Platelet gel: a new therapeutic tool with great potential

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Abstract

Chronic wounds, such as diabetic foot ulcers, represent a serious clinical problem for patients and clinicians. Management of these wounds has a strong economic impact worldwide. Complications resulting from injuries are a frequent cause of morbidity and mortality. Chronic wounds lead to infections, painful dressings and prolonged hospitalisation. This results in poor patient Quality of Life and in high healthcare costs. Platelet concentrates (PC) are defined as autologous or allogeneic platelet derivatives with a platelet concentration higher than baseline. PC are widely used in different areas of Regenerative Medicine in order to enhance wound healing processes; they include platelet-rich plasma (PRP), platelet gel (PG), platelet-rich fibrin (PRF), serum eye drops (E-S), and PRP eye drops (E-PRP). This review highlights the use of platelet-rich plasma (PRP) and platelet gel (PG) preparation for clinical use.

Keywords: wound healing process, platelet growth factors, platelet concentrates, platelet-rich plasma, platelet gel.

Introduction

Wounds represent a serious clinical problem for patients and clinicians. Management of wounds has a strong economic impact worldwide. The complications resulting from injuries are a frequent cause of morbidity and mortality[1,2]. In fact, acute wounds can convert into chronic wounds that are more difficult to manage because of their association with skin fibrosis. Chronic wounds lead to infections, painful dressings and prolonged hospitalisation. This results in poor patient Quality of Life and in high healthcare costs[3]. Diabetic foot ulcers are the best example of such hard to heal wounds and are by far the largest cause of hospital admissions and lower leg amputation[4]. They occur in 15% of all patients with diabetes and are found in 84% of all diabetes-related lower leg amputations[5]. Because of the lack of consensus on their treatment, many chronic wounds persist longer than necessary[6]. Because of the increased life expectancy of the ageing population, it is very likely that the prevalence of chronic wounds will increase, resulting in higher social costs[7]. Therefore, wound healing processes and new developments in this field need to be improved and should be encouraged[8]. This review highlights the use of platelet-rich plasma (PRP) and platelet gel (PG) preparation for the treatment of wounds.

The wound healing process

A wound is defined as "a simple break in the continuity in the skin epithelial layer or it can be deeper and reach subcutaneous tissue, causing damage in other structures such as tendons, muscles, vessels, nerves, parenchymal organs, etc."[8].

Wounds can be classified into acute and chronic wounds: i) acute wounds heal through an orderly and timely reparative process that restores the anatomic and functional integrity; ii) chronic wounds fail to proceed through the normal stages of healing and they cannot be repaired in an orderly and timely way, so that a sustained anatomic and functional result is not achieved[6]. A chronic wound is also defined as a tissue with an impaired ability to heal due to different clinical conditions, such as diabetes, obesity, chemotherapy, pressure, etc. Cutaneous wounds have the tendency to deviate from the normal healing course of acute wounds[9]. In addition, some wounds may be complicated by the combination of an infection and a tissue defect[2]. They are more likely to occur after a wide surgical tissue resection (e.g. in tumour management) and are also more likely to become infected[8]. A good understanding of the physiology of wound healing is a prerequisite for any therapeutic intervention[10].

Wound healing involves keratinocytes, fibroblasts, endothelial cells, macrophages and platelets[11]. Even if the process is continuous, it is conventionally divided into four phases: i) coagulation, which takes place immediately after injury to prevent bleeding; ii) inflammation, which allows an immune barrier to be established against micro-organisms; iii) proliferation, (the key to the healing process) which begins within days of injury and lasts for approximately two weeks; iv) a remodeling process,

which represents the final phase and is of a longer duration. In this phase, new epithelium develops until scar tissue is formed[12]. Wound healing processes are regulated by a complex cocktail of growth factors and cytokines released by platelet-α granules[11]. Therefore, platelets (PLT) not only prevent blood loss, but also promote tissue regeneration, enhance collagen synthesis, and trigger angiogenesis and the immune response by releasing growth factors and cytokines (Table I)[13].

Platelet-derived growth factors

Growth factors can be macromolecules, such as proteins, or small molecules, such as hormones[14]. During wound healing processes, platelet-derived growth factors (PDGF) are attractive agents that stimulate tissue repair and cell proliferation, influence extracellular matrix deposition[15], and support cell proliferation and differentiation[16] (Figure 1). Moreover, PLT promote the migration of keratinocytes (which have morphometric and mitogenic effects) within the wound, ensuring the formation of a skin barrier[14]. PLT are usually quiescent and become activated after endothelial injury by direct contact with the collagen exposed to bloodstream[17] (see below). Activated platelets release α-granules, which are carried inside PDGF and bioactive proteins[18] (Table I) that play fundamental roles in many aspects of haemostasis, including vessel constriction, leukocyte recruitment and vessel repair. PDGF is primarily produced upon injury and plays a role in each stage of wound healing. It abounds in acute wounds[19] and is decreased in chronic wounds[20]. It binds to transmembrane tyrosine kinase receptors (PDGF receptor-β), causing many intracellular events that culminate with the activation of target genes such as c-fos and junB[21]. Along with IL-1, PDGF attracts neutrophils to the wound site to remove contaminating bacteria[22]. In addition, transforming growth factor-β (TGF-β) activates monocytes into macrophages increasing inflammatory response and tissue debridement. TGF-β is mostly found in inflammation, angiogenesis and re-epithelialisation processes during wound healing; it also promotes matrix formation and remodeling[11]. Together with fibroblast growth factor (FGF), PDGF encourages the recruitment and the activation of inflammation cells such as neutrophils, endothelial cells and macrophages that are involved in tissue restoration[16]. FGF increases in the acute wounds and promotes tissue formation, re-epithelialisation and tissue remodeling[23]. Epidermal growth factor (EGF) is a key factor of tissue repair, promoting keratinocyte proliferation, cell migration and re-epithelialisation in acute wounds[24]. Vascular-endothelial growth factor (VEGF) has a crucial part in the healing process by increasing vessel permeability and angiogenesis[9]. Connective tissue growth factor (CTGF) promotes platelet adhesion, white blood cell migration and angiogenesis, and regulates collagen synthesis[25]. It is important to emphasise that during reparative processes growth factors are produced and released by keratinocytes, fibroblasts and PLT[4,26] (Table I).

Platelet concentrates

Platelet concentrates (PC) are defined as autologous or allogeneic platelet derivatives with a platelet concentration higher than baseline. In human blood, normal platelet counts range between 150×10^9/L and 350×10^9/L, with an average of approximately 200×10^9/L[27]. There is still lack of consensus over the correct terminology to define, classify and describe the different PC[28]. PC are widely used in different areas of Regenerative Medicine in order to enhance wound healing processes; they include platelet-rich plasma (PRP), PG, platelet-rich fibrin (PRF), serum eye drops (E-S) and PRP eye drops (E- PRP)[16]. The reason for the widespread clinical use of PC lies in the abundance and accessibility of growth factors contained in PLT that promote soft/hard tissue regeneration[29]. Furthermore, in addition to their regenerative properties, PC also have anti-inflammatory and analgesic effects[30-32], and an anti-bacteria effect against some micro-organisms[29]. In particular, PRP and PG are used for the treatment of chronic wounds such as diabetic foot and leg ulcers[33].

Table I - List of the most important platelet-derived growth factors (PDGF) and their relevant functions.

Platelet-derived growth factor	Most representative functions	References
PDGF	Promotes chemotaxis of macrophages and neutrophils; re-epithelialisation, matrix formation and remodeling	De Pascale *et al.*[16], Barrientos *et al.*[11]
TGF- β	Inhibition of macrophages and lymphocyte proliferation, mesenchymal stem cells proliferation, neutrophil and monocyte chemotaxis, matrix formation	Crovetti *et al.*[43], Ramos-Torrecillas *et al.*[58], De Pascale *et al.*[16]
FGF	Mitogenic effect on fibroblasts, endothelial cells, mesenchymal stem cells chondroblasts, osteoblasts; it promotes angiogenesis	Sonmez *et al.*[60]
EGF	Promotes fibroblast migration and proliferation	You *et al.*[61]
VEGF	Promotes angiogenesis and increases vessel permeability	Bao *et al.*[9]
CTGF	Promotes platelet adhesion, white blood cell migration and angiogenesis; it also regulates collagen synthesis	Kubota *et al.*[25]

TGF-β: transforming growth factor-β; FGF: fibroblast growth factor; EGF: epidermal growth factor; VEGF: vascular-endothelial growth factor; CTGF: connective tissue growth factor.

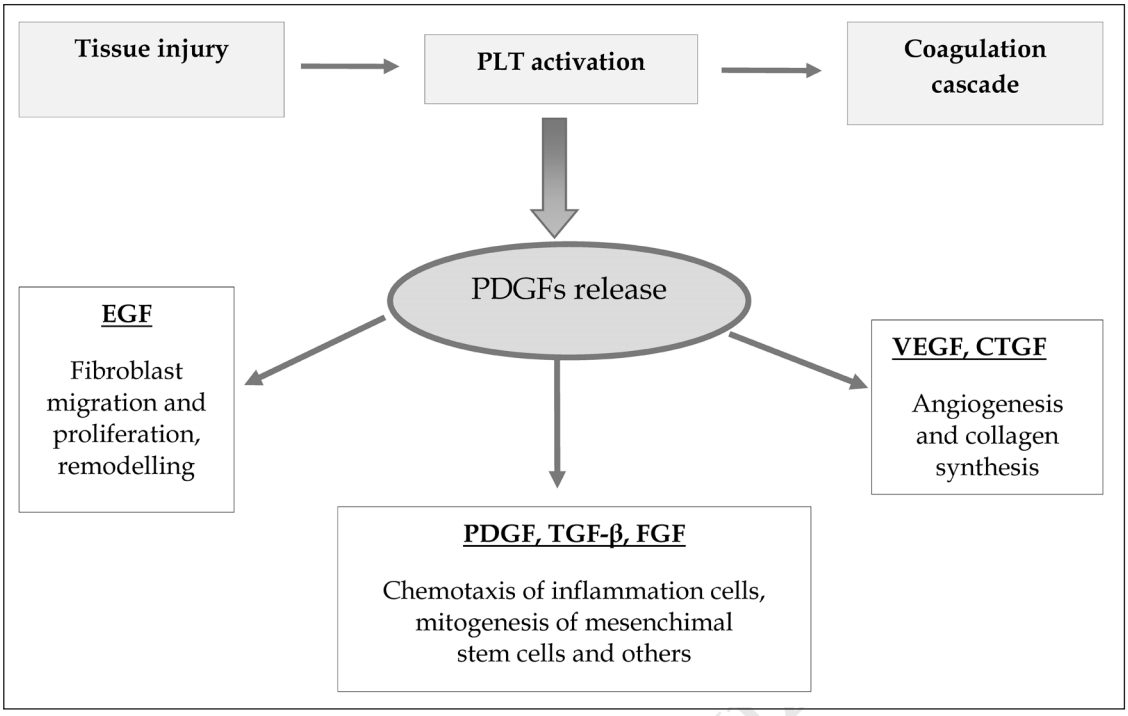

Figure 1 - Role of platelet-derived growth factor (PDGF) in wound healing processes.
PLT: platelet; EGF: epidermal growth factor; PDGF: platelet-derived growth factor; VEGF: vascular-endothelial growth factor; CTGF: connective tissue growth factor; TGF-β: transforming growth factor-β; FGF: fibroblast growth factor.

Many techniques are in place to obtain PC and each method leads to a final product with different biological features (e.g. for the quantities of growth factors) and potential uses[34]. The lack of standardisation and the lack of a detailed description of the procedures adopted make it difficult to compare results[35]. Clinicians need to evaluate the possible risks (mainly transfusion-transmitted disease) associated with the choice of autologous or allogeneic PC. Clearly, the use of autologous PLT concentrates is better accepted by patients because there is no risk of cross infections[36,37], but it is not recommended when the underlying disease may impair the efficacy of the PC products, such as in the case of cancer, haematologic malignancies, diabetes, etc. Moreover, autologous preparations have varying quality of PC compared to allogeneic preparations provided by blood banks according to standardised criteria, making it problematic to compare methods and results[38]. Current systems for blood donor screening using nucleic acid testing (NAT) have dramatically reduced the risk of viral infection transmission, leading to a larger use of allogeneic PC.

Key platelet functions

Platelets circulate in the bloodstream in a quiescent state. They undergo rapid activation after vessel wall damage or in particular conditions (e.g. nitric oxide depletion) and participate actively in haemostatic and thrombotic processes (Figure 2). To facilitate a rapid regenerative action, PLT are enriched in mRNA and surface receptors, allowing direct protein synthesis and PLT-aggregate formation. Under normal conditions, PLT are unable to form stable contacts on endothelial cells, but this can occur on diseased or activated endothelial cells because of the lack of nitric oxide synthesis. Adhesion of PLT to endothelial cells promotes local inflammatory events that can initiate plaque formation, endothelial activation and damage. Lesions in the endothelial layer lead to exposure of subendothelial matrix proteins, particularly collagens and membrane surface-expressed tissue factor, which can initiate powerful platelet activation and coagulation, respectively.

The initial event that occurs after a vessel injury is the tethering or capture of PLT through the interaction of von Willebrand factor (VWF) with the GPIb-IX-V complex. The interaction between VWF and GPIb is made possible thanks to a conformational change induced in VWF as a consequence of its binding to collagen. This interaction alone is not sufficient to give rise to rapid stable adhesion but activates a collagen receptor, GPVI, which in turn leads to the activation of integrins αIIbβ3 (GPIIb/IIIa) and α2β1 (GPIa/IIa). The latter bind to VWF/fibrinogen and collagen, respectively, allowing stable adhesion and further activation of GPVI. The signals from GPVI and the two platelet integrins induce PLT spreading on the matrix and release of the feedback messengers

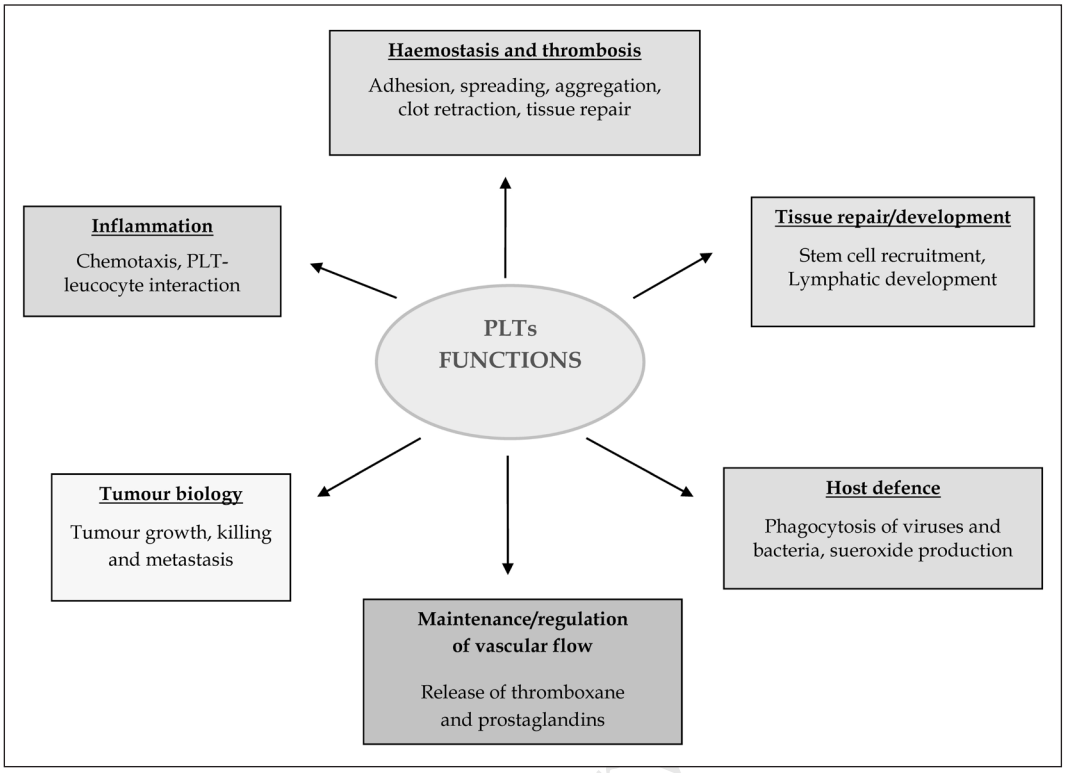

Figure 2 - Functional roles of platelets (PLT).

ADP and TxA2. In this way, VWF and fibrinogen in combination with ADP, TxA2 and thrombin, mediate thrombus formation (aggregration) and stabilisation (clot reaction).

Platelet-rich plasma

Platelet-rich plasma (PRP) consists of autologous plasma with a platelet concentration five times higher than basal level due to an extraction and concentration process[39] (Figure 3). In the past, PRP was the name given to the standard platelet concentrate for transfusion in patients with severe thrombocytopenia and classically contained 0.5×10^{11} platelets per unit. The use of PC for wound healing processes has been explored in the last decade. Most of these products are often improperly called PRP like the original transfusion platelet concentrates, making it difficult to distinguish between different protocols[34]. Marx gives a working definition of PRP as a concentration of 1,000,000 platelets/μL in 5 mL volume of plasma; a lower concentration cannot be relied upon to improve wound healing and greater concentrations have not yet been shown to further enhance wound healing[27].

Criteria for platelet-rich plasma preparation

Current literature contains several protocols to obtain PRP. Each of them aims to optimise conditions such as temperature, and centrifugation speed and duration. However, the final products have different PLT concentrations according to the protocol used, underlining the lack of a standardised approach to these procedures[40].

Despite these variations, each protocol provides a blood collection and a double centrifugation at different forces to concentrate platelets. The steps listed below describe the highlights in PRP preparation (Figure 3).

1) Blood collection: at the time of treatment, around 30 mL of the patient's venous blood is drawn in tubes with anticoagulant sodium citrate in order to prevent platelet activation prior to its use.
2) First centrifugation step: whole blood is first centrifuged with low forces (soft spin from 200 *g* to 600 *g*) and separated into three layers: an upper layer that contains mostly platelets and white blood cells (WBC) called platelet-poor plasma (PPP); an intermediate thin layer of whitish color called buffy coat (BC), rich in WBC; a bottom layer that consists mostly of red blood cells (RBC). The upper layer and superficial buffy coat are recovered and then transferred into another sterile tube without anticoagulant[28].
3) Second centrifugation step: the tube is centrifuged at higher speed (hard spin from 700 *g* to 2,300 *g*) to concentrate platelets. After the second spin step, soft pellets (erythrocyte-platelet) are formed at the bottom of the tube. The upper two-thirds of the volume (PPP) is discarded, while the lower one-third

(5 mL of plasma) is homogenised by gently shaking the tube to create PRP[28].

4) Activation: if a platelet activator is added to the obtained platelet concentrate, a platelet aggregation occurs and so PRP is transformed into what is known as PG. The choice of the PLT activator affects the timing and level of growth factor release[41]. There is no consensus as to whether PLT must be previously activated before their application and, if so, with which agonist. Some technicians activate platelets with thrombin or calcium, while others claim that better results are obtained applying PLTs directly without them being activated[42]. However, PG is commonly obtained by mixing PRP with thrombin (the most potent platelet activator) and calcium gluconate so that the final result is the gelation of the platelet concentrate[16]. PLT-gel gives an exogenous *in situ* addition of GF allowing homeostasis restoration, tissue reparation and regeneration[43].

The quality of PRP can be evaluated according to certain parameters, such as high platelet recovery, platelet integrity and vitality, which in turn depend on centrifugal acceleration and duration of centrifugation. Moreover, platelet concentration gradient is influenced by the size of platelets, the biological difference among individuals, and haematocrit variability[28].

Ethical issues concerning the use of platelet-rich plasma and platelet gel

Under Italian legislation, PRP and PLT-gel should not be considered as drugs *per se*[44], even if they are rich in growth factor and other substances. Instead,

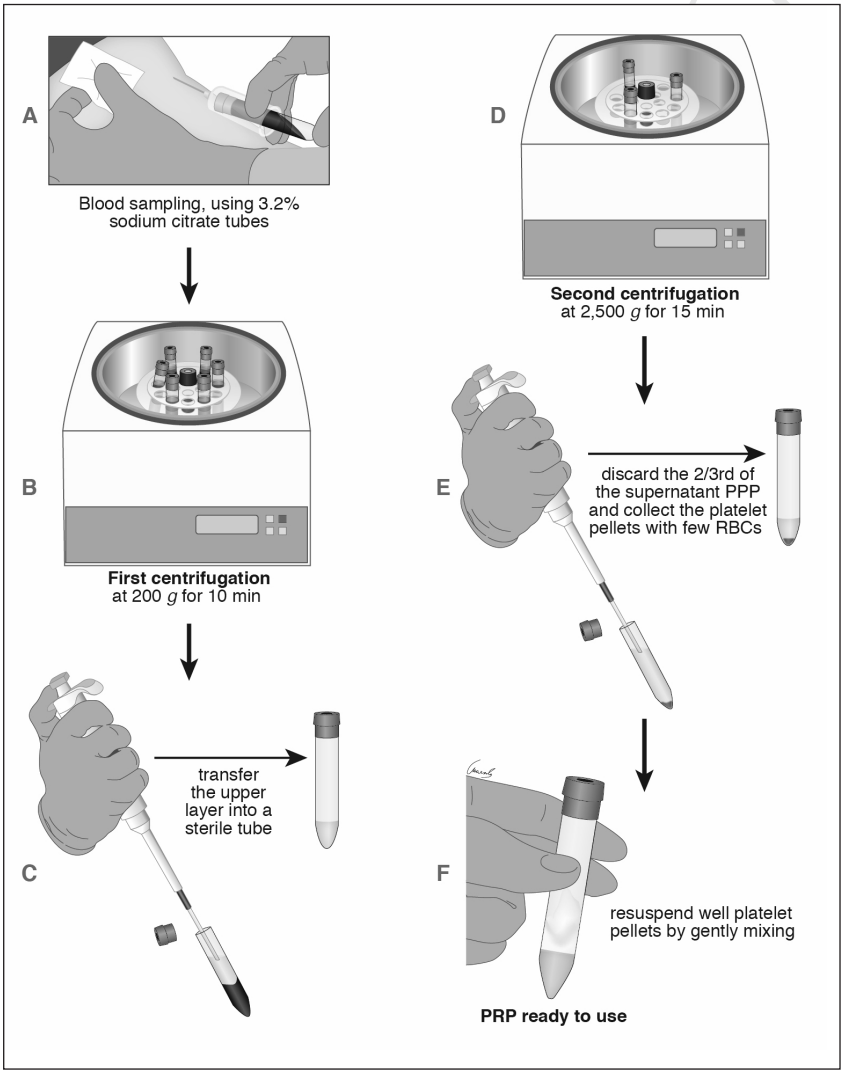

Figure 3 - Flow-chart in platelet-rich plasma (PRP) preparation. PPP: platelet-poor plasma; RBC: red blood cell.

both substances should be considered as "haemo-derived products" and placed in the category of blood components[45]. For these reasons, there have only been a few clinical studies on PRP and/or PLT-gel, with suitable ethical approval and scientific design (phase I, II and III studies)[46,47]. Nevertheless, the literature is not clear on this issue. Furthermore, because the preparation of autologous PLT-gel is not complicated, there is a real risk of indiscriminate use of these blood-derived products for aesthetic proposals (lifting, air loss, etc.) outside the clinical scientific setting. Also, the use of these products is relatively new, and therefore knowledge on possible associated side effects (such as secondary malignancies) is very limited. In addition, PLT-gel preparation is not a standardised procedure, making comparisons of results quite difficult. Therefore, we believe that studies in this field are required, in particular, to further clarify the properties of these products and to study their clinical applications within clinical trials, ensuring ethical approval at hospital level. Recently, the use of cord blood PLT-gel (CBPG) for regenerative medicine has been proposed. The Italian Health Authority has deliberated that CBPG is equivalent to adult derived PLT-gel. Recently, Rebulla and co-workers have also standardised clinical trials using CBPG[45].

Conclusions and future perspectives

Several studies on the clinical use of PRP have already been conducted. Most of the literature ranges from dental medicine[48] to maxillofacial surgery[49], from dermatology and aesthetic medicine[50,51], to orthopaedics and sports medicine[52], and even neurology[53]. PRP is also a promising therapeutic tool for the treatment of chronic mucositis[54] and for diabetic foot and leg ulcers[55]. In addition, PRP not only enhances the rate of wound healing, but it also reduces neurological and neuropathic pain associated with injuries[56,57]. Use of autologous platelet concentrates accelerates healing in dental implant surgery, orthopaedic surgery, muscle and tendon repair, skin ulcers, hole repair in eye surgery and cardiac surgery[13]. All these therapeutic properties are possible because PLT blood products are rich in growth factors and cytokines that stimulate and accelerate the wound healing process. Although there has already been much research, there is still a lot to be done. For example, the absence of a standardised protocol for PRP preparation and the lack of universally accepted terminology make it difficult to compare studies and their results. Furthermore, the available studies remain controversial as to the true benefit, especially in ulcer treatment[58] and there is no agreement on the duration of therapy because PGF are quickly released and require several applications in order to maintain their therapeutic effect. Finally, there is no information about PRP therapeutic doses and about the possible interference of some drugs on PGF release[16]. A future perspective could be the use of PLT-gel derived from cord blood. Parazzi *et al*. demonstrated that high levels of angiogenic factors, hormones and molecules able to support tissue growth are present in the cord blood PLT-gel releasate if compared with peripheral blood[59]. Further investigations are required to define standardised protocols for the preparation of high quality PRP suitable for different clinical applications, thus making it possible to compare results. In addition, future studies should aim to better clarify the "ingredients" within PG, for example, the presence of activated microparticles[62], and of mRNA and their role.

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Authorship contributions

AP and AMDP contributed equally to this work.

The Authors declare no conflicts of interest.

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