LEG ULCERS ORIGINAL ARTICLE

Clinical efficacy of an autologous whole blood clot matrix in the management of hard-to heal leg wounds, a retrospective observational study

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submitted: Oct 19, 2024, accepted: Dec 15, 2024, EPub Ahead of Print: Dec 27, 2024, published: Dec 31, 2024
Conflict of interest: None

DOI: 10.24019/jtavr.219 - Corresponding author:Dr. Rosario Miranda, rosmir@tiscali.it

Abstract Objective. The objective of this retrospective observational study was the clinical evaluation of the efficacy of an autologous whole blood clot matrix in patients with hard-to heal leg wounds. Methods. In this study have been evaluated retrospectively all patients all patients received at the Studio Medico Farina, from March 2021 to December 2021, with hard-to-heal wounds of various etiologies treated with whole blood red clot dressing (ActiGraft Red Dress). All selected subjects received, with a frequency ranging from 7 to 10 days, a topical dressing based on a whole blood red clot made extemporaneously with a venous blood sample, anticoagulated and activated with calcium gluconate and kaolin. Results. Seven patients were included: 5 males and 2 females. At the first whole blood red clot application the wounds had median surface area of 13.07 cm^2 (range 5.42-94.20 cm^2), six patients completed the treatment, all healed and median treatment period was 56 days (range 47-123 days), with good scar quality and immediate improvement in pain. One patient died for external causes. Conclusions. This series of cases, albeit with small numbers, highlights the clinical efficacy of the autologous whole blood clot matrix, as well as good pain control and a remarkable simplicity of implementation and application.

Keywords ActiGraft Red Dress, whole blood clot matrix, chronic wound, clinical efficacy.

Funding disclosures

Study materials - the kits were offered by the manufacturer (RedDress - 822 A1A North Ste 310, Ponte Vedra Beach, FL 32082).

Introduction

The first event that occurs immediately after an acute wound is the development of a clot, which, in addition to stopping the bleeding from the injured vessels, is also the first stage of the tissue repair process. This phase will be followed by the so-called inflammatory, proliferative and remodeling phases of wound healing[1,2]. Chronic wounds are trapped in the inflammatory phase of the reparative process for local, regional or systemic reasons[1]. In order to promote the healing of chronic wounds, many procedures have been put in place to overcome this inflammatory phase and restart healing.

For this purpose, dressings based on platelet concentrates or on platelet-enriched white clots have been extensively studied and have shown interesting results in promoting the healing of hard-to-heal wounds[3,4]. The use of platelet concentrates in the treatment of hard-to-heal wounds arises from the fact that these blood components contain granules rich in growth factors, which in the

coagulation phase of tissue repair, initiate the healing process[3]. For this purpose, a variety of platelet concentrates have been developed over time[3].

Recent scientific evidences show also the implication of other blood components in the process of wound repair: fibrin scaffold[5,6], monocytes / macrophages[7,8], and stem cells[9,10,11]. In platelet concentrates, erythrocytes are removed as these elements are not yet considered useful for the healing process, however in acute wounds the clot formation always occurs with a significant amount of red blood cells.

Red blood cells play a role in coagulation, but little is known about their role in wound healing and their potential therapeutic effects[12,13].

Erythrocytes modulate thrombin generation, fibrin formation, structure, and viscoelastic properties of the clot[14]. Clot retraction is determined by the stretching of the platelets and occurs with the participation of factor XIIIa with a complex interaction between fibrinogen concentration, platelet count, and hematocrit[14]. Erythrocytes tend to occupy the center of the clot with the platelets and the fibrin network prevalently on the surface[14]. The erythrocytes massed in the center of the clot acquire a polygonal geometry (polyhedrocytes) minimizing the interface area between cells and decreasing the clot permeability[14]. It also reduces the permeability to plasminogen activators, increasing the resistance to fibrinolysis. Red clot is mechanically very more fragile than the those obtained from platelet concentrates[15], however this could not to be an absolute impediment to the realization of dressing with whole blood red clot[14].

Regarding the possible influence on wound healing it is known that erythrocytes are a source of catalase[16], which is an antioxidant, they can modify the responses of innate immunity[17] and are a source of iron that can influence reparative processes[18]. Whole blood red clot entraps erythrocytes with a physiological platelet concentration[19]. Even if centrifugation, increasing platelet concentration[20,21], increases growth factor availability, there is currently no systematic work that evaluates the clinical efficacy of the various platelet concentrates, very different in preparation method and in platelet concentration[3], and there is no experimental proof that increasing the number of platelets the effectiveness increases[15].

There are only very few data on comparison between PRF / PRP and the whole blood red clot[15]. In an in vitro study, TGF-β activity and inflammation modulation activity were not significantly different between PRP and whole blood red clot[22].

A recent wound dressing method is based on the continuous weekly application of a whole blood red clot obtained from a peripheral vein sample. The first data, based on a limited number of case series[23,24], are very encouraging, due to the simplicity and safety of the method. However data are still incomplete and the quality of the scars at the end of the healing process has not been evaluated yet.

The aim of this observational work is to evaluate retrospectively whether this simple method of using blood can be useful to dress hard-to-heal wounds. This could be useful for example, when platelet concentrates are not available because these require specific instrumentation and generally cannot be performed at the patient's bed.

It can be interesting therefore to evaluate whether the use of a fresh whole blood clot, which is the type of clot closest to the pathophysiology of the acute wound, can be useful in the treatment of chronic skin wounds that do not respond to proper care.

Study Objective

The objective of this small retrospective observational work was to evaluate the clinical efficacy, the feasibility and safety of the continuous weekly application of a whole blood clot matrix on healing of hard-to heal chronic wounds and the quality of scars in healed wounds.

Methods

Patient eligibility and enrollment

In this study have been evaluated all patients with hard-to-heal wounds treated with whole blood red clot dressing all patients received at the Studio Medico Farina, from March 2021 to December 2021. Hard-to-heal wounds are defined as wounds that have not healed after suitable treatment for at least three months[24]. Vascular assessment and identification of ischemia was performed with color Doppler ultrasonography and ABI measurement. Exclusion criteria were: wounds with an area > 120 cm^2, osteomyelitis, a life expectancy < 6 months, neoplastic patients or with suspected cancerous wounds, systemic sepsis, anemia < 9 g/dL.

Materials

The whole blood red clot dressing was obtained with the ActiGraft Red Dress kit (RedDress - 822 A1A North Ste 310, Ponte Vedra Beach, FL 32082) as already described in literature[22,23]. Briefly each kit contains 1) two 10 mL tubes with Anticoagulant Citrate Dextrose solution (ACD-A); 2) one 30 mL sterile syringe; 3) a 5 mL sterile ampoule containing 3.6 mL of 10% calcium gluconate; 4) a single-use clotting tray containing a medical cotton gauze stretched across the bottom of the tray and 20 mg of pharmaceutical grade kaolin; 5) a 3 mm polyurethane foam dressing.

The two ACD-A anticoagulated tubes were filled with the blood withdrawn from a peripheral vein, and then all the blood was collected from the same tubes with a 30 mL syringe. Immediately after 3.6 mL of

10% calcium gluconate provided in the kit were aspirated with the same 30 mL syringe already containing the blood and finally all the contents of the syringe was slowly injected into the clotting tray containing kaolin. The time needed to complete blood clotting is about 12 minutes, even for subjects on anticoagulant or antiplatelet treatment.

The gauze, embedded into the bottom of the clot during the coagulation process, allows for easy transfer to the wound. The clot can be cut with its gauze support using sterile scissors to adapt it to the size and the shape of the wound, making sure the size of the clot is slightly larger than that of the wound. The dressing was then immediately applied with the top of the clot adhering on the wound bed and slightly overhanging the wound margin (photo1). The secondary dressing, a 3 mm polyurethane foam, was finally applied. After a time ranging from 7 to 10 days the clot matrix was removed and a new clot was created and applied. After removing the previous dressing, the wound was gently cleansed with gauze and saline.

At each reapplication, the wound was visually assessed, mainly evaluating the presence of clinical signs of infection (color, heat, odor, swelling, and pain). This procedure was repeated at every reapplication until the wound healed. All wounds were digitally photographed after cleansing at each dressing change. The main diameters have been acquired with a tailor's tape, the measurement of the wound area is correlated by approximation to the ellipse (diameter x diameter x $\pi/4$).

Wound assessment included the wound depth and dimensions, the presence of necrosis, slough, granulation and epithelialization, the type and amount of exudate.

Pain evaluation

Pain was assessed according to the NRS scale.26[27].

Scar evaluation

A physician of the research group not involved in patient care performed scar evaluation. The evaluation criteria included the size of scar, any keloid or hypertrophic characteristics, adherence, irregularities, coloration, limitation of functions caused by scars (observed or referred by the patient) or change in sensation referred by the patient. Based on these qualitative criteria, the scars were classified in a scale from 0 (no scar, normal skin) to 5 (very poor quality scar). The level of satisfaction in scar quality or the importance attributed by patients to the quality of the scar was not collected.

Ethics

Each patient received detailed information and gave written informed consent for study participation prior to the patient's inclusion in the study. They consented to the processing of their data and authorized the publication of photos of their wounds for scientific purposes in accordance with Italian law. The kits were offered by the manufacturer. Observational retrospective studies do not require Ethic Committee authorization according to Italian law.

Results

Seven patients, 5 males and 2 females were included in the study. The median age was 75 years (range 65-83 years). During the first visit, an accurate anamnesis was collected and all patients underwent routine blood chemistry examinations. Pharmacologic therapies in place and main comorbidities and multimorbidities (hypertensive cardiomyopathy, cerebral and peripheral vascular disease, type 2 diabetes, hypertension, leukocytoplastic vasculitis, etc.) and are listed in table 1.

All patients had one wound under study. Etiology included diabetic foot, ischemic, venous and vasculitic ulcers. In particular patient 1 (Figure 1), suffering from atherosclerotic arteriopathy, had been treated with femoro-distal bypass followed by trans-metatarsal amputation and the amputation stump healed regularly. Following the closure of the by-pass, the patient developed an ischemic lesion on the scar of the amputation stump. Treated with vasoactive drugs, the pain was reduced, but the wound showed no signs of healing after 4 months of therapy.

Patient 7, suffering from atherosclerotic arteriopathy, following acute ischemia was treated a few years ago with a femoral-distal bypass, with remission of symptoms. In subsequent checks, the by-pass was occluded, but no signs of critical ischemia appeared. Subsequently, the patient developed leukocytoclastic vasculitis (biopsy) with the appearance of an ulcer on the left leg. The ABI was just above 0.6 and we can't exclude that the wounds had an ischemic component even if this did not play a predominant role. After one year of therapy with vasoactive and immunomodulatory drugs, the lesion showed no sign of healing, therefore the patient was referred to the dressing with whole blood clot. Unfortunately, patient 7 subsequently contracted COVID with serious complications that led to his death.

Patient 6 was treated with Negative Pressure Wound Therapy (NPWT), patients 1 and 5 were treated with hydrosurgical units, the others were treated only with advanced dressings appropriate to the needs of the wound. The principles of wound bed preparation[2] were carefully applied and periodically re-evaluated, but they did not produce the start of healing. All wounds had an adequately prepared bed at the start of the dressing with red clot from whole blood. If compression therapy was indicated (patients 4 and 5) a short-stretch bandage was applied.

Etiologies, age, and diameters of the wounds are listed in detail in table 1. Median age of the wounds was 4 months (range 3-12 months). Before the first whole blood red clot dressing application, each wound bed was prepared according to the wound bed preparation principles. Infected wounds were evaluated with culture on biopsy sampling with subsequent systemic antibiotic administration, if necessary. At the first whole blood red clot application the wounds had a median surface area of 13.07 cm^2 (range 5.42-94.20 cm^2).

All clot preparation sessions resulted in adequate clot formation and in no case it exceeded the scheduled time (12 minutes) also in patients under anticoagulation or antiaggregation. Clots too big to be placed directly on the wound were cut by means of a sterile scissors to remove the excess of biomaterial.

	Pat. 1	Pat. 2	Pat. 3	Pat. 4	Pat. 5	Pat. 6	Pat. 7
sex	m	m	m	f	f	m	m
age	79	82	75	68	65	74	80
Comorbidities and multi-morbidities	Diabetes II HHD CAD *(bypass)* PAD *(femoral-distal bypass)*	HHD CAD	Diabetes II Obesity HHD Larynx cancer *(laryngeectomy)*	Hypertension	Phlebo-lymphedema	Chronic CLI HHD CAD	Diabetes II Type B AoD HHD CAD *(bypass)* PAD *(femoral-distal-bypass)*
Anticoagulant or antiplatelet therapy	DOAC	DOAC	antiplatelet	-	-	antiplatelet	DOAC + antiplatelet
Wound etiology	diabetic foot (ischemic)	ischemic	diabetic foot (ischemic)	venous (atipic)	veno-lymphatic	diabetic foot (ischemic)	vasculitic
Wound age	4 months	4 months	4 months	3 months	>12 months	6 months	12 months
Wound area at 1st application	13.07 cm^2	5.42 cm^2	94.20 cm^2	5.35 cm^2	38.78 cm^2	10.05 cm^2	47.10 cm^2
NRS scale at 1st application	5	4	5	4	6	5	8
Total number of applications	18	5	8	6	15	7	5
Days of treatment	123	51	61	47	104	48	35
Quality of scar (0-5)	3	3	2	1	3	3	-

Table 1: Key data of the patients included in the study. Patient 7 abandoned the treatment because he contracted the COVID-19 infection and later died. HHD: hypertensive heart disease; CAD: coronary heart disease; PAD: peripheral arterial disease; CLI: critical limb ischemia; AoD: aortic dissection; DOAC: direct oral anticoagulant.

Table 1 summarizes the clinical outcomes of wounds treated with the whole blood red clot. One patient abandoned the treatment because he contracted the COVID-19 infection and later died. Complete healing was achieved in all remaining wounds. Median treatment period was 56 days (range 47-123 days), with a median of 7.5 applications (range 5-18 applications). At the 4th treatment, all wounds reduced their diameter of at least 30%. During the treatment period, no exudate imbalance was observed and there were no sign any adverse reaction in the wound and in the perilesional area. None of the patients had infections at the time of initiation of therapy and clinical control of infection was carefully performed at each dressing change.

No patient showed signs that could indicate critical colonization or infection, so no culture was performed.

Pain was assessed according to the NRS scale. At the first whole blood red clot application the median NRS value of pain was 5 (range 4-8) All patients had a reduction in their pain from the first application of whole blood red clot and after the 4th application none experienced any pain (figure 2). On a scale between 0 (normal skin) and 5 (very poor quality scar) the average score of scars was 3 (range 1-3). Scars are shown in figure 3.

No change in the extent of arterial obstructions, either in the morphology of the Doppler signal or in the ABI was found.

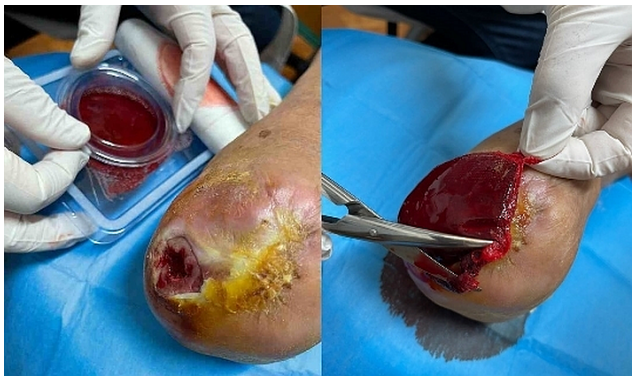

Figure 1 - Whole blood red clot application with adaptation to the size of the wound (patient 1)

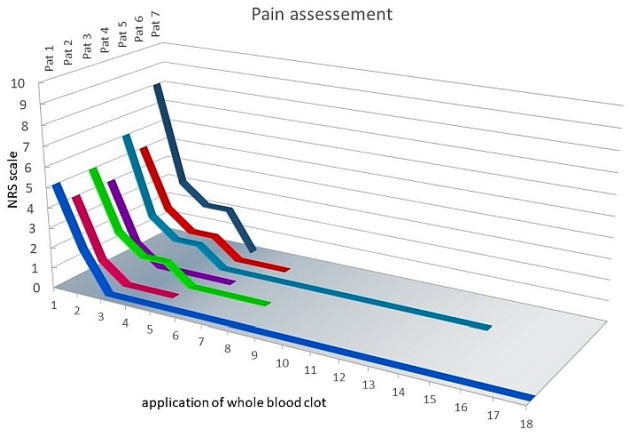

Figure 2 - Pain assessment, evolution of the NRS score over the course of treatment. Patient 7 abandoned the study because he contracted the COVID-19 infection and later died.

Limitations

A case series is a collection of patients who share common characteristics used to describe some clinical, pathophysiological, or operational aspect of a disease[26]. Case series rank low in the hierarchy of evidence, but have a role in recognizing new diseases or in the unusual presentation of known diseases, in identifying new risk factors or the adverse effects of new drugs or therapies and finally are useful for generating hypotheses. Although they provide data on the effects of a therapy, they are not useful to test hypotheses. Wounds may arise from a variety of etiologies such as peripheral arterial disease, venous hypertension, diabetic neuropathy, pressure, vasculitis, burns and others and for this reason studies have to be adequately dimensioned. Our results from a very small sample of hard-to-heal wounds should not be overestimated and require further testing with randomized controlled and adequately dimensioned studies. Further studies should be performed on the follow-up of scars and to evaluate the probability of relapse in wounds treated with this method.

Discussion

The whole blood clot was effective and safe in treating the patients with hard-to-heal wounds included in this study. Healing process was activated by autologous whole blood clot application in spite of comorbidities, included diabetes, witch delay the healing process.

All treated subjects were affected by important comorbidities, listed in table 1, which can interfere with healing. In particular, diabetic patients (1, 3 and 6) presented with a diabetic foot. The diabetic foot is a complication of complications as it includes ischemic, neuropathic and infectious components. In all patients under examination, the infectious component, if present in the past, was adequately treated. When in the genesis of the wound, the ischemic factor was prevalent, they were indicated as ischemic. For a necessary completeness of information, however, it was indicated in the table that they were diabetic feet. Other factors interfering with healing such as drug treatments (eg corticosteroids) were not present or had been eliminated long ago.

The medication procedure is safe and simple because the whole blood red clot is obtained from autologous blood extemporaneously sampled, does not require any specific instrumentation nor training and can be performed at the patient's bed both in hospital and at home. Platelet Rich Fibrin (PRF) is based on a white clot with an increased platelet concentration and is obtained by centrifugation that requires adequate staff training and specific instruments.

The whole blood red clot matrix is well tolerated by the patient: the only case of abandonment of the treatment was caused by an event independent of the wound and the dressing (COVID19 infection in the course of a pandemic).

The formation of the clot is physiologically the first phase of tissue repair following an injury and creates the first temporary matrix that replaces the one that has been destroyed. In acute wounds the fibrin scaffold is the first provisional matrix that modulates inflammation, supports cell migration and hosts cytokines and growth factor[5]. Blood clot is a natural healing agent and when applied on a chronic wound recreates an ambient similar to an

acute wound, and provides the growth factors necessary for restarting or accelerating the healing process.

In addition, it exerts a mechanical and thermal protective action and a regulation of the exudate, creating the environmental conditions that favor the repair process as seems confirmed by the good control of the exudate, the absence of infectious complications and the improvement of pain. Finally, the clot is dissolved and substituted by the new provisional matrices. Also the satisfactory quality of the scars can be a consequence of modulation of inflammation produced by the whole blood clot.

No change in color Doppler ultrasound was recorded. It appears implausible that topical treatment could improve regional perfusion.

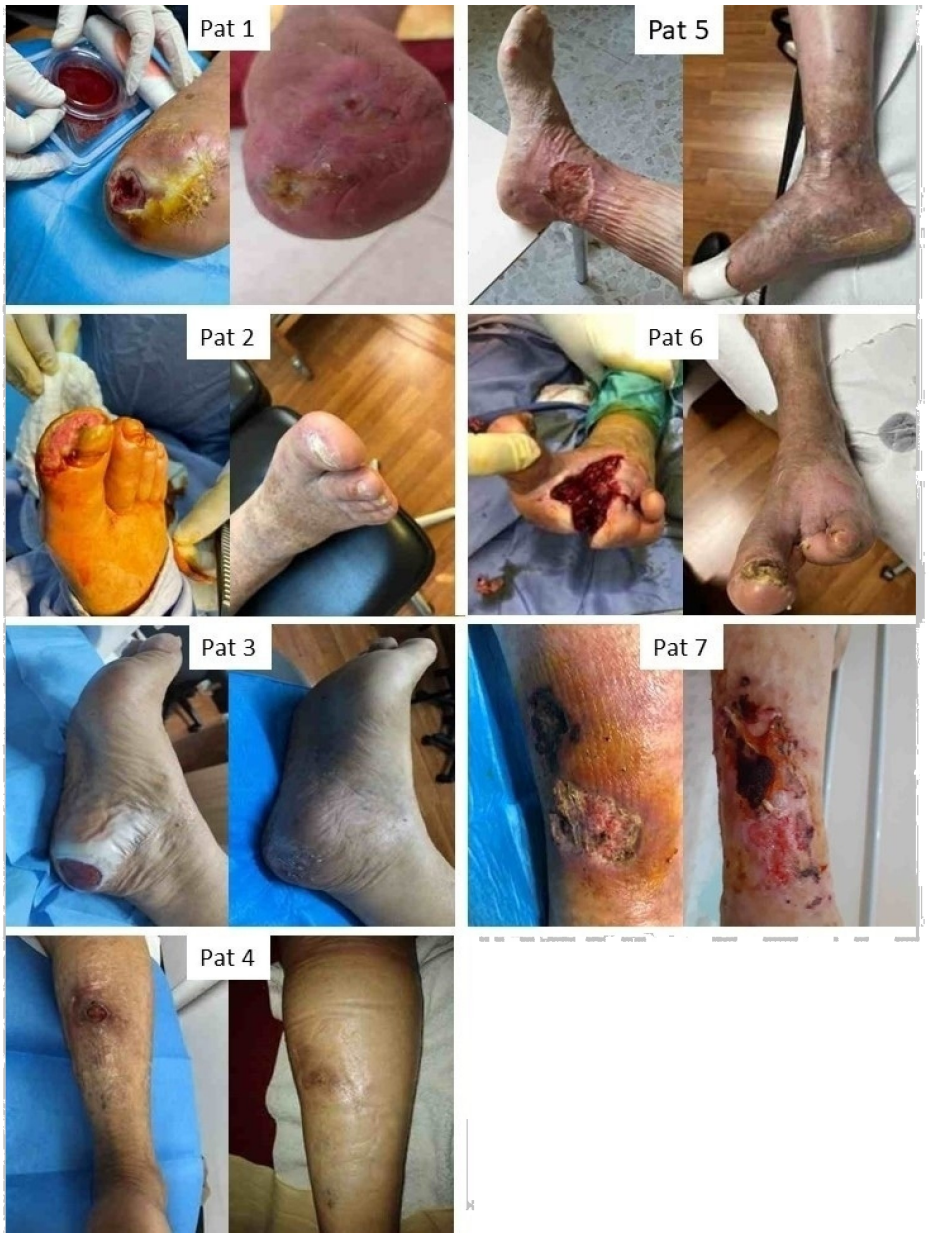

Figure 3 - Wounds at the time of the first application of whole blood red clot (left) and the respective scars at the end of treatment (right). For the patient 7, who died during treatment because he contracted the COVID-19 infection, is shown the last recorded image.

Conclusion

This study, even if on a very limited sample, makes us appreciate the simplicity of preparation of the method that can also be carried out at the patient bed and by nurses with minimal training. The biological material belongs to the same patient and does not undergo any significant manipulation other than the activation of the natural coagulation process. The whole blood red clot is also a matrix whose mechanical properties allow it to be cut out to adapt it to the various dimensions and morphology of the wounds. The good response obtained in subjects with ulcers of various etiologies and the absence of adverse events makes it a useful future tool in the treatment of wounds.

Implications for Practice

The whole blood clot is a natural and inexpensive method of wound dressing that can be used at point of care and does not require any medical support at reapplication. Moreover, this method requires just a very short a training time for nurses. Whole blood red clot dressing has high potential for cost effectiveness:

1. requires no medical staff, but can be practiced by trained nurses with a short course;
2. can be done in bed or at the patient's home without the need for complex equipment with minimum costs and training time;
3. it is safe as the red clot from whole blood is prepared extemporaneously with the patient's own blood with minimal manipulation, which reduces the risk of infection;
4. the kit for the preparation of the whole blood clot is inexpensive and not bulky;
5. preliminary results from this case series indicate a good patient response in terms of pain and reactivation of the wound healing process in ulcers of different etiology;
6. this dressing could be used in subjects who have chronic wounds that respond unsatisfactorily to therapies properly conducted and periodically re-evaluated.

Recommendations for Future Study

Controlled studies are needed to evaluate the real effectiveness of using the whole blood clot in the treatment of skin ulcers by comparing it with platelet concentrates or "advanced dressings". Due to the considerable variability of the causes of skin ulcers, these studies will have to be adequately sized or conducted on selected groups of patients. It would also be interesting to know from pathophysiological and histological studies what may be the role of red blood cells in the whole blood red clot dressing, which is the only method that requires their presence in the dressing.

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