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ORIGINAL ARTICLE

Regenerative Surgery: Use of Fat Grafting Combined with Platelet-Rich Plasma for Chronic Lower-Extremity Ulcers

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Abstract
Background The authors present their experience with reconstructive surgery of the lower extremity for chronic ulcers, evaluating the effects related to the use of a platelet-rich plasma combined with fat tissue.
Methods A total of 20 patients, 25 to 50 years of age (median age, 40 years), have been managed with platelet gel in the Plastic and Reconstructive Surgery Department at the "Tor Vergata," University of Rome. The patients were affected by both lower-extremity chronic ulcers and vascular disease.
Results The authors observed that 16 of 20 chronic lower-extremity ulcers reepithelialized during an average of 9.7 weeks, with platelet releasate suspended on a collagen base (platelet-derived wound-healing factor), compared with 2 of 10 similar wounds treated with medication based on hyaluronic acid and collagen. Collectively, these data provide evidence for the clinical use of platelet technology in the healing of both soft and hard tissue wounds.
Conclusions Currently, plastic surgery with autogenous fat grafts can be performed for stabilization of chronic lower-extremity ulcers. The objective of this study was, through the presentation of clinical cases, to suggest a therapeutic plan formed by two sequential treatments: acquisition of platelet gel from a small volume of blood (9–18 ml) followed by the Coleman technique for reconstructing the three-dimensional projection and superficial density of tissues. The results proved the efficacy of combining these two treatments, and the satisfaction of the patients confirmed the quality of the results.

Keywords Lipostructure · Lower-extremity ulcers · Platelet-rich plasma

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Chronic venous insufficiency is the cause of ulcerations for 60% to 80% of patients with chronic leg ulcers [1–3]. Probably, more than 500,000 people in the United States experience these painful and debilitating ulcerations [4, 5]. Although it is true that the peak prevalence occurs after the age of 60 years, ulcers develop in early adulthood for many patients [6]. The annual cost of medical care for one of these difficult-to-heal lesions often runs into thousands of dollars. The disease manifests itself in older women or men, influencing the soft and hard tissue structure of the involved side of the lower extremity [7, 8] (Figs. 1, 2, 3, 4, 5 and 6).

Elevated venous pressure is the underlying cause of skin and tissue changes that lead to chronic ulceration. This elevated venous pressure results from the failure of venous valves in deep or superficial veins or both. The elevated venous pressure, transmitted to the venules and capillaries of the skin and subcutaneous tissues, causes a series of clinical changes, including edema, lipodermatosclerosis, or stasis dermatitis; the development of hyperpigmentation, hyperkeratosis, and atrophie blanche; and ultimately, a chronic nonhealing ulcer [8, 9].

On a microscopic level, lymphatic vessels are enlarged, and capillaries are elongated and dilated, with the functional number of capillaries reduced [10]. Capillaries often show occlusive microthrombi and sludging of white blood cells [11]. Plasma proteins and red blood cells leak into the

Fig. 1 Preoperative situation

Fig. 2 Intraoperative situation

Fig. 3 Postoperative situation after 6 weeks

Fig. 4 Postoperative situation after 10 weeks

Fig. 5 Coleman Kit (cannula for placement)

Fig. 6 Coleman Kit (cannula for harvest)

interstitium, where hemosiderin and fibrin deposition occurs at the same time as fibrinoblastic activity [12]. These microscopic changes result in a reduction and stagnation of blood flow, decreased oxygen levels at the capillary level in the preulcer skin, and increased flow and arteriovenous shunting in the nearby tissues [13]. The protein-rich edema, acting as a diffusion barrier for oxygen transport, probably plays a major role in tissue hypoxia [13]. This results in an area of the skin in which capillaries are either missing or severely damaged and altered. In this setting, a slight trauma or infection can lead to a nonhealing ulcer.

It is important to stress that other causes of leg ulceration must be considered before the treatment is initiated. Arterial insufficiency must be ruled out before treatment because compression bandaging is contraindicated in such cases. Diabetic foot ulcers, which usually result from neuropathy, may be combined with arterial insufficiency from distal small vessel arteriosclerotic occlusions. Decubitus ulcerations, infectious causes, and vasculitis also should be considered. Venous ulcers are found in patients with superficial, deep, or perforator incompetence; a combination of two; or all three. Duplex scanning has documented the importance of superficial venous reflux and the relatively infrequent finding of deep reflux in patients with venous ulcers [8, 14].

Materials and Methods

The analysis involved 20 patients (13 women and 7 men) treated with platelet gel combined with fat tissue centrifuged in the Department of Plastic and Reconstructive Surgery at the "Tor Vergata," University of Rome. The patients ranged in age from 25 to 50 years (mean, 40 years). They were treated with Coleman technique [15–17].

The preoperative study (Fig. 1) was conducted through a complete clinical examination, a photographic examination in four projections (frontal, lateral, three-fourths, and axial), and through magnetic resonance (MR) of the tissue. In addition, for the more complex cases, a high-resolution computed tomography (CT) scan with three-dimensional (3D) imaging for a better view of the anatomic structures was performed.

The patients were treated with a platelet-rich plasma (PRP) combined with fat tissue in the lower-extremity region. The PRP was obtained using 18 ml of blood from a peripheral venous access collected in two sterile 9-ml tubes. The blood was extracted using the Cascade Kit[1] and subjected to centrifuging for 10 min at 1,100*g* [7, 18].

After centrifugation, the two tubes of inactivated PRP were switched with two 10-ml tubes containing CaCl [7]. From this point, a window of 4 to 5 min existed for including the PRP in the centrifuged fat. Indeed, 4 to 5 min after the addition of CaCl (which determined the activation of PRP), a product with increased consistency was obtained, making it more difficult to combine the PRP with the centrifuged fat.

We harvested fat tissue in the abdominal region using some specific cannulas, with diameters of 2 to 3 mm and 1.5 mm, for grafting (Coleman Kit, Tucson, AZ) [15–17]. Maintaining asepsis, we took the plungers of syringes and closed them with their caps, then positioned them flatly in the sterile centrifuge. The syringes were processed for 3 min at 3,000 rpm/min [15].

This purified body fat combined with PRP was put in 1-ml syringes and aseptically reinjected using the specific microcannulas (Coleman Kit, Tucson, AZ) [15–17] to implant it into the bed around the margins of the ulcers. Skin incisions about 2 mm in diameter to permit passage of the cannula were made using a no. 11 scalpel blade.

The implant location on the lower extremity destined to receive the implant was selected by an accurate study of the necessary corrections [19]. Fat tissue combined with PRP was implanted at different levels in small tunnels around the margins created earlier by forcing the cannula with precise controlled movements (Fig. 2). A small quantity of fat cells was laid, one or two at a time, during the exiting movement of the cannula to create a large grid to correct the vascular development around each fat cell.

Layers of the aligned single cells were laid to increase the contact surface between the receiving tissue and the implant. This technique was of fundamental importance in allowing each single layer deposited to survive through the few days necessary for the growth of the blood vessels that would nourish them permanently [9].

We closed the access incisions with 5–0 nylon stitches, and a compressive bandage was not applied. The bed of the ulcer contained the PRP solid gel fixed with nylon points. The control group was treated with medication-based collagen and hyaluronic acid. Postoperative follow-up assessment took place after 2 and 5 weeks, after 3, 6, and 12 months, and then annually.

Results

The authors observed that 16 of 20 chronic lower-extremity ulcers reepithelialized an average of 9.7 weeks (Figs. 3, 4) after treatment using fat grafting combined with platelet-rich plasma, compared with the 10 similar wounds treated using medication based on hyaluronic acid and collagen. After crossover of the control group, 11 wounds achieved 100% epithelialization in an average of 7.1 weeks.

Our results are displayed in Table 1. We observed 13 patients with the tissues restored in 7.5 weeks after a single treatment (PRP and fat grafting). Five patients presented the same results during two treatments (PRP and fat grafting) after an average of 5 weeks from the last treatment. We treated two ulcers with PRP alone, and complete epithelialization was obtained in an average of 10.5 weeks.

[1] Cascade Medical Enterprises LLC, 20 Greenup Court, 07470 Wayne, NJ, USA (cascade@optonline.net) is the tissue lab for exclusive marketing in Italy.

Table 1 Volume–quantity of platelet-rich plasma (PRP) and fat used in each case, the number of treatments, the time from the last treatment to complete healing, and any recurrences

Patient	PRP quantity (ml)	Fat quantity (ml)	No. of treatments	Time from last treatment (weeks)	Recurrences
1	10	20	2	2.5	0
2	5	10	1	6.5	0
3	10	17	1	7	0
4	9	18	1	8.5	0
5	10	23	2	3	0
6	10	25	1	9	0
7	9	21	1	7.5	0
8	10	19	1	8.5	0
9	10	18	1	8	0
10	5	17	1	7	1 after 3 months
11	9	15	1	8.5	1 after 1 year
12	10	16	2	4.5	0
13	10	18	1	6.5	0
14	9	19	1	7	1 after 7 months
15	4	8	1	6.5	0
16	7	12	2	7	0
17	10	19	2	8	0
18	50	0	1	12	0
19	10	23	1	8	1 after 1.5 years
20	30	0	1	9	0

In the control group, we observed that only 5 of 10 chronic lower-extremity ulcers reepithelialized during an average of 8.4 weeks (Table 1).

Discussion

In plastic surgery, the use of autologous blood-derived products has been limited largely to fibrin glues, used primarily to obtain hemostasis and adherence of skin flaps. The authors used platelet-rich plasma for lower-extremity venous chronic ulcers, demonstrating that the use of platelet gel combined with fat tissue in this case resulted in numerous advantages [20, 21].

Platelet gel is a mixture of autologous proteins [22, 23] that contains more than 300,000 to 350,000 platelets whose action consists of stimulating skin fibroblasts when injected (the authors use 0.5 ml of activated platelet gel, on the average, combined with 1 ml of centrifuged fat tissue). This mixture favors tissue growth by a new synthesis of collagen.

As a laboratory product, platelet gel [7, 12] is easy and safe to use. It cannot be contaminated, and it lacks surface antigens responsible for potential allergic reaction.

The platelets contain active substances involved in tissue repair mechanisms such as chemotaxis, cell proliferation and differentiation, angiogenesis, intracellular-matrix deposition, immune modulation, antimicrobial activity, and remodeling. Some of the numerous growth factors released by the platelets after activation identified to date are platelet-derived growth factor, transforming growth factor alpha and beta, epidermal growth factor, fibroblast growth factor, keratinocyte growth factor, insulin growth factor, platelet-derived epidermal growth factor, interleukin-8, tumor necrosis factor alpha, connective tissue growth factor, and granulocyte macrophage colony-stimulating factor [24, 25].

During the wound-healing process (4 phases: hemostasis, inflammation, proliferation, and remodeling), platelet growth factors serve as messengers to regulate a well-orchestrated and complex series of events involving cell–cell and cell–matrix interactions that promote the proliferation of mesenchymal and other stem cells at the wound site [26]. The authors, on the basis of this concept, have added fat grafting (containing mesenchymal stem cells) to PRP. Autologous platelet-derived wound-healing factors were proposed to regulate wound healing of chronic ulcers by promoting the formation of granulation tissue in the early healing phase.

In addition to their tissue-forming and proliferative effects, growth factors exhibit a chemotactic effect that causes the migration of neutrophilis and macrophages, adding an antimicrobial component to the wound site. Each patient received 9 to 10 ml (volume–quantity) of platelet gel after centrifugation. This amount combined with fat grafting is used throughout treatment in most cases

(Table 1). However, for some patients, the authors have used only a part of the PRP available, depending on the site of the injection, which in some cases may be small and thus not appropriate for the entire amount available.

We observe that the fat grafting creates a 3D projection and that the platelet-rich plasma increases the maintenance during the period of observation. The PRP can favor neo-angiogenetic vascularization and activity of fibroblasts that in turn favor adipose tissue survival and 3D organization. In addition, compared with lipofilling, if fat cells are laid in rows without a solution of continuity, survival of the implants is more probable for reduction of fat necrosis due to improved vascular development in the implanted area. The time taken for complete healing is reported as the time from the last treatment (Table 1).

We harvested fat tissue in the abdominal region using some specific cannulas, with diameters of 2 to 3 mm (Fig. 6) and 1.5 mm (Fig. 5), for grafting. Maintaining asepsis, we took the plungers of the syringes and closed them with their caps, then positioned them flat in the sterile centrifuge. The syringes were processed for 3 min at 3,000 rpm/min. This procedure obtained highly purified fat tissue, preserving the integrity of the adipocyte wall but separating the fluid fat portion from the serous bloody part [27].

The literature contains several important articles that mention the use of fat grafting for various diseases using different methods [16, 17, 27]. The authors, using the technique of Coleman in the preparation of fat for his injection, reproduces the benefits obtained by adding the PRP to prolong maintenance of the grafted fat tissue and its effects in tissue regeneration. According to the findings of recent studies [28, 29], administration of the stromal-vascular component of normal adipose tissue, documented to be rich in stem cells, would elicit the excretion of angiogenic factors. This would lead to the production of new microvessels, which ultimately would ameliorate the circulation.

On the basis of these reported results, we agree with the idea that the chain of events leading to mesemchymalization [27] of the tissue would be the following: targeting of damaged areas, release of angiogenic factors, formation of new vessels, and oxygenation. This process would favor the development of stem cells in mature adipocytes and in a newly formed microcirculation, replacing the existing seriously damaged one.

Alternatively, the authors used conservative treatment that included elevation, skin grafting, local treatment (biosynthetic material), and Vac (vacuum) therapy. Until recently, this conservative approach was considered the mainstay of the treatment, and its success in achieving ulcer healing has been documented [30]. The currently available alternatives for treating ulcers are autologous skin (harvest, graft), skin from a bank (omologous, taken from cadaveric or living subjects), engineered skin (autologous or omologous expanded in vitro), and biosynthetic materials.

Conclusions

We planned sequential treatments involving platelet gel combined with centrifuged fat tissue followed by the Coleman technique for reconstructing the 3D projection of the lower-extremity contour, restoring the superficial density of the tissues. Collectively, these treatments provide existing evidence for the clinical use of platelet technology in the healing of both soft and hard tissue wounds. The results prove the efficacy of combining these two treatments. The satisfaction of the patients equally confirms the quality of the results.

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