Is the Use of Autologous Platelet-Rich Plasma Gels in Gynecologic, Cardiac, and General, Reconstructive Surgery Beneficial?

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Abstract: Tissue repair at wound sites begins with clot formation, and subsequently platelet degranulation with the release of platelet growth factors, which are necessary and well-regulated processes to achieve wound healing. Platelet-derived growth factors are biologically active substances that enhance tissue repair mechanisms, such as chemotaxis, cell proliferation, angiogenesis, extracellular matrix deposition, and remodeling. This review describes the biological background and results on the topical use of autologous platelet-rich plasma and platelet gel in gynecologic, cardiac, and general surgical procedures, including chronic wound management and soft-tissue injuries.

Keywords: Platelets, platelet-rich plasma (PRP), platelet gel (PG), growth factors, wound healing, cardiac surgery, gynecology, general surgery, adipose graft.

1. INTRODUCTION

The physiologic cascades of soft tissue wound healing and bone growth are only partially clarified, but it is clear that cellular and hormonal factors have fundamental roles in these processes [1]. In particular, platelet-derived growth factors (PDGFs), which are stored in various platelets vesicles, are pivotal healing factors [2, 3]. Currently, platelets can be retrieved and isolated from a volume of fresh, autologous whole blood with point-of-care devices which intraoperatively fractionate the blood into platelet-poor plasma (PPP), platelet-rich plasma (PRP), red blood cells, and other biological mediators [4]. In most cases, the PRP is a so-called buffy coat product and therefore consists of a small volume of plasma in most instances, in which the number of platelets and leucocytes in excess of baseline values can be measured. In general, PRP is a term used to describe a variety of techniques to produce blood components, which are enriched in platelets with the growth factors contained therein. The term PRP is a bit of a misnomer since the end product is not always a plasma fraction, but can also be a gel.

Most of the platelet growth factors are stored in the alpha granules of platelets and are inactive upon platelet activation. Platelet aggregation and activation can be accomplished with platelet agonists (e.g., thrombin, calcium, or other proteins) to create a viscous solution frequently termed platelet gel (PG). This platelet coagulum can be applied exogenously as a spray or as a solid gelatinous mass to soft tissues, chronic wounds, bone, or synthetic bone. The reason for applying PG to tissues is the delivery of platelet growth factors and other biological mediators (e.g., adhesive proteins, fibrinogen, fibronectin, vitronectin, and thrombospondin-1) to mimic and accelerate physiologic wound healing and regenerative tissue repair processes [5, 6]. This article reviews the use of PG in gynecologic, cardiac, and general and reconstructive surgery.

2. PREPARATION OF PRP AND MECHANISMS OF ACTION

2.1. PRP Preparation and Growth Factor Release

In our institution, blood is drawn in the patient holding area or in the operating room prior to the induction of anesthesia, depending on the type of surgery. To draw blood, a venous infusion catheter is placed in the patient’s antecubital vein. Blood is collected in syringes or blood bags containing an anticoagulant to prevent the blood from clotting. Thereafter, the pre-donated blood is sequestered with point-of-care devices, including blood cell savers/separators or table top devices, to produce PRP. In our opinion, the preparation of PRP by blood banks through discontinuous plasmapheresis methods should be limited because of higher production costs and delayed availability of PRP compared to bedside devices. Furthermore, blood bank–prepared PRP is not accessible by the clinician, and demands a highly controlled logistic system to avoid product mismatch before administration to the patient.

With cell savers/separators, larger pre-donation blood volumes (150 to > 500 mL of whole blood) can be obtained, resulting in a PRP volume ranging from 15 to > 50 mL. Tabletop centrifuges have been used to manufacture smaller volumes of PRP from lesser amounts of whole blood (50–150 mL). The choice for system is mainly dependent on the type of surgical procedure, and thus the anticipated amount of PRP to be produced.

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PRP is stored in the patient’s operating room at room temperature on a shaker until use. The PRP is placed in the surgical field in a sterile specimen cup when appropriate during surgery. To release platelet growth factors, the PRP needs to be activated. When platelet activators, such as thrombin, interact with PRP, a sticky platelet gel will be formed. At this stage, the semi-viscous PG can be applied to wounds or used during surgical wound closure.

2.2. Mechanisms of Action

Tissue repair and surgical wound healing are well-orchestrated, and a complex series of events involving cell–cell and cell–matrix interactions in which platelet growth factors serve as messengers to regulate various regenerative processes.

Initially, tissue repair begins with activation of the coagulation cascade, platelet clot formation, platelet aggregation, and degranulation. During this degranulation period, the platelets release a pool of biologically active proteins (PDGFs) and other substances into the extracellular milieu. In this environment, the biologically active proteins might bind to specific platelet growth factor receptors present in surgical tissues. Released growth factors interact and bind with the platelet tyrosine kinase receptor (TKR), which is present in the cell membranes of tissue cells (ligand-receptor interaction) [10]. Therefore, the actual binding site is on the outer surface of the cell membrane, and thus not directly on the cell nucleus. The TKR is a membrane spanning protein that extends into the cytoplasm of cells. After the platelet growth factor interacts with the external part of the TKR, activation of inactive messenger proteins occurs in the cytoplasm. Thereafter, the messenger proteins become activated and bind to the TKR cytoplasmic tail. Activated proteins are generated via an active signaling cascade in the cell nucleus where the genes responsible for control of cell division are triggered. Thus, transcription of messenger RNA is induced, producing a biological response that starts cascades, which in turn provoke tissue repair and tissue regeneration [11, 12].

2.3. Platelet Growth Factors in PRP

A variety of platelet growth factors are located in the alpha granules of platelets present in the PRP. Some of the most relevant platelet growth factors and their specific characteristics are summarized in Table 1.

Platelet-derived growth factor was one of the first growth factors to be identified in platelets. Subsequently, additional platelet growth factors have been identified, including transforming growth factor (TGF)–α and –β, fibroblast growth factor (FGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF) [13,14]. The platelet growth factors have their own specific function and biological activity. Cell studies in wound care have shown that multiple growth factors tend to be more effective than the use of a single growth factor [15]. The concept of harvesting and concentrating autologous platelets with subsequent transfer and fixation to the wound site within the medium of an autologous soft tissue graft allows access to higher concentrations of multiple growth factors placed directly into the wound site [16].

2.4. Infection Prevention

In addition to the PG delivery of growth factors, limited data are available that deal with the role of leukocytes present in PLG to act as an antimicrobial component. We and others have reported that buffy coat-prepared PRP not only comprises a high concentration of platelets containing platelet growth factors, but that it is also rich in concentrated leukocytes, in particular neutrophilic granulocytes, monocytes, and lymphocytes [17,18]. Neutrophilic granulocytes and monocytes contain numerous granules full of myeloperoxidase, which catalyzes the oxidation of chloride to generate hypochlorous acid and other reactive oxygen derivatives that act as potent bactericidal oxidants toxic to micro-organisms and fungi [19, 20]. Furthermore, Yeaman et al. [21] and Tang et al. [22] have maintained the idea that platelets are also involved in microbial activity, suggesting that platelets take part in the platelet host defense mechanism by releasing a variety of platelet microbicidal proteins. The platelet microbicidal proteins have been shown to be released after platelet activation, demonstrating potent activities against pathogens that have a tendency to enter the bloodstream [23].

2.5. Wound Healing

During the initial days of wound healing, an inflammatory process is initiated by migration of neutrophils, and subsequently macrophages, to the wound site. In turn, activated macrophages release multiple growth factors, including platelet-derived growth factor, TGF-α and -β, interleukin-1, and FGF [24]. Angiogenesis and fibroplasia start shortly after day 3, followed by collagen synthesis on days 3-5. This process leads to an early increase in wound-breaking strength, which is the most important wound-healing parameter of surgical wounds, followed by epithelialization and the ultimate remodeling process leading to a tissue scar [25].

Based on the actions of the various platelet growth factors during the different stages in the wound healing cascade, the use of autologous PG to stimulate wound tissue repair and tissue regeneration is an interesting proposition. Clinicians have also used recombinant growth factor to stimulate wound healing [26]. However, as compared with recombinant single growth factor applications, PG has the advantage of being autologous. In addition, in PG the multiple platelet growth factors and other biological and adhesive proteins work together synergistically and promote mitogenesis of mesenchymal stem cells and growth factors at the surgical wound site, and therefore have the potential to accelerate and boost tissue healing [27].

3. PLATELET-RICH PLASMA GELS IN GYNECOLOGIC, CARDIAC, AND GENERAL AND RECONSTRUCTIVE SURGERY

3.1. Methods

Few articles are published on the use of autologous platelet growth factor applications to support wound healing, tissue regeneration, or tissue growth in gynecologic and cardiac surgery. Therefore, we performed a review of the literature, as recommended by the Cochrane Collaboration with studies
In the gynecology literature, we could only retrieve three systematic reviews from the last 5 years. Data on diabetic foot ulcers, neuropathic foot ulcers, and the exception of the use of PRP in wound care management. For these reasons, we searched for appropriate studies using the same methodology as described above, with the exception of the use of PRP in wound care management. Data on diabetic foot ulcers, neuropathic foot ulcers, and chronic diabetic foot ulcers treated with PRP-like products are being presented as a summary and conclusion of existing systematic reviews from the last 5 years.

3.2. Gynecology

In the gynecology literature, we could only retrieve three relevant articles and one case report on the use of autologous prepared PRP, PG, or recombinant platelet growth factors on wound healing and tissue regeneration.

Fanning et al performed a prospective, non-randomized trial in 55 consecutive patients undergoing major gynecologic surgery [28]. The treated patients were compared with a control group consisting of 55 matched patients from the previous 6 months by surgeon and surgical procedure. They conducted a phase I/II trial of autologous platelet tissue graft in gynecologic surgery to evaluate toxicity and efficacy on decreasing pain. There were no adverse effects recorded in this study related to the application of the autologous platelet tissue graft. Median pain on the day of surgery and on post-operative day 1 was significantly less in the autologous tissue graft. Median pain on the day of surgery and on post-operative day 1 was significantly less in the autologous tissue graft. Likewise, the median use of morphine per hospital stay was significant for the autologous platelet tissue graft-treated patients.

Shackelford et al. conducted a double-blind, randomized, placebo-controlled trial using topical recombinant human PDGF gel after abdominal wound separation [29]. They used the recombinant growth factor to treat the wound and studied the effects on wound healing. The patients in the placebo group closed 54 ±/− 26 days post-operatively, whereas the wounds of patients in the treatment group closed in 35 ±/− 15 days (P = .05). The preliminary study suggests that the topical application of 0.01% recombinant human PDGF gel accelerates healing of separated surgical wound significantly, as determined by Kaplan-Meier analysis.

Table 1. Sources of Growth Factors and their Biological Actions on Wounds

<table>
<thead>
<tr>
<th>Platelet Growth Factor Type</th>
<th>Growth Factor Source</th>
<th>Biological Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Derived Growth Factor, PDGF(a-b)</td>
<td>Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells</td>
<td>Mitogenic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glia/ smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis</td>
</tr>
<tr>
<td>Transforming Growth Factor TGF (α-β)</td>
<td>Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/monocytes and neutrophils</td>
<td>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</td>
</tr>
<tr>
<td>Vascular endothelial growth factor, VEGF</td>
<td>Platelets, endothelial cells</td>
<td>Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells</td>
</tr>
<tr>
<td>Epidermal Growth Factor, EGF</td>
<td>Platelets, macrophages, monocytes</td>
<td>Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis</td>
</tr>
<tr>
<td>Fibroblast Growth Factor, FGF</td>
<td>Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts</td>
<td>Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal cells, chondrocytes and osteoblasts</td>
</tr>
<tr>
<td>Connective tissue growth factor, CTGF</td>
<td>Platelets through endocytosis from extracellular environment in bone marrow.</td>
<td>Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion</td>
</tr>
<tr>
<td>Insulin-like growth factor-1, IGF-1</td>
<td>Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, osteoblasts, bone matrix</td>
<td>Chemotactic for fibroblasts and stimulates protein synthesis. Enhances bone formation by proliferation and differentiation of osteoblasts</td>
</tr>
</tbody>
</table>

N.B. PRP also contains other proteins like fibrin, fibronectin, vitronectin, serotonin and thrombospondin, which are known to act as cell adhesion molecules, important for the migration of osteoblasts, fibroblasts, and epithelial cells [11].

published and indexed in the Cochrane, Embase, and PubMed databases until April 2010. The following criteria were established for the selection and inclusion of articles: clinical trials, complete articles, and articles published in international and national journals indexed in the databases mentioned above. Exclusion criteria were articles, editorials, and letters published in abstract form. Only sources available in English were used. The databases were searched for literature on PRP using the keywords platelet-rich plasma, PRP, platelet releasate, platelet gel, platelet concentrate, platelet-derived growth factor, gynecology, gynecologic surgery, and cardiac surgery.

The search for articles on PRP use in general surgery was more complex because this is a very diverse medical specialty, including many sub-specialties, like vascular surgery, laparoscopic surgery, oncologic surgery, and wound care management. For these reasons, we searched for appropriate studies using the same methodology as described above, with the exception of the use of PRP in wound care management.
In an in-vitro study, PRP was used to assess its ability to seal an iatrogenic fetal membrane defect [30]. If during pregnancy these membrane defects do not seal spontaneously, it is most likely that fluid leakage through the vagina may occur, resulting in infections and pregnancy loss.

The authors evaluated the sealing capability of PRP plugs in an in vitro model that mimics a fetoscopic membrane defect. Furthermore, the effect of PRP on membrane repair and cell proliferation in monolayer cell cultures and amnion-chorion tissue explants was determined. The fetal membranes were obtained from uncomplicated singleton pregnancies undergoing elective caesarean section, and PRP was obtained from healthy volunteers and produced by laboratory techniques, although we could not clearly elucidate a true definition of the PRP plugs used in the study. The results showed that PRP plugs persisted in amniotic fluid for a median of 7 weeks; they also demonstrated waterproof sealing of a fetoscopic membrane defect. In addition, PRP stimulated cell proliferation in a monolayer cell culture and resulted in a good matrix for cell proliferation and migration in amnion-chorion tissue explants. Sipurzynski-Budrass et al. published a case report in which a pregnant woman with ruptured membranes after genetic amniocentesis in the 16th gestational week was successfully treated with a platelet concentrate [31]. Complete closure of the rupture was realized 10 days after placement of the platelet plug.

Several endometrial tissue remodeling studies with the involvement of PDGFs have been performed [32]. In a study of Matsumoto et al. the effects of PDGF isoforms (PDGF-AA, PDGF-AB, and PDGF-BB) on the proliferation, motility, invasiveness, and gel contractility of cultured human endometrial stromal cells (ESC) were studied in well-established in-vitro models [33].

### 3.3. Cardiac Surgery

#### 3.3.1. Blood Component Platelet Plasmapheresis

Peri-operative platelet-rich plasmapheresis with cell-saver devices has been used for decades in cardiac surgery in order to control bleeding. The rationale for employing these blood sequestration techniques has been that coagulopathy after cardiopulmonary bypass (CPB) and platelet dysfunction are the most common causes of non-surgical bleeding. Repeatedly, peri-operative transfusion of both allogeneic red blood cells and platelet concentrates are used to overcome these life-threatening situations. Pre-CPB whole blood sequestration, in order to produce autologous blood components (PRP, PPP, and red blood cell concentrate) is thought to be one of the potential solutions to the problem of bleeding and blood product transfusions in cardiac surgery. A meta-analysis of clinical outcomes and costs has been well-described by Mahoney in 1998 [34]. The concept of removing platelets from a patient immediately before CPB, thereby potentially sparing platelets due to the avoidance of these platelets with foreign surface materials of the extracorporeal circuit, followed by post-CPB platelet re-infusion, seems to be a reasonable approach to the problem of post-CPB platelet dysfunction and bleeding.

Carless et al., in a Cochrane systematic review, suggested that PRP infusion therapy in general is effective in reducing allogeneic RBC transfusion in adult patients undergoing elective surgery [35]. However, there was considerable heterogeneity in treatment effects and the trials were of poor methodological quality. Their conclusion was therefore that the available studies provided inadequate data for firm conclusions with respect to the impact of PRP infusions on clinically important endpoints.

#### 3.3.2. Platelet Gel and Platelet-Poor Plasma Application

The production of these fresh blood components at point of care was the starting point to apply activated PRP (i.e., PG) as an element of a blood management program in cardiac surgery on wound tissues to affect bleeding and contribute to improved wound healing and prevention of infection in select patients.

In cardiac surgery, PG is only used after CPB and antagonization of the systemic heparin effects in order to have it stick to the tissues. PG is applied on the cut sternum when both sternal sites are re-approximated and tightened, using either a single syringe technique or a dual spray tip catheter. Thereafter, PG can be subcutaneously applied prior to skin closure. Eventually, the vein harvesting donor site of the leg can also be injected with PG.

#### 3.3.3. Clinical Studies

The efficacy and safety of PG and PPP use during sternal and saphenous vein harvest site closure was recently addressed in a retrospective analysis by Khalafi et al. [36]. Forty covariates were collected for each patient to determine the effect on infection and drainage of the sternal and leg wounds. Propensity scoring was used to adjust for baseline imbalance. The application of platelet-rich and platelet-poor plasma significantly reduced the rates of chest wound infection, chest drainage, and leg wound drainage. Additionally, no treatment-related adverse events were recorded. Englert et al. were the first to report on the use of autologous PG as a byproduct of platelet-rich plasma sequestration during cardiac surgery [37, 38]. The purpose of their prospective randomized pilot study was to examine the effects of PG on post-operative sternal and leg pain and tissue bruising, comparing the pre- and post-operative situation. A retrospective follow-up study by the same group revealed that the PG treatment group had significantly shorter intensive care unit and total hospital lengths of stay with less post-operative blood loss when compared with control patients. Furthermore, they reported less incisional wound infections in PG-treated patients. Another group also reported less wound infections in patients undergoing cardiac surgery in a large sample (n = 2259) retrospective study following PG applications [39]. The incidence of superficial infection was significantly lower in patients in whom PG was applied compared with non-PG-treated patients and a historical control group. A similar result occurred for deep sternal wound infections, and they concluded that the application of PG in patients undergoing cardiac surgery seems to present a level of protection against infection. An increased resistance to infection, and a significantly better hemostatic wound healing success rate was reported by Gunaydin et al. when PG was used in a prospective, randomized study in coronary artery bypass surgical patients [40]. Several studies also mention no effect of PG in cardiac surgical patients. A prospective, dou-
ble-blind study in 44 patients at risk for wound complications at thoracotomy, as well as at the site of saphenous vein harvesting, was conducted using a table top platelet separator [41]. The incidence of major and minor wound complications was not enunciated in either of the groups. Pain sense, blood loss, and intensive care were not significantly either. Intensive care unit stay and in-hospital mortality were also comparable for both groups. In similar studies, the incidence of post-operative wound healing disturbances was studied in PG and control patients. Buchwald and co-workers also determined PDGF AB and EGF levels originate from the PG [42]. In this study, wound healing was photographically documented after surgery, and the patients were monitored until the 50th post-operative day to obtain information on wound healing status. During the hospital stay, no statistically significant differences were recorded in the number of hematomas, post-operative leg swelling, or pain level, although large-area hematomas were less frequently observed in the PG group, whereas both PDGF AB and EGF concentrations were significantly higher when compared to whole blood levels. Vang et al. also reported no significant data on blood loss and wound bruising, although these parameters scored less in the PG-treated groups on the 2nd post-operative day [43].

The outcomes of multiple studies on the efficacy of PG treatment in cardiac surgery have been published. Proponents of PG application refer to improved wound healing, beneficial effects on pain, blood loss, and bruising. A reduction in the development of severe post-operative wound infections with the application of PG during incisional wound closure has also been reported. These observations indicate that this is a promising technique, with the result that the delivery of autologous platelet growth factors and vital neutrophilic leucocytes is now gaining more popularity. However, randomized controlled studies to support the use of PG in cardiac surgery are mandatory, but it is also difficult to execute such a study because these patients have a number of confounding factors which need to be compensated for, and therefore large patient groups are necessary order to achieve statistical significance [44].

3.4. General and Reconstructive Surgery

General surgery focuses on skills in a variety of medical areas, such as the abdomen and its contents, the vascular system, skin, breast, trauma, soft tissues, and reconstruction procedures. Despite large general surgical expertise with PRP-application, few well-documented studies are available in the literature. Therefore, we are limited in addressing the use of PRP gels in general surgery. We will describe PRP/PG applications in inguinal herniorrhaphies, to treat complications following endovascular surgery and diabetic wound care management. Furthermore, we would like to introduce the bioengineered adipose tissue (BEAT) graft as a recent development of PRP use. This graft is a combination of fat tissue, PRP, and a mixture of calcium chloride with autologous-prepared thrombin. The rationale to employ these grafts is to augment tissue regeneration in vascular-deprived areas (e.g., after radiotherapy following breast cancer), diabetic foot ulcers, and soft tissue deficits.

3.4.1. General Surgery: Inguinal Herniorrhaphy

Meshes are frequently used in inguinal herniorrhaphies and they have dramatically reduced the recurrence rate. However, chronic pain has become the main post-operative complication, probably due to the sutures or staples used to fix the mesh. A glue technique is an alternative for suturing to avoid these complications. De Hingh and co-workers were the first to publish a feasibility study in which they used autologous platelet-rich fibrin (PRF) Fig. (1), a prepared product similar to PRP without leucocytes, in order to study its ability to glue the mesh instead of using sutures and staples [45]. They assessed post-operative pain and impaired daily activities. The conclusion of the study was that it is technical feasible to use PRF to fix the mesh, and the visual analogue scale and disability pain scores were lower than they were pre-operatively for all patients with no chronic pain, sensory disorders, or discomfort at long-term follow-up. One patient underwent re-operation due to discomfort. If glue fixation becomes the standard to repair inguinal hernias with mesh, than autologous prepared materials should be further studied in randomized trials, with a focus on direct postoperative pain and costs.

![Fig. (1). Platelet-rich fibrin (white haze) is sprayed during inguinal hernia repair for mesh fixation. A total of 3 mL is applied.](image)

3.4.2. Vascular Surgery: Endovascular Repair

Endovascular repair (EVAR) is an alternative technique for open surgical procedures in various vascular fields, including abdominal aortic aneurysms (AAAs) [46]. With EVAR it is necessary to use a percutaneous catheterization technique via the common femoral arteries.

Due to this technique, wound-related complications have been reported, including hematomas, seromas, infections, pseudoaneurysm formation, and arterial bleeding. Saratzis et al. conducted a patient- and assessor-blinded controlled trial involving 100 patients undergoing EVAR of AAAs [47]. A subcutaneous injection of autologous, non-activated PRP was injected bilaterally into the subcutaneous tissues during wound closure, and a final volume of PRP was injected per-
cutaneously while closing the skin. Safety and efficacy of PRP injections were evaluated in terms of duration of post-operative hospital stay and wound-related complications.

The post-operative hospitalization was significantly lower in the PRP-treated patient groups. The overall surgical wound-related complications rate was also significantly lower in the PRP group. In addition to this, the complications observed in the control group were of greater extent and severity than in the PRP group.

3.4.3. Vascular-Reconstructive Surgery: Chronic Wound Management

Diabetic foot ulcers represent a major medical, social, and economic problem in many countries [48]. Approximately 15% of diabetics will develop at least one foot ulcer during their lifetime, and in 5%-8% of diabetics a major amputation is to be expected within 1 year [49]. The triad of vasculopathy, neuropathy, and immunopathy outlines the fundamental point on which the chronicity of diabetic wounds rests. Most of these wounds are typified by increased protease levels, particularly matrix metalloproteinase’s (MMPs) and neutrophil elastases. Furthermore, tumor necrosis factor–alpha has been proven to increase the production of MMPs, while hindering the production of tissue inhibitory metalloproteinase [50]. The goal of diabetic foot ulcer treatment is to obtain wound closure as promptly as possible. Accepted standards of care for diabetic foot ulcers include pressure relief in the wound area, appropriate wound management, infection and ischemia management, management of co-morbidities, and wound debridement as needed. Aggressive sharp wound debridement is believed to convert chronic wounds to acute wounds and allows growth factors to function more effectively. This allows the wound to progress through the normal phases of wound healing (inflammatory, proliferative, and remodeling). These phases involve complex paracrine-mediated growth factors which influence mitogenic and cellular differentiation activity. In addition, Cooper et al. illustrated that a number of growth factors were strikingly reduced in wound fluids from chronic wounds as compared with acute wounds [51]. Moreover, FGF and TGF-β concentrations are significantly down-regulated in chronic wounds when compared with acute wounds.

Emerging cellular therapies, such as PRP, can have an adjunctive role in the standardized, patient treatment plan. The use of platelet growth factors for the topical treatment of chronic wounds is based on the fact that PRP growth factors aid the three phases of wound healing in the newly created “acute” wound Figs. (2 and 3).

Platelet releasates, PRP, and PGs, including multiple growth factors, have been used to treat chronic wounds since 1985 [52]. Since this period, a variety of studies have been published on the application of PRP gels in wound care management.

In 2001, Margolis published a retrospective study analyzing the treatment results of 26,599 patients with diabetic neuropathic foot ulcers who had been treated with an autologous platelet releasate [53]. One of the conclusions was that platelet releasate applications are more effective than standard therapy, and the effect is more pronounced in more severe wounds. Crovetti et al. performed a technique based on once-weekly application of PG [54]. They enrolled patients with single or multiple cutaneous ulcers with a different ethio-pathogenesis. In each case, granulation tissue formation increased following the first PG applications, while complete re-epithelization was obtained later. An interesting observation was that pain was reduced in every patient treated with PG.

A prospective, randomized, controlled, blinded, multicenter clinical study was conducted by Driver and associates to evaluate the efficacy and safety of autologous PRP gel for the treatment of non-healing diabetic foot ulcers [55]. The primary study objective was the proportion of patients with a healed wound. Seventy-two patients were enrolled the study. The proportion of completely healed wounds was significantly higher in the PRP gel group when compared to the control group (81.3% and 42.1% in the PRP gel and control group).
treatment groups, respectively). Furthermore, no treatment-related adverse effects were noted, indicating safe PRP gel preparation and application. A variety of reviews have addressed the efficacy of autologous platelet growth factor applications in chronic and diabetic wounds [56, 57]. In general, all authors concluded that diabetic foot ulcers are a major health care problem, and that complications of foot ulcers are a leading cause of hospitalization and amputation in diabetic patients. In most of the reviewed papers, it was concluded that there is a rising body of evidence suggesting that wound healing in chronic diabetic foot ulcers is growth factor-dependent, and that the topical therapeutic delivery of these growth factors to wounds has the potential capability to speed up wound healing in combination with conventional wound care. In a systematic review by Villela and Santos, 18 studies were selected, from which 7 (39%) were randomized clinical trials, and 5 of which studied ulcers of diabetic etiology [58]. The results of a meta-analysis showed that PRP favors the healing process (95% CI: 2.94-20.31), demonstrating that there is scientific evidence regarding favorable outcomes of the use of PRP for the treatment of diabetic ulcers.

3.4.4. Reconstructive and Plastic Surgery: Adipose Tissue Grafting with PRP

Despite modern advances in wound care treatment protocols, reconstructive surgical procedures, and cosmetic surgery, there is still a significant requirement for new methods to enhance healing processes, or to restore soft tissue contour defects. Clinically implemented tissue engineering protocols have emerged as a promising alternative to current clinical treatment plans. Many of these new therapies have included the use of human growth factors, with their known biological activities. One of the promising, yet clinically challenging areas of recent therapeutic development involves the injection of adipose tissue derived from a modified lipoculture technique [59]. However, successful fat graft techniques are frequently limited by the sometimes low, and often unpredictable, survival rates. As a consequence, clinicians tend to initially overcorrect fat grafts, and/or perform multiple operations to meet the recipient site volume and contour requirements. In an attempt to increase fat graft survivability, Cervelli and co-workers were the first to report on the enhancement of fat grafting with PRP during in-vivo tissue engineering applications [60, 61]. They applied the PRP-fat graft in plastic, reconstructive, and maxillofacial surgical procedures, and as a treatment option in patients with chronic lower extremity ulcers. The authors observed that in 16 of 20 patients with chronic ulcers, re-epithelialization occurred during an average period of 9.7 weeks when PRP was mixed with fat tissue with an improved functional fat graft. According to the authors, this underlying principle for applying PRP to fat tissue grafts is the delivery of autologous platelet growth factors to mimic and accelerate physiologic wound healing and reparative tissue processes. The platelet alpha granules release their growth factors into the extracellular milieu of the fatty tissue. In this environment, the growth factors bind to specific platelet growth factor receptors. Released platelet growth factors interact and bind with the platelet TKR on the cell membranes of fat cells (ligand-receptor interaction). The release of VEGF, TGF-ß, FGF, and IGF has been shown to stimulate human adipose-derived stem cells and human dermal fibroblast proliferation and differentiation [62]. Therefore, PRP might be suitable for clinical cell-based, soft-tissue engineering protocols in order to promote wound healing, when the appropriate ratio between activated PRP and fat tissue was used. Furthermore, Blanton et al. suggested that an important component of wound healing was induced by the combination of adipose stem cells and PRP, promoting enhanced vascularization of wound repair in an experimental wound model [63]. Increased VEGF levels were found when adipose stem cells were combined with PRP, contributing to a higher content of arterioles formed in healed wounds, resulting in neovascularization, an important process in the healing of wounds.

Despite the positive effects of PRP on fat survivability, Por and associates reported an animal study in which they did not observe statistically significant changes with regard to weight, volume, and histological parameters when PRP was mixed with fat tissue when compared to a mixture of fat tissue with a saline solution [64]. Closer analysis of the methodology revealed that the final platelet concentration in the fat graft was lower than the circulating whole blood platelet concentration. The therapeutic contribution of platelet growth factors might therefore be questionable.

3.4.5. Bio-Engineered Adipose Tissue (BEAT) Graft

Based on the available results in the literature, and our long-term experience on PRP applications, we decided to develop a modified fat-PRP grafting, the bio-engineered adipose tissue (BEAT) graft [65]. Tumescent fluid infiltration of the donor sites is carried out with xylocaine and epinephrine. Microcannulas are used to harvest the fat tissue. During fat tissue harvesting, low-negative pressure is applied by limiting the plunger movement of a 10 mL syringe to one-half when the cannula is inserted. The syringe will be filled with fat and tumescent fluid, which will be placed in a holder for approximately 20 minutes to separate the fatty tissue by gravity in fluid and oil, which will be removed, leaving fat tissue behind.

Prior to the induction of anesthesia, a volume of anticoagulated whole blood is drawn from the antecubital vein for blood component sequestration. This process is performed manually in order to collect PRP with a high platelet count, approximately 5-6 times the baseline value.

Both, PRP and PPP are collected separately. When the fat harvesting is performed, these blood products are aseptically transferred to the sterile field. At the sterile OR table, PPP is mixed with 10% calcium chloride and placed in a glass Petri dish in order to create a viscous PPP cloth. After 25-40 min, a clot was formed inside the glass dish and compressed manually. The cellular clot debris was left behind on the dish and the liquid fraction containing, the thrombin is then aspirated with a syringe. The separated fat tissue will be transferred in a 60 mL syringe, to which PRP will be added in a ratio of 1:0.5. The mixture is gently, but frequently, agitated for good mixing Fig. (4). Prior to graft injection, 3 mL aliquots are drawn from the 60 mL syringe and mixed with 0.15 mL of autologous prepared thrombin in order to induce platelet degranulation shortly after the graft has been injected, as advocated by Kakudo. The activated BEAT graft is
injected in a fan-shaped manner Fig. (5). Rather than focusing on the mature adipocytes, it is our belief that the BEAT graft bioactivation aims to improve fat graft survival rates by stimulating the early ischemic phase with new mature adipocytes from the differentiation of cells under the influence of platelet growth factors by mechanisms, such as neoangiogenesis, neovasculogenesis, and adipogenesis.

![Fig. (4).](image) PRP is transferred in a syringe containing adipose tissue and then both components are gently but frequently mixed in order to achieve a homogenous graft mass.

![Fig. (5).](image) After activation with thrombin a small portion of BEAT graft is injected with a large bore cannula during a reconstructive surgical procedure of a skin grafting procedure of the lower extremity following an accident.

4. CONCLUSION

From the literature it is clear that autologous PRP and PG have a wide and safe application within a variety of operative procedures as a tissue regenerative agent. Its application has extended to patients that are prone to higher surgical complications, and to patients suffering from chronic, often diabetic, wounds. The ability of PRP to deliver multiple growth factors with synergistic effects to wound sites is an attractive proposition. Activated PRP will result in a platelet plug, which acts as a barrier to microorganism invasion of wounds, achieved with the help of highly concentrated leukocytes present in the PG if prepared from the buffy coat volume. Platelet growth factors and other platelet cytokines promote mitogenesis of a variety of cells, such as macrophages, other circulating growth factors, and mesenchymal stem cells at wound sites. Ultimately, these mechanisms might boost primary wound healing during surgical wound closure, especially in patients who are at risk for wound healing disturbances, or they might contribute to wound healing in patients with chronic lesions.

However, the overall efficacy of PPR gels in treating wounds is likely to be a function of many variables, such as the platelet concentration of the PRP, PRP preparation device used, platelet activation, application volume to a wound, and the overall health status of the patient.

As a three-dimensional volumetric soft connective tissue replacement, the combination of thrombin-stimulated PRP with fat tissue provides a unique active tissue matrix for cell migration, proliferation, differentiation, and finally, tissue granulation formation.

Until now, few well-designed studies are available in a variety of procedures related to gynecology, cardiac and general surgery. Therefore, more randomized, controlled, blinded, studies on autologous growth factor applications are needed to demonstrate its effects in supportive healing during primary and delayed wound healing. Furthermore, cost-effectiveness studies of PRP therapy benefits are lacking in all medical disciplines.

5. REFERENCES

The page contains a list of references related to PRP in Gynecology, Cardiac and General, Reconstructive Surgery, Current Pharmaceutical Biotechnology, 2012, Vol. 13, No. 1. The references are cited in the text without visible pagination or sectioning, indicating a continuous flow of information. The text is a collection of scientific articles, studies, and reviews on the use of platelet-rich plasma (PRP) in various medical applications, including gynecology, cardiac surgery, and general surgery. The references cover a range of topics from basic research on platelet function and growth factors to clinical trials and case studies evaluating the efficacy of PRP in wound healing, tissue regeneration, and other medical conditions. The documents are from reputable sources such as journals, books, and conference proceedings, indicating a well-researched and scientifically validated approach to the use of PRP in medical practice.


