Use of platelet growth factors in treating wounds and soft-tissue injuries

P. Rožman and Z. Bolta

Summary

Tissue repair begins with clot formation and platelet degranulation, which release the growth factors (GFs) necessary for wound repair. Platelet-derived GFs 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 of the topical therapy of wounds and soft-tissue injuries with platelet gel (PG) and PG-derived GFs as well as the success of the clinical studies performed so far. Some other interesting topical applications of PG are also described. Platelet-derivatives represent a promising therapeutic modality, offering opportunities for treatment of wounds, ulcers, soft-tissue injuries, and various other applications in regenerative medicine.

Introduction

In addition to their well-known function in hemostasis, platelets also release substances that promote tissue repair, angiogenesis, and inflammation (1). At the site of the injury, platelets release an arsenal of potent inflammatory and mitogenic substances that are involved in all aspects of the wound-healing process (2). Based on this, platelet releasate in the form of activated PG has been extensively used for the topical therapy of various clinical conditions, including wounds and soft-tissue injuries (3). The purpose of this article is to review the current knowledge about the tissue regenerative abilities of PG and to review the clinical results of the topical use of PG therapy for treating wounds, ulcers, and soft-tissue injuries.

Platelet structure and physiology

Platelets are non-nuclear cellular fragments derived from megakaryocytes in the bone marrow through controlled cellular fragmentation. They are specialized secretory cells that release the contents of their intracellular granules in response to activation. Platelets contain a complete array of pre-synthesized protein molecules, among which the high presence of cytoskeletal proteins, signaling proteins, membrane proteins, protein-processing proteins, and cytoskeleton regulatory proteins is noted (4). When platelets are activated, they exocytose the granules; this process is mediated by molecular mechanisms homologous to other secretory cells, uniquely coupled to cell activation by intracellular signaling events (5).

Platelet secretory granules contain GFs, coagulation

Keywords

allogeneic platelets, platelet gel, platelet-rich plasma, regenerative medicine
proteins, adhesion molecules, cell-activating molecules, cytokines, integrins, inflammatory molecules, and some other molecules, which are synthesized in megakaryocytes and packaged into the granules through vesicle-trafficking processes. Three major storage compartments in platelets are alpha granules, dense granules, and lyzosomes (6). The majority of the substances are contained in alpha granules (see Table 1).

For their numerous functions, platelets have developed a set of platelet receptors that are the contact between platelets and their surroundings. They determine the reactivity of platelets with a wide range of agonists and adhesive proteins. Some of these receptors are expressed only on activated platelets. Certain biological mechanisms present in the platelets are shared with other cells, and therefore they contain some common cytoplasmic enzymes, signal transduction molecules, and cytoskeletal components (5).

The platelet lifespan is approximately 7 to 9 days, which they spend circulating in the blood in their resting form. When adhered to exposed endothelium or activated by agonists, they change their shape and secrete the contents of the granules (including ADP, fibrinogen, and serotonin), which is followed by platelet aggregation. Initiation of the signaling event within the platelet leads to the reorganization of the platelet cytoskeleton, which is visible as an extremely rapid shape change (see Fig. 1).

Aggregation of platelets is mediated by molecules of fibrinogen or vWF, which connect platelets by bridging the complexes of glycoprotein IIb/IIIa (integrin)a,b) on adjacent platelets, forming a platelet aggregate. Each platelet contains about 50,000 to 80,000 glycoprotein IIb/IIIa (GP IIb/IIIa) molecules on its surface. In order to bind fibrinogen and vWF, GP IIb/IIIa must initially be converted from a low affinity/avidity state to a high affinity/avidity state by a process described as "inside-out signaling" that is initiated during and after platelet activation (5) (see Fig. 2).

Platelets can be activated by several mechanisms. Platelets are activated either by adhesion to the molecules that are exposed on an injured endothelium, such as vWF, collagen, fibronectin, and laminin, or by physiologic agonists such as thrombin, ADP, collagen, thromboxane A2, epinephrine, and platelet-activating factors,

Figure 1: Platelets in their resting form (A) and at the beginning of activation (B).

Figure 2: Schematic representation of intracellular events during platelet activation. A: "inside-out" signaling, B: "outside-in" signaling.

Figure 3: Platelet gel for topical application. A: Platelet gel on a hyaluronic acid carrier prepared for topical application, B: Patient with diabetes and a traumatic vasculopathic ulcer, treated for 6 months with no effect (left). The same patient after 15 applications of autologous platelet gel, once per week (right).
which interact with specific extracellular membrane receptors, and which via G-protein–coupled or other mechanisms induce intracellular signaling. This process is described as “outside-in” signaling (see Fig. 2). Some of the substances released by these cells – in particular adenosine diphosphate, serotonin, thromboxane, and others – can, in an autocrine and paracrine fashion, further enhance platelet activation and aggregation. Under these conditions there is also activation of the coagulation cascade; therefore thrombin is formed, which also markedly stimulates platelet activation in a positive feedback loop fashion (5).

Platelet growth factors

There are at least 60 different biologically active substances in the platelet that are involved in tissue-repair mechanisms such as chemotaxis, cell proliferation and differentiation, angiogenesis, intracellular matrix deposition, immune modulation, antimicrobial activity, and remodeling (3). Among these, GFs are the most important (see Table 2). They are contained in the alpha-granules and other granular bodies and released from the cell following activation (7–9). They exhibit extensive tissue-forming abilities, such as the initiation and modulation of wound healing in both soft and hard tissues (10).

Some of the numerous GFs released by the platelets after activation identified so far are PDGF (platelet-derived growth factor), TGF-α & β (transforming growth factor alpha & beta), EGF (epidermal growth factor), FGF (fibroblast growth factor), KGF (keratinocyte growth factor), IGF (insulin growth factor), PDEGF (platelet-derived epidermal growth factor), IL-8 (interleukin-8), TNF-α (tumor necrosis factor alpha), CTGR

---

Table 1. The contents of the platelet alpha-granules (modified from Anitua et al. (10)).

<table>
<thead>
<tr>
<th>Category</th>
<th>Term</th>
<th>Biological activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive proteins</td>
<td>VWF + propeptide, Fg, Fn,Vn,TSP-1, laminin-8</td>
<td>Cell contact interactions, clotting, extracellular matrix composition</td>
</tr>
<tr>
<td>Clotting factors and associated proteins</td>
<td>Factor V/Va, factor XI, multimerin, gas6, protein S, high-molecular weight chinoinogen, antithrombin, tissue factor pathway inhibitor (TFPI)</td>
<td>Thrombin production and its regulation, angiogenesis</td>
</tr>
<tr>
<td>Fibrinolytic factors and associated proteins</td>
<td>Plasminogen, PAI-I, u-PA, osteonectin, alpha2-antiplasmin, histidine-rich glycoprotein, TAFI, α2-macroglobulin</td>
<td>Plasmin production and vascular remodeling</td>
</tr>
<tr>
<td>Proteases and anti-proteases</td>
<td>Tissue inhibitor of metallopeptase-4 (TIMP-4), metallopro tease-4, platelet inhibitor of FXI, protease nexin-2, C1 inhibitor, α1-antitripsin</td>
<td>Angiogenesis, vascular modeling, regulation of coagulation, regulation of cellular behavior</td>
</tr>
<tr>
<td>Growth factors, cytokines, and chemokines</td>
<td>PDGF, TGFβ1 and 2, EGF, IGF-1, VEGF (A and C), bFGF and FGF-2, hepatocyte GF, RANTES, IL-8, MIP-1α, growth regulated oncogene-α, ENA-78, MCP-3, angiopoietin-1, II-β1, IGF BP-3, neutrophil chemotactic protein</td>
<td>Chemotaxis, cell proliferation and differentiation, angiogenesis</td>
</tr>
<tr>
<td>Basic proteins and others</td>
<td>PF4, β-thromboglobulin, platelet basic protein, connective tissue activating peptide III, neutrophil activating peptide-2, endostatins</td>
<td>Regulation of angiogenesis, vascular modeling, cellular interactions</td>
</tr>
<tr>
<td>Anti-microbial proteins</td>
<td>Thrombocidins</td>
<td>Bactericidal and fungicidal properties</td>
</tr>
<tr>
<td>Others</td>
<td>Chondroitin 4-sulfate, albumin, immunoglobulins</td>
<td>Diverse</td>
</tr>
<tr>
<td>Membrane glycoproteins</td>
<td>αIIbβ3, αβ3, GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonists, CD40L, tissue factor, P-selectin</td>
<td>Platelet aggregation and adhesion, endocytosis of proteins, inflammation, thrombin generation, platelet-leukocyte interactions</td>
</tr>
</tbody>
</table>
Table 2: Platelet growth factors (modified from http://www.copewithcytokines.de/cope.cgi).

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Wound-healing and tissue-forming ability</th>
</tr>
</thead>
</table>
| **EGF (epidermal growth factor)**, β-Urogastron | - Stimulates the proliferation of epidermal and epithelial cells, fibroblasts, and embryonic cells  
- Chemotactant for fibroblasts and epithelial cells  
- Stimulates re-epithelialization, augments angiogenesis  
- Influences the synthesis and turn-over of extracellular matrix |
| **PDGF (platelet-derived growth factor)** | - A and B isoforms are potent mitogens for fibroblasts, arterial smooth muscle cells, chondrocytes, and epithelial and endothelial cells  
- Potent chemotactant for hematopoietic and mesenchymal cells, fibroblasts, and muscle cells, stimulates chemotaxis toward a gradient of PDGF  
- Activates TGF-β, stimulates neutrophils and macrophages, mitogenesis of fibroblasts and smooth muscle cells, collagen synthesis, collagenase activity, and angiogenesis |
| **TGF-α (transforming growth factor alpha)** | - Resembles EGF, binds to the same receptor  
- Stimulates mesenchymal, epithelial, and endothelial cell growth, endothelial chemotaxis, controls the epidermal development  
- Stimulates the proliferation of endothelial cells, more potent than EGF  
- Promotes the generation of osteoblasts, influencing them to lay down bone matrix during osteogenesis  
- Affects bone formation and remodeling by inhibition of the synthesis of collagen and release of calcium |
| **TGF-β1 (transforming growth factor beta)** | - Stimulates fibroblast chemotaxis and proliferation and stimulates collagen synthesis  
- Decreases dermal scarring  
- Growth inhibitor for epithelial and endothelial cells, fibroblasts, neuronal cells, hematopoietic cell types, and keratinocytes  
- Antagonizes the biological activities of EGF, PDGF, aFGF and bFG |
| **KGF or FGF-7 (keratinocyte growth factor)** | - Most potent GF for skin keratinocytes, playing a role in tissue repair following skin injuries  
- Promotes wound healing via proliferation, differentiation, angiogenesis, and cell migration  
- Mitogen for many epithelial cells but not for fibroblasts and endothelial cells |
| **aFGF or FGF-1 (fibroblast growth factor; acidic)** | - A potent GF for skin keratinocytes, dermal fibroblasts, and vascular endothelial cells |
| **bFGF or FGF-2 (fibroblast growth factor; basic)** | - Promotes the proliferation, differentiation, angiogenesis, and cell migration  
- Mitogen for skin-derived keratinocytes, dermal fibroblasts, and vascular endothelial cells |
| **VEGF/ VEP (vascular endothelial growth factor)** | - Stimulates the proliferation of macrovascular endothelial cells.  
- A strong angiogenic protein, induces neovascularisation  
- Induces the synthesis of metalloproteinase, which degrades interstitial collagen type 1, 2, and 3 |
| **CTGF (connective tissue growth factor)** | - Induces the proliferation, migration, and tube formation of vascular endothelial cells and angiogenesis  
- A potent stimulator for the proliferation and differentiation of osteoblasts, stimulates the matrix mineralization |
| **GM-CSF or CSFα (granulocyte/macrophage colony-stimulating factor)** | - Stimulates proliferation and differentiation of osteoblasts  
- Synergizes with Epo in the proliferation of BM progenitor cells  
- Strong chemotactant for neutrophils |
| **IGF (insulin-like growth factor)** | - Growth factor for normal fibroblasts, mitogenic in vitro for a number of mesodermal cell types  
- Promotes the synthesis of collagenase and prostaglandin E2 in fibroblasts  
- Stimulates collagen and matrix synthesis by bone cells, regulating the metabolism of joint cartilage |
| **TNFα (tumor necrosis factor alpha)** | - Growth factor for fibroblasts  
- Promotes angiogenesis |
| **IL-1β (interleukin 1 β)** | - Inhibits the growth of endothelial cells and hepatocytes  
- Activates osteoclasts, suppresses the formation of new bone. In low concentrations, however, promotes new bone growth  
- Enhances inflammatory reactions and collagenase activity |
| **IL-8 (interleukin 8)** | - Supports angiogenesis  
- Mitogenic for epidermal cells |
(connective tissue growth factor), and GM-CSF (granulocyte macrophage colony stimulating factor), as reviewed by several authors (11–14).

Platelet-rich plasma (PRP) has been traditionally used as a source of platelet GFs. The platelets in the PRP are activated by the addition of thrombin and excess calcium, which promotes both platelet activation and a coagulation cascade in a positive loop fashion and finally results in the formation of a thrombus-like gelatinous substance (platelet gel), in which the activated platelets are trapped on the fibrin network, where they continue to excrete their contents, whereas the bioactive substances slowly diffuse into the surroundings. The activation can be alternatively performed by using other substances, such as the serine protease enzyme batroxobin from Bothrops atrox snake venom (15, 16), which have a thrombin-like effect on the platelet activation. The final result, platelet gel, can be shaped according to need, put on different vehicles such as medical gauze or even advanced types of biocompatible carriers and scaffolds, such as fibrin or hyaluronic acid, and used topically (see Fig. 3).

Autologous PRP was developed in the early 1970s as a byproduct of multicomponent apheresis. Although various platelet GFs had been discovered by then, the clinical use of activated PRP (platelet gel), was rarely reported in the 1980s (17–24). It became more popular after presentation to the maxillofacial community by Whitman et al. in their 1997 article (25). The authors postulated that, through the activation of platelets within the gel and the resultant release of GFs, enhanced wound healing should be expected. PRP enjoyed a great increase in popularity after 1998 and it is now widely accepted that the correct strategy to promote the wound-healing cascade is to prepare an autologous PRP/PG that contains GFs, and to administer it directly (i.e., topically) to the sites of surgical interventions, wounds, or injuries (3, 26). The provision of PG in chronic wound and ulcer treatment nowadays is mostly based on the preparation of autologous PRP with various apheresis machines, which collect PRP with continuous flow centrifugation. Several companies recommend their devices that provide PRP, which is subsequently activated with autologous thrombin to degranulate the platelets. However, the equipment and the protocols show different harvesting capacities for the collection of concentrated platelets, which is potentiated by inter-individual variability of platelet counts in the PRP products that is not yet fully understood (26, 27).

**Clinical use of platelet-gel derived growth factors**

The use of PG is most widespread in dentistry and oral-maxillofacial surgery, where it is used for implantation and bone regeneration (28, 29). Similarly, the use of PG is largely expanding in bone surgery (30–32). In this review, however, we shall focus on the use of PG in wound healing and soft-tissue injuries.

**Wound healing**

The wound-healing process is a complex mechanism that includes coagulation, inflammation, ground substance and matrix synthesis, angiogenesis, fibroplasia, epithelialization, wound contraction, and remodeling. Wound healing has four distinct but overlapping phases: a) hemostasis, b) inflammation, c) proliferation, and d) remodeling (2). The underlying physiological processes begin immediately upon tissue injury.

a) Hemostasis. At the moment of wounding with a vascular injury, tissue factor and intracellular calcium are released, activating factor VII and initiating the extrinsic coagulation cascade. Simultaneously, the coagulation cascade, the arachidonic acid pathways, and the creation of GFs and cytokines work together to initiate and maintain the inflammatory phase and the sequence of cells involved in the process. Concomitant reflex vasoconstriction occurs to aid in hemostasis. Hemostasis is ultimately secured by the end product of the coagulation cascade, the fibrin plug. The fibrin fibers in the plug become a provisional wound matrix and are the lattice on which platelets aggregate (2).

b) Inflammation. Activated platelets are the most abundant cells in the wound in the early post-injury period. They are a source of GFs that act on inflammatory cells, fibroblasts, and endothelial cells to direct the processes involved in wound healing, including chemotaxis of neutrophils, monocytes, and fibroblasts into the wound (2, 33, 34). During the first two days of wound healing, an inflammatory process is initiated by the migration of neutrophils, which are responsible for debris scavenging, complement-mediated opsonization of bacteria, and bacteria destruction via myeloperoxidase (MPO)-driven oxidative burst mechanisms (i.e., superoxide and hydrogen peroxide formation). The neutrophils kill bacteria and decontaminate the wound from foreign debris. The next cells attracted in the wound are the lymphocytes and the macrophages (monocytes). The macrophages secrete enzymes including collagenases, which debride the wound, as well as additional GFs, including transforming growth factors-alpha and -beta (TGF-α, TGF-β), which stimulate keratinocytes and platelet-derived growth factor (PDGF), interleukin-1 (IL-1), fibroblast growth factor (FGF), and tumor necrosis factor (TNF), which stimulate fibroblasts to produce collagen and promote angiogenesis. This step marks the transition of inflammation into the process of tissue reconstruction; that is, the proliferative phase.

c) Proliferation. The proliferative phase of wound healing begins approximately 2 to 3 days after wounding and is signaled by the arrival of fibroblasts into the
wound. Fibroblasts migrate from the wound margins using the fibrin-based provisional matrix established during the inflammatory phase. Within the first week after wounding, fibroblasts are driven by a macrophage-derived buff, TGF-β, and PDGF to proliferate and synthesize glycosaminoglycans and proteoglycans, the building blocks of the new extracellular matrix of granulation tissue, and collagen (2, 33). Angiogenesis and fibroplasia start after day three, followed by the beginning of collagen synthesis on days three to five (35).

d) Remodeling. Because macrophage numbers have begun to diminish in the acute wound by this time, fibroblasts start to produce bFGF, TGF-β, and PDGF. They also begin producing KGF and IGF-1. Fibroblasts become the dominant cell type, reaching their peak numbers at 7 to 14 days. After the secretion of collagen molecules, fibroblasts then assemble them extracellularly into collagen fibers. These fibers are then cross-linked and organized into bundles. Collagen is the major component of acute wound connective tissue, with net production continuing for the next 6 weeks. The increasing content of wound collagen correlates with increasing tensile strength (33, 34), which is the most important wound-healing parameter of surgical wounds, followed by epithelialization, and ultimately wound remodeling.

During the wound-healing processes, platelet GFs serve as messengers to regulate a well-orchestrated and complex series of events involving cell-cell and cell-matrix interactions, and it is generally accepted that they play a key role during the various phases of the wound-healing process (21, 33, 36). Therefore, the local application of PG to stimulate wound repair was an appealing proposition. Compared to recombinantly derived single GFs, PG has the advantage that it offers numerous synergistically working GFs promoting the proliferation of mesenchymal and other stem cells at the wound site (37, 38).

Based on this, platelet-derived GFs in the form of activated PG were tested in several settings. Autologous platelet-derived wound-healing factors were proposed to regulate wound healing of chronic cutaneous ulcers by promoting the formation of granulation tissue in the early healing phase. This conclusion was based on a randomized, prospective, double-blind, placebo-controlled study in 18 patients, who showed improved healing compared to the usual treatments (39).

Foot ulcers as a common complication of diabetes are often multifactorial, but arise in the setting of peripheral neuropathy and/or vascular complication. Platelet releasate has been used on thousands of such cases over a ten-year period in the US, and analysis of results allowed Margollis et al. to conclude that use of platelet releasate was of proven efficacy. Treatment of diabetic wounds was even more effective in patients with deeper wounds than superficial lesions (40). Tarroni et al. (41) successfully avoided leg amputation by using a PG for a chronic ulcer in a 62-year-old diabetic man whose ulcer recovered after 8 platelet applications. The addition of PG to treatment significantly improved healing efficacy and amputation reduction in a study of 54 diabetic patients with 86 wounds (42).

In another study by Mazzucco et al., patients with chronic non-healing wounds showed substantial improvement when treated with PG dressings (43).

There is an increasing cause for optimism in the treatment of diabetic and other chronic wounds (44). Therefore, topical PG applications have been performed to treat chronic non-healing wounds and to support healing after incisional wounds in diabetic patients that were at risk of impaired wound healing. In chronic wound care management, PG has been successfully used for patients that suffer from chronic non-healing and often painful (diabetic) ulcers (43, 45). Pain reduction following PG application was observed in a study by Crovetti et al., an effect that is still not understood (46). Improved wound healing has been encountered when PG was applied during wound closure after total knee arthroplasty (47).

In a recent prospective study on eight healthy individuals, Horn et al. (2007) have shown that full-thickness dermal wounds treated topically with autologous PG healed faster compared to those treated with antibiotic ointment and a semiocclusive dressing. After 42 days, the PG-treated wounds had an 81.1% closure compared to the other treatment (with 57.2% closure). Histologically, the PG-treated sites had similar cellularity, cellular replication, granulation tissue, vascularity, and epithelialization compared with controls (48).

### Healing of soft tissues

In addition to assisting wound healing, autologous platelets are especially useful for the soft-tissue and bony reconstruction encountered in facial plastic and reconstructive surgery (49). Their use results in a decrease in operative time, the necessity for drains and pressure dressings, and incidences of complications. Reduced infections and length of hospital stay in plastic surgery was the conclusion of Valbonesi, who used autologous PRP in 14 patients with skin and soft-tissue losses caused by recent trauma or chronic pathology (50). This points to useful bactericidal properties as well as cell-proliferation promoting properties of PG. Anti-inflammatory properties with reduced edema and ecchymosis were associated with the use of autologous PG in eight women after deep-plane rhytidectomy (face lifting) (51). PG was also shown to be effective in stopping capillary bleeding in the surgical flaps of a series of 20 patients undergoing various cosmetic surgery (face lifts, breast size changes, or neck lifts) (52).

Soft-tissue trauma (tendon and ligament ruptures), joint capsular injuries, and tendonitis occur frequently in sports medicine. Current applications involve the use of PG in various conditions, such as tendonitis, tendon,
and muscle repair (53). In an Achilles tendon injury rat model, PG-treated animal tendons gained an approximately 30% increase in tensile strength and stiffness after the first week when compared to control animals (54). In humans, anterior cruciate ligament surgery is routinely performed to reconstruct the ligament with an autologous graft. Injecting calcified autologous PRP may facilitate anterior cruciate ligament reconstruction and reattachment of knee articular cartilage (55). Sanchez et al. reported enhanced healing with fewer complications and improved fixation of the graft within the bone tunnels in a retrospective clinical trial involving 100 patients (53). Recently, Mishra and Pavelko used PG in the treatment of chronic elbow tendonitis. Treated patients had less pain and better function when compared to conservative standardized physical therapy protocols and a variety of other non-operative treatments (56).

The reported advanced healing following PG applications in these soft tissues might be explained by higher concentrations of VEGF at the tissue-injury wound site, released from PG, which stimulated angiogenesis. Subsequently, the blood supply to the injured tendon, which is mandatory for the tendon-repair process, improved (57). Furthermore, the high concentrations of leukocytes in the PG might reduce tissue inflammation with increased macrophage migration. Future research should provide evidence of whether transcutaneous PG injections, rather than peri-articular corticosteroids injections or surgery, could be indicated in the treatment of tendonitis and peri-arthritis (Everts 2007, in press).

The future of platelet-gel–based therapies

PG-based therapies are especially useful for treating chronic wounds and ulcers; however, they remain an underestimated means of therapy in both dermatology and surgery. Evidence-based medicine introduces new demands for the best treatment modalities. Based on the amount and quality of clinical study results, therapies with PG are expected to expand both in the extent and in the varieties of indications, diagnoses, and application techniques. There are several questions left to be solved in the future of this exciting therapy. First, we shall have to explore which of the plateletlet products is best for PG technology. The choices include single random platelet units, pooled platelets, platelet releasates, and apheresis platelets (32). Also, the content of leukocytes might be of crucial importance. Here, two paradigms confront each other: the use of platelet leukocyte gel containing the complete leukocyte population (31), or the use of leukocyte-depleted products, which are also irradiated in order to minimize any DNA- or RNA-related biological action in the final therapeutic product (32). It seems that, for the purpose of scientific evaluation, the leukocyte-depleted products offer a more standardized product that is easier to evaluate in prospective clinical studies because the leukocyte population can add immense multifactorial variability to the clinical setting, including allogeneic immune reaction, cytotoxicity, inflammatory cytokine induction, generation of graft versus host components, and so on. The complete absence of such biological effects is of key importance in the evaluations. The next crucial variable is the technique of platelet activation, which results in the amount of secreted GFs at the site of injury, as well as in the efficiency of the granule release during the activation. In the absence of good quantitative studies of the numerous GFs, the best activators still have to be determined, mainly ranging between autologous thrombin, heterologous or recombinant thrombin, and other substances, such as hatroxobin (15, 16). The next question relates to the standardization of PG as a therapeutic product. Here, many technical and safety-related questions must be solved (26). In the vast majority of clinical applications, autologous platelets were used for PG formation. Recently, we have shown that allogeneic PRP products can effectively be used for the treatment of soft-tissue and bone defects in diabetic patients with PG. We used allogeneic single donor platelet units from the blood bank that were ABO and RhD matched, leukocyte depleted, irradiated, and activated by human thrombin and we stated that they had several advantages compared to autologous platelets because they are available in larger quantities, are safe and affordable, and are highly standardized in terms of platelet, residual leukocyte, and red blood cell content, and the techniques of separation and processing (32). Last but not least, costs should also be taken into consideration, which positively illustrates the additional advantages of mass-produced allogeneic platelet units in comparison to the individual autologous products.

Conclusion

It is presumed that numerous polypeptide GFs serve as potent inducers of normal tissue repair. These GFs are released by activated platelets, macrophages, fibroblasts, and endothelial cells. Tissue repair begins with clot formation and platelet degranulation, which release the GFs necessary for wound repair. In addition to their tissue-forming and proliferative effects, GFs exhibit chemotactic effects that cause the migration of neutrophils and macrophages, adding an antimicrobial component to the wound site. Recent in-vitro studies have established that platelets and their derivatives accelerate proliferation of an array of cells involved in soft and bony tissue regeneration. These effects have been evaluated, both in vitro and in vivo, in animals and humans.
The outcomes of in-vivo studies are considerably less homogeneous than the outcomes of in-vitro investigations. The resultant discrepancies reflect not only differences of technical protocols, but also the greater complexity of healing vital tissues compared with circumscribed in-vitro studies (3, 12). Therefore, PG represents a promising biological therapy, offering opportunities for various applications in the treatment of wounds, ulcers, sports injuries, and other forms of regenerative medicine.

REFERENCES


33. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83:835–70.


45. Henderson JL, Capp CL, Ross EV, Shick PC, Keefe MA, Wester DC, Hannon T, McConnell D. The effects of


AUTHORS’ ADDRESSES

Primož Rožman, MD, PhD, ass. prof., head, Department of Immunohematology, Blood Transfusion Centre of Slovenia, Šljajmerjeva 6, SI-1000 Ljubljana, Slovenia, E-mail: primoz.rozman@ztm.si

Zoran Bolta, MD, same address, E-mail: zoran.bolta@ztm.si