L-PRP/L-PRF in Esthetic Plastic Surgery, Regenerative Medicine of the Skin and Chronic Wounds

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Abstract: The use of platelet concentrates for topical use is of particular interest for the promotion of skin wound healing. Fibrin-based surgical adjuvants are indeed widely used in plastic surgery since many years in order to improve scar healing and wound closure. However, the addition of platelets and their associated growth factors opened a new range of possibilities, particularly for the treatment of chronic skin ulcers and other applications of regenerative medicine on the covering tissues. In the 4 families of platelet concentrates available, 2 families were particularly tested and used in this clinical setting: L-PRP (Leukocyte- and Platelet-rich Plasma) and L-PRF (Leukocyte- and Platelet-Rich Fibrin). These 2 families have in common the presence of significant concentrations of leukocytes, and these cells are importantly involved in the local cleaning and immune regulation of the wound healing process. The main difference between them is the fibrin architecture, and this parameter considerably influences the healing potential and the therapeutic protocol associated to each platelet concentrate technology. In this article, we describe the historical evolutions of these techniques from the fibrin glues to the current L-PRP and L-PRF, and discuss the important functional properties of the platelet growth factors, the leukocyte content and the fibrin architecture in order to optimize the numerous potential applications of these products in regenerative medicine of the skin. Many outstanding perspectives are appearing in this field and require further research.

Keywords: blood platelet, fibrin, growth factors, plastic surgery, platelet-rich fibrin (PRF), platelet-rich plasma (PRP), regenerative medicine, wound healing.

1. INTRODUCTION

The use of fibrin-based adjuvants in order to improve soft tissue healing is an old strategy in plastic surgery [1]. The first studies on fibrin glues were done on the skin [2], and the first clinical tests of platelet concentrates were done on chronic skin ulcers [3-5]. These products were first of all fibrin gels and had a clear beneficial impact on skin healing [6, 7]. Indeed, the fibrin matrix has a strong effect on soft tissue healing through neangiogenesis and cell proliferation and migration for wound closure [8, 9]. The use of platelet concentrate gel was therefore a natural evolution of the fibrin adhesives [10]: the addition of autologous platelets and their growth factors was a way to reinforce the fibrin matrix and stimulate the cells on the wounded site. If the craze for “growth factors” started and developed strongly in oral and maxillofacial surgery [11, 12], these technologies were always of particular interest in the field of plastic surgery and regenerative medicine of the skin [13].

In the literature, the exact content of the platelet concentrates is often neglected, particularly the leukocyte content and the fibrin architecture [1, 14]. Several authors indeed pointed out that most platelet gels were in fact leukocyte-platelet gels [15-17], and that leukocytes may have a strong influence on the healing equation [18-23], and this is particularly true in the field of skin healing [24]. Like in many fields of science [25], the lack of proper classification and characterization of the various technologies was the source of many misunderstandings. Recently, a complete classification system was proposed [1] and reinforced [14], based on the leukocyte content and fibrin architecture of the numerous products. In the 4 families of platelet concentrate technologies [1], 2 families were particularly investigated in this clinical setting: L-PRP (Leukocyte- and Platelet-rich Plasma) and L-PRF (Leukocyte- and Platelet-Rich Fibrin). These 2 families contain significant concentrations of leukocytes, but their fibrin architecture is different: the L-PRP is an injectable leukocyte-platelet suspension [26, 27] that can be activated as a gel on the wounded site, and L-PRF is on the contrary a solid fibrin-based biomaterial that can only be used as a fibrin bandage [28]. These 2 forms of products require necessarily completely different methodology of clinical use.

In order to use these new technologies in the best possible way, it is first necessary to understand their intrinsic biology and their potential interactions with the tissues. This is the key step to transform these blood concentrates into reliable therapeutic options.

2. WOUND HEALING PROCESSES

Wound healing, whether from accidental injury or surgery, is a complex process which is composed of interactions between different types of cells and mediators including growth factors. The wound healing process consists of two components: regeneration and repair, and the difference be-

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between them is based on the final tissue formation [29, 30]. During the regeneration process, the tissue is replaced by the proliferation of surrounding undamaged cells, while in repair, the tissue defect is replaced by granulation tissue which promotes scar formation. Noncomplicated wounds heal by primary adhesion (sanatio per primam intentionem) within 6 to 8 days, as epithelial closure with sparse connective tissue formation in smooth-edged wounds what results in a small scar formation. In extensive soft tissue defects, especially with infection coexistence, wound tissue healing passes by granulation and secondary adaptation of the wound edges (sanatio per secundam intentionem), and often take as long as weeks to heal, resulting in a clearly visible scar [29-31].

Physiological wound healing process consists of four distinct phases: hemostasis, inflammation, proliferation, remodelling, although this process is continuous and each phase overlap the next [32]. First phase - hemostasis - begins at the moment of wounding when different tissue factors and intracellular calcium are released, with factor VII activation and coagulation cascade initiation [32, 33]. In this phase, a 5-10 minutes period of vasoconstriction is followed by vasodilation. Hemostasis is secured by fibrin plug with fibrin fibers which become a wound matrix. The plasma-coagulation cascade is usually divided into two branches for convenience of discussion and coagulopathy testing. The intrinsic and extrinsic branches can be separately potentiated, but merge into a common pathway leading to thrombin (FIIa). FIIa hydrolyses fibrinogen into fibrin units that oligomerize into a fine mesh which, in turn, causes plasma to gel or clot. The extrinsic pathway is responsible for hemostatic control and response to vascular injury [33].

Platelets play an essential role in hemostasis. When adhered to exposed endothelium or activated by agonists, they change their shape and secrete the contents of the granules (including ADP, fibrinogen and serotonin), what is followed by platelet aggregation Fig. (1) [34]. Aggregation of platelets is mediated by molecules of fibrinogen or vWF, which connect platelets by bridging the glycoprotein IIb/IIIa complexes on adjacent platelets, forming a platelet aggregate. During this phase of the coagulation cascade, arachidonic acid pathways, growth factors and cytokines initiate an inflammatory phase [34, 35].

![Fig. (1). Platelet processes after their activation.](image)

At the beginning, an inflammatory phase is initiated by neutrophils responsible for bacteria opsonization and de-

struction and removing foreign debris from the wound, providing the first line of defense against infection [36]. Lymphocytes and macrophages are the next cells attracted in the wound Fig. (2). In the late inflammatory phase, monocytes are converted into macrophages which destroy bacterial pathogens and remaining neutrophils. Macrophages begin the transition from wound inflammation to repair by secreting enzymes and transforming growth factors (TGF α and β), platelet-derived growth factor (PDGF), interleukin 1 (IL-1), fibroblast growth factor (FGF) and tumor necrosis factor (TNF). These factors induce angiogenesis and stimulate fibroblasts to produce collagen, and therefore cell migration, proliferation and tissue matrix formation [37].

![Fig. (2). Evolution of the number of cells at the wound site after injury.](image)

Proliferation phase begins 2-3 days after wounding and is determined by two processes: granulation tissue formation and epithelialization. Growth factors which are released from platelets and macrophages, stimulate migration and activation of wound fibroblasts. In early proliferation phase, fibroblasts activity is limited to cellular replication and migration. Fibroblasts migrate from wound margins using the fibrin matrix created in the inflammatory phase [37, 38]. During the first week after wounding, fibroblasts are stimulated by macrophages for proteoglycans and glycosaminoglycans synthesis. The growth factors stimulate the production of the extracellular matrix of the granulation tissue and particularly the collagen. About 3 days after wounding, the growing mass of fibroblasts begins to synthesize collagen, which levels rise during 3 weeks. Collagen produced in this period determines the tensile strength of the wound. Moreover, three days after wounding starts angiogenesis followed by collagen synthesis, which is also responsible for vascular integrity and strength of the capillary beds [37, 38].

The final phase of wound healing is the remodelling phase and it lasts from 3 weeks to 12 months. Fibroblasts start to produce TGFβ, FGF, KGF, IGF-1 and PDGF and are then the cells with the highest number between 7-14 days after wounding. These cells stimulate collagen fibers and then bundles forming. The increasing content of collagen correlates with increasing tensile strength in the wound. The remodelling process continues up to two years, achieving 40-70 percent of the strength of the undamaged tissue at 4 weeks [39, 40].
3. FROM AUTOLOGOUS FIBRIN GLUES TO AUTOLOGOUS PLATELET CONCENTRATES

Since many years, scientists are looking for an ideal way to accelerate tissue healing. To minimize edema and scarring, they try to obtain hemostasis and closed wound edges by using sutures and autologous tissue glues. At first, the use of autologous blood components in plastic and esthetic surgery was limited to fibrin glues, which were used to obtain hemostasis and skin flaps adherence. Fibrin's adhesive properties were discovered by Bergel in 1909. Grey described fibrinogen first application in liver and cerebral hemorrhage in 1915. However, the first controlled clotting reaction with thrombin usage was performed in 1944 by Tidrick and Warner [41]. Fibrin glue essentially involves the collection of concentrated fibrinogen in the presence of factor XII and pooled plasma proteins [42]. Compared to plasma, the commercial fibrin adhesive contains a 30-fold higher concentration of fibrinogen and up to a 50-fold higher concentration of factor XII, which is responsible for fibrin conversion in vivo. When the fibrin adhesive is set, interlacing fibrin strands form a fibrin mesh resembling a coarse clot. This fibrin structure is said to form the natural environment for an unimpeded fibroblast proliferation and thus should enhance wound healing by conductive properties [43]. Commercial fibrin adhesive has been used to repair osteochondral fractures, meniscal lesions, nerves, tendons and also in soft tissues [43]. Since 1970s, fibrinogen was produced in high concentrations and fibrin-based tissue adhesives became popular. Tidrick, Warner and Cronkite started to use the mixture of fibrinogen and thrombin to fix skin grafts, and this fibrin adhesive slowly became a sealant, to achieve hemostasis and adherence of skin flaps [41, 43, 44].

Fibrin glue is composed of concentrated fibrinogen and fibrin stabilizing factor - factor XIII, fibronectin and cold insoluble globulin. After mixing with thrombin, calcium chloride and one of the fibrinolysis inhibitors (tranexamic acid, E-amino caproic acid or aprotinin), the glue formation is initiated [42]. The conversion of fibrinogen into a cross-linked gel occurs in several steps. Thrombin as an enzyme cleaves the amino-terminal regions of the Aα and Bβ chains of fibrinogen and releases 2 moles each of fibrinopeptide A (FPA) and fibrinopeptide B (FPB) for each mole of fibrin monomer produced [45-47]. The release of FPA has been shown to be more rapid than that of FPB [46]. The fibrin monomers first spontaneously polymerize end-to-end to form protofibrils, which then associate laterally to form fibrin fibers [46-48]. These fibrin fibers form a network and the final fibrin solution is converted to a gel when about 25% of the fibrinogen is converted to fibrin [49].

In facial plastic and reconstructive surgery, fibrin adhesives are widely used as hemostatic agents in rhytidectomy flaps, blepharoplasties, brow flaps, skin grafts and sutures for the closure of incisions. In 1982, Bruck reported his experience with using fibrin glue in 82 rhytidectomies [41]. He noticed in perioperative period a decrease of swelling and an increase in patient comfort. In 1994, Marchac and Sándor evaluated the use of aerosolized fibrin glue in a group of 100 consecutive patients undergoing face lift procedures and compared to a similar group of 100 consecutive patients who had face lifts without fibrin glue [50]. There was a statistically significant decrease in the rate of major hematoma formation and ecchymosis associated with the use of fibrin glue. The incidence of total complications was unchanged between the two groups, despite the fact that there were no drains or postoperative dressings used in the fibrin glue treated group. Patients found the absence of drains and dressings to be most convenient. In the same year, Oliver performed a prospective, randomized, double-blind trial after using fibrin sealant in 20 patients with face lifts, and reported significant decrease of drainage, bruising and swelling [51]. He did not observe incidence of hematoma. Similar results were presented by Fezza in 2002, who used fibrin glue in 24 patients after face lift surgery [52]. He reported less bruising and swelling, no incidence of hematoma and shorter operative time in comparison with another group of 24 patients without fibrin glue.

Fibrin sealant has been used with increasing frequency in a variety of surgical applications for its unique hemostatic and adhesive properties. However, in some fields, where induction properties are required, a more stimulating biomaterial is needed. By the centrifugal separation of autologous whole blood, the leukocyte- and platelet-rich plasma was developed. This wound sealant contains an array of cytokines and mediators that have been demonstrated in vitro and in vivo to increase revascularization, accelerate epidermal and epithelial regeneration, promote angiogenesis, enhance wound strength, hasten hemostasis, improve tissue regeneration, decrease dermal scarring, and facilitate wound remodeling [53, 54].

First attempts of platelet concentrate production were performed in the 1970s, and these first products were then considered as reinforced autologous fibrin glues [55-57]. The first clinical demonstration that platelet concentrate promotes healing processes locally was reported by Knighton et al. in 1986 [4]. They treated 49 patients with chronic non-healing cutaneous ulcers with good outcomes. The critical difference between gelling platelet concentrate and fibrin glue is the platelet presence in high amount and the native concentration of fibrinogen [54], and both parameters influence the adhesive properties of the material. Moreover, by concentrating platelets, high levels of growth factors are reached to stimulate the healing processes. Finally, some studies showed the presence of a substantial concentration of leukocytes in many platelet concentrates.

The use of platelet concentrate techniques and protocols was massively launched first in maxillofacial surgery in the 1990's [11, 12], and from this moment, these techniques developed considerably in plastic surgery [13]. In the course of time, plastic surgeons started to use leukocyte- and platelet-rich plasma (L-PRP) instead of fibrin glue in esthetic surgery (flaps, face and neck lifts, breast reductions and augmentations), with satisfying results and advantages such as reduction of postoperative hematoma, edema and pain and also faster wound healing [53, 54]. L-PRP became more popular and now is widely accepted as an autologous platelet-based wound sealant and hemostatic agent. However, the quantity of published data remained quite limited in plastic surgery.
4. THE EFFECTS OF L-PRP: ANIMAL STUDIES

L-PRP has been used in wound medicine for acceleration of re-epithelialization and improvement of the dermal healing processes. There are only a few studies on animals evaluating the L-PRP properties in cutaneous wound healing and soft tissue regeneration.

Kimura et al. performed an experiment on 60 rats [58]. Six centimeters full-thickness parallel linear cutaneous wounds were created on the back of animals. In the first group, the fibrin glue was applied to the edge of one wound, and the other received no treatment. In the next rats, the wounds were filled up by fibrin glue and platelet-rich plasma respectively. Using histopathological examination, a new formed epidermis at a skin margin on the 3rd day of experiment was observed in the fibrin glue and L-PRP groups. In the 7th day, the collagen network and epidermis growth were significantly increased in L-PRP group in comparison with other groups (i.e. fibrin glue and control group). The breaking strength examination was also performed on the 3rd and 7th day, but no difference between groups was observed. However, after 14 days, a rich neovascularization was found only in the L-PRP-treated group, and the L-PRP group also showed significantly higher mean breaking strength than the fibrin-treated wound. The mean breaking strength of the fibrin glue-treated group was also significantly higher in comparison to the control group.

Another investigation confirmed Kimura’s observations. Two wounds were made in the skin on the back of 20 rabbits: one served as control, and L-PRP was applied on the other [59]. Seven days after wound treatment, the re-epithelialization processes were significantly more intense in L-PRP group than in the control. However, in contrast to Kimura’s study, no difference was observed in the last period. Significant correlation regarding resolution of the inflammatory process was found at the 7th day; 40% wounds from the L-PRP group had resolved it, while none of the control demonstrated full resolution. After 28 days, no significant difference was noted.

We recently performed similar investigations. 64 in-breeding rats have been divided into two groups. In the control group, dorsal incisions were made and then the wounds were sutured with no biomaterial [60]. In the experimental group, L-PRP was placed into the wound via percutaneous application. In all cases, the wound was created by rectangular skin resection. On the base of clinical estimation, wound healing differences were not observed between the groups. However, pathological and immunohistochemical examinations of CD31 immunoexpression showed a wound healing acceleration in the L-PRP group in comparison with the controls, but differences were not statistically important. Molecular analysis of the tissue samples and evaluation of the TGFβ, EGF, VEGF, IL-1α and CD34 gene expressions were also performed, but we did not show large discrepancies. The VEGF gene expression was higher in the L-PRP than in the control group at the 2nd and 3rd days, what may be the evidence of a neovascularization stimulation. However, CD34 gene expression was lower in the L-PRP group, during the whole experimental time except on the 3rd day: CD34 is a marker of the quantity of fully-developed vessels [61]. Therefore, we concluded that L-PRP does not accelerate the healing processes in noncomplicated sutured skin wounds. By using a new form of computer analyzer on the tissue samples Fig. (3), this investigation also opened new perspectives for the evaluation of the L-PRP effects.

Other authors tried similar studies in larger animals. To evaluate the effect of L-PRP on wounds, 3 skin incisions were created on metacarpal regions in 6 horses [62]. 18 wounds were treated with L-PRP biomaterial and bandaged, whereas 18 control wounds were similarly bandaged with no prior topical treatment. Tissue samples were taken from wounds at 1 week for histological examination and measurement of the TGFβ1 concentrations, and at closure for histological examination, biomechanical evaluation and measurement of collagen type I and type III mRNA. Histological, biomechanical and gene expression data did not differ significantly between treated and control wounds. However, TGFβ1 had a 1.6-fold higher concentration in

![Fig. (3). Evaluation of the neovascularization in a wounded tissue using the average optical density (AOD) of a sample (Image-Pro® Plus 3.0 version for Windows).]
treated wounds, compared with untreated wounds. The authors concluded, that topical application of autologous L-PRP did not accelerate or improve the quality of repair of small granulating wounds on limbs of horses. Moreover, they noted that this treatment may better suit wounds with massive tissue loss or chronic wounds.

In contrast to the physiological healing process, the vascularization is disturbed in the surrounding tissues of chronic nonhealing wounds, and the addition of growth factors may induce its development and recovery. However, a relevant chronic wound animal model is difficult to create. Therefore, only case report exists. Kim et al. used L-PRP in a nonhealing wound with tissue necrosis of the skin in dog occurring after follicular cyst excision [63]. On a base of clinical observations, they noted improvement of the local state in the 2nd day after L-PRP gel application. After the 4th week, they observed a decrease of tissue edema and no necrosis.

The clinical application of recombinant growth factors has been reported for many years and presented a positive effect on wound healing. The usage of recombinant growth factors allows particularly to trace the impact of a selected factor on healing processes [64, 65], and could be therefore useful in various animal models. The exogenous application of recombinant growth factors may also be used to prevent acute wound failure. However, the limited clinical benefits do not justify to use of these very expensive molecules, and they are therefore not used widely. Moreover, recombinant factors are not really comparable to the autologous growth factors of L-PRP, because of the variety of the interacting substances contained in the concentrate.

Finally, animal experiments can give us many clues about the effects of L-PRP and their underlying mechanisms, but the differences between human and animal healing (and between human and animal platelet concentrates) and the complexity of the wound models, considerably limit the impact of these studies. For the evaluation of platelet concentrates in plastic surgery, the evaluation of the clinical benefits in human is finally the best method.

5. CLINICAL APPLICATIONS OF L-PRP

The use of L-PRP was widespread in maxillofacial surgery and dentistry, particularly in bone regeneration procedures to accelerate and improve bone graft healing. The scientists also noted soft tissue healing enhancement and fast re-epithelialization of the oral mucosa and gingival flaps [66]. Nowadays, the use of L-PRP is also largely developed in many other fields: orthopaedics, neurosurgery, thoracic surgery, but also in surgical wound regeneration [20, 67-69]. L-PRP is particularly used in plastic and aesthetic surgery in skin resurfacing, blepharoplasty, face lift, forehead lift, cervicofacial liposuction and rhinoplasty procedures [13], as accelerating wound healing biomaterial, and it is also used in extensive post-traumatic wounds [24] and in patients with leg ulcers related to chronic venous insufficiency [70].

5.1. Plastic and Esthetic Surgery

The major complication in plastic surgery is hematoma formation, which has a reported incidence of 1.86-3.9% [71]. The risk of hematoma occurrence is related to the formation of a large raw surface under the skin flap. If the pressure from the hematoma is not reduced sufficiently, it may lead to skin necrosis and further complications.

Marchac and Greensmith performed prospective, nonblinded, randomized, controlled trial in 30 patients undergoing face lifts, and they established the efficacy of fibrin glue in reducing postoperative wound drainage, hematomas and, in particular, the degree of ecchymosis and edema at 24 hours and at 8 days [72]. These differences were statistically significant numerically, but was not thought to be clinically significant. Comparing scores among grades of hematomas, ecchymosis and edema, there were minimal differences between the glued and unglued sides. Similar results were noted in Por et al.'s meta-analysis of randomized controlled trials registered in the Medline database and the Cochrane Central Register of Controlled Trials using key words "fibrin tissue adhesive", "tissue sealant", "platelet-rich plasma", "face-lift" [73]. They reported that the primary effect of tissue sealant was clot formation, binding of the tissue planes together, and diminution of fluid or hematoma collection. The result was a reduction in both wound drainage and postoperative ecchymosis. In another study, the platelet-poor and platelet-rich plasma were combined with a thrombin-calcium chloride solution to produce autologous fibrin glue and autologous platelet-leukocyte gel respectively, and they were applied in 20 patients: 15 patients underwent face and neck lifts and 5 breast reductions. They advocated the usage of L-PRP in procedures associated with capillary bleeding (mainly face lifts, forehead lifts, breast surgery and abdominoplasty), and illustrated the biological relevance of L-PRP for the prevention of complication occurrence. The L-PRP application also optimized the gluing and repositioning of the flaps and reduced postoperative pain and swelling [13]. This effect of pain reduction was already pointed out in many different studies, and is both related to the fibrin bandage and the release of various agents such as the serotonin from the platelet alpha granules [20].

Clevens reviewed 250 facial plastic and reconstructive surgery cases, where L-PRP was applied directly to the resurfaced facial skin as a fine mist with a spray applicator [54]. The L-PRP was allowed to gel, and then acellular plasma was applied over the L-PRP, creating a gelatin-like mask. To provide a measurable method to evaluate the potential benefits of this platelet gel, time to re-epithelialization after CO2 laser skin resurfacing was examined. More rapid re-epithelialization of wounds after laser skin resurfacing was observed with L-PRP. With the application of L-PRP, the mean time for completion of re-epithelialization was 7.8±2.6 days as compared with 11.4±2.1 days for cases in which L-PRP was not used. Clevens concluded that platelet gel represents an opportunity to optimize wound healing in patients undergoing facial plastic and reconstructive surgery. L-PRP significantly minimizes bruising, decreases postoperative pain, speeds healing, enhances flap and graft survival, and hastens re-epithelialization in laser skin resurfacing.

In plastic surgery, the skin flaps are not the sole tissues that may require a stimulation of healing. Fat grafts are often used for the filling of cavities in esthetic surgery. Normal adipose tissue is rich in stem cells that could be particularly receptive to L-PRP stimulation [74]. Therefore some authors
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tried to induce tissue regeneration by adding L-PRP to fat grafting. Cervelli et al. used L-PRP mixed with fat in 15 patients affected by facial aging (characterized by the atrophy of the subcutaneous tissue and the loss of volume)[75]. After 18 months, they obtained excellent aesthetic improvement in these patients. Moreover, further in vitro experiments confirmed that L-PRP stimulates adipose tissue regeneration. In addition, it can stimulate neangiogenesis and fibrogenetic activity of fibroblasts. The authors concluded, that L-PRP sustains an optimal microenvironment that allows adequate adipocytes distribution, better cell-to-cell interaction, adipose tissue growth and differentiation from adipose stem cells (ASCs).

These various publications report interesting results in plastic and esthetic surgery, but it is probable that many other potential applications will be developed in the near future in this field.

5.2. Chronic Wounds

The chronic wounds induced by venous or arteriovenous insufficiency in patients with diabetes or immunological defects such as acquired immunodeficiency syndrome (AIDS), and also in patients with extensive traumatic lesions and burns, cause considerable problems. No fully efficient therapeutic solution exists and many wounds are almost impossible to close. Therefore the research for new treatments is very active, particularly in the use of new biomaterials to treat these lesions.

Chronic nonhealing wounds or skin ulcerations are the result of insufficient repair and the destruction of the local healing potential. The most common etiology of cutaneous ulcers is decreased skin perfusion caused by arterial stenosis and additional infection. Venous hypertension, extensive tissue trauma or pressure also causes this decreased skin and subcutaneous perfusion, what leads to tissue ischemia and necrosis. Traditional treatment of these nonhealing wounds has consisted of a passive attempt to protect the local environment to favor repair of the tissue loss [60]. Oral and parenteral antibiotic are administered to decrease bacterial count in the wound, protective dressings are used to decrease tissue trauma and augment repair, and also topical agents, debridement and removal of the wound exudates are attempted [24, 76]. The chronicity and poor results of conventional therapy, persuade surgeons to look for new methods of treatment. Although randomized controlled studies are rare, L-PRP is increasingly used in therapeutic tissue regeneration in patients with venous insufficiency, pressure ulcers, peripheral vascular disease, autoimmune disorders and injuries, as described in several published clinical and experimental reports in human medicine.

Crovetti et al. used platelet-rich plasma in 24 patients with single or multiple cutaneous ulcers with different etiopathogenesis; diabetes, venous insufficiency, infection and post-traumatic, neuropathic or vasculitis origin [70]. The median initial wound size was 67.4 cm² (range 0.5-560 cm²) with a median depth of 0.69 cm (range 0.2-3 cm). Ulcer duration prior to L-PRP use ranged from one month to 30 years. The authors formed their conclusions on a base of clinical observations. After 10-fold L-PRP application, complete wound closure was reached in 9 patients, 2 were subjected to cutaneous graft, 4 stopped treatment, 9 had partial response and were still receiving the treatment. However, after the first use, the granulation tissue formations were increased in all cases, and the complete re-epithelialization needed different times due to the different size and duration of the ulcers. The authors noted pain relief in all patients. They concluded that topical hemotherapy with L-PRP may be considered as an useful adjuvant treatment for cutaneous ulcers in a multidisciplinary process.

In another study, Bielecka et al. applied L-PRP in 12 patients with venous leg ulcers after performing debridement [60]. Unlike postoperative wounds where percutaneous injection can be done and the whole content of L-PRP can be located in a closed area (and therefore keep the substances active continuously) Fig. (4), the external application on a skin defect may only operate for a limited time. After L-PRP spray application, a dressing is used and may absorb the serum enriched with the active substances of the L-PRP, therefore interfering with the healing potential of the platelet concentrate. For this reason, Bielecka proposed to use first an impermeable dressing fixed with a special glue and then the filling up with L-PRP of the closed space between the wound and the dressing Fig. (5A and 5B). The glued dressing is then kept for 120 hours. In a previous investigation, we used a resorbable membrane as dressing; however, the strength of the membrane decreased with time and after a few days L-PRP was leaking outside. We noted a total wound closure in all cases. The ulcer healing processes were evaluated with clinical observations, but also with histopathological, immunohistochemical and molecular tests of tissue samples (obtained with the ethic committee agreement) taken before and at the 10th day after treatment. The positive effects of L-PRP were obvious.

Fig. (4). L-PRP application into a postoperative wound in a patient suffering from a double mandibular fracture.

The reasons of these positive results may be found in the nature of the nonhealing ulcers. The wound healing processes of these lesions were most time described and analyzed using noninvasive procedure, particularly sample fluid harvesting from skin lesion. Several studies have demonstrated that the extravasated fluid from a venous leg ulcer has elevated levels of cytokines e.g. IL-1, IL-6 and TNFa [77, 78], and unusual low concentrations of growth factors [78]. The
local regulation of the mediators has a great influence on the outcomes of the chronic wounds, and the plasma constituents released following blood vessel disruption are of great importance for the stimulation of wound healing [79]. Logically, the application of platelet concentrates in these wounds can only have beneficial effects on these lesions.

![Image](image.png)

Fig. (5). A. Impermeable dressing with a special glue at the margins to cover the wound. B. L-PRP application into the closed wound in patient with a large chronic ulcer.

Although the chronic venous insufficiency has a low prevalence in a general population, primarily affecting the elderly, it is a major health problem for middle-aged persons who have chronically abused injected drugs [80]. Drug abuse is a risk factor for HIV (human immunodeficiency virus) contamination as well as chronic venous insufficiency and leg ulcers. Chronic venous or arteriovenous insufficiency in HIV positive patients is heralded by varicose veins, edema, stasis dermatitis, indurated discolored skin and leg ulcers, and impairs lower extremity function. Ulcers in HIV infected patients often do not heal with the conservative treatments, even after autologous skin grafting [81]. Although venous ulcers are usually small at the beginning, they often enlarge. Regenerative medicine has begun to adopt new treatments for these skin ulcers. Autologous and allogeneic cultured skin substitutes and growth factors concentrates have been used successfully in various cases, promoting the formation of a granulation tissue and epithelialization [81, 82]. There is no report in the literature concerning the influence of L-PRP on nonhealing wounds in HIV infected patients. However, we covered extensive leg ulcers with L-PRP gels in 3 patients with AIDS Fig. (6). We did not observe positive effect of the L-PRP gel on soft tissue healing with our clinical examinations. Nevertheless, immunohistochemical analysis was performed in the 10th day after L-PRP application and established a statistically significant increased number of blood vessels compared to tissue sample taken before gel covering at day 0. Molecular examinations also showed the decrease of IL-1A gene expression, what can be an evidence of the inflammatory reduction and the beginning of neoangiogenesis.

![Image](image.png)

Fig. (6). L-PRP application in patient suffering from AIDS and chronic leg ulcer.

Driver et al., carried out the first reported prospective, randomized, controlled multicenter trial regarding the use of L-PRP for the treatment of diabetic foot ulcers [83]. 129 patients were screened; 72 completed a 7-day screening period and met the study inclusion criteria. Patients were randomized into two groups: the standard care with platelet-rich plasma gel or the control (saline gel) dressing group. Wounds were evaluated biweekly for 12 weeks or until healing. Healing was confirmed 1 week following closure and monitored for another 11 weeks. An independent audit led to the exclusion of 32 patients from the final analysis because of protocol violations and failure to complete treatment. The authors noted that 68.4% (13/19) of cases in L-PRP group and 42.9% (9/21) in the control group healed. The authors suggested that the majority of nonhealing diabetic foot ulcers treated with autologous platelet-rich plasma gel can be expected to heal.

Patients with extensive traumatic lesions suffer from chronic infected wounds that are resistant to conventional therapy. In these cases, it is important to develop new effective treatment methods, which combine healing inductive and antimicrobial properties. It seems that L-PRP/L-PRF
have these characteristics and can be a breakthrough in the treatment of these infected chronic wounds [19, 21, 23]. There are only a few articles in the literature documenting an influence of L-PRP on post-traumatic lesions. The most important objective in the open injury management is to obtain an adequate soft tissue covering. These soft tissue covering procedures are performed to provide wound closure, to promote revascularization of injured bone and soft tissue, and to prevent late infection and nonunion that may occur secondary to persistent ischemia [84]. The options for covering of traumatic soft tissue defects include skin flaps, skin transplantsations or biological dressings [85]. In 2006, Bielecka and Gazdzik first described the case of a patient with tibia nonunion and soft tissue lesion complicated by infection [86]. Because of the nonhealing wound and nonunion presence, the surgeons performed a radical wound purifying with simultaneous garamycin sponge application. 4 months after revision, MSSA (*methicillin susceptible Staphylococcus aureus*) was present in the culture from the fistula. After application of the L-PRP gel into the soft tissue defect, wound healing was obtained in about 30 days only. After 5 months, union was noted. In another study, Yuan et al. performed 19 operations based on debridement, garamycin application and muscle transplants in patients with post-traumatic chronic femoral ostemyelitis, without positive outcomes before L-PRP gel application into the soft tissue defect [87]. Afterwards they administered twice 5 mL L-PRP gel with thrombin into the wounds, and they obtained finally a satisfying outcome.

Guo et al. evaluated 47 patients suffering from chronic traumatic wounds of lower limbs [88]. With conventional treatments, the wounds did not undergo recovery. Debridement and injection of autologous L-PRP gel were used twice in an interval of 2 months. Two months after the first L-PRP injection, chronic wounds already contracted significantly in 34 patients, purulence and necrosed tissue had disappeared, microcirculation in the soft tissue was improved and the exposed bone or muscle tissues were covered with a proliferative tissue, but no patient was completely cured. However, 2 months after the second L-PRP injection, the average covering rate was 79.3% and the total cure rate was 29.8%.

Bielecka et al. used L-PRP in 6 patients with extensive post-traumatic defects associated with chronic bone and soft tissue bacterial infection [60]. In 2 cases, where bone infection after open fracture occurred, the healing processes were slower; in one patient, plastic surgery intervention was necessary for a thin cutaneous free flap reconstruction. Histopathological and immunohistochemical evaluation in the 10th day confirmed the positive effect of L-PRP on healing processes. Molecular studies also found a statistically significant increase in the VEGF-A gene expression.

Application of platelet-rich plasma has been reported to facilitate wound healing in acute wounds, including burns. To assess the benefits of the L-PRP usage in the treatment of acute limb soft tissue wounds, Kazakos randomized 59 patients with open fractures, closed fractures with skin necrosis and also friction burns, into two groups [89]. The wound healing rate was significantly higher and faster in the L-PRP group in comparison to the control during the whole experimental period; the mean time before plastic surgical recon-

struction in these groups was 21.26 days and 40.6 days respectively. The treatment of burns is therefore a good indication for the use of platelet concentrates, as it was already shown in other fields of research, such as ophthalmology. Marquez-de-Arcena et al. used L-PRP in 10 patients suffering from ocular burns [90]. Statistically significant differences were found between the group treated with subconjunctival autologous platelet concentrate and the group treated with conventional topical medications, with a reduced time for corneal and conjunctival healing with the L-PRP.

Finally, these numerous studies reported the positive impact of L-PRP for the treatment of chronic ulcers, and it is interesting to see that no significant controversy exists in this specific application. However, the definition of efficient clinical methodologies for the use of the L-PRP gel in these applications is still an important issue.

6. THE USE OF L-PRP

L-PRP (mainly the Choukroun’s method) is a more recent technique than the various L-PRP, and is often considered as a second-generation technology [10, 91, 92]. Therefore the number of publications on L-PRP are less extensive than on the PRPs in general. L-PRP clot or membrane is a solid fibrin-based biomaterial, with a specific three-dimensional distribution of the leukocytes and platelet aggregates [28]. The fibrin architecture of the L-PRP is denser than in a PRP or a fibrin glue, and this parameter considerably influence the biological kinetics of this material. This fibrin matrix releases growth factors (such as Transforming Growth Factor β1 (TGF-β1), Platelet-Derived Growth Factor-AB (PDGF-AB) and Vascular endothelial Growth Factor (VEGF)) and matrix proteins (fibronectin, vitronectin and thrombospondin-1) during at least 7 days [93-97]. These properties are of particular interest, because L-PRP can then be considered as a bioactive fibrin bandage that can last several days on a wounded site and release significant amounts of healing factors directly in the wound during the whole time period. These properties were also confirmed during *in vitro* cell cultures, where the L-PRP membrane stimulated proliferation and differentiation in many different cell types, particularly osteoblasts, fibroblasts, adipocytes, keratinocytes and bone mesenchymal stem cells [98-100]. The effects on fibroblasts, adipocytes and keratinocytes are particularly important for the potential applications in plastic surgery and regenerative medicine of the skin [101].

Most publications on L-PRF focused on oral and maxillofacial applications [102-104], particularly implant dentistry [105-111] and periodontology [112]. However, several articles reported the use of L-PRF in some interesting interventions of plastic surgery. Mixed with an adipocyte graft, L-PRF was reported to improve the stability of the graft during the facial Coleman’s liposuction [113]. Used as filling material after superficial parotidectomy, this material was able to limit the post-surgical pain and edema, and to stabilize the facial volumes after these delicate surgeries [114]. All these applications are mainly related to the filling properties of L-PRF, indeed contrary to PRP gels, L-PRP is a solid material that presents a significant volume and can fill small cavities. However, the main perspectives of L-PRF in plastic surgery are clearly the
surgery are clearly the treatment of skin wounds in general, and chronic skin ulcers in particular. Indeed, with its intrinsic properties of slow release of stimulating factors and its strong fibrin architecture, L-PRF may seem the ideal bioactive fibrin bandage. If no complete studies were still published, our own results are outstanding, particularly in the treatment of chronic wounds or infected post-surgical wounds in diabetic patients Fig. (7). The growth factors promote a local cell stimulation and the apparition of proliferative buds on the wound surface, and the antimicrobial properties (from the platelets and the leukocytes) are of great interest to clean the wound, stop the local infection and regulate the local inflammatory mechanisms. It is also interesting to note that in one of the rare studies about P-PRF (Pure Platelet-Rich Fibrin), it was shown that this PRF without leukocyte had also a significant potential for closing small chronic leg ulcers which had failed to heal with conventional methods [115]. This field of research is however still new, and further researches are needed to validate the numerous potential protocols.

7. CONCLUSIONS

As promoters of wound healing, L-PRP and L-PRF are powerful tools for soft tissue reconstruction in cosmetic, plastic and reconstructive surgery. The use of these materials results in a reduction in operating time and postoperative pain, enhances flap and graft survival, hastens re-epithelialization, decreases the necessity of drains and pressure dressings and the incidences of complications. L-PRP and L-PRF are also effective in stopping capillary bleeding in the surgical flaps, like the fibrin glues. Their inflammatory-regulating properties promote edema and ecchymosis reduction. Finally, they can be used as bioactive fibrin bandages, which stimulate healing of deep post-traumatic wounds and vast soft tissue ulcerations, and their associated antibacterial effects are also very important for a positive clinical outcome in many complex clinical situations. This field of research offers great perspectives for new therapeutic options, and requires an extensive evaluation of all the potential clinical methodologies.

DISCLOSURE OF INTEREST

The authors declare no competing financial interests.

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