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New Trends

The role of autologous fibrin-platelet glue in plastic surgery: A preliminary report

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ABSTRACT: To promote wound healing, we used autologous fibrin-platelet glue in 14 patients with skin and soft tissue losses caused by recent trauma or chronic pathology. The level of improvement was scored, arbitrarily, from 0 to 4. Very favourable results (score 3-4) were seen in 11 out of 14 patients. The glue preparation is very easy, inexpensive and creates excellent and stable hemostasis. From a general point of view, we have confirmed the utility of fibrin-platelet glue in terms of reduced infections and length of hospital stay. (Int J Artif Organs 2002; 25: 334-8)

KEY WORDS: Plastic surgery, Autologous fibrin-platelet glue, Growth factors

INTRODUCTION

Autologous platelet gel is currently being used in many surgical specialities with apparent clinical success. In plastic surgery it has been most frequently described as an autologous alternative to fibrin glue (1-3): this kind of application has proved especially useful in the United States where homologous fibrin glue was not approved by the FDA. In maxillofacial surgery it has been demonstrated that platelet gel mixed to autologous bone fragments accelerates the process of alveolar bone regeneration (4, 5) and is therefore effective for closure of alveolar defects and oral-antral fistulas. This effect can be attributed to the presence of at least two growth factors (i.e. Platelet Derived Growth Factor, PDGF, and Transforming Growth Factor-ß1, TGF-ß1) within platelet gel (5, 6). PDGF is one of the earliest growth factors discovered: it is a small molecular weight glycoprotein that serves as a potent mitogen for mesenchymal cells involved in wound healing (7, 8). Recombinant PDGF effectiveness for topical treatment of long-standing ulcers has been demonstrated by a multicenter, double blinded, placebo controlled trial (9). Due to its high content of PDGF and TGF-ß1, along with the hemostatic and glue-like properties when used along with fibrin glue, autologous platelet gel seems ideally suited for a wide range of applications in plastic surgery, ranging from controlling intraoperative bleeding or sealing widely undermined flaps to improving the healing of difficult wounds. Several studies (10) report distinct advantages deriving from the application of biologic glue to recent or long-lasting wounds: among these the most important advantages seem to be prevention of hematoma, reduced blood loss, early graft adherence and improvement of graft take, antibacterial action, shorter hospital stay. A further advantage is the fact that fibrin glue is completely reabsorbable (which is not the case for other surgical adhesives commercially available).

The aim of the present paper is to describe how we have recently incorporated the use of autologous fibrin-platelet glue in our practice, and to compare our preliminary results with what could be expected on the basis of our previous clinical experience and available data.

Autologous fibrin-platelet glue preparation

Platelet glue is usually obtained adding some milliliters of thrombin-calcium chloride solution to concentrated platelet glue suspended in 15-20 mL of plasma. When these components are mixed, thrombin transforms fibrinogen to fibrin monomers. Factor XIII is activated by thrombin in the presence of calcium ions, causing cross-linking and further stabilization of the fibrin coagulum. A

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firm coagulum is therefore produced that can be easily handled and, according to the specific clinical requirement, can be applied to raw surface or inserted in cavities to obliterate them. Unfortunately the clinical use of bovine thrombin is under debate in Europe due to the risks of transmission of prions. Even the use of human thrombin is questionable if the theoretical risks of virus disease transmission are considered. As a consequence, in an attempt to avoid these risks, in the preparation of the platelet gel, we have substituted thrombin with batroxobin, a Russel's viper venom, commercially available under the name of Botropase (Ravizza Farmaceutici Spa Milano, Italia) in vials containing 1±0.2 U thrombin equivalents (NIH). Batroxobin is a thrombin-like enzyme that is present in the venom of the snake *Bothrops atrox* (11) and has been used as a procoagulant for many years (12): it is still commercially available all over Europe. Its action is due to activation of the coagulation factor X (13) as well as to induction of platelet aggregation (14). To increase the sealing/healing efficacy of the platelet gel we have also used an autologous cryoprecipitate obtained along with platelets during the same autopheresis session.

Briefly, autologous platelets anticoagulated with ACD-A were collected according to the dry-platelet collection technique employing an Excel apparatus (Dideco Spa Mirandola, Italy), as previously described (15). From 3.6 to $5x10^{11}$ platelets were collected in 20-25 mL of plasma, along with 450-500 mL of autologous plasma subsequently used for obtaining 35-45 mL of cryoprecipitate. After collection the plasma unit was immediately frozen at -80°C in a mechanic refrigerator, the frozen plasma was then put at 4°C for 18-24 h for spontaneous thawing. Cryodepleted plasma was removed and the residual cryoprecipitate dissolved in 35-40 mL of plasma, divided in aliquots and frozen for subsequent use. The apheresis platelet concentrate was aliquoted as well, and those not immediately used frozen at -80°C, without any further manipulation. Each aliquot contained $0.8x10^{11}$ platelets.

Immediately prior to the end of operation or medication one platelet aliquot is mixed with one aliquot of cryoprecipitate in a sterile Petri plastic dish. After mixing, 2-5 vials of Botropase are used for dissolving 0.5-1 g of calcium gluconate: the solution is aspirated into a syringe and spread under pressure onto the cryoprecipitate-platelet mixture. The content of the Petri dish is evenly mixed until it becomes gel-like, which usually takes from 2 to 4 minutes, depending on the amount of Botropase added to platelets and cryoprecipitate. This is the fibrin-platelet glue that can now be manipulated, moulded, cut into small pieces and used as needed. The glue can also be prepared as a "spray" for intraoperative usage in the surgery of skin grafts and flaps.

When using the fibrin glue spray, what was done in 6 cases, it was applied on both the dermal side of the skin graft and the recipient side; at this point gentle, uniform pressure was applied over the entire unit for a few minutes immediately and a few absorbable sutures were placed at the corners of the grafts, thus providing a simpler and quicker application.

RESULTS

Between February 2001 and October 2001, a total of 14 patients with soft tissue losses caused by recent trauma or chronic pathologies were treated by application of autologous fibrin glue and platelet gel. As shown in Table I, the caselist includes patients with acute traumas (mostly road accidents) and patients with chronic pathologies such as diabetic ulcers, pressure sores (4^{th} grade), venous and arterial ulcers and a rare case of radiation injury in the outer malleolar region. All pathologies involving the lower limbs were complicated by exposure of tendon and in one case by exposure of tendon and bone stumps of the left tibia. Age of the patients ranged from 18 to 79 years (average 56.4 years).

Bacterial infection was assessed in every lesion and treated by local antiseptics and, when necessary, by a targeted antibiotic therapy. In most cases bacterial infection was due to *Staphilococcus Aureus* and/or *Pseudomonas Aeruginosa*.

Autologous fibrin glue and platelet gel, obtained according to the previously described method, were applied one or more times to the wounds.

The mean treatment frequency was one application every 7 days, repeated several times, occasionally suspended for some time. The mean number of applications per patient was 3, with a minimum of 1 application in three cases of pressure sores and in one case of trauma of the knee with a deep soft tissue loss. In these cases, the gel was used to file the cavity. Once the cavity was obliterated by the gel, the skin edges were advanced by a few skin sutures thus closing the opening. Skin sutures were left in place until the gel inside the cavity had been substituted by granulation tissue, which in our experience, took place in 28 to 35 days. The mean

TABLE I - CLINICAL CHARACTERISTICS OF THE 14 PATIENTS TREATED WITH FIBRIN OR FIBRIN-PLATELET GLUE

Pt	Age	Sex	Cause	Location	Size of the wound cm	Exposure of tendon	Infection +/-	Previous treatment
1	48	M	diabetes	right foot	6 x 9	+	+	debridement
2	65	F	diabetes	left foot	5 x 9	+	+	debridement
3	72	F	diabetes	left foot	10 x 12	+	+	debridement
4	68	M	diabetes	left foot	4 x 7	+	+	debridement
5	61	M	diabetes	right foot	6 x 11	+	+	debridement
6	72	F	venous ulcer	right leg	9 x 7	+	+	debridement
7	75	F	venous ulcer	left leg	12 x 8	+	+	debridement
8	18	M	pressure sore	ischiatic	2 x 3	+ and bone	+	debridement
9	60	M	pressure sore	ischiatic	1 x 4	+ and bone	+	debridement
10	48	F	pressure sore	sacrum	2 x 4	+ and bone	+	debridement
11	42	M	trauma	left leg	12 x 9	+ and bone	+	debridement
12	18	M	trauma	right knee	3 x 5	+	+	debridement
13	74	F	radiation injury	right leg	6 x 3	+	+	debridement
14	69	F	arterial ulcer	right leg	2 x 6	+	+	debridement

length of treatment was 45 days and the covered surfaces ranged from 4 to 120 square cm.

The fibrin-platelet gel was used to stimulate the tissue in the following cases: fracture of the tibia, to regenerate bone tissue; exposure of tendon, to have sufficient granulation tissue to receive sheet grafts or meshed grafts or flaps. From 1 to 11 applications (5 on average) were required to produce a visible and undeniable improvement in most patients. The level of improvement was scored, on an arbitrary base, from 0 (no improvement) to 4 (complete healing). In this scale score 1 meant only perilesional tissue improvement, score 2 meant graft take under 80% for poor granulation tissue and score 3 graft take over 80% with adequate granulation. Very favourable results (score 3-4), as the one shown in Figure 1, were seen in 11 out of 14 patients as summarized in Table II.

TABLE II - CLINICAL IMPROVEMENT AFTER FIBRIN OR FIBRIN-PLATELET GLUE APPLICATION, SCORED FROM 0 (NO IMPROVEMENT) TO 4 (COMPLETE HEALING) ON AN ARBITRARY SCALE

Pt	Number of applications	Length of the treatment (days)	Improvement Score
1	5	30	4
2	7	45	3
3	9	60	3
4	4	30	3
5	7	45	4
6	5	40	2
7	8	57	3
8	1	15	4
9	1	15	4
10	1	15	4
11	12	120	4
12	1	28	4
13	6	90	1
14	10	90	1

DISCUSSION

This study demonstrated that the use of autologous fibrin-platelet gel in plastic and orthopedic surgery with procedures involving flaps, mesh and sheet grafts as well as in the treatment of difficult wounds had many advantages. The use of autologous material eliminates the risk of transmission of viral disease and the formula for platelet gel and fibrin glue preparation is simple and standardised. The glue is inexpensive and creates excellent, stable hemostasis and its use as tissue stimulant is unrivalled on the market: the cost of 4U of fibrin glue doesn't exceed 300 US$ which is much less than the cost of a single fibrine-glue (500$) or Tissucol (1000$) preparation and is marginal if compared with the cost of recombinant PDGF to get the same results.

No study was carried out on platelet quality or viability since platelets were frozen without any cryoprotectant since, in reality, their function after thawing is the provision

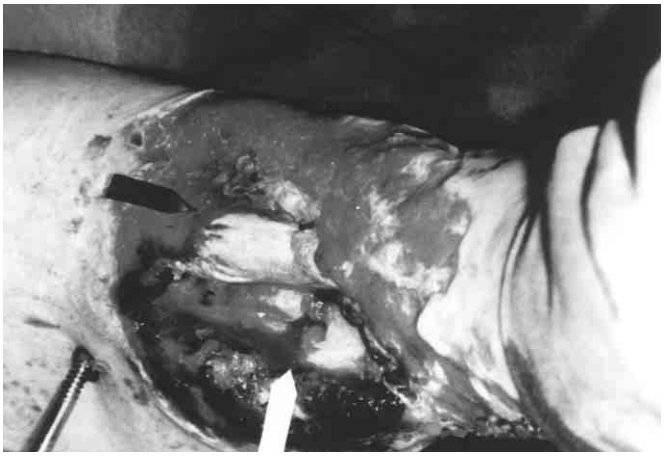

Fig 1a - *Left leg trauma injury. The black arrow indicates the tendon exposure. The white arrow indicates the bone exposure.*

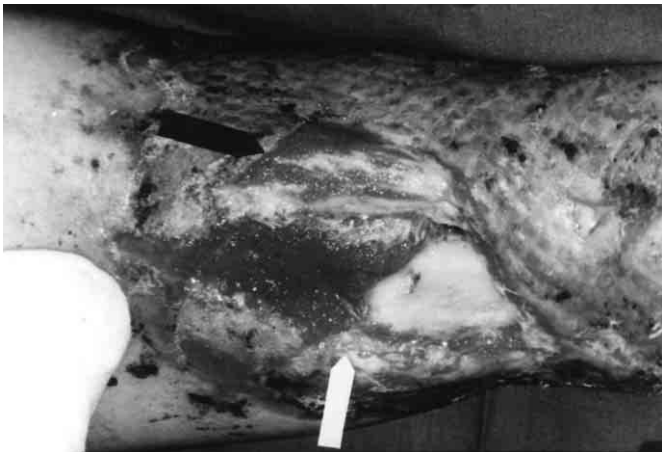

Fig.1b - *Left leg trauma injury after plastic surgery and application of autologous platelet-fibrin glue. The black arrow indicates the tendon exposure. The white arrow indicates the bone exposure.*

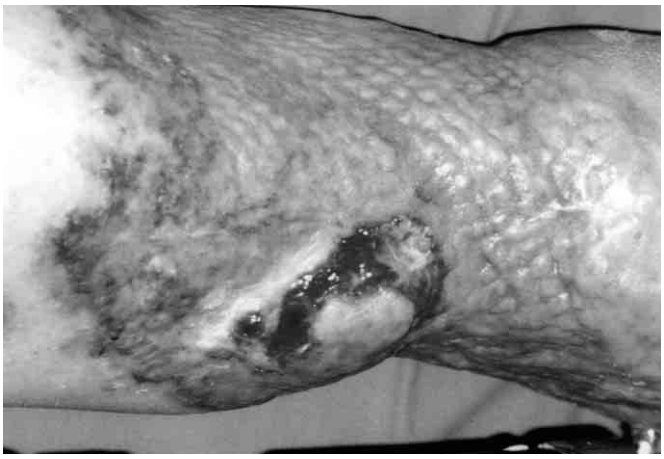

Fig. 1c - *Left leg trauma injury: residual lesion*

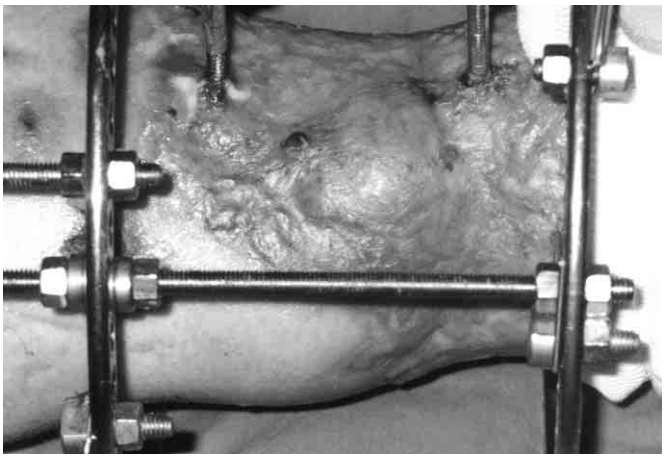

Fig.1d - *Left leg trauma: complete healing after a new application of autologous platelet-fibrin glue.*

of phospholipidic membranes along with the provision of preformed growth factors to be incorporated into the gel.

Nonetheless from a clinical point of view and using as a comparison the results obtained in the same or comparable patients seen at our facility who were treated conventionally, we are convinced of the absolute utility of the fibrin-platelet glue also in terms of reduced infections and length of hospital stay. Our preparations may not be as sophisticated as the ones produced and studied by Zimmermann et al (16). We don't believe that the knowledge of the WBC contamination, aggregability, activation and release of the content of α granules is of importance since we have intentionally frozen the platelets without any cryoprotectant, getting clinical results which, probably, do not differ from the ones produced in Zimmermann's experience. What appears to be of importance in the incorporation of platelet lysate in the cryoprecipitate is phospholipidic substrate for orienting the fibrin strands and the presence in the lysate of growth factors. There is thus no need for fresh or viable platelets and there is no need for storing them in a viable status, as also shown by the studies of Landesberg et al (17) who confirmed that freezing and thawing is the best system to get growth factors released from platelets. On the basis of our experience we think that fibrin-platelet glue is different from and has different indications from fibrin glue (which

lacks high levels of cytokines and growth factors), from platelet gel (which has minimal adhesive properties) as well as from recombinant grown factors (even if FDA approved). We also feel that Snyder's editorial, published in Transfusion (18), has a misleading title, since fibrin-glue cannot be contained in the definition "of lotions and potions" but may become another successful field of application in therapy, especially when production is limited to the autologous setting.

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