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Case Report

A case report on the effect of micrografting in the healing of chronic and complex burn wounds

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Abstract: Different approaches can be used to repair extensive burn injury and chronic wounds, including full and split thickness skin grafts, temporising matrices and scaffolds, and composite cultured skin products. The use of non-cultured or autologous skin cells suspension in chronic burn is well established, but despite this no significant literature has been realized. The Rigenera micrografting technology is an innovative technique allowing to obtain a suspension of autologous micrografts that can be applied over the wounds in a combined methodology specifically developed and based on the both injections of the wound edges and spraying over the wound bed of this suspension. A black male patient with open wounds on the back already treated with a traditional split skin graft, present a 10% of wounds not healing. Then, the patient was treated with micrografts suspension obtained by mechanical disaggregation of small split skin biopsies using the Rigeneracons medical device. Micrografts were directly injected and sprayed in the wounds. The combination of injection and sprayed micrografts solution over the wounds achieved full closure over 10% over a period of 6 months. The follow up more than 2 years showed stable wounds with no breakdown in the epidermis. The final cosmetic and functional results obtained with micrografting on chronic burn wounds is a valid alternative when all the other options cannot provide wound closure.

Keywords: Micrografts, burns, non-healing wounds, autologous, chronic

Introduction

Despite multiple and recent advance in the field of treatment of chronic wounds, there is a growing interest to highlight new therapies in order to improve the healing potential for these type of wounds [1]. Furthermore, a prolonged wound delay due for example to surgical complications is a significant toll on patients' quality of life with profound psychological implication in the normal life activities [2]. Also burn injuries, in several ways, have a significant economic impact on patients and represent a severe cost on health sectors especially in developing countries leading to disruptive socio-economical outcome [3, 4].

The wound healing phase in response to burn injury is similar to that of a normal wound, consisting of inflammation, proliferation, and remodelling. However, in burns, there is a higher degree of inflammation, resulting in increased capillary permeability, persistent vasodilation, and edema. All these factors are responsible for a prolonged wound healing process with a delayed remodelling peaking if compared to a normal wound [5].

The dermal wound healed by three main mechanisms, connective tissue deposition (during the proliferation period), contracture (during the phase of proliferation) and epithelization (during the phase of remodelling). The major mechanism for wound healing primary intention is the connective tissue deposition during the proliferation period in which there is interaction between the fibroblast and the matrix to slowly advance from the edges of the wound to the centre [6]. Chronic wounds begin the healing process with a similar scale, but they have a prolonged inflammatory phase during the which there is a significant destruction of the matrix element and release of high levels of proteolytic enzymes that not allow progression in proliferation phase and keep these wounds in a persistent inflammatory circle [7]. Chronic and acute wounds are also associated with elevated levels of MMPs that may directly contribute to non-

healing by degrading proteins necessary in normal wound healing such as extracellular protein, growth factors and protease inhibitors [8].

Different strategies are available today to manage chronic or acute wounds and regenerative medicine has emerged as an alternative to provide additional therapeutic options to potentially improve wound healing and restore normal skin architecture [9, 10]. In this case report, was described a clinical experience in the management of non-healing chronic wounds due to extensive burns on the back using a new approach based on the injection and spraying of autologous micrografts suspension obtained by mechanical disaggregation of autologous skin tissue.

Case report

The study protocol complied with the Declaration of Helsinki of 1975 and the patient provided written informed consent before participating in the study.

A man 39 yrs. old, was referred to us with open wound post flame burn accident occurred to him 6 months previous hospital admission in May 2015 in our Burn Unit. Patient had no-comorbidity, and presented wounds over the scalp, neck, back, both arms and both hands, scattered burn areas over both legs and chest. His burn area was assessed to be 42% TBSA. After 6 months of treatment the only area that not healed was the back. Multiple pus-swabs and wounds biopsy showed no bacterial growth, and multiple histology showed presence of granulation tissue. Combination of multiple surgical techniques were performed in the following months to improve the non-healing wound on the back, including multiple debridement, silver ion dressing, artificial dermis with negative wound pressure therapy, plated derived growth factors, Meek grafting. All the different surgical procedures were unsuccessful and a join team composed by burn, plastic and dermatology was created to manage this situation. The team decided to treat the non-healing wound on the back with a conservative treatment through dressing alternative days with a combination of corticosteroid, antifungal and sulphonamides. Eight months later, the patient was discharged with 9% open wounds on the back. After 7 months from discharge, the patient was readmitted with 9% non-healing wounds on his back (**Figure 1A**). Based on these considerations, we took the decision to treat the patient with Rigenera micrografting technology, based on the application of autologous micrografts suspension, performing both injection and spraying procedures.

Rigenera micrografting technology

Practically, this technology is based on the use of the Rigeneracons device (Human Brain Wave, Turin, Italy) that is a masher able to disaggregate different samples of human tissue selecting a specific cell population which can be used as autologous micrografting. All the steps of the procedure are described in the **Figure 2**. Briefly, a small skin biopsy (2×2 cm) was cut in stump of 2×2 mm and inserted into Rigeneracons device adding 4 ml of saline solution for each stump disaggregated (**Figure 2A**, **2B**). After rotation of Rigeneracons by Sicurdrill medical device (Human Brain Wave, Turin, Italy), the micrografts suspension was collected (**Figure 2C**). The micrografts obtained through this procedure can be injected or sprayed directly on the wounds. Following the micrografts application, the wounds were dressed with petroleum gauzes and the first dressing was performed 8 days post application. The Rigenera micrografting procedure was repeated twice in an interval of 30 days for a total of three micrografts applications.

Clinical follow-up

We observed an initial remission of the wounds one month after the first application with the closure of small areas and remission over the large areas (**Figure 1B**). After 30 days, was performed a second micrografts application and the wound closure was evaluated after 2 months. After 30 days from the second application, we performed a third micrografts application and we showed the progression of healing wound process at 4 months (**Figure 1C**). We then observed a full healing at 6 months from the first micrografts application (**Figure 1D**) reporting an improvement of wound closure also after 12 and 24 months later (**Figure 1E**, **1F**). We did not report side effects or complication during the procedures and over the following dressing changes.

Discussion

The management of burn wounds is still a big challenge for burn and plastic surgeons, and

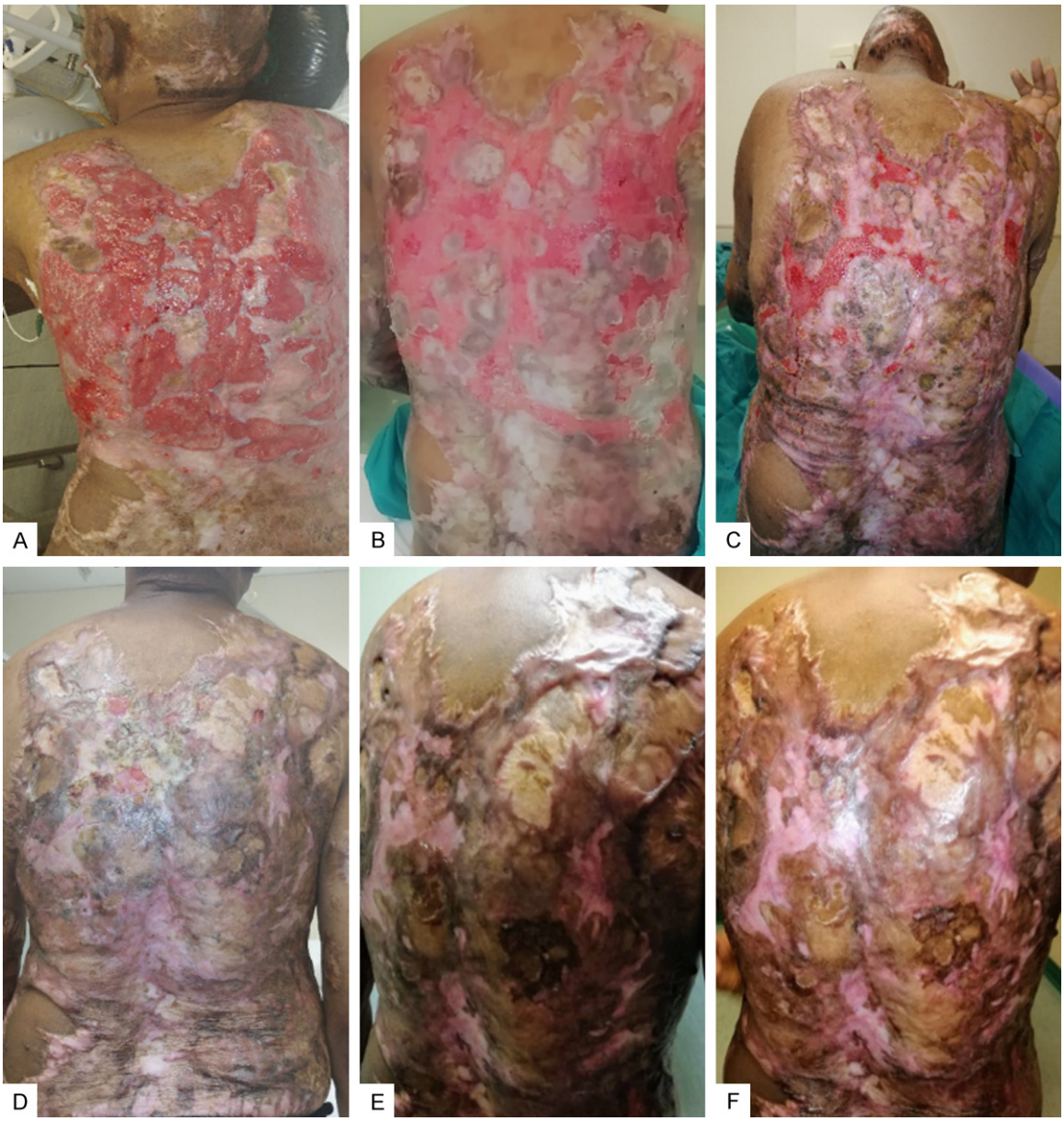

Figure 1. Representative images of chronic burn wounds. Patient with 9% non-healing wounds on the back readmitted 7 months after first discharge (A). Evaluation of wound closure after 30 days from first micrografts application (B) and evaluation after 4 months (C). Full healing after 6 months from first micorgrafts application (D) and follow up after 12 (E) and 24 months with no skin raptures (F).

among the therapeutic options to date available, the use of epithelial cells or keratinocyte transplantation has been reported as effectiveness for patients with extensive burns [11, 12]. Further, therapeutic cell delivery options with hydrogels and spray devices are always assuming a growing interest even if an optimisation of application techniques and carrier type has not yet been reached [13, 14]. In this case report, we demonstrated the effect of autologous micrografting to promote the healing of complicated wounds in which all the other conventional surgical approaches have failed. In particular, we proposed a dual micrografts application performing both the injection and spraying with a spray nozzle on the edges and over the wound bed, observing a full wound healing after 6 months from treatment.

These data are in line with our recent clinical experience, where the sprayed micrografts have been applied to massive and chronic full

Collection of a small piece of skin tissue (2x2 cm) from an intact area of the body where skin was not harvested or where graft was placed.

↓

Cut the sample in small skin stump 2x2 mm (panel a) and placed in the Rigeneracons device adding 4 ml of saline solution for each cycle of disaggregation (panel b).

↓

Mechanical disaggregation by rotation with Sicurdrill device for 3 minutes.

↓

Collect micrografts suspension by 20 ml syringe (panel c).

↓

Micrografts application by perilesional infiltration in the wound's margins and over the wound's centers and by spray nozzle on the wound (Mad nasal 300, USA).

↓

Micrografts were following applied after 30 and 60 days from the first application (for a total of 3 applications).

↓

Clinical evaluation of wounds healing was performed after 1, 2, 4, 6, 12 and 24 months

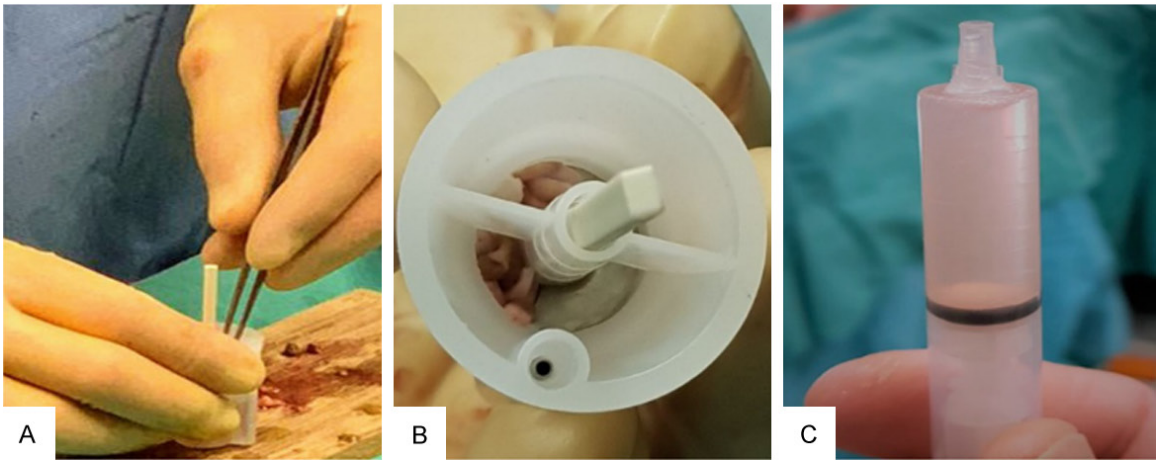

Figure 2. Schematic illustration of the micrografting procedure. The autologous micrografts were collected by mechanical disaggregation of a small piece of health skin (A). After the disaggregation (B), micrografts suspension was collected by a syringe (C) and applied directly on the wounds by injection on the edges and spraying with a spray nozzle on the wound bed. The micrografts were applied for a total of 3 times, at basal condition, after 30 days and after 60 days from first application.

thickness burns successfully promoting the re-epithelization process [15]. In addition to clinical outcome, the micrografts have the big advantage to be harvested and prepared in theatre during surgical procedure with no wasting time.

To support the efficacy of micrografts in the skin repair, previous studies have been reported their ability to improve the healing of complex and post-operative wounds, or chronic ulcers even if in area smaller than that one we treated [16-20]. Furthermore, a very recent published Italian multi-centre study on a cohort of 70 patients affected by traumatic wounds of the lower and upper limbs, characterized by extensive loss of skin substance reported a good clinical outcome stimulating skin regeneration and reducing the outcome scar [21].

In fact, previous studies also have suggested that dermal micrografts in combination with growth factors and cytokines could be able to induce angiogenesis, decreasing inflammatory process and allowing a fibroblast migration and normal collagen production from disaggregated human tissue. In particular, autologous micrografts were successfully used to treat hypertrophic scars [22]. In this case report and in general for all burns treated in our Unit we never reported the appearance of keloid scars, even if a retrospective study has confirmed the high frequency of keloids in dark skin [23]. However, frequently we observed the presence of hypertrophic scars but not strictly related to burns. In the patient described in this case report was observed the onset of mild hypertrophic scars on regenerated tissue but the scars were less prominent with reduced itch if compared with the scars on the tissue spontaneously regenerated. Furthermore, the scars were more elastic and painless when inspected.

Finally, a recent study has reported in vitro and in vivo data showing that micrografts are able to reduce several inflammatory pathways and promote cell migration and wound closure, suggesting a potential biological mechanism for which micrografts induce wound healing [24].

Another advantage of clinical application of micrografts is that they are ready to use and do not require further cell manipulation or a period of delay to allow time for the cells culture process, given that the culture time will vary from laboratory to laboratory depending on the area that need to be treated.

In conclusion, despite of the limitation of a case report, micrografting technology can offer a valid alternative to close difficult wounds such as burn wounds not responsive to existing surgical approaches and to treat cases with no disappoint results as with sheets of cultured cells. We suggest that this approach may benefit and may be useful for those patients whose skin graft have previously failed or in patients with long standing open wounds.

Disclosure of conflict of interest

None.

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