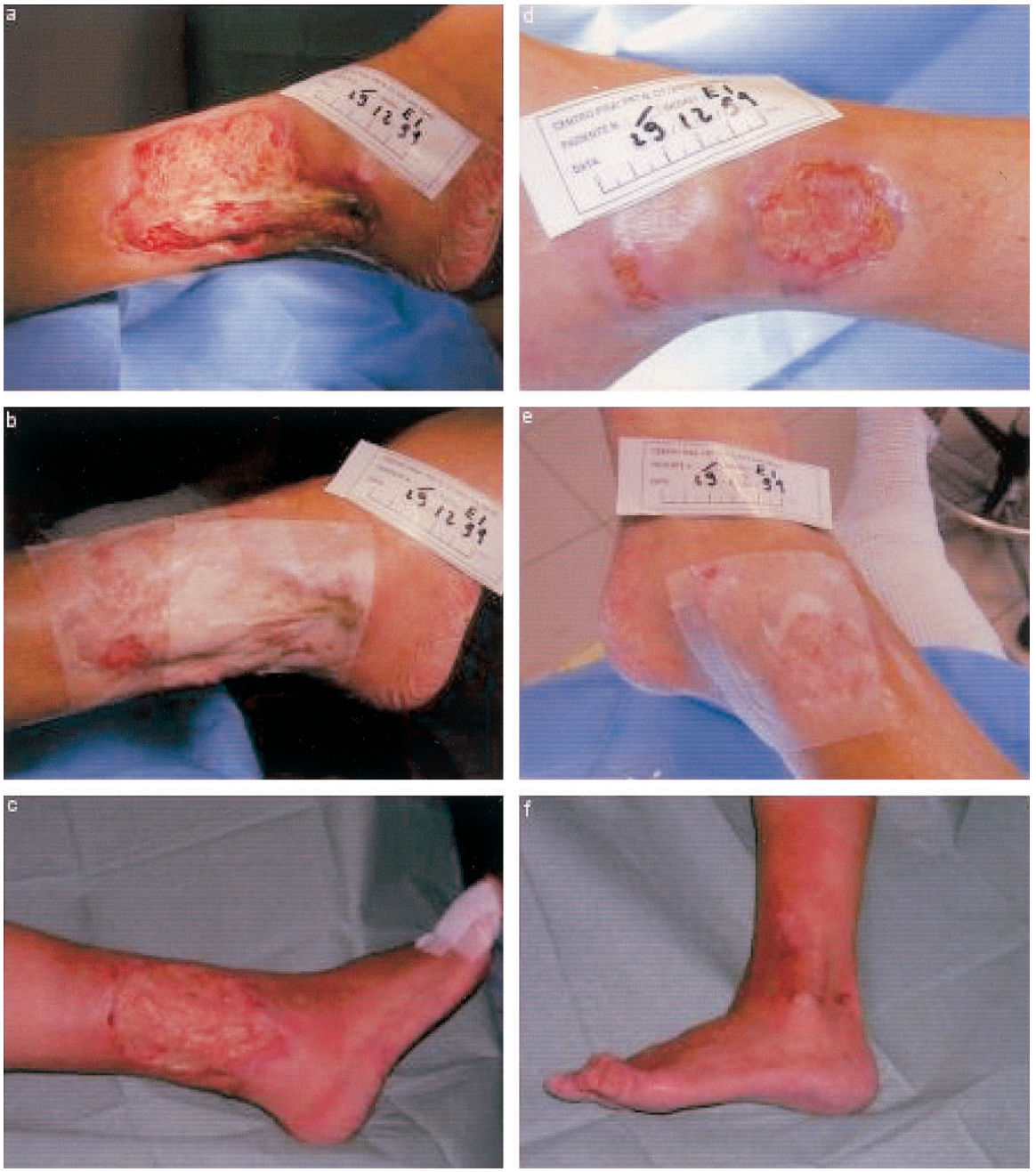

FIG. 1. Skin ulcers at the right and left malleolus before treatment (a and d), at the time of Hyalograft 3D application (b and e) and 6 months after skin grafting (c and f).

[8]. The ulcers were thoroughly debrided and cleansed, and Hyalograft 3D (containing autologous fibroblasts) was applied to the ulcer bed 15 days after the initial excision. The ulcers were covered with non-adhering gauze that was kept in place by a conventional secondary dressing, and antibiotic treatment was administered for 20 days (Fig. 1b and e).

Seven days after grafting, the ulcers were cleaned accurately and a Laserskin autograft was applied. Clear improvement of the ulcers was observed 30 days after grafting, with evident re-epithelialization from the margins. The left ulcer healed and the right one healed completely during the subsequent 6-month period (Fig. 1c, f). This clear-cut therapeutic result persisted after 2-yr of follow-up.

This is the first case of an SSc patient with skin ulcers resistant to traditional therapies that were successfully treated by skin autografting using biopolymers to produce a scaffold for cultured keratinocytes. The wide skin lesions observed in our patient had been unresponsive to previous therapeutic attempts with vasoactive drugs, steroids and immunosuppressors. The autograft

system using Hyalograft 3D and Laserskin was able to resolve the scleroderma malleolar ulcers; the improvement of the concomitant anaemia after erythropoietin therapy might have contributed, at least in part, to the success of the autologous grafting. It is of interest that the complete healing of the skin ulcers remained stable for a long period of follow-up.

Ischaemic skin lesions are a frequent complication of SSc, varying from digital pitting scars to wide ulcers, more prevalent on the lower limbs. These manifestations are particularly difficult to treat due to the severe microvascular involvement and possible concomitant venous disturbances and/or local superinfection [14]. During recent years there have been some anecdotal case reports of SSc patients treated with artificial skin grafting [15, 16]. In the first attempt at autologous skin grafting in patients with skin ulcers, seeding efficacy was only 8% [15].

In 1997, Hafner *et al*. [16] described a patient with non-healing skin ulcer who was usefully treated by surgical debridement followed by split skin grafting.

On the whole, the use of tissue-engineered skin, especially autologous skin grafting, appears to be a promising treatment for non-healing ulcers; in particular, the present engraftment technique has been employed more recently in the treatment of skin ulcers of different aetiology [5, 10, 11, 17]. When applied to the wounded area, Hyalograft 3D elicits no adverse reactions and is fully integrated and well vascularized by 1–3 weeks. Immunohistochemical analysis reveals consistent macrophagic infiltration around the biomaterial fibres and deposition of newly synthesized extracellular matrix is evident [17]. After gentle abrasion of the newly formed dermal area, the keratinocytes carried by Laserskin show a good take and are able to promote the neoepithelialization of the wound surface after 2 weeks [17]. The macrophage-mediated resorption of the biomaterial is complete in 5–7 weeks.

The result of treatment of scleroderma cutaneous ulcers is often discouraging; moreover, this complication may severely reduce the quality of life of SSc patients. The frequent concomitance of arterial and venous deficiency in the pathogenesis of leg ulcers in SSc, needing a combined (surgical and grafting) therapeutic approach, should also be taken into account [14]. The complete, rapid recovery of malleolar ulcers observed in our patient suggests that this novel technology, which has been employed successfully in skin lesions of different aetiology, can also be used in refractory scleroderma skin ulcers.

D. Giuggioli, M. Sebastiani, M. Cazzato, A. Piaggesi, G. Abatangelo[1], C. Ferri

Department of Internal Medicine Rheumatology Unit, University of Pisa and [1]*Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy*
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Correspondence to present address: C. Ferri, Rheumatology Unit, University of Modena, Via del Pozzo 71, 41100 Modena, Italy. E-mail: clodoveo.ferri@unimore.it

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