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Review

# Applications of blood plasma derivatives for cutaneous wound healing: A mini-review of clinical studies

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ABSTRACT

Skin injuries are a global healthcare problem. Chronic ulcers do not heal in a timely fashion, so it is essential to help the body with skin repair. There are some treatments that have been applied to chronic ulcers. One of these treatments is growth factor (GF) therapy. Platelet-rich plasma (PRP) and Platelet-poor plasma (PPP) are two types of plasma derivatives containing many GFs important for wound healing. Several works have reported their application in wound healing and tissue regeneration. The use of autologous PRP is now an adequate alternative in regenerative medicine. It was also demonstrated that PPP is a hemostatic agent for wounds. This review has studied the latest clinical studies, which have applied PRP and PPP to patients with chronic wounds.
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**Contents**



*Abbreviations:* GF, Growth factor; TGF-β, Transforming Growth Factor β; PRP, Platelet-Rich Plasma; PPP, Platelet-Poor Plasma; ECM, Extracellular matrix; PU, Pressure Ulcer; DFU, Diabetic Foot Ulcer; CLU, Chronic leg ulcer; VLU, Venous leg ulcer; PLU, Platelets gel; RCT, Randomized Control Trial; VEGF, Vascular Endothelium Growth Factor; PDGF, Platelet-Derived Growth Factor; FGF, Fibroblast Growth Factor; EGF, Epidermal Growth Factor; HGF, Hepatocyte Growth Factor; IGF, Insulin-like Growth Factor.

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## 1. Introduction

Skin injuries are a global healthcare problem, with approximately 300 million chronic and 100 million traumatic wound patients [1]. Based on Statistics in 2012–2013, the British healthcare system managed 11,200 wounds, and it was predicted that 23,000 wounds would be managed by 2022 [2]. The incidence of non-healing dermal lesions in the United States alone is 5–7 million cases annually, and more than 20 billion dollars are spent annually. This amount of money is paid annually, while the standard wound therapy methods have 50%effectiveness [3]. Pressure, venous, arterial ulcer, and non-healing wounds due to obesity or diabetes and age-related disease, which do not heal in a timely fashion, are called chronic wounds. Wounds caused by trauma or surgical operation and burn are called acute wounds that cannot heal if the patient has malnutrition. Both types of wounds could have a significant burden on any healthcare system. These kinds of skin wounds may cause millions of dollars in expenditure on health care, although they might not recover well and finally lead to fibrosis tissue [4]. Wound healing requires the complex coordination of several different cell types in successive stages. It involves the spatial and temporal synchronization of different cell types and chemokines with specific roles in the various stages [5]. Following injury, the body does some actions. First, it should stop bleeding by platelet activation and clotting cascade initiation. Second, the inflammatory cells respond to platelet-released chemokines and migrate to the injury site. So, the Inflammation phase commences. It would continue 24–48 h in normal wound healing unless encumbered by local infection or various pathological states such as diabetes, poor arterial perfusion, venous hypertension, malnutrition, or sepsis. The late inflammatory phase overlaps with a period of proliferative phase, in which fibroblast and endothelial cells migrate to the wound site. The dominant cell population is still the inflammatory cells. Third, in the Proliferation phase, 5–7 days after injury, Fibroblasts are the chief cells that produce and deposit collagen for granulation tissue formation. The development of mature granulation tissue also includes an ingrowth of endothelial cells. Simultaneously, the last phase occurs in which Fibroblasts and macrophages produce Matrix Metalloproteinases (MMPs) in order to break down the collagen in a remodeling manner. This is a regenerative process to soften and lighten the pigment of scar tissue. The Remodeling phase can continue for several months and depends on the depth and extent of the wound [6,7]. The ultimate goal of wound healing is to close the wound and have the proper functional and aesthetical scar tissue [8].

With advanced therapies like growth factors, extracellular matrices, synthetic skin, and negative pressure wound therapy; chronic wound care would have become its specialty [9–11]. Platelets and their derivatives have a considerable role in tissue regeneration. Platelet α-granules contain Platelet Derived Growth Factor (PDGF), Transforming Growth Factor β (TGF-β), Vascular Endothelial Growth Factor(VEGF), Insulin Like Growth Factor 1(IGF-1), Epidermal Growth Factor (EGF), and basic Fibroblast Growth Factor (b-FGF) that cause the effect of tissue regeneration [12]. α-granules have many proteins with a wide range of functions, such as; chemokines, clotting factors, proteases, anti-proteases, and anti-microbial proteins [13]. Plasma has been extensively researched and used in a variety of settings to speed up healing by improving tissue adhesion and hemostasis [14]. A gel made from human blood is increasingly being used to help people with chronic wounds heal more quickly [15]. Platelet-rich plasma (PRP) is a small amount of plasma containing an autologous concentration of human platelets [16].

In contrast, Platelet-poor plasma (PPP) is an autologous plasma with a low platelet concentration utilized as a sealant [17]. As a result, PRP and PPP can be characterized as plasma fractions having platelet concentrations above and below baseline in whole blood, respectively. PRP is a medicinal material containing fibrin and high concentrations of growth factors that have the potential to assist chronic wound healing [18]. This review studies the properties and efficacies of blood-derived productssuch as PRP and PPPin the wound healing processes. In addition, it evaluates the most relevant studies published of research in this area.

## 2. Skin structure

The skin is the biggest organ in the human body and serves as the main interface between the interior and exterior environments. It serves as an important barrier that protects the body against a variety of environmental stresses. It also acts as a homeostasis maintenance agent by reducing electrolyte imbalance and water evaporation. The epidermis, dermis, and hypodermis are three separate structural top layers of the skin [19].

The epidermis is the uppermost layer of human skin (Fig. 1). The epidermis is made up of five layers: the Stratum Basale, the Stratum Spinosum, the Stratum Granulosum, the Stratum Lucidum, and the Stratum Corneum. At the dermo-epidermal interface, the basal lamina separates the Stratum basale from the dermis. Melanocytes and keratinocytes are present in the basal layer. The cuboidal to columnar mitotically active stem cells that constantly create keratinocytes to proliferate, move superficially, and differentiate to form the stratified epidermis are the most common cells in this layer. These cells expand, form strong intercellular connections, and lose their capacity to proliferate as they approach the surface and develop into the spinosum layer.

Desmosomes contact nearby cells in the Stratum Spinosum, which has irregular, polyhedral cells with cytoplasmic processes reaching outward. Stratum Granulosum contains elongated and diamond-shaped keratinocytes with keratohyalin granules and lamellar bodies. Keratin precursors in keratohyalin granules cluster,

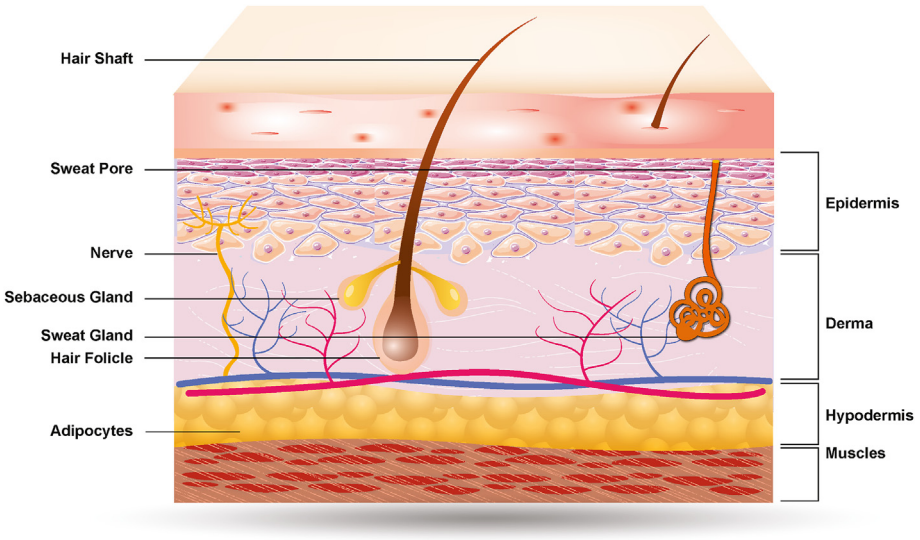


**Fig. 1. Human skin structure and its appendix.** Skin layers include Epidermis, Dermis, Hypodermis, and other appendices (i.e., nerves, veins, glands, cell population, etc.).

crosslink, and form stacks over time. The glycolipids (profilaggrin and loricrin) that are secreted to the cell surface and act like glue to bind the cells together are found in the lamellar granules. Stratum Lucidum is a thin translucent layer made up of eleidin, a transformation product of keratohyalin, found in thicker skin found in the palms and soles. The topmost layer is a 10–20 μm thick layer, the Stratum Corneum, which is made up of keratin and dead keratinocytes, also known as anucleate squamous cells, which are composed of terminally differentiated, flattened cells known as corneocytes. This layer varies most in thickness, especially in callused skin [19–21].

The dermis is another layer of skin; the hat epidermis relies on it for support. Blood and lymph vessels penetrate the dermis, unlike the epidermis. Hair follicles, nerve endings, and secretory glands, which provide energy and nutrients to the epidermis, are located next to them. The fibroblast is the most common cell type in the dermis. They produce extracellular matrix (ECM) proteins. The primary component of the dermis is collagen, fibronectin, and elastin, which are responsible for skin elasticity and tensile strength. The dermo-epidermal junction connects the dermis and the epidermis, permitting regulated substance exchange and basal keratinocyte polarity [22].

The hypodermis is a loose connective tissue that connects the skin to the underlying fascia under the dermis. It has a structure that extends into the dermis and contains collagen and elastic fibrils. This layer also contains hair roots, adipocytes, vasculature, and nerves. It has a critical role in maintaining both the mechanical and mobility properties of the whole skin. Also, it plays a main role in regulating the upper layer and wound healing [23].

## 3. Wound healing

All multi-cellular creatures have the ability to respond to harm and repair tissue as a fundamental property. Numerous intracellular and intercellular pathways must be activated and organized immediately after an injury to retort to the loss of tissue integrity and restore homeostasis. Cellular and molecular components of the immune system, blood coagulation, and inflammatory pathways are all stimulated. After exposure to practically any damaging irritant, the wound healing process occurs in almost all tissues [24]. Numerous cell types, including immune cells (neutrophils, monocytes,etc.), endothelial cells, keratinocytes, and fibroblasts, experience significant alterations and exhibit distinct gene expression and phenotypic features, resulting in cell proliferation, differentiation, and migration [8]. The typical mammalian reaction to injury occurs in three phases that overlap but are distinct in all organ systems: inflammation, proliferation, and remodeling.

### 3.1. Inflammatory phase

In the initial phase of wound healing, inflammation occurs shortly after tissue injury, and components of the coagulation cascade, inflammatory pathways, and immune system are recruited to prevent further blood and fluid loss. It has the ability to eliminate dead and devitalized cells as well as prevent infection. Hemostasis is achieved first by the formation of a platelet plug; during this stage, an enzymatic cascade results in the conversion of fibrinogen to fibrin, which temporarily seals the injured blood vessels and provides a provisional fibrin matrix for inflammatory cell invasion and later fibroblast and epidermal cell migration. In response to platelet degranulation and bacterial breakdown products, neutrophils are attracted to the wound site [25,26] (Fig. 2). They secrete ROS in response to the invasion of bacteria. Monocytes emerge in the wound site after 2–3 days and differentiate into macrophages. Macrophages are thought to be vital for organizing subsequent events in the aftermath of an injury [27]. Pro-inflammatory macrophage (M1 phenotype) removes cellular debris, invading microbes, and damaged ECM components. They also secrete pro-inflammatory cytokines and growth factors such as; Interleukin 1 (IL-1), b-FGF, PDGF, and VEGF, which recruit more immune cells and promote the proliferative phase. M1 Macrophages are transformed to an anti-inflammatory (M2) phenotype during the new tissue formation phase, and they begin to secrete anti-inflammatory cytokines, including TGF-β, which promote wound contraction [28].

#### 3.1.1. Inflammatory phase in chronic wound

But, in chronic wounds, the inflammatory phase returns, resulting in a pro-inflammatory condition that persists (Fig. 2). High numbers of inflammatory cells and biofilm formation prevent the restoration of tissue homeostasis in chronic wounds. Increased numbers of neutrophils lead to ahigh concentration of Reactive Oxygen Species(ROS) and MMPs, which can result inthedegradation of growth factors and ECM proteins, result in senesces of

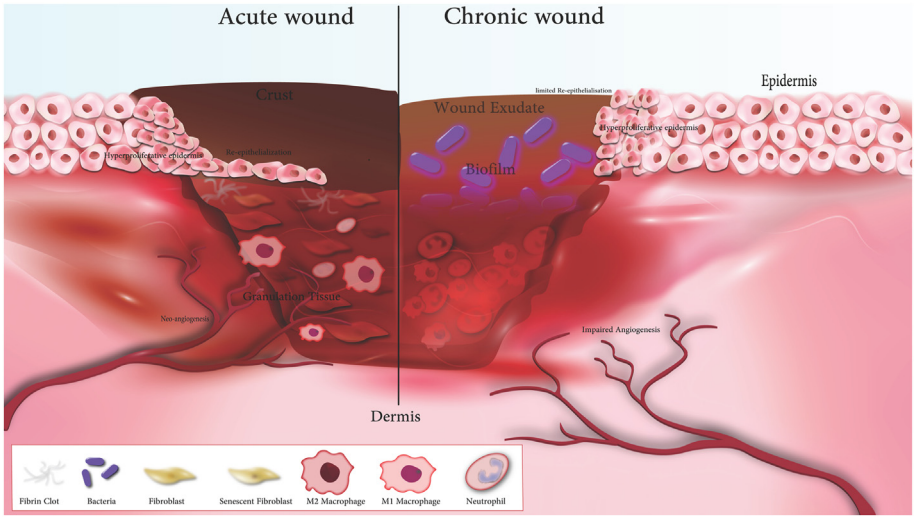


**Fig. 2. Wound healing in acute and chronic wounds**, In acute wounds, after the formation of crust, the hyperproliferative epidermis grows and moves forward on the surface of the defect site(*Re-epithelialization*) using fibrin clot formed in the hemostasis phase. Beneath this layer, the inflammatory cells penetrate the defect site to clear the dead cells and tissue, combat the invading pathogens, and control the healing process by releasing a different kind of chemokines (Inflammation). Meanwhile, the fibroblasts migrate to the wound site and release chemokines to manage wound healing. They produce and release ECM as well. The microenvironment, which different kinds of cells and chemokines have prepared, makes the healing process such a way to regenerate the lost tissue by forming the *granulation tissue* (Proliferation). But in a chronic wound, poor condition of the ulcer micro-environment halts the healing process in the inflammatory stage. Because of biofilm, the inflammatory cells' load raisesin the ulcer environment, turning it into a devastating condition.

fibroblasts, and prevent macrophage phenotype conversion. These processes lead to a feed-forward loop that prevents wound healing progression [28].

### 3.2. Proliferative phase

The second phase of wound healingoccurs in 2–10 days following injury. The proliferation and migration of various cell types characterize it. Keratinocytes first migrate over the damaged dermis. Angiogenesis occurs, and the formation of granulation tissue by the germination of capillaries associated with fibroblasts and macrophages replaces the fibrin matrix, producing a new substrate for keratinocyte migration later in the repair process. Behind the leading edge of the wound, keratinocytes proliferate and mature, eventually restoring the epithelium's barrier function. Vascular endothelial growth factor A (VEGF-A) and fibroblast growth factor 2 (FGF2; also known as b-FGF) are the essential positive regulators of angiogenesis [29]. The recruitment of bone marrow-derived endothelial progenitor cells can also result in angiogenesis. However, this contribution is low, at least in non-ischemic wounds (in which the oxygen concentration is average) [30]. Macrophages stimulate fibroblasts recruited from the wound margin or bone marrow in the later stages of this phase. Some of these fibroblasts differentiate into myofibroblasts [31]. Myofibroblasts are contractile cells that help to close wound edges over time. ECM is produced by fibroblasts, and myofibroblasts interact with it, primarily in the form of collagen, which eventually forms the bulk of mature scar tissue [32].

### 3.3. Remodeling phase

The remodeling phase of wound healing begins 2–3 weeks after injury and might span a year or longer. All processes that were engaged after the injury terminate and haltduring this stage. The majority of endothelial cells, macrophages, and myofibroblasts undergo apoptosis orleave the wound site, leaving a mass of collagen and other extracellular matrix components that contain few cells. Skin integrity and homeostasis are most likely constantly regulated by epithelial-mesenchymal interactions [33]. Additional feedback loops are required in order to maintain the various cell types in the skin. Furthermore, the ECM is actively remodeled from a major type III collagen backbone to one largely consisting of type I collagen during the course of 6–12 months [34]. Matrix metalloproteinases, which are released by fibroblasts, macrophages, and endothelial cells, help to reinforce the healed tissue. The tissue, on the other hand, never regains the features of healthy skin [24].

## 4. Platelet

Megakaryocytes produce platelets, which are small discoid blood cells. Several internal structures, including glycogen, lysosomes, and two types of granules, can be found within the platelet. The first type is dense granules, which have a high concentration of low molecular weight compounds like; ADP, ATP, serotonin, and calcium [35]. α-granules, which contain clotting factors, growth factors, and other proteins, are the second type. When platelets are activated, their morphologies change (developing into pseudopodia), promoting platelet aggregation and subsequent granule content release through the open canalicular system [36]. VEGF, PDGF,FGF,EGF, Hepatocyte Growth Factor (HGF), and insulin-like growth factor (IGF) are some of the growth factors stored in α-granules, and they all help with angiogenesis and wound healing. Other pro-angiogenic mediators found in granules include angiopoietin, CXCL12 (SDF-1), and matrix metalloproteinases (MMP-1, -2, and -9) [35,37].

## 5. Blood derivatives with different platelet concentrations

### 5.1. Plasma

Blood plasma is a light amber liquid component of blood that is devoid of blood cells but has a suspension of proteins and other components of whole blood. It accounts for around 55% of the body's total blood volume. Miroshnychenko et al. investigated three types of samples: plasma, platelet-rich plasma, and platelet-poor plasma. In the samples, about 320 proteins were found. In all

three fractions, about half of the proteins were determined to be the same. Based on proteomic analysis of the PRP fraction, there are 129 unique compounds that do not exist in plasma or PPP, and 133 compound is joint in both PRP and PPP [38].

### 5.2. Platelet rich plasma (PRP)

PRP is a plasma fluid containing concentrated platelets obtained by separating and concentrating venous blood. Calcium can be used to activate it, resulting in the release of a vast variety of growth factors [39]. Platelet-rich plasma (PRP), platelet-rich growth factors (PRGFs), platelet-rich fibrin (PRF) matrix, and platelet concentrate are all terms for platelet-rich plasma (PRP) [40,41]. Platelet gel (PG) is formed when PRP is activated [42]. Due to deposited growth factors(see Table 1) in platelets, which can stimulate a tissue regeneration process, clinical evidence suggests that PRP could have favorable therapeutic effects on hard and soft tissue healing [43–45]. PRP also contains other intra- and extra-platelet components that aid in regeneration. Fibrinogen is an example of this component, as it generates a fibrin mesh that is required for cellular implantation and subsequent cellular proliferation [46,47]. In a study performed by Xu P et al. [48], they observed that the PRP-treated group had higher neovascularization, increased VEGF production, and lower IL-17 and IL-1β, which led to better granulation tissue formation, enhanced angiogenesis, and moderate inflammatory conditions in the wound environment, respectively. Also, they observed that PRP improved epithelial cell proliferation, which enhanced re-epithelialization.

### 5.3. PRP-derived exosomes

Recent studies have focused on the effectiveness of PRP-derived exosomes (PRP-Exos) in promoting diabetic wound healing due to their low immunogenicity and excellent stability. Exosomes serve as an optimal delivery mechanism, transporting bundled cargoes within a phospholipid bilayer to specific locations while protecting the contents from degradation and turnover. PRP-Exos, the concentrated form of PRP, have identical functional components but in a higher concentration. Exosomes are composed of proteins, microRNAs, growth factors, and sphingosine-1 phosphate (S1P), a biologically active lipid produced by sphingosine kinase 1/2. S1P functions as an intracellular second messenger, transmitting small molecule signals to specific locations by binding to S1P receptors (S1PR1–5) on the cell surface. S1P is essential for controlling blood vessel growth and regeneration. Studies have found higher levels of S1PR1 in the skin of diabetic lower extremities compared to other lipids [49].

### 5.4. Platelet poor plasma (PPP)

During PRP extraction, there will be a large amount of discarded PPP. It has been demonstrated to contain large amounts of various proteins, such as; fibrinogen, hepatocyte growth factor-like protein(HGFl), and some other growth factors shown in Table 2 [38]. PPP can be obtained from the patient's blood in an autologous manner, which has a lower risk of viral disease and allergic reaction. Due to a large amount of fibrinogen that can be converted to fibrin by adding calcium salts, PPP can be solidified and is thought to act as a scaffold for migrating cells implicated in wound healing and tissue regeneration [50]. PPP has properties that are similar tocommercial fibrin glue, with adhesive and hemostaticpowers. Still, PPP has lower fibrinogen levels than these glues, although it does have adhesive action [42]. Glasbey et al. observed that the HGFl level in the acute wound is higher than in chronic wounds or normal skin. rhHGFlhas enhanced the migration of HaCat cells. It can be concluded that the application of PPP andthe presence of HGFL in chronic wounds can lead to re-epithelialization [51].

## 6. Preparation of PPP and PRP

There is various PRP preparation protocol. The release of growth factor content differs according to the preparation process. Many parameters, including centrifugal force and time, influence the quality and quantity of PRP [39]. Numerous protocols for preparing plasma have been proposed, and several of them can isolate the platelet-rich plasma (PRP) and the platelet-poor plasma (PPP) simultaneously (Fig. 3). In Table 2, some of them areillustrated.

## 7. Applications of PRP in the wound healing studies

Chronic wounds have a proinflammatory biochemical environment that impedes healing. Furthermore, it has a strong protease and ROS activity, which lowers the effective concentration of Growth Factors. Because PRP is a source of GFs and hence

**Table 1**
Lists some of the known active ingredients contained in the PRP and PPP.

| Type | Exist in | Biological actions | Ref. |
|---|---|---|---|
| *PDGF* | Only PRP | It promotes chemotaxis and mitogenesis of mesenchymal stem cells, and fibroblasts. It stimulates angiogenesis, wound contraction, the formation of granulation tissue, and wound remodeling. | [45, 46] |
| *TGF-β* | Only PRP | It has an autocrine and paracrine role in long-term healing; it inhibits macrophage and lymphocyte proliferation; stimulates mesenchymal stem cell proliferation; regulates endothelial, fibroblastic cell mitogenesis, and collagen synthesis and collagenase secretion; and it inhibits macrophage and lymphocyte proliferation. | [47, 48, 49, 50] |
| *VEGF* | PRP PPP | It stimulates the proliferation and migration of endothelial cells to form immature vasculature. | [51, 52] |
| *IGF-1* | PRP PPP | It stimulates protein synthesis and collagen gene expression in fibroblasts. | [53] |
| *HGF* | PRP PPP | It's an anti-inflammatory protein that promotes granulation tissue development, wound angiogenesis, and re-epithelialization. | [54] |
| *Angiopoietin-1* | Only PRP | It stimulates the migration and proliferation of endothelial cells | [55] |
| *SDF-1α* | Only PRP | It promotes the proliferation and differentiation of CD34+ cells into endothelial progenitor cells. | [56] |
| *EGF* | Only PRP | It is a potent mitogen that elevates the expression of several genes leading to DNA synthesis and cell proliferation; it regulates mitogenesis of mesenchymal stem cells and epithelial cells mitogenesis; it promotes chemotaxis of endothelial cells, which leads to angiogenesis. | [48,57,58] |
| *b-FGF* | PRP PPP | It is a multifunctional protein with mitogenic effects and regulatory, morphological, and endocrine roles; it regulates endothelial cells, mesenchymal stem cells, and fibroblastic cells; it promotes angiogenesis and the formation of new blood vessels from the preexisting vasculature. | [59,60] |
| *HGFl* | Only PPP | It is a pleiotropic factor effective in tissue homeostasis and response to insult, including tissue repair and epidermal wound healing | [44] |

**Table 2**
Summarizes some studies that use PRP/PPP extraction and preparation protocols.

| Volume (milli-liter) | Autologous or Heterologous | Anticoagulant | Soft/first spin | Hard/second spin | Activation method | Ref. |
|---|---|---|---|---|---|---|
| 60 | H | Sodium citrate | 400g 10min | 1200g 20min | Calcium gluconate | [37] |
| 20–45 | A | Sodium citrate | 2500 rpm 10min | 3500 rpm 5min | Calcium chloride 10% | [61] |
| 10–20 | A | Not reported | 1500 rpm 10min (6 cm) | 1500 rpm 10min (6 cm) | Thrombin + calcium | [62] |
| 20 | A | Citrate dextrose | 3600 rpm | 2400 rpm | Calcium chloride 20% | [63] |
| 10 | A | Sodium citrate | 2000 rpm 5min | Not reported | Not reported | [64] |
| 20–100 | A | Not reported | 313g 4min | 1252g 6min | Calcium gluconate and thrombin | [65] |
| Up to 20 | A | Citrate dextrose | 2000–3200 rpm 10–15min | Not reported | Calcium chloride or thrombin | [66] |

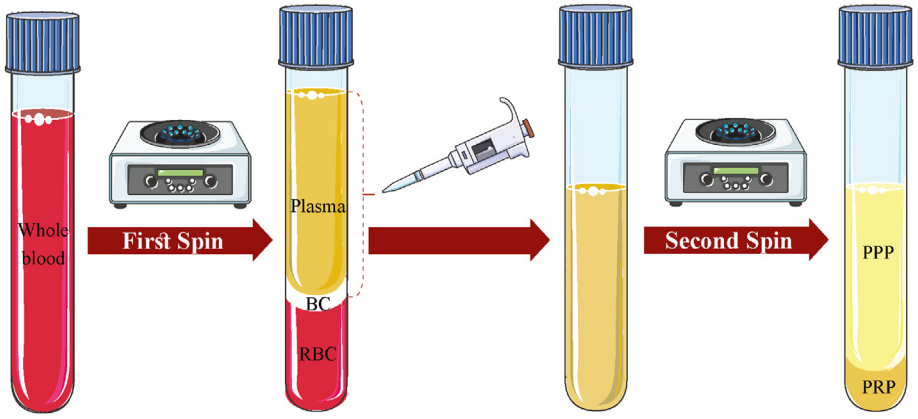


**Fig. 3. Extraction and Preparation of PRP and PPP.** Peripheral blood of patients could be taken from veins and poured into an anticoagulated centrifuge tube. After the firstspin, the blood would be divided into three layers, the uppermost layer is PPP, the red blood cell layer is at the bottom, and the buffy coat layers at the middle layer. In the next step, by the collection of the two uppermost layers in a new tube and centrifugation for the second time (second spin), the plasma layer would be separated into two layers: PRP and PPP.

possesses mitogenic, angiogenic, and chemotactic qualities, it is a popular alternative treatment for non-healing wounds [40]. Here, we overview all clinical studies from January 2015 up to January 2023 in which PRP was administered for cutaneous ulceration (Table 3).

These results demonstrated that PRP has excellent potential in different clinical applications. But still, it has to be further investigated by performing more precise and larger Randomized Control Trials (RCTs) to gather enough data with low bias, which assure the uses of PRP in wound management.

**Table 3**
Summarizes the last clinical studies available for topical PRP treatment for skin ulcers.

| Author | Sample size | Age | Etiology | Follow up | Treatments | Study design | Adverse effect | Outcomes |
|---|---|---|---|---|---|---|---|---|
| Helmy(67) | 80 | 27–64 | CLU | 12 months | I: PRP injection C: conventional therapy | RCT | N/P | PRP>*control |
| Rainys(68) | 69 | 65.08 | CLU | 8 weeks | I: PRP gel C: Standard treatment | Open-label RCT | N/P | PRP>*control |
| Li(65) | 117 | 62.8 ± 11.6 | DFU | 12 weeks | I: APG C: standard therapy | RCT | N/P | APG > control |
| Elsaid(63) | 24 | 55 ± 6 | DFU | 20 weeks | I: PRP gel C: saline dressing | RCT | N/A | PRP>*control |
| Singh(66) | 55 | 30–82 | DFU | 4 weeks | I: PRP C: standard treatment | prospective study | N/P | PRP>*control |
| Saad setta H(69) | 24 | 40–60 | DFU | 20 weeks | I: PRP C: PPP | comparative study | N/A | PRP>*PPP |
| Liu (62) | 102 | 39–80 | PU | 21 days | I: PRP C: NPWT | N/A | N/P | PRP>*control |
| Ramos thorecillas(70) | 100 | 64–90 | PU | 36 days | I1: PRGF and HA I2: two dose PRGF I3: one dose PRGF C: standard treatment | Open-label RCT | N/A | I3**>I2*>I1>C |
| Uçar Ö(64) | 60 | 68 | PU | 60 days | I: PRP gel C: physiological serum | RCT | N/A | PRP>*control |
| Tsai(71) | 28 | 37–84 | various | 12 weeks | I: PRP injection and PRP patch C: silver impregnated, collagen-based foam dressings | CT | N/P | PRP>*control |
| Suthar M(72) | 24 | 62.5 ± 13.53 | various | 24 weeks | I: subcutaneous injection | case series | N/P | potential safety and efficacy |
| Xuan Liao(37) | 60 | 57 ± 10 | various | 6 months up to 4 years | I: PRP and skin graft C: conventional therapy and skin graft | Randomized-single center | N/P | PRP>*control |
| Miłek T(73) | 100 | 50–89 | VLU | 1 month | I: PRP C: hydrocolloid dressing | prospective observational study | N/A | PRP>*control |
| Elbarbary(61) | 90 | 22–66 | VLU | 12 months | I1: intralesional PRP injection I2: PRP application on wound site C: compression therapy | randomized study | N/P | injection > application > control |
| Cardeñosa(74) | 102 | 64.15 | VLU | 24 weeks | I: PRP C: standard treatment | CT | N/P | PRP>*control |
| Elgarhy(75) | 60 | 30–60 | VLU | 6 weeks | I1: topical platelet gel (PG) I2: PRP injection C: saline dressing | Randomized case-control | N/P | topical PG>*control |

*A*: Autologous, *H*: Heterologous, *N/A*: Not Available, *N/P*: Not Reported, *: significant. *Sample size*: It includes either patients or their wounds quantity. *Age*: It has either "Mean age (lowest and highest reported ages)" or "Mean ± SD" (which were available in mentioned articles). *Outcomes:* based on the reported healing rate.

## 8. Application of PRP-Exo in wound healing studies

A clinical study conducted by Johnson J et al. examined the use of Ligand-based Exosome Affinity Chromatography Purification of platelets Extracellular Vesicles (LEAP-purified pEVs) on 11 healthy volunteers. The study aimed to assess the safety and bioactivity of non-autologous EVs produced by clinical grade isolation and purification facilities in a phase I clinical trial using a human model. The results have shown that LEAP-purified pEVs did not induce any negative effects or mortality in humans, indicating their safety. However, no significant difference was observed in the time it took for wound closures in humans between the arms that were treated with EVs or Placebo injection [52].

## 9. Applications of PPP in the wound healing studies

Man et al. [17] performed a study on 20 patients who underwent plastic surgery; theyreceived PPP(fibrin glue) as a hemostatic agent and then PRP(platelet gel) at the end of surgery as ahealing agent. The experiment group observed decreased bleeding time(15-−45sec) and improved healing results. However, they did not define a control group in order to compare whether their intervention improved wound healing or whether other factors were implicated.

In Saadsetta H et al. [53] study on DFU patients, the experimental group received PRP, the control group received PPP, the PRP group's mean healing time was 11.5 weeks, and the PPP group was 17 weeks. It was better to recruit a group with no intervention as a control. So, the result would have been more valuable. In conclusion, bothgroups' ulcers healed, but the PRP group had a significantly faster healing rate.

Pietrzak W et al. [54] performed a study to compare the hemostatic effect of PRP and PPP. The results demonstrated that PRP has a significantly better hemostatic effect than PPP, and the PPP group has a better one than untreated wounds.

Yang L et al. [50] applied PPP gel with amnion as a pathogen protective agent on full-thickness skin wounds in an in vivo study. The result demonstrated that the experiment group had more significant wound contraction than the untreated group. However, PPP gel alone did not apply to evaluate whether it was implicated in wound contraction or whether the amnion membrane had an auxiliary role. So, a lack of PPP gel alonetreatment limits the interpretation of the results.

## 10. Conclusion and future direction

Wound healing is a multi-step physiological process that involves multiple molecular, cellular, and biochemical mechanisms. The b-FGF, IGF-1, TGF-1, VEGF, HGF, and PDGF are just a few of the molecules involved in tissue regeneration and wound healing. These growth factors' chemotaxis, proliferation, and differentiation effects on diverse cells are well-known. Platelets provide a cocktail of many of these factors, which act as a key player in tissue regeneration and wound healing strategies. PRP is indeed an excellent source of GFs and cytokines, and several works have been reported on their application in wound healing and tissue regeneration. The use of autologous PRP is now an adequate alternative in regenerative medicine. The majority of research has used autologous PRP.

Nonetheless, studies with infectious and safety issues on patients with chronic wounds are likely to be performed in the future to evaluate heterologous(allogenic) PRP. However, depending on the preparation technique, the PRP composition and concentration may be affected, requiring more research to develop standardized protocols. In addition, given the increasing number of studies with good outcomes, the absence of well-designed research to evaluate the effect of PRP on wounds of various etiologies is notable. It was demonstrated that PPP is a hemostatic agent for wounds, but it was not compared with a non-treated group to evaluate tissue regeneration effects. Consequently, in vivo investigations to determine the efficacy of PPP in normal and chronic wound healing are envisaged in the future. Platelet-Exosand pEVs also are.

## Author contributions

Keyvan Mehdipour Chari: Investigation, Methodology, Conceptualization, Writing.

Reyhaneh Nassiri Mansour, Atefeh Hojjat, Mohamadfoad Abazari, Peyman Asadi, and Elham Hasanzadeh: Methodology.

Seyed Ehsan Enderami: Investigation, Methodology, Conceptualization, Writing − review & editing, Supervision.

## Declaration of competing interest

The authors have no conflicts of interest to declare.

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