Provisional chapter

Autologous Platelet-Rich Plasma and Mesenchymal Stem Cells for the Treatment of Chronic Wounds

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Additional information is available at the end of the chapter

Abstract

Emerging autologous cellular therapies, utilizing platelet-rich plasma and mesenchymal stem cell applications, have the potential to play an adjunctive role in a standardized wound care treatment plan in patients suffering from chronic and recalcitrant wounds. The use of platelet-rich plasma growth is based on the fact that platelet growth factors can support the three phases of wound healing and then ultimately contribute to full wound closure. Mesenchymal stem cell-based therapies are also an attractive approach for the treatment of these difficult-to-heal wounds. This field of regenerative medicine focuses primarily on stem cells, which are specialized cells with the ability to self-renew and differentiate into multiple cell types. Mesenchymal stem cells can be isolated from bone marrow and adipose tissue via minimally manipulative and cell-processing techniques, at point of care. Both platelet-rich plasma and mesenchymal stem cell applications have the potential to become an effective and ideal autologous biological cell-based therapy, which can be applied to chronic wounds to effectively change the wound bed microenvironment to enable and accelerate wound closure.

Keywords: chronic wounds, microenvironment, wound healing, clinical platelet-rich plasma, platelet-rich plasma gel, mesenchymal stem cells, bone marrow concentrate, adipose tissue

1. Introduction

In the Western world, approximately 1–2% of the population will develop a chronic wound during their lifetime. These numbers will increase worldwide as a result of the aging population, increase in diabetes and obesity, and cardiovascular disease as well [1–3]. In particular, chronic leg wounds represent the largest fraction, with venous and diabetic foot ulcers (DFUs)

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accounting for 70–90% of these ulcers [4]. Concomitantly, the costs of wound care services are rising, with the market of wound care products surpassing $15 billion annually.

There is an unmet need to stimulate the healing of acute and chronic wounds to a level that is not possible with the current standard care measures and therapy approaches.

An area of medicine that holds promise for the treatment of recalcitrant and difficult-to-heal wounds is regenerative medicine. Therefore, it is critical to use more effective and efficient treatment options from patient and cost perspectives. The use of autologous biologics, such as platelet-rich plasma (PRP)- and mesenchymal stem cell (MSC)-based therapies, holds substantial promise to enhance tissue regeneration and repair in many different diseases and could therefore also be potentially effective in chronic wound care management strategies.

This review aimed to describe the scientific rationale and clinical experiences of two different autologous biological therapies to support the healing of chronic and recalcitrant wounds. First is the use of clinical PRP, prepared at point of care using a dual spin buffy coat device, and second is the local application of MSCs, derived from either bone marrow concentrate or adipose tissue.

2. Skin layers

The skin consists of three layers. The epidermis is the most outer layer, consisting of multilayered epithelium extending from the basement membrane, which separates the dermis from the air. The basement membrane contains progenitor stem cells, which undergo continuous self-renewal and differentiate into keratinocytes. The keratinocytes migrate towards the surface of the skin where they normally undergo terminal differentiation and maturation [5]. The dermis is the thickest layer, just below the epidermis. The dermis is a connective tissue, composed of the extracellular matrix (ECM), fibroblasts, vascular endothelial cells, and skin appendages such as sweat glands and hair follicles [6]. Fibroblasts are cells that secrete molecules including collagen and elastin, which provide mechanical strength and elasticity to the skin. The third layer is the hypodermis, which is underneath the dermis and composed of adipose tissue, providing insulation and cushioning between the skin and bone, muscle, tendon, and other skeletal structures [6]. A skin defect is repaired through cutaneous wound healing processes to recover loss of integrity, facilitate tensile strength, and provide a barrier for the skin [7]. Normal cutaneous wound repair is a multifaceted process.

3. Normal wound healing and cellular mechanisms

Wound healing is a well-orchestrated and complex series of events involving cell-cell and cell-matrix interactions, with platelet growth factors (PGFs), their dedicated receptors, and stem cells serving as messengers to regulate the various processes involved. The “wound healing” process as a whole has to be considered from the point of view of the type of lesion, which will in turn dictate the degree of healing that can be obtained. A partial thickness skin abrasion heals almost entirely by epithelialization, whereas deep pressure chronic ulcers rely mainly on matrix synthesis, angiogenesis, fibroplasia, and wound contraction.

3.1. Platelet clot and degranulation

With wounds and also after surgical incisions, repair begins with platelet clot formation, activation of the coagulation cascade, and subsequent platelet degranulation, releasing PGFs. After tissue damage, specific growth factors, including platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), are already being produced by the injured tissue cells [8]. Once a platelet plug is in place, platelets will get trapped in the fibrin mesh and start to degranulate, releasing PGFs, among other molecular components. Different growth factors have different characteristics and thus biological activities. Chemotactic and mitogenic capabilities have been demonstrated with regard to inflammatory cells (i.e., neutrophils, monocytes, and macrophages) [9]. At wound sites, PDGF subunits AB and transforming growth factor-β (TGF-β) are the most important growth factors initiating the wound healing process.

3.2. Inflammatory cell mechanisms

During the first 2 days of wound healing, an inflammatory process is initiated by the migration of inflammatory cells (neutrophils, macrophages, and T-lymphocytes) to the wound site to accomplish phagocytosis with the removal of bacteria, cellular debris, and damaged tissue. After the early inflammatory phase subsides, the predominant macrophage population assumes a wound healing phenotype that is characterized by the production of numerous growth factors and cytokines, including PDGF, transforming growth factor β1 (TGF-β1), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor-a (VEGF-a), which promote cell proliferation and blood vessel development [10, 11]. Activated macrophages can be classified into different phenotypes. M1-type, with antimicrobial and antitumor properties, is activated upon wound formation by inflammatory signals from interferon-y (IFN-y) and tumor necrosis factor-α (TNF-α) or when pathogen-associated molecular patterns or endogenous danger signals are recognized. Their main role is host-defense mechanisms in the early healing process, releasing IL-12, promoting pro-inflammatory Th1 immune responses [12]. Conversely, the M2 macrophage phenotype downregulates inflammation and initiates tissue repair by releasing anti-inflammatory cytokines, such as IL-10 [13, 14]. Apoptotic wound neutrophils are ingested by these M2 macrophages, which release cytokines to promote macrophage recruitment and synthesize mediators critical to remodeling and angiogenesis, including TGF-β, VEGF, and epidermal growth factor (EGF) [15, 16].

3.3. Proliferative cell activity

Angiogenesis and fibroplasia are the next phases of wound healing, the proliferative phase. New blood vessel formation and the migration of fibroblasts, which deposit new ECM, are facilitated by EGF, keratinocyte growth factors (KGFs), and TGF-α [17, 18]. Keratinocytes migrate from the wound edges between the dermis facilitated by the production of collagenase and other proteases in the epidermis. Fibroblasts migrate, proliferate, and produce ECM in the wound bed, resulting in early granulation and tissue formation [19]. This process leads to an early increase in wound breaking strength, which is an important wound healing parameter of surgical wounds.

3.4. Epithelialization

The final phase of wound closure is epithelialization, characterized by the exit of inflammatory cells, a decrease in growth factor release, an increase in the ratio of collagen deposition to fibroblasts, and the cross-linking and organization of collagen molecules. Remodeling takes place over a much longer period of time in which the newly formed tissue is reorganized for higher tensile strength [17]. Hence, the normal wound healing process constitutes a delicate balance of cells secreting and regulating the many cytokines, chemokines, proteins, and growth factors.

4. Chronic wound healing characteristics

A vast majority of chronic wounds begin as minor traumatic injuries, such as penetrating injuries, insect bites, or even simple scratches of dry skin. Normally, these wounds heal within a few days/weeks. However, aging and underlying pathologies, such as diabetes-induced and nondiabetic neuropathies, can lead to the development of poor or non-healing wounds [20]. Furthermore, arterial and venous vascular pathologies with hyperglycemia could further complicate the wound healing process. Chronic wounds are chronically inflamed and can be characterized by dysfunctional cellular events and aberrant cytokine and growth factor activities, leading to failure of normal wound closure with the potential for infections [19, 21]. Wound infections trigger extensive recruitment of inflammatory cells, particularly resulting in high concentrations of neutrophils, serine elastase, and inflammatory macrophages, while cell extravasation is facilitated by disproportionate expression of vascular cell adhesion molecules and interstitial cell adhesion molecules by resident endothelial cells. The accumulated inflammatory cells in the wound bed lead to protease activity, with elevated levels of matrix metalloproteases (MMP) 2, 8, and 9, successively prolonging inflammation [22]. Moreover, tissue inhibitor of MMP 1 is decreased in non-healing wounds, thereby increasing collagenolytic activity. Furthermore, neutrophils also produce various reactive oxygen species (ROS), inducing considerable oxidative stress and thus damaging structural elements of the ECM and wound biochemical microenvironment [23]. Nonetheless, together with proinflammatory cytokines, an abnormally prolonged inflammatory phase will result in wound chronicity, which might lead to premature cell senescence [24]. Tissue hypoxia and repeated wound infections will continue to promote MMP enzyme activity, resulting in decreased growth factor functions, and fibrin deficits will transpire. It has been demonstrated that a chronically inflamed wound microenvironment subjects proteins and cytokines to degradation and sequestration, in particular the growth factors PDGF, EGF, and TGF-β [25, 26]. In addition, Cooper et al. demonstrated that a number of growth factors were markedly reduced in wound fluids from chronic wounds as compared to acute wounds [27]. Moreover, FGF and TGF-β concentrations were significantly downregulated in chronic wounds. Decreased growth factor levels and upregulation of proinflammatory cytokines and chemokines will worsen normal progression of wound healing and consequently the potential for full wound closure. In chronic wounds, the microenvironment must be modified to be an active and effective intervention, eliminating the factors that impede healing.

To succeed in the reparative phase of wound healing, chronic wound care treatment strategies should have a dual approach. This includes the treatment of any underlying systemic disease and wound-microenvironmental tissue therapy. Evidence-based principles for local and systemic wound care management exist in the literature but are not further discussed in this chapter [28, 29]. In these traditional wound care treatment options, the application of autologous cellular biologics, such as platelet-rich plasma (PRP) growth factor therapy and MSC applications, is not anticipated, but discussed in detail here below.

5. Platelets in platelet-rich plasma therapy

PRP therapies have been used for a variety of indications, for more than 30 years. More than 10,000 references are currently in PubMed, using the search term platelet-rich plasma. These countless applications have given rise to considerable interest in the potential of autologous PRP in numerous regenerative medicine indications. In the last decade, numerous studies and reviews have been published on PRP therapies as a biological, adjunctive, therapy option in the management of chronic wounds.

5.1. Platelets and their intracellular content

Platelets are formed from megakaryocytes and are synthesized in bone marrow by pinching off from their progenitor cell. Thereafter, platelets are released into the peripheral circulation. Platelets are small, anucleate, discoid blood cells (1–3 μm), with an in vivo half-life of 7 days. The average platelet count in adults ranges from 150 to 350 × 10^6/mL of circulating blood. Platelets have a ring of contractile microtubules (cytoskeleton) around their periphery, containing actin and myosin. Inside platelets, there are a number of intracellular structures, including α-granules comprising PGFs and angiogenesis regulators and dense granules containing ADP, ATP, serotonin, histamine, calcium, and mitochondria. Other complex platelet biological components include adhesins and coagulation and immunological molecules. These molecules serve a multitude of functions, first within the clotting cascade and finally as initiators of tissue-healing processes. Platelets are equipped with an extensively invaginated membrane with an intricate canalicular system, which is in constant contact with the extracellular fluid [30]. Normally, platelets are in a resting state, non-thrombogenic. They require a 'trigger' before they become a potent and an active player in hemostasis and an accelerator of the wound healing cascade, depending upon the microenvironmental effectors.

5.2. Platelet-rich plasma gel, growth factors, and platelet receptors

When PRP is indicated to treat recalcitrant wounds, in the vast majority of these applications, PRP is delivered as a topical semiviscous coagulum so that concentrated platelets and various cytokines can adhere to the surface of the wound bed. For platelets to stick to a prepared wound bed, the PRP sample needs to be activated, thereby changing from a resting, inactive state to an active form. The platelet discoid shape changes, with the development of pseudopodia (**Figure 1**). This change in platelet shape and configuration is facilitated by the

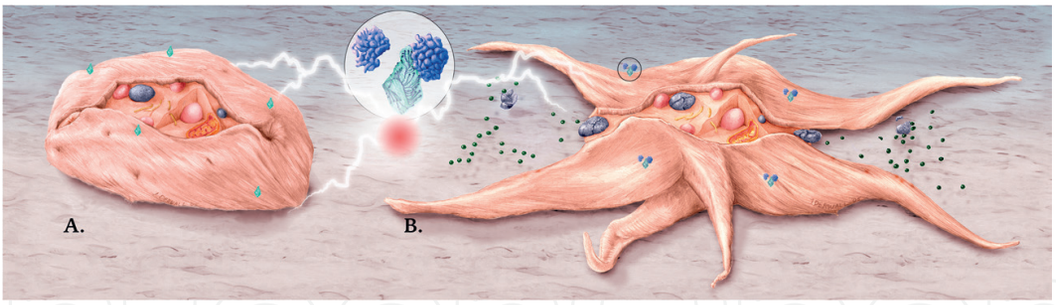

Figure 1. Graphic illustration of non-activated and an activated platelet. (A) A normal, discoid, resting platelet in a non-activated state, with platelet glycoprotein surface receptors on the outside of the platelet. (B) Following activation, the platelet shape is changed, with the development of pseudopods and the release of platelet granules and other intracellular storage vesicles via the opened canalicular system into the local microenvironmental milieu.

addition of platelet agonists (e.g., autologous or bovine thrombin, calcium, tissue factor, or other platelet-activating proteins) to a volume of PRP. Platelet activation and aggregation then leads to the creation of the semiviscous coagulum, that is, platelet clot, referred to in the literature as platelet-rich plasma gel (PRP-G). In this constitution, PRP-G can then be exogenously applied to soft tissues and chronic wounds.

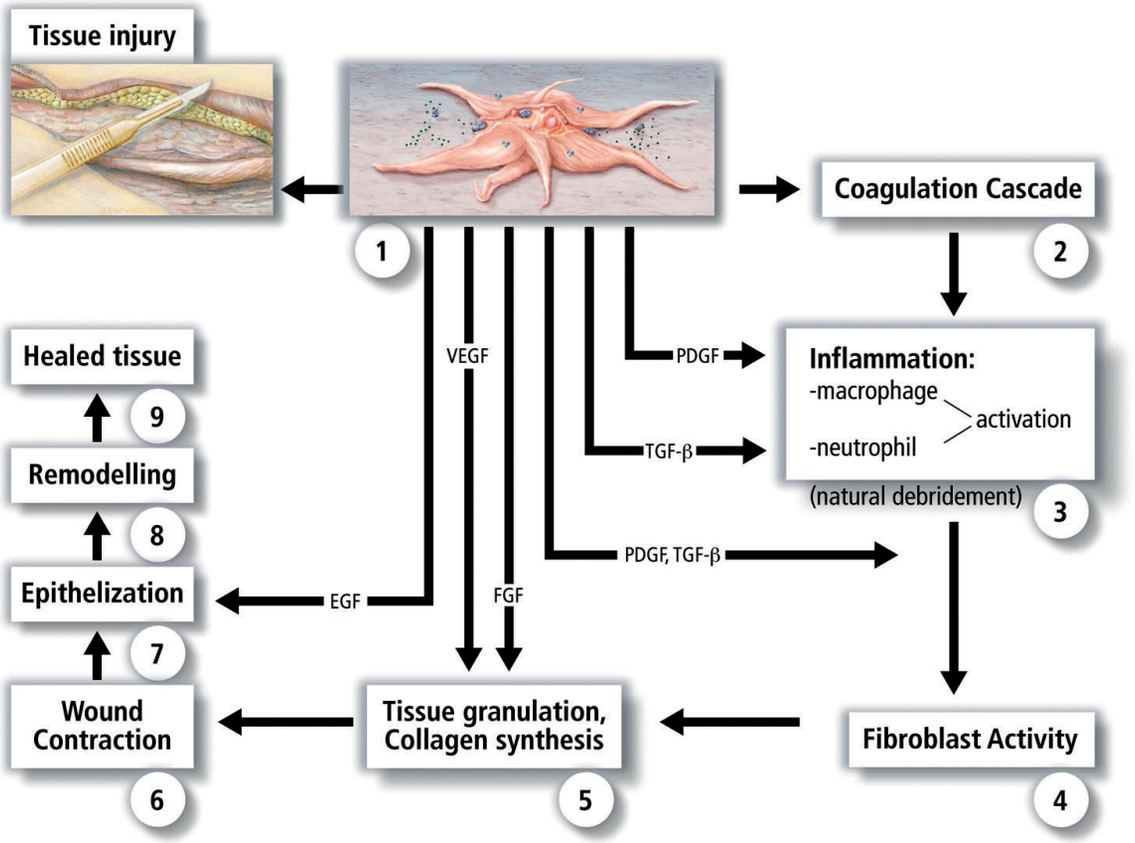

Figure 2. Schematic illustration of the activities of platelet growth factors during the different stages of the wound healing cascade. The numbers indicate the sequence of the phased stages of the wound healing process in which platelet growth factors have pivotal roles (EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet derived growth factor; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor).

Following a PRP-G application on a debrided wound bed, fibrinolysis occurs over time and the platelets start to disintegrate, subsequently releasing their PGFs and other plasma proteins. This is the onset of PGF-mediated stimulation of cell proliferation, promotion of cell differentiation and chemotaxis, and induction of migration of various (stem) cells to the wound area [31, 32]. The rational for applying PRP-G to wound bed tissues is the delivery of a diversity of concentrated platelet-derived growth factors and other biological mediators (e.g., adhesive proteins, fibrinogen, fibronectin, vitronectin, and thrombospondin-1) to mimic, and accelerate, physiologic wound healing cascades and regenerative tissue repair processes (**Figure 2**) [33].

After disintegration of the topical semiviscous coagulum, PGFs and other platelet molecules accumulate in the ECM and the released growth factors interact and bind with a specific platelet tyrosine kinase receptor (TKR), present on the outer surface of cell membranes (ligand-receptor interaction). TKRs are membrane spanning proteins that extend into the cytoplasm of the cell. After growth factors interact with their specific cell membrane TKR, activation of (inactive) messenger proteins in the cytoplasm occurs. The activated TKR cytoplasmic tail now serves as a binding site for the messenger proteins. An activated protein is generated through a signaling cascade, capable of entering the cell nucleus, where it triggers the genes responsible for controlling cell division. Subsequently, transcription of mRNA is induced, producing a biological response that initiates cascades that induce tissue repair and regeneration (**Figure 3**) [34, 35].

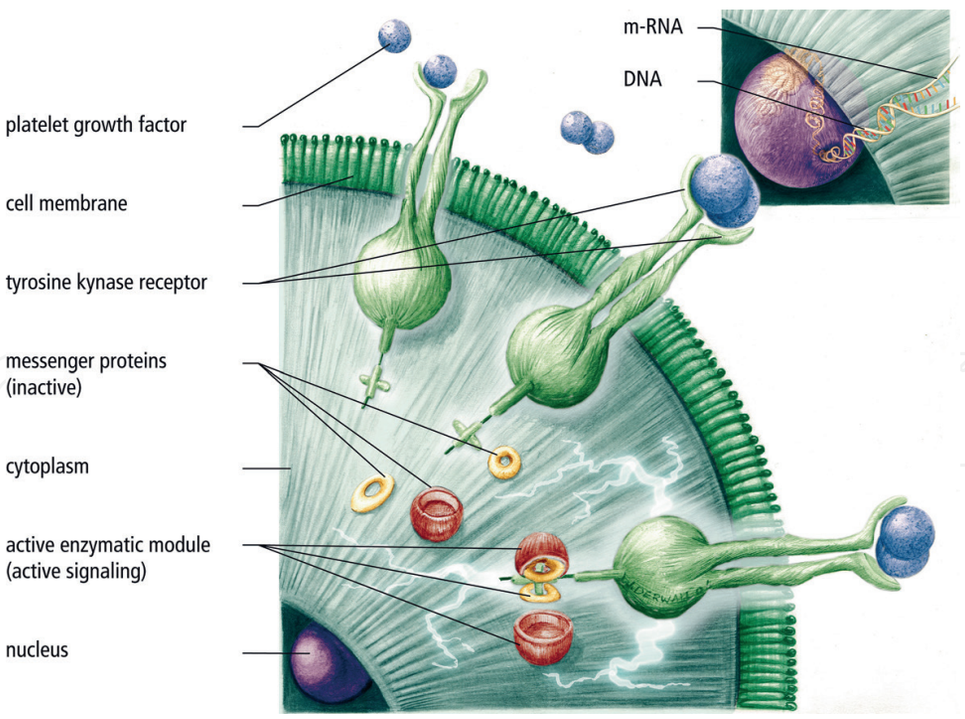

Figure 3. Illustrative representation of the mechanisms involved in platelet growth factor binding to their receptor. Specific platelet growth factors find their dedicated cell membrane tyrosine kinase receptor on the outside cell membrane. Following coupling, active enzymatic intracellular signaling occurs, with transmission to the cell nucleus via messenger ribonucleic acid.

Platelet growth factor	Growth factor sources	Biological activities
Platelet-derived growth factor, PDGF(a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis
Transforming growth factor, TGF(α-β)	Platelets, extracellular matrix of bone, cartilage matrix, macrophages/monocytes, and neutrophils	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells
Epidermal growth factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
Fibroblast growth factor, FGF(a-b)	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
Connective tissue growth factor, CTGF	Platelets through endocytosis from extracellular environment in bone marrow	Promotes angiogenesis, cartilage regeneration, fibrosis, and platelet adhesion
Insulin-like growth factor-1, IGF-1	Plasma, epithelial cells, endothelial cells, fibroblasts, osteoblasts, bone matrix	Chemotactic for fibroblasts and stimulates protein synthesis. Enhances bone formation by proliferation and differentiation of osteoblasts
Interleukin-8, IL-8	Platelets, macrophage, epithelial cells	Stimulates mitosis of epidermal cells and supports angiogenesis

Adapted from Everts [130].

Table 1. Comprehensive description of the most known platelet α-granule components as they appear in PRP.

A synopsis of the most well-known PRP growth factors is provided in **Table 1**, along with a description of the growth factor sources and their individual specific functions [36–47]. Besides the numerous activities of their growth factors, platelets also contribute to many adjunctive and supportive activities (**Table 2**) via paracrine, autocrine, and endocrine modes of actions [35, 37]. Because of these unique modes of action, PGFs are capable of exerting effects on multiple cell types, showing a series of morphometric and mitogenic functions. The morphometric growth factors, involved in bone growth, can turn undifferentiated multipotent MSCs into immature and mature osteoprogenitor cells through the presence of the so-called bone morphogenetic proteins (BMPs) [48]. Most PGFs have mitogenic actions that increase the population of healing cells and degranulate by mitogenesis.

Proteins-chemokines-cytokines	Biological activities
Adhesive proteins	Cell contact interactions
	Extracellular matrix composition
Proteases and anti-proteases	Angiogenesis
	Vascular remodeling
	Cellular regulation
	Cellular behavior
Mitogenic factors	Increases angiogenesis
	Cell proliferation
	Chemotaxis
Chemokines and cytokines	Cellular interaction
	Vascular remodeling
	Bone formation
Membrane glycoproteins	Platelet aggregation
	Platelet adhesion
	Inflammation
	Platelet and leukocyte interaction
Granules	Capillary permeability
	Vascular local regulation

Table 2. Non-platelet growth factor-related adjunctive effects of PRP therapy.

6. PRP device technology and cellular formulations of clinical PRP

PRP treatment protocols have evolved immensely over the past 20 years. Through laboratory, experimental, and clinical research, followed by more recent meta-analyses, physicians, medical practitioners, and scientists have gained a better understanding of platelets in PRP cellular physiology. The platelet secretome consists of all the proteins that are released upon platelet activation, which can be measured through proteomic-based techniques [49]. This proteomic profiling has increased our current understanding of the functional importance of the platelet granule contents [50], especially with regard to the biological cellular functions of the multifaceted platelet secretome and other plasma constituents, affecting PRP treatment outcomes.

6.1. Autologous blood predonation and PRP processing devices

The starting point for any PRP preparation is whole blood. At point of care, a fresh unit of autologous blood is drawn via a phlebotomy, following standard operating procedures.

The median cubital vein is often used as this is an easily accessible and superficial vein, enabling the introduction of 18- to 21-gauge butterfly systems. Blood is collected in a syringe

containing an anticoagulant to prevent clotting. The blood predonation volume depends on the PRP device of choice to prepare PRP and the volume needed for specific single, or multiple, wound care treatments in the same patient. Directly after blood collection, the PRP centrifugation process should be initiated in order to produce a sample of PRP.

Currently, physicians can choose from more than 30 PRP processing systems. However, a lack of consensus on standardizing PRP has contributed to the variation in PRP devices, which produce dissimilar platelet concentrations and cellular compositions [51, 52].

Optimal blood separation is best safeguarded by so-called double-spin PRP centrifuges with dedicated disposable platelet concentration devices. These double-spin PRP devices create a layered buffy coat stratum based on different centrifugal forces and specific gravities and densities of the individual blood components (**Figure 4**). Single-spin devices, or plasma-PRP

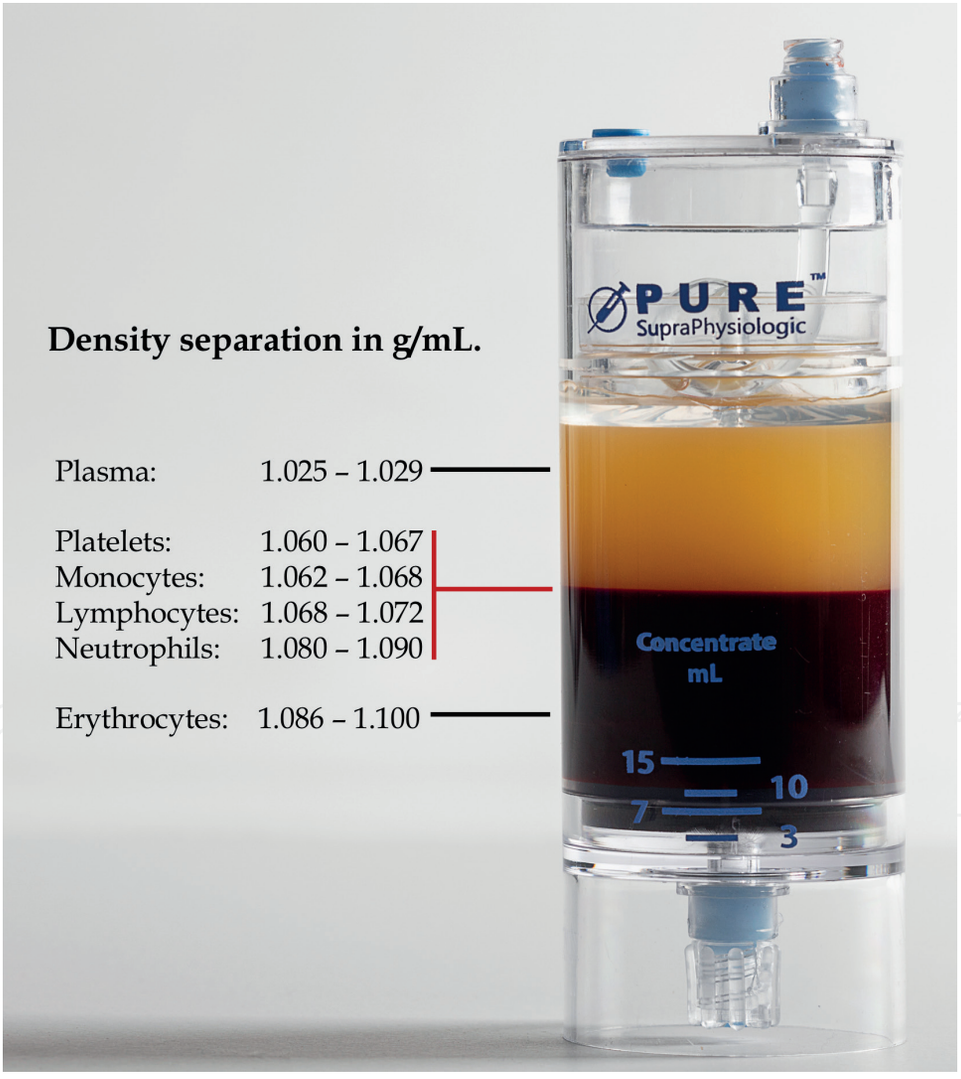

Figure 4. Cellular density separation of whole blood by centrifugation. After the first centrifugation procedure, the whole blood components are separated in the PRP device from the plasma as a result of the different densities in two basic layers. The top layer is the platelet plasma suspension, consisting of plasma and the multicomponent buffy coat layer, containing platelets, monocytes, lymphocytes, and neutrophils. The second layer consists of erythrocyte pack. The range of the specific cell densities varies between individuals.

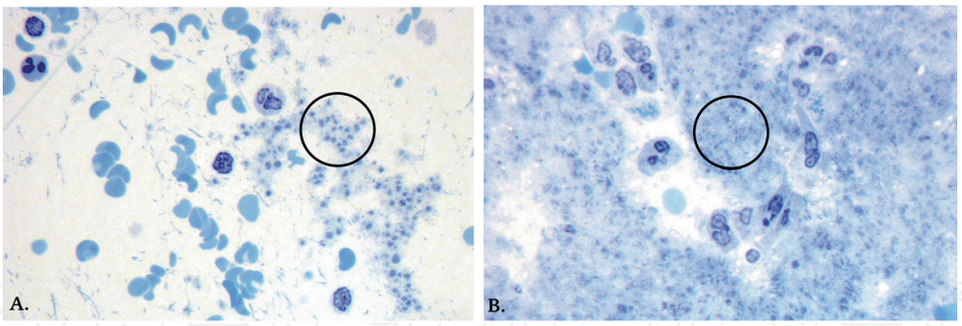

Figure 5. Whole blood and PRP smears with non-activated platelet. (A) Peripheral blood smear of whole blood inside the circle, platelets are visible (platelet count of the smear is 276,000/μL), next to white blood cells, erythrocytes, and fibrin. (B) Platelet-rich plasma smear. A high density of platelets inside the circle, with minimal leukocytes and erythrocytes (platelet count of 2,208,000/μL, prepared with the EmCyte System).

devices, prepare a product from the acellular plasma layer, excluding erythrocytes and leukocytes, while collecting as many platelets as possible from the plasma layer [53]. These differences in cellular compositions, and thus PRP characteristics, have recently been recognized in the literature [54]. Marques et al. found that inferior treatment outcomes following PRP applications correlated directly with poor quality and inconsistent PRP products [55]. Therefore, PRP devices should be versatile and compliant to enable the production of different PRP formulations, while maintaining supraphysiologic platelet numbers (**Figure 5**). More specifically, the final cellular PRP treatment sample should be tailored to serve treatment protocols contingent to wound bed condition, wound size and depth, and undermining tissue.

6.2. Definition of clinical-PRP and platelet dose

PRP can be characterized as a complex composition of autologous multicellular components in a small volume of plasma, with a substantial supraphysiologic concentration of platelets compared to baseline values, with minimal red blood cell contamination.

Clinical PRP (C-PRP) contains a clinical dose of concentrated platelets in a treatment sample. Marx demonstrated enhancement of bone and soft tissue healing with a minimum platelet count of 1×10^9/mL [56]. Furthermore, Giusti et al. revealed in an experimental study that 1.5×10^9 platelets/mL are needed for inducing a functional angiogenic response, via endothelial cell activity, in tissue repair mechanisms [57]. Therefore, to significantly induce an angiogenic response in circulatory compromised chronic wounds, C-PRP should contain at least 7.5 billion deliverable platelets in a 5-mL treatment sample. These platelets should then be able to release their entire content after (tissue) activation has occurred, as visualized in **Figure 6** by electron microscopic imaging. Furthermore, this platelet dose will correspondingly induce cell proliferation and cell migration. Ultimately, an increase in wound bed microcirculation would contribute to ECM remodeling and wound epithelialization [58]. Consequently, C-PRP containing a platelet dose of 1.5×10^9 platelets/mL has the ability to stimulate (neo)angiogenesis and elicit the healing of chronic wounds.

6.3. Leukocytes in C-PRP

Leukocytes have a great impact on the intrinsic biology of chronic wounds because of their immune and host-defense mechanisms. Therefore, the presence of leukocytes in

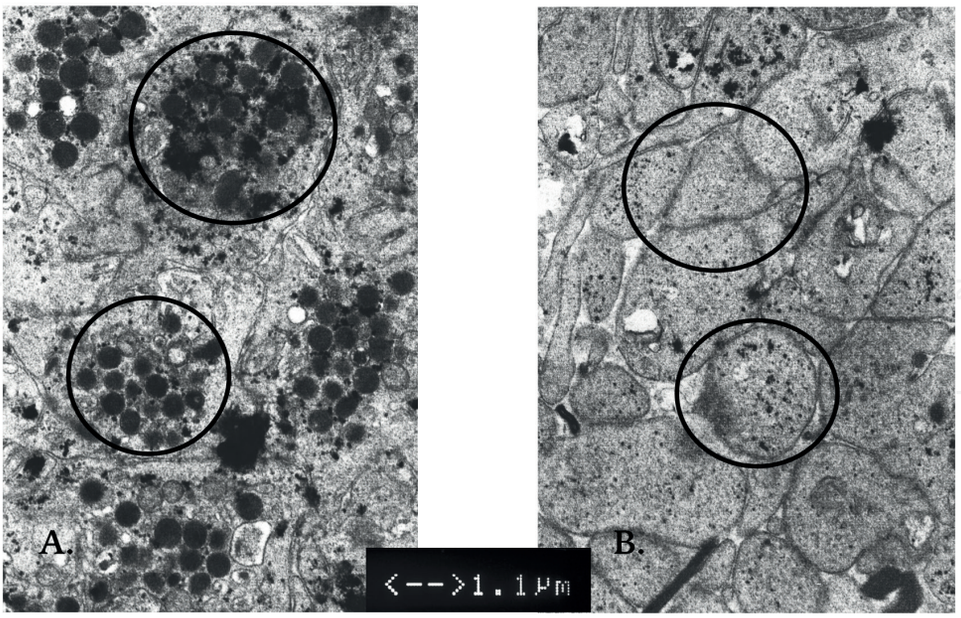

Figure 6. (A) Electron microscopic image of a single platelet in the circles. The internal platelet α and dense granules (black and gray structures, respectively) and lysosomes are visible with intact cellular membranes. (B) Electron microscopic image of activated platelets in the circles. The platelet membranes are ruptured, and their granular content is no longer visible. The platelet growth factors and other vesicles have been released to the extracellular matrix.

re-establishing wound healing attempts in chronic non-healing wounds can be turntables in the wound healing process [59].

The eventual presence of leukocytes in PRP specimen depends on the operating and design principles of PRP devices. Most ideally, PRP processing devices should be able to produce different PRP cellular formulations, including leukocyte composition and concentration. PRP formulations should be based on a disease-specific pathology, medical condition, and tissue types.

Leukocytes develop from multipotential hemopoietic stem cells in the bone marrow and mature along several differentiation pathways. Via common myeloid progenitor cells and myeloblasts, they become differentiated granular (neutrophils, eosinophils, basophils) and a-granular cells (lymphocytes and monocytes) [60]. However, during PRP preparation, the cell membranes of eosinophils and basophils are destroyed following the centrifugation procedure. Interactive wound healing processes involve mediators, extracellular matrix components, resident cells, including platelets, and infiltrating leukocytes. They participate in the classical pathway of wound healing: hematoma, inflammation, tissue formation, and ultimately tissue remodeling.

In PRP, lymphocytes are more concentrated than other leukocytes. They produce insulin-like growth factors, and they may contribute to tissue remodeling [61].

Monocytes are non-inflammatory white blood cells and are the precursors to macrophages. Macrophages are important cells of the immune system that, similar to neutrophils, are formed to fight infection or engulf accumulating damaged or dead cells. Unlike neutrophils, monocytes do not lead to a prolonged inflammatory condition but play important roles in tissue healing.

M1 macrophages are responsible for producing several inflammatory cytokines that support host defense through pathogen clearance, necrotic tissue clearance, and reactive oxygen species. Furthermore, the M1 phenotype produces growth factors such as VEGF and FGF. M2 macrophages have anti-inflammatory capacities and generate precursors for collagen and fibroblast stimulating factor, thus supporting their role in extracellular matrix deposition. Generally, the plasticity of monocytes is dependent on the microenvironment in which they are present. Monocytes and macrophages release additional pro-regenerative growth factors that lead to neovascularization, proliferation of myogenic precursor cells, and stimulation of the activity of satellite cells, playing key roles in wound repair and inflammatory control [21, 62].

Neutrophils have a clear function in healing cascades since they form a dense barrier against invading pathogens and counteract infections [63]. Their presence in PRP can be desirable in wound care treatment to functionally destroy and clear bacteria from the wound bed, in certain types of open surgical procedures to prevent wound infections, or within specific treatment protocols that require higher levels and longer periods of inflammation [64]. However, when PRP samples containing very high neutrophil concentrations are used, for example, in non-infected and granulating wound beds, this neutrophil-rich PRP poses a potential risk of progressive and persistent microenvironmental inflammation via the secretion of proteases and toxic oxygen metabolites. PRP products containing elevated levels of proinflammatory neutrophils facilitate a strong leukocytic chemotaxis to induce a phagocytic response, not contributing to wound epithelialization [65, 66].

6.4. Erythrocytes in C-PRP and effects of eryptosis on the wound microenvironment

Detrimental consequences of erythrocytes or red blood cells (RBCs) on tissues have been studied by several groups. In a study by Hooiveld and coworkers, chondrocytes and synoviocytes were exposed to RBCs causing tissue degeneration and destruction, including apoptosis [67]. In another study, it was postulated that erythrocytes inhibit fibroblast proliferation in a collagen scaffold. These findings indicate potential negative effects on the healing of soft tissue cellular structures when using PRP that contains high concentrations of erythrocytes [68]. Indeed, the use of PRP containing RBCs should be avoided in wound healing strategies to prevent wound breakdown.

Another rare phenomenon occurs when a PRP preparation including RBCs is applied to tissues. Under normal physiological circumstances, erythrocytes are removed from the circulatory system by the process of senescence after approximately 120 days. In tissues treated with PRP-containing erythrocytes, natural mechanisms of erythrocyte elimination are no longer valid, and erythrocytes undergo eryptosis before they reach their full lifespan [69]. Typical features of eryptosis are similar compared to apoptosis: membrane blebbing and cell shrinkage, resulting in the release of platelet activating factor (PAF). PAF plays a role in control mechanisms of inflammation and stimulating ceramide release and intracellular stress response, while eryptotic RBCs bind to endothelial cells and impede microcirculation [70]. Therefore, the application of PRP containing RBCs in the chronic wound microenvironment finally leads to tissue inflammation and an intracellular stress response, causing oxidative destruction in the wound vasculature.

7. PRP preparation protocol to produce PurePRP®SP

In this paragraph, a detailed and specific PRP preparation procedure is described to produce Pure Platelet-Rich Plasma-Supra Physiologic (PurePRP®SP, EmCyte Corporation, Fort Myers, FL, USA). This autologous cellular platform technology is able to generate C-PRP with high concentrations of platelets; there are protocol options to produce neutrophil-poor or -rich PRP, with minimal erythrocyte contamination (**Figure 7**). Furthermore, this platform technology enables clinicians to also concentrate bone marrow aspirate to retrieve, among other cells, concentrated mesenchymal cells [71]. Additionally, the same technology is capable of creating concentrated and viable adipose tissue complex. Both bone marrow and adipose biological tissue types will be discussed in another paragraph to emphasize the ability to use viable MSCs for wound care treatment.

7.1. PRP preparation and procedural therapy application steps

At point of care, 54 mL of fresh whole blood is predonated in a 60-mL syringe preloaded with 6 mL of 3.8% sodium citrate (anticoagulant). The PurePRP®SP device is loaded from the top and placed in a centrifuge with pre-programmed settings. Following a first centrifugation of 1.5 min, the whole blood is sequestered in a Platelet-Poor Plasma Suspension (PPS) containing a buffy coat layer and RBCs. Using a syringe, the PPS is aspirated until a band of RBCs, which holds mature platelets, is captured with the PPS. This volume is then transferred to the bottom part of the same device, the concentration chamber, and placed in the centrifuge for a 5-min second spin. During this period, a final cell PPS separation is achieved, with the concentrated platelets pelleted at the bottom of the chamber. Excessive platelet-poor plasma (PPP) is removed, leaving a PurePRP®SP volume, generally between 3 and 7 mL. This PPP

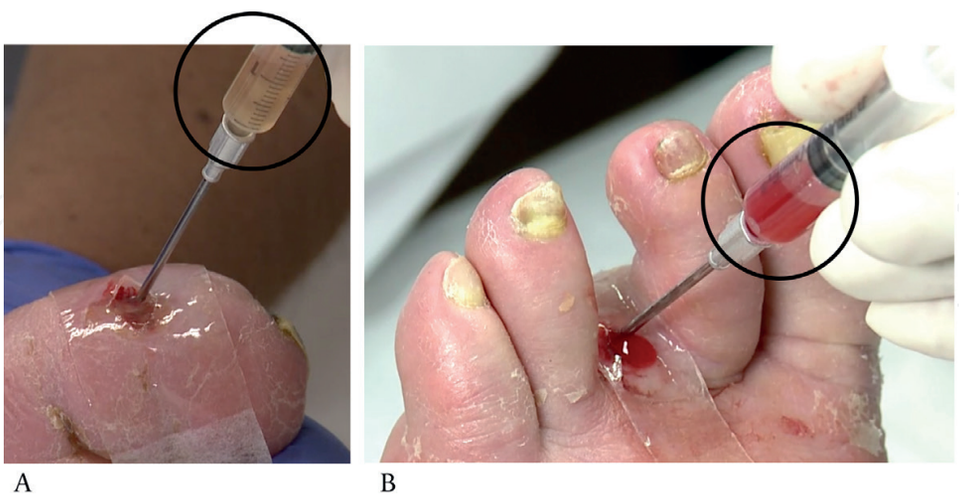

Figure 7. (A) Typical aspect of a neutrophil-poor PurePRP®SP sample, with a more yellow coloring. This PRP is intended to treat a wound that does not require proinflammatory PRP stimulation. (B) Typical aspect of a neutrophil-rich PurePRP®SP sample. This formulation is defined as a full buffy coat PRP, containing a significant concentration of platelets, neutrophils, monocytes, and lymphocytes. The red color in this preparation is due to the erythrocytes present in the PRP, as the collected neutrophils are on top of the red cells when they are from the platelet-plasma suspension (PurePRP®SP, pure platelet-rich plasma supraphysiologic; PRP, platelet-rich plasma).

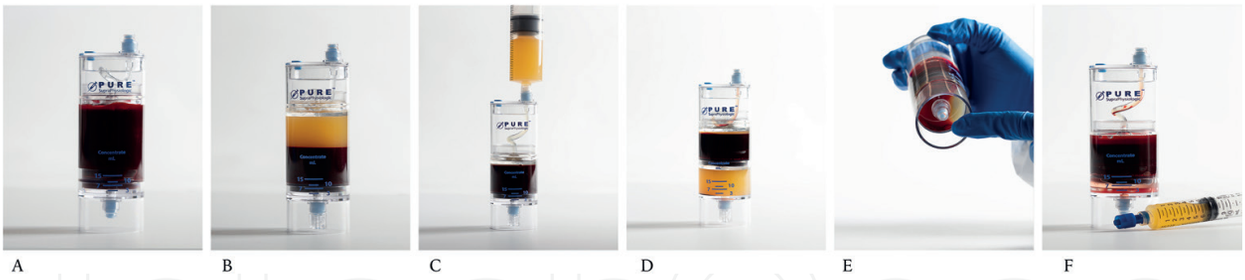

Figure 8. PurePRP®SP preparation procedure. (A) The PRP device is loaded with anticoagulated whole blood. (B) After first spin, the PPS is created following gravity centrifugal separation. (C) The PPS evacuated from the top chamber and meticulously injected in bottom part of the PRP device for the second spin procedure. (D) After the second spin, the PPS is further refined in a PPP fraction and concentrated platelets. (E) PPP has been softly removed with a syringe, leaving a desired volume behind to gently resuspend the supraphysiologic platelet concentrate, which is attached to the bottom of the second chamber of the device. (F) The platelets are aspirated in a small volume of plasma and the PurePRP®SP product is collected in a syringe prior to application (PPS, platelet plasma suspension; PRP, platelet-rich plasma; PPP, platelet poor plasma; PurePRP®SP is a registered trademark of EmCyte corporation, Fort Myers FL, USA).

volume is used to resuspend the platelets from the bottom of the device back into the PPP by gentle swirling of the device. When the bottom part is clear, all platelets are resuspended in the plasma and the PurePRP®SP is then withdrawn with a 12-mL syringe (**Figure 8**).

Depending on the biological wound treatment strategies, different PRP application techniques can be used. Before starting any PRP procedure, a meticulous sharp wound debridement should be done. Microfracturing the wound bed, with removal of cellular/plasma debris or dead tissue, allows PGFs to function more effectively while being resistant to rapid degradation by proteolytic wound activities.

The application of PRP, or PRP-G, can be done using different techniques. First, PRP can be injected intralesionally, including the wound edges [72]. This technique delivers the platelets directly into the deeper tissue structures. The objective of this technique is to stimulate tissue regeneration faster in more stagnant wounds and wound edges or to prepare the wound bed for a final reconstructive procedure [73]. Second, PRP mixed with bovine or autologous thrombin creates a PRP-G coagulum, where this topical "primary" biological wound dressing covers and sticks to the wound bed. The PRP-G can be applied to a wound bed via a single syringe technique or delivered using a double syringe spray device to ultimately generate a solid graft (**Figure 9**). Lastly, wounds with undermining can be filled with PRP-G using a single syringe and blunt needle approach. Furthermore, the same technique using a sharp needle is suitable for injecting the wound perimeter with adipose tissue, with or without PRP (**Figure 10**).

After PRP has been applied to the wound bed, wound undermining areas, and wound edges, platelets will slowly start to lyse, releasing their PGFs, cytokines, and other proteins; inducing cell signaling processes; and initiating regeneration and tissue healing [74].

The literature is not clear on the number of PRP treatments needed to treat a wound and the associated outcomes. Several studies indicate multiple treatments over a period of time. Everts et al. performed initially two treatments weekly for 2 weeks. Thereafter, the procedure was bi-weekly, until final wound closure was expected. In the same study, the PRP procedure was followed by using a naturally derived porcine intestinal submucosa matrix graft to support building the

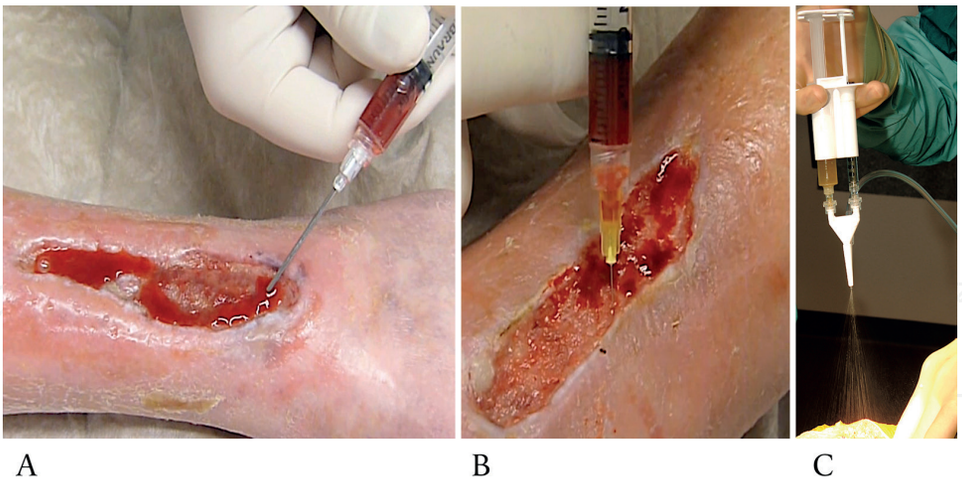

Figure 9. PRP application techniques. (A) A semiviscous PRP-G coagulum is topically placed on a wound bed via a single syringe technique. PRP and thrombin are mixed in the same syringe and delivered via a blunt needle covering the entire wound bed. (B) Intralesional injection of PRP with a 30-gauge needle in the wound bed and/or wound edges. (C) Spray application using an aerosol delivery technique, with PRP and autologous thrombin in separate syringes. The content mixes at the tip of the spray catheter, where after PRP-G is formed at the tissue site (PRP-G, platelet-rich plasma gel; PRP, platelet-rich plasma).

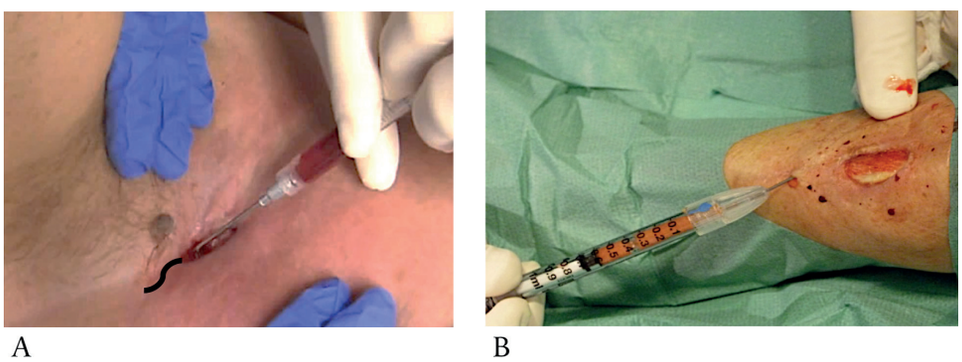

Figure 10. (A) Filling an undermining of a wound with PRP-G, using a single syringe technique in which both PRP and thrombin have been mixed to a semiviscous coagulum (the black line indicates the direction of the undermining). (B) The wound perimeter of the chronic wound is injected with a mixture of PRP and concentrated adipose tissue to deliver PGFs and adipose tissue constituents like MSCs (PRP-G, platelet-rich plasma gel; PRP, platelet-rich plasma; PGFs, platelet growth factors; MSCs, mesenchymal cells).

ECM and limited permeability to keep the lysing platelet fluids in place (OASIS® Wound Matrix, Cook Biotech, Inc., West Lafayette, IN, USA), followed by a hydrocolloid secondary dressing (DuoDERM® Extra Thin Dressing, ConvaTec, Greensboro, NC, USA) [73]. Others have used, for example, a non-absorbent sterile transparent sheet (Tegaderm™, 3M Medical Inc.) or a knitted cellulose acetate non-adherent dressing impregnated with a petrolatum emulsion (Adaptic, Systagenix Wound Management Limited, North Yorkshire, UK) [75].

Recent review articles do not provide clear information on post-PRP treatment protocols [76, 77]. This author's experiences with PRP wound care treatments included no dressing changes for 5 days post-treatment. Thereafter, minimal wound cleaning and no sharp debridement are standard wound care activities, until the next PRP application. During all

patient visits, the wounds were assessed according to the TIME wound grading system [78], which was designed for tissue evaluation, infectious condition, and moisture evaluation, and the condition of the wound edges was checked at every visit to monitor progress and regression of wound healing.

8. Overview of some of the most relevant studies using autologous PRP to treat chronic wounds

The characteristics of biological PRP and PRP-G suggest that they might be a beneficial tool in the surgical armamentarium. PRP-G has been successfully used in maxillofacial surgery, orthopedics, cosmetic surgery, and dental implantology. Furthermore, several randomized controlled clinical trials studied the effect of PRP-G in wound rehabilitation and tissue engineering. Eleven studies were identified involving the use of different PRP formulations in venous and diabetic leg ulcers between 2007 and 2018 [79–89]. A summary of all the studies is shown in **Table 3**. A general comment from these studies is that some of them were underpowered [79, 81]. The PRP interventions were highly variable with regard to platelet dosing, formulations, the total number of PRP applications, and the interval between applications. PRP-G was produced using bovine thrombin and/or $CaCl_2$ or calcium gluconate to initiate a platelet coagulum. The presence of leukocytes in PRPs and the platelet dose relative to peripheral blood were hardly described. The frequency of application varied between twice weekly and weekly. Time to wound healing or wound size reduction was the most common outcome measurement. Six trials involved predominantly diabetic patients [81–83, 87, 88], while mixed ulcer etiology was included in the other studies. Outcome results favored experimental treatments with PRP, in all studies presented. Furthermore, Carter et al. conducted a review in 2011, analyzing published prospective and retrospective studies and meta-analyzed the use of PRP and PRP-G in wound healing in acute and chronic conditions [90]. Their paper included 24 studies, from which 3 studies were systematic reviews and 9 studies were included in the meta-analysis. The systematic review and meta-analysis stated that PRP applications in cutaneous wounds exposed complete and partial wound healing when compared to control wound care. Furthermore, the presence of infection was reduced in acute wounds treated with PRP. Martinez-Zapata and co-workers presented their results from a systematic review, including10 randomized controlled trials (RCTs) in chronic wounds in their meta-analysis [91]. Three of these RCTs involved DFU and three studies involved venous leg ulcers. Their results indicated that autologous PRP can enhance DFU healing when compared with standard care.

A condensed summary review by Everts et al. revealed the efficacy and safety of PRP-G treatments when used by different institutions [92]. Picard et al. published a literature review, comprising 12 studies, to summarize evidence-based data regarding the treatment of diabetic chronic wounds with PRP. In 87.5% of controlled studies, they found a significant benefit for the use of PRP therapy to treat chronic diabetic wounds, which remained unhealed after standard wound care treatment [93]. However, more studies remain necessary to produce strong evidence eliminating poor design and high bias [90, 91].

Year; author [reference]	Study design	N patients in study; indication	Duration of wound	Outcomes
2007; Kakagi [77]	RCT	51; foot tissue defects	>3 months	Ulcer reduction in treatment group
2010; Jeong [77]	RCT	100; DFU	>4 weeks	Complete wound healing
2011; Saad Setta [79]	RCT	24; non-healing DFU	>8 weeks	PRP treated group healed significantly faster
2015; Karimi [80]	RCT	50; DFU	No limit	PRP significantly reduced wound surface and depth in 3 weeks
2015; Li [81]	RCT	117; DFU	>2 weeks	PRP significant better healing than standard care
2016; Pravin [82]	RCT	31; 22 VLU and DFU; 9 others	>8 weeks	Leukocyte free PRP healed better, 86% ulcer healing
2017; Moneib [83]	RCT	40; venous ulcers	>6 months	Significant ulcer reduction
2017; Obolensky [84]	CT	100; non-healing, mixed etiology	>6 weeks	Earlier epithelialization; shorter hospitalization; less total costs
2017; Babaei [85]	PT	150; DFU	>3 weeks	Full closure after 8.8 weeks
2017; Milek [86]	CT	100; DFU	>6 months	Full wound closure treatment group controls only small wounds
2018; Etugov [87]	PT	23; VLU	>4 weeks	Significant ulcer size reduction compared to control

RCT, randomized controlled trial; CT, controlled trial; PT, prospective trial; DFU, diabetic foot ulcer; VLU, venous leg ulcer; PRP, platelet-rich plasma.

Table 3. Overview of some of the most relevant studies using autologous PRP technology to treat chronic wounds.

Presently, more studies are ongoing to clarify optimized PRP protocols to improve its angiogenic and regenerative properties to be implemented as a standard practice of care in advanced wound care treatment plans.

9. Comprehensive background on stem cells

In any regenerative tissue microenvironment, there are essentially stem cells, growth factors, and a biological scaffold to provide the necessary biological milieu for cell-tissue regeneration and cell renewal. MSCs originating from either bone marrow or adipose tissue are now extensively being used in a variety of patients who have an indication for minimally invasive, regenerative medicine therapies to enhance tissue repair and regeneration. Traditionally, bone marrow aspirate (BMA) has been utilized as a source of bone marrow-derived mesenchymal stem cells (BM-MSC), hematopoietic stem cells (HSCs), progenitor cells, and platelets. Lately, MSCs derived from adipose tissue have emerged in a variety of regenerative treatment protocols. However, in chronic wound care strategies, autologous, non-cultured, MSC therapies are rarely used. However, Hocking reported from preclinical and clinical trials that MSC therapy has the potential to effectively treat wounds with

delayed healing, resulting in accelerated wound closure [94]. A stem cell is, by definition, the one cell capable of duplicating itself (self-renewal) and resuming its undifferentiated status, while also originating progeny that can differentiate into one or more final products that are physiologically defined by their specific functions. Stem cells can be classified on the basis of their origin and their potential to proliferate and differentiate. According to Wagers and Weissman, the classification of stem cells is based on their plasticity and potential for differentiation [95]: totipotent, able to give rise to all embryonic and extraembryonic cell types; pluripotent, able to give rise to all cell types of the embryo proper; multipotent, able to give rise to a subset of cell lineages; oligopotent, able to give rise to a restricted subset of cell lineages; and unipotent, able to contribute only one mature cell type. Adult stem cells have a multipotent lineage and are able to transdifferentiate into various progenies, forming cells of multipotent lineages, such as HSCs and MSCs [95]. HSCs are pluripotent cells that further differentiate via hematopoiesis into distinct progenitor cells which mature into blood cells of myeloid lineages (monocyte, granulocyte, erythrocyte, and megakaryocyte/platelets) and lymphoid cells (B, T and NK cells) [96].

10. Mesenchymal stem cells

MSCs are multipotent adult stem cells and can be obtained from various adult tissues, including bone marrow stroma, adipose tissue, and other tissue types. According to the International Society of Cellular Therapy, MSCs are defined as those cells that are able to adhere to plastic and express a number of cell surface markers (including CD73, CD90, and CD105) while undergoing multilineage differentiation. Furthermore, MSCs should have the ability for self-renewal [97]. MSCs can also be identified as specialized populations of mural cells/pericytes. They provide a niche for HSCs and have the ability to differentiate into various mesodermal lineages. Under appropriate conditions and an optimal microenvironment, MSCs can differentiate into mesodermal lineage cells such as osteoblasts, endothelial cells, adipose tissue, and smooth muscle cells [4]. These capabilities have led to the use of MSC as a potential strategy for treating various diseases since they promote biological processes, such as angiogenesis and cell proliferation and differentiation [98]. Furthermore, they synthesize mediators (cytokines and trophic factors) that participate in tissue repair processes, immune modulation, and the regulation of inflammatory processes. [99]. The trophic effects are facilitated by the MSC secretion of reparative cytokines and growth factors, including TGF-β, VEGF, and EGF, to contribute to local tissue repair [100]. Caplan also suggested that the modulation of inflammation is instigated by the suppression of inflammatory T-cell proliferation and inhibition of monocyte and myeloid cell maturation [101]. Based on above characteristics, one can see that MSCs are able to establish a regenerative microenvironment at the site of release, which could improve the recruitment, activation, and differentiation of endogenous stem cells with the potential for repair in wound healing. Currently, clinical research is investigating MSCs as a therapy to treat difficult-to-heal wounds.

10.1. Bone marrow mesenchymal stem cells

BM-MSCs from adult bone marrow tissue were first isolated by Pittenger et al. [102]. Since then, BM-MSCs are frequently used successfully as a biological product, like PRP, in regenerative

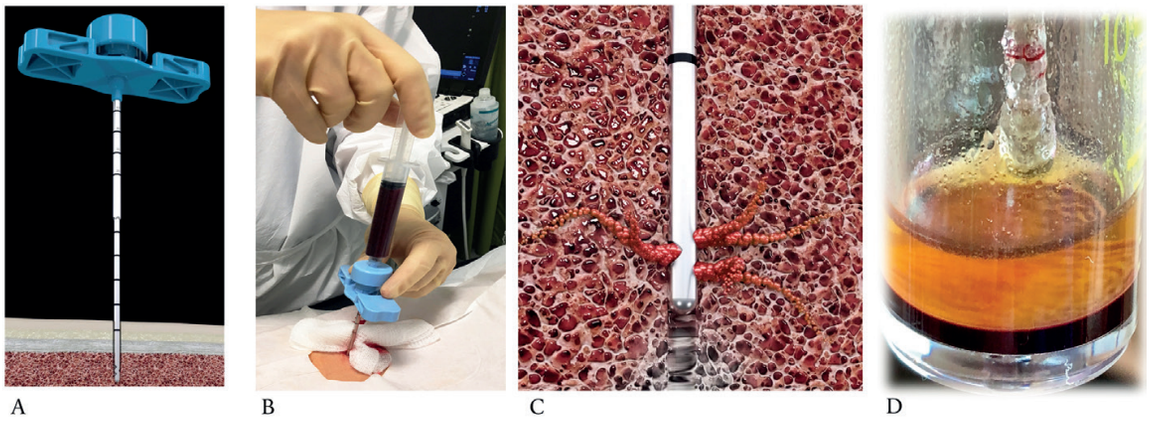

Figure 11. Aspire bone marrow aspiration from the posterior superior iliac spine area. (A) The introducer and aspiration needle are placed through the skin, sub cutaneous layer, and cortical bone into the marrow cavity. (B) The BMA device is placed in the PSIS. Bone marrow is meticulously aspirated via suction vacuum applied to a syringe, through the aspirator needle. (C) Bone marrow cells, including purified mesenchymal stem cell, hematopoietic stem cells, total nucleated cells, platelets, and progenitor cells, are collected through the fenestrated aspirator needle with a blunt tip from the cancellous bone. (D) The final BMC sample is produced following a 2-step proprietary centrifugation protocol. Inside the blue circle, concentrated bone marrow cells are visible, on the top of the erythrocyte layer (BMA, bone marrow aspirate; PSIS, posterior superior iliac spine; BMC, bone marrow concentrate; Aspire™ bone marrow aspiration system is trademark of EmCyte Corporation, Fort Myers FL, USA).

medicine therapies to treat a variety of musculoskeletal disorders, such as chondral defects, osteoarthritis, and rotator cuff lesions [103, 104]. BM-MSCs are relatively easy to acquire via a BMA procedure. Bone marrow can be harvested from a variety of anatomic sites during a surgical procedure in the operating room, or an office setting, with minimal morbidity. A variety of donor locations are available, including the anterior or posterior iliac crest, calcaneus, tibia, distal femur, and proximal humerus. The iliac crest is used frequently and known to be a rich source of BM-MSCs (**Figure 11**). BM-MSCs are transplanted autologously, therefore avoiding any ethical issues. Furthermore, the relatively simple preparation and separation and high genetic stability of BM-MSCs allow for their easy use in vitro and as an injectate. Imperative for an effective BM-MSC injection is the quality of the initial bone marrow aspiration procedure with regard to minimizing trauma to cellular content of the bone marrow niche, such as platelets, progenitor cells, and leukocytes, while maximizing cellular yields and minimizing peripheral blood infiltration [105].

Furthermore, the collected bone marrow cells should be viable, with no presence of disintegrated erythrocytes (hemolysis), as this would have a profound negative effect on tissue regeneration [106]. The author believes that a BMA sample should always be preceded by a 2-step centrifugation procedure to concentrate the sample to a bone marrow concentrate (BMC). This will concentrate the indispensable cellular content, such as MSCs (measured by CFU-f), HSCs, total nucleated cells, and platelets, above the baseline counts of these cells. Nonetheless, the centrifugation procedure will decrease hemolytic parameters as well as RBC levels. The effects of concentrating BMA with regard to some of the most important constituents and factors are shown in **Table 4**. Erythrocytes should also be avoided in a BMC specimen, for the same reasons as discussed in the above paragraph on C-PRP and effects

Laboratory parameters	BMA	BMC	Concentrating effect
TNC (−nRBCs) × 10^6/mL	28	142.8	5.1 × BL
Platelets × 10^6/mL	96	614	6.4 × BL
CD34+ cells × 10^5/mL	1.68	9.2	5.5 × BL
CFU-f (MSCs) × 10^3/mL	1.05	5.59	5.3 × BL
Hematocrit %	40.7	6.8	−83% × BL
Hemolysis %	6.3	1.8	−73% × BL
Cell viability %	95.9	97.3	+ 0.6% × BL

BMA, bone marrow aspirate; BMC, bone marrow concentrate; × BL, effects times baseline values; TNC, total nucleated cells; −nRBCs, minus red blood cells; CD34+, stem cell marker/expression on hematopoietic progenitor cells found in bone marrow; CFU-f, fibroblast colony-forming units: assay for bone marrow mesenchymal stem cell analysis; MSCs, mesenchymal stem cells.

Table 4. Effects of bone marrow aspirate concentration on cell counts, hematocrit, and the elimination of hemolytic red cells.

of eryptosis on the wound, as this will cause profound inflammation and compromise the microcirculation [69].

10.2. Adipose mesenchymal stem cells

Similar to BM-MSCs, adipose-derived mesenchymal stem cell (AD-MSC) has been used in regenerative medicine applications. AD-MSCs can be isolated following an adipose tissue (mini) liposuction procedure of subcutaneous fat tissue, mostly from the abdomen.

Various preparation techniques, including centrifugation, exist to collect, wash, and rinse adipose tissue to generate a concentrated adipose tissue concentrate (ATC). Adipocytes constitute almost 90% of adipose tissue volume and nearly 65% of the total cell number [107]. When enzymatically digested, adipose tissue yields a heterogeneous population of many cell types (pre-adipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes), which upon isolation are termed the stromal vascular fraction (SVF) [108]. AD-MSCs have a multilineage cell differentiation potential, that is, they are capable of differentiating into adipogenic, chondrogenic, myogenic, osteogenic, and neurogenic cells [109]. Thus, AD-MSCs might be indicated in clinical applications for the repair of damaged tissues, as well as for angiogenic therapy to improve neovascularization [110].

The popularity of AD-MSCs in regenerative medicine treatment protocols and recently in a biological wound care treatment protocol as well is due to an abundance of MSCs, with a high proliferation capacity and differentiation potential, when compared to MSCs derived from bone marrow [111, 112]. Furthermore, Yun et al. described AD-MSC-mediated effects on the reduction of proinflammatory cytokines, chemokines, cellular apoptosis, and collagenases [113]. Moreover, AD-MSCs have been shown to be immune-privileged [114].

11. MSCs in cutaneous wound healing

Currently, cell-base therapy is an attractive approach for the treatment of recalcitrant chronic wounds. MSCs from adipose and bone marrow tissues are being investigated as a therapeutic strategy for a distinct group of pathological conditions, including chronic hard-to-heal wounds [115]. The orchestrated process of wound healing entails cellular and hormonal physiological processes of inflammation, epithelialization, proliferation, collagen matrix formation, and particular neoangiogenesis, regulated by various growth factors such as TGF-β, VEGF, PDGF, granulocyte macrophage colony-stimulating factor, the interleukin family, EGF, FGF, and TNF-α [116, 117]. However, the activity of these cytokines in chronic wounds is often reduced due to a prolonged inflammatory state, decreasing the neoangiogenic potential.

BM-MSCs and AD-MSCs have been studied as potential solutions for these major issues. Both types of MSCs have been shown to be effective in augmenting wound healing by modulating the immune response and secreting paracrine factors which promote therapeutic (neo) angiogenesis and thereby providing biological ingredients for wound tissue regeneration, and they are ultimately capable of inducing full wound closure (**Figure 11**; [118–121]).

Optimal wound bed preparation encompasses not only debridement and proper management of the bacterial load but also correction of the wound matrix and reconditioning of phenotypically altered resident cells which are present in chronic wounds. Based on their characteristics and biological activity, MSCs are capable of interacting with resident wound cells to transform resident cells to functional matrix building cells [122]. This might be of particular importance for the dermal rebuilding process to stimulate keratinocytes to accomplish epithelialization.

Given their higher isolation yield, ease of harvesting, and abundance of adipose tissue, some groups believe that AD-MSCs might be more clinically attractive. Not only because of their angiogenic capability, but they may also function in situ as pericytes providing vascular stability and they might communicate with endothelial cells in response to environmental stimuli [123, 124]. However, experienced clinicians may dispute the cited potential risk for complications with BMA, as they feel comfortable in performing BMA procedures in medical-office settings using local anesthetics and imaging to perform the aspiration. Shapiro and coworkers performed a prospective, single-blind, placebo-controlled trial on 25 patients with bilateral knee osteoarthritis and reported that the BMA, production, and use of BMC is a safe procedure [125].

11.1. Critical limb ischemia

BM-MSCs are frequently being studied in patients with critical limb ischemia, who also might suffer from chronic wounds and who are not eligible for the revascularization procedure due to several comorbidities, namely high operative risk, multiple failures of revascularization, and high rate of restenosis. These patients are suitable for biological cell-based therapy with MSCs. In particular, BM-MSCs protocols are newly emerging therapies to treat CLI in this

subset of patients, promoting the regeneration of impaired endothelium and neoangiogenesis in ischemic tissues [126, 127]. The effects of several types of bone marrow cell therapy (e.g., bone marrow-derived mononuclear cells, CD34+ bone marrow cells, and mesenchymal stromal cells) have been studied in CLI patients. The outcomes of several cell-based therapy trials demonstrated that the rate of major amputation was significantly decreased [128]. It can be concluded that MSC application can be considered a promising target for future biological therapies in CLI patients [129].

12. Conclusions

Regenerative medicine technologies offer solutions to a number of compelling clinical problems that have not been able to adequately result in a solution through the use of drugs, surgery, or permanent replacement devices.

The purpose of this chapter was to review multiple aspects of both PRP and MSC biocellular therapies as part of a wound care treatment plan to support in the healing of chronic and recalcitrant wounds.

Numerous significant aspects that are still not well understood or standardized have been discussed, as well as the rationale for cell-based therapies. For platelet-rich plasma preparations, specific formulations, platelet dosing, processing, and the differences between systems were discussed. With regard to bone marrow and adipose tissue, as cell sources for obtaining high quality mesenchymal cells, some technicalities were provided.

Among both tissue-based cellular therapies, bone marrow mesenchymal cells have been the most frequently employed and reported on. In this review, evidence is shown on results from several clinical studies in which autologous biologics have been applied in patients with chronic wounds. The outcomes of these studies suggested that the application of biocellular products can reverse the microenvironment in chronic wounds, achieving the ultimate goal: full wound epithelialization in the shortest possible time. Furthermore, it was revealed that these treatments are safely executed without adverse effects for patients.

Conflict of interest

The author served also as Chief Scientific Officer of EmCyte Corporation.

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