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Micrografting chronic lower extremity ulcers with mechanically disaggregated skin using a micrograft preparation system

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ABSTRACT

Objective:

The Rigenera system is a new standardised micrograft preparation system. It works by means of automated mechanical disaggregation of small tissue samples, extracting only the smallest cells (<50µm). The aim of this study was to retrospectively evaluate patients affected by chronic ulcers and who were treated with the micrograft preparation method.

Method:

Chronic ulcers have been included regardless of the cause. The specimen was collected with a 3mm diameter biopsy punch and immediately dissociated by means of the Rigenera System. The obtained suspension was placed on a scaffold of equine collagen.

Results:

We included 15 patients (four males, 11 females) with a mean age of 72.2±8.41 (mean±standard deviation) years. In seven patients the ulcers were related to the complications of diabetes, post-traumatic in a further three diabetic patients, vasculitis in one patient, and four patients had venous leg ulcers (VLUs). The median main diameter was 5.0cm and the median estimated area was 43.96cm^2. The ulcers were present from a mean of 4.50±2.30 months before inclusion in this study. At the second week the wounds were reduced by 37.33%±19.35%, at the week eight, nine patients (60.0%) were healed, and at week 16, 13 (86.7%) were healed. The quality of scars was good and did not deteriorate at the six month follow-up.

Conclusion:

The simplicity of the approach, the minimal invasiveness of the specimen collection, and the good quality of scarring of healed wounds, confirmed in the follow-up, makes this micrograft preparation method a useful tool to use on large or complex wounds.

Chronic non-healing wounds can be defined as those that usually have a multifactorial pathogenesis and do not follow the normal healing process, remaining unhealed for at least 12 weeks.[1] Chronic wound healing is different from acute healing because of underlying cellular dysfunction and dysregulation, alteration of matrix molecules, and abnormal prolongation of the inflammatory and proliferative stages of healing.[2]

Autologous skin graft is considered the gold standard of graft materials, but this approach is still limited due to small amount of tissue that can be collected, and to the necesssary sample manipulations (grinding, centrifugation, enzymatic or mechanical separation methods) that reduce viability of the obtained cells and increase the execution time. In most studies, micrografting has proven to be valid, effective and less invasive than the main grafting procedures as it uses small amounts of tissue from the donor site.[3] An autologous skin suspension, created with a sheet of split-thickness skin whipped with a conventional kitchen blender,[4] could achieve very large expansion ratios and be readily available, however, because of the poor histological, functional and cosmetic results,[5,6] the method fell from use. An ideal graft should be immediately available, non-immunogenic, permanent and safe.[3]

The Rigenera system is a standardised micrograft preparation system.[7] It works by means of automated mechanical disaggregation of small tissue samples, it extracts from them only the smallest cells (<50 micron) and cuts, without damaging, extracellular matrix (ECM) constituents, which can help to reduce the inflammatory process. Cell characterisation by flow cytometry analysis shows a heterogeneous pool of cells, including endothelial cells and mesenchymal stem cells.[7]

Mesenchymal stromal or stem cells, of particular interest in wound healing, are multipotent with trophic and support functions. They are able to release anti-inflammatory cytokines, trophic and anti-apoptotic molecules, and have become of great interest in recent years in the field of regenerative medicine.[8] A stem cell-like subpopulation, named ‘side population cells’,[9] was identified in mouse bone marrow[10] and then in many human tissues including skin.[11] This method allows substantial levels of cell viability after disaggregation.[7]

The aim of this study was to retrospectively evaluate patients treated with the Rigenera method who were affected by chronic ulcers.

Methods

Patient selection

In this paper, we report the results of a retrospective evaluation of chronic leg ulcers treated with standardised micrograft preparation system (Rigenera method). Ulcers with a medical history of at least 12 weeks[1] were included, regardless of the cause. Patients with critical leg ischaemia or with severe systemic diseases were excluded. All wound sizes were included and measured with a measuring tape.

Wound bed preparation

Comorbidities were assessed and treated, by specialists such as diabetologists, nephrologists, cardiologists, and offloading footwear was produced for neuropathic leg ulcers. Elastic compression (elastic stockings or bandage) was applied to patients with venous leg ulcers (VLU).

Before applying the micrograft preparation, all ulcers were treated according to the principles of wound bed preparation. The leg ulcers were treated, according to the treating health professional, with either non-selective

surgical debridement by means of hydrosurgery (Versajet, Smith & Nephew)[12,13]or with selective surgical debridement, followed by a dressing according to the principles of wound bed preparation, until the achievement of an optimal wound bed.

Wound bed preparation is adapted to the wound state at a particular time and comprises of tissue management (removing necrotic tissues and slough), inflammation and infection control, moisture balance (reducing oedema and exudates, avoiding exsiccation) and epithelial (edge) advancement.[14]Advanced dressings such as polyurethane, hydrocolloids, alginates, hydrocolloids or hydrogel were chosen by the physician depending on the state of the wound.

Specimen (skin sample) collection

The donor area was in the third proximal of the lateral region of the lower leg. The specimen was collected with a 3mm diameter biopsy punch, as already described in the literature.[15] The volume was approximately 1mm^3 and contained the dermis and epidermis.

Micrograft production

The collected skin samples were immediately dissociated by means of the Rigenera System (Human Brain Wave, Turin, Italy), composed by the Rigeneracons (tissue disruptor) and the Rigenera machine (tissue disruptor system). According to the manufacturer's instructions and as already described in the literature,[7,16,17] the collected skin samples were placed within the Rigeneracons along with 1.5ml of injectable sterile saline solution. The Rigeneracons is placed into the Rigenera Machine providing the mechanical disruption of the inserted skin samples by means of two metal blades. The disaggregation time is programmable (from a minimum of 10 seconds to a maximum of four minutes). In this experience, a 30-second disaggregation time was programmed for all skin samples, as it was empirically considered most suitable for skin micrografting.

A grid with 100 hexagonal holes of 50µm was placed at the bottom which allows filtration by gravity. At the end of the process, fragments of less than 50µm remaining in the liquid suspension were collected with a syringe.

Scaffold and micrograft application

The obtained suspension was placed on a scaffold of non-denatured and non-cross-linked type I equine collagen (Salvecoll Avascoll, Como, Italy) to form a bio-complex to be applied on the ulcer. The suspension was adsorbed on the scaffold, then the obtained complex was applied on the ulcer, and covered with a gauze impregnated with hyaluronic acid sodium salt.

Scar evaluation

Scar evaluation was performed by the same treating physician. The evaluation criteria included size of scar, any keloid or hypertrophic characteristics, irregularities, colouration, limitation of functions caused by scars (observed and referred by the patient), change in sensation referred by the patient. On the basis of these qualitative criteria, the scars were classified as good, mediocre or poor. No patient information was collected on the level of satisfaction in scar quality or the importance attributed by patients to the quality of the scar.

Results

The 15 patients studied had a mean age of 72.2±8.41 (mean±standard deviation) years, four were male and 11 female (Table 1). In seven patients, ulcer aetiology was related to complications of diabetes, including three ischaemic ulcers and four neuropathic diabetic foot ulcers (DFU). In the three other patients with diabetes, the leg ulcers were post–traumatic (Table 1), one patient had a systemic lupus erythematosus (SLE)-related vasculitis ulcer, and the remaining four patients had a VLU. In six patients, the ulcer started as a post-traumatic lesion.

Table 1. Patient charcteristics

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Patient, age, sex	Type of wound	Diabetic	Comorbidties
1 64-year-old male	Diabetic (neuropathic)	Yes	Hypertension, obesity, venous disease
2 57-year-old male	Diabetic (ischaemic)	Yes	Renal failure (dialysis treatment), PAD
3 68-year-old male	Diabetic (ischaemic)	Yes	Hypertension, PAD
4 74-year-old male	Diabetic (neuropathic)	Yes	Hypertension, PAD (previous PTA)
5 77-year-old female	Post-traumatic	Yes	Hypertension, PAD
6 75-year-old female	Post-traumatic	Yes	Hypertension, venous disease
7 75-year-old female	VLU (post-traumatic)	No	Hypertension, allergy
8 57-year-old female	Vasculitis	No	SLE
9 70-year-old female	VLU (post-traumatic)	No	Hypertension, PAD, ischaemic heart disease
10 73-year-old female	Diabetic (neuropathic)	Yes	Hypertension, ischaemic heart disease, obesity, renal failure
11 78-year-old female	VLU (post-traumatic)	No	Hypertension
12 80-year-old female	Post-traumatic	yes	Hypertension, ischaemic heart disease, atrial fibrillation, COPD
13 82-year-old female	VLU (post-traumatic)	No	Hypertension, PAD, ischaemic heart disease
14 62-year-old female	Diabetic (ischaemic)	Yes	Hypertension, PAD (previous PTA)
15 82-year-old female	Diabetic (neuropathic)	Yes	Hypertension, PAD, ischaemic heart disease

VLU—venous leg ulcer; PAD—peripheral artery disease; PTA—percutaneous transluminal angioplasty; COPD—chronic obstructive pulmonary disease; SLE—systemic lupus erythematosus

All patients had at least one comorbidity, 13 had hypertension, two patients were obese, two had renal failure, of whom one was having dialysis treatment. Peripheral artery disease (PAD) was found in eight patients, five of which had diabetes, and five were affected by ischaemic heart disease, three of which had diabetes.

The wounds had a median largest diameter of 5.0cm (range: 2–25cm). The ulcers were present from a median of 16 weeks (range: 12–48 weeks) before they were first referred to the study group (Table 2).

Table 2. Treatment and healing times

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Patient	Wound diameter (cm)	Infection	Duration before treatment	WBP	WBP duration before MG	% diameter reduction at week two	Healed at week 8	Healed at week 16	Healing time (after MG)
1	2 x 3cm	Yes	16 weeks	AWD	4 weeks	70	Yes	Yes	13 days
2	10 x 8cm	Yes	12 weeks	AWD	0	30	No	Yes	118 days
3	5 x 2 cm	No	16 weeks	Hydrosurgery	0	50	Yes	Yes	40 days
4	2 x 2 cm	No	12 weeks	Hydrosurgery	0	50	No	Yes	69 days
5	25 x 5cm	Yes	20 weeks	AWD (only hydrogel)	4 weeks	30	No	Yes	83 days
6	3 x 2cm	No	12 weeks	AWD (only hydrogel)	4 weeks	70	Yes	Yes	16 days
7	3 x 3cm	Yes	12 weeks	Hydrosurgery	8 weeks	50	Yes	Yes	72 days
8	2 x 2cm	Yes	16 weeks	AWD	8 weeks	30	Yes	Yes	47 days
9	4 x 3cm	Yes	16 weeks	Hydrosurgery	0	30	Yes	Yes	64 days
10	6 x 5cm	Yes	20 weeks	AWD (only hydrogel)	4 weeks	5			Deceased
11	9 x 5cm	Yes	48 weeks	Hydrosurgery + AWD	4 weeks	30	Yes	Yes	74 days
12	8 x 7cm	Yes	24 weeks	AWD	4 weeks	5	No	No	–
13	2.5 x 2cm	No	16 weeks	AWD	4 weeks	30	Yes	Yes	84 days
14	10 x 12cm	No	20 weeks	Hydrosurgery	0	30	No	Yes	118 days
15	3 x 3cm	No	12 weeks	Hydrosurgery	0	50	Yes	Yes	43 days

WBP – wound bed preparation; MG – micrografting; AWD – advanced wound dressing (polyurethane, hydrocolloids, alginates, hydrocolloids, hydrogel, chosen by the treating physician)

Non-selective hydrosurgical debridement was performed in seven patients, immediately followed by the application of the micrograft preparation, except in one case where the patient was treated with advanced medications (polyurethane foam, hydrocolloids, hydrofibers, chosen according to the characteristics of the ulcer, to obtain a good wound bed after surgical debridement). The remaining eight patients were treated with advanced dressings according to the WBP criteria for an average of 5.00±1.85 weeks (range: 4–8 weeks). In three cases, the entire pretreatment was conducted with hydrogel dressings, in the other cases the dressings were chosen according to the characteristics of the ulcer. At the time of observation, nine ulcers were clinically infected and after a cultural examination *Staphylococcus* ssp. and *Pseudomonas aeruginosa* were identified. These were treated with antibiotics (ciprofloxacin 500mg orally every 12 hours for 10 days or clarithromycin 500 mg orally every 12 hours for 10 days) and cleaned with diluted antiseptics (iodine or sodium hypochlorite) (Table 2). Selective surgical debridment was performed on the eight patients treated with advanced dressings.

Each punch was enough to cover a surface of $2cm^2$ and the median number of punches was three. The healing of the punch sites occurred quickly with the application of a medicated gauze and without complications in all cases.

On examination, two weeks after the application of the micrograft preparation the ulcers were reduced on average by 37.33±19.35% in largest diameter (range: 5–70%; median: 30%). At week eight, nine patients (60.0%) were healed and at week 16 only two patients had not healed (86.7% healed). In the first case, at week 16 the ulcer was reduced by about 30%, however the patient died from cardiovascular causes just after the follow-up visit. In the second case, the ulcer was reduced by about 20% at week 16. The mean time of healing in the patients who healed within 16 weeks was 64.92±34.10 days (range: 13–118 days, median: 70 days).

Figs 1–3 show examples of the cases treated. For example the Case 4 (Fig 1), a 74-year-old male, already treated with multiple distal percutaneous transluminal angioplasty, had a neuropathic diabetic foot ulcer (DFU). Cell suspension with the collagen scaffold was applied just after treatment= with hydrosurgery and the ulcer healed 69 days after the micrograft procedure.

Fig 1. Case 4, a 74-year-old male with a neuropathic diabetic foot ulcer (DFU), already treated with multiple distal percutaneous transluminal angioplasty. At presentation (**a**), application of cell suspension with a collagen scaffold (immediately after hydrosurgical debridement) (**b**), at the second week (**c**), and at day 69 after the micrograft procedure (**d**)

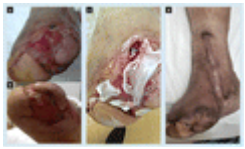

Fig 2. Case 14, a 62-year-old female presented with an ischaemic diabetic foot ulcer (DFU) having undergone a previous percutaneous transluminal angioplasty. At presentation, dorsal (**a**), and plantar (**b**), application of cell suspension with a collagen scaffold (immediately after hydrosurgery) (**c**), and at day 118 after the micrograft procedure (**d**)

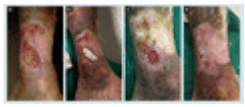

Fig 3. Case 11: a 78-year-old female with a venous leg ulcer (post-traumatic). At presentation (**a**), application of cell suspension with a collagen scaffold (after hydrosurgery followed by advanced wound dressing) (**b**), at the first week (**c**), and at day 74 after the micrograft procedure (**d**)

Case 14 (Fig 2) was a 62-year-old female, already treated with percutaneous transluminal angioplasty, with an ischaemic DFU. At presentation, she was treated with hydrosurgery, immediately followed by cell suspension application and the ulcer healed 118 days after the micrograft procedure.

Case 11 (Fig 3) was a 78-year-old female who developed a venous ulcer after a trauma. In this case, the ulcer was treated with hydrosurgery followed by advanced wound dressing and finally by the application of cell suspension. The ulcer healed 74 days after the micrograft procedure.

No signs of recurrence were found at the six months follow-up. The quality of scars was good in all subjects at healing time and did not deteriorate at the six months follow-up. No patient complained of scar problems.

Discussion

Allografts, xenografts or engineered artificial skin offer a rapid and costly approach to achieving wound coverage. An ideal graft should be readily available, non-immunogenic, permanent and comfortable for the patient.[3]

The micrograft preparation method used in this study is a new procedure that relies on the breakdown of a very small amounts of donor tissue, obtainable by one or more punches. As the patient is both the donor and the acceptor of the tissue at the same surgical moment, so this procedure does not represent a tissue or cell transplantation, but a graft (autologous transplantation). The micrografts have been applied alone or in combination with common biological scaffolds, like collagen sponges, to optimise the efficacy of micrografts implants.[16–18]

In the absence of a control group the healing times were relatively short, considering the healing time of skin ulcers in general (for example, the Swedish Registry of Ulcer Treatment,[19] median duration: 12 weeks; mean: 117 weeks) and in view of the long clinical history of the ulcers treated. The quality of the scars was good and no relevant aspects of retraction and fibrosis were observed. It is conceivable that this result could have been

favoured by the use of non-cross-linked type I collagen scaffold of equine origin. Further studies are needed to assess the healing time and the quality of scars comparing various types of scaffolds.

The micrografting method closest to the Rigenera method is the ‘autologous skin suspension’, a controversial method experimented exclusively on animal models but abandoned because of the poor quality of the scars.[5,6] This means it is difficult to compare our results with those of others using similar methods

Limitations

A case series is a collection of patients sharing common characteristics used to describe some clinical, pathophysiological or operational aspects of a disease, treatment or diagnostic procedures.[20] Although they rank low in the hierarchy of evidence, case series have a role in recognising new diseases or in the unusual presentation of known diseases, in identifying new risk factors or the adverse effects of new drugs or therapies. They are also useful for generating hypotheses, they provide data on the effects of a therapy, but they are not useful to test hypotheses. From this point of view, our results should not be overestimated and require further testing with controlled studies.

Conclusion

In this study we retrospectively evaluated chronic ulcers of various aetiology and extension. In most cases, ulcers had very large dimensions and patients were affected by multiple systemic comorbidities. The overall results were good for the high proportion of healed ulcers, with good quality scars confirmed at follow-up. The simplicity of the approach, its minimal invasive nature and the good quality of scarring of healed wounds makes the micrograft preparation method a useful tool to use on large or complex wounds.

Reflective questions

- Can the Rigenera method speed up healing time for ulcers that are hard-to-heal?
- How would you describe the quality of the scar obtained with the Rigenera method with standard dressings?

Declaration of interest: None to declare.

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