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## The Role of Platelet Gel in Regenerative Medicine

Primož Rožman<sup>1</sup>, Danijela Semenič<sup>2</sup> and Dragica Maja Smrke<sup>2</sup>

<sup>1</sup>*Blood Transfusion Centre*

<sup>2</sup>*Department of Surgical Infections, University Clinical Centre Ljubljana  
Slovenia*

### 1. Introduction

For decades, platelet concentrates have been a standard transfusion component for the treatment of clotting disorders. They are prepared from the whole blood within 24 hours from collection from voluntary blood donors by means of differential centrifugation of platelet rich plasma (PRP) or by the apheresis and stored at room temperature with a maximum shelf life of 7 days. They are transfused to the patients who suffer from thrombocytopenia or some other platelet disorder in order to sustain the haemostatic function. Freezing platelets is feasible but results in poor recovery, although the platelets retain their clotting functions after thawing. The use of platelets is permanently increasing, due to advanced surgical and other therapies and they are considered vital products in blood banking today (Brecher, 2005).

Apart from their clotting functions, platelets have been shown to possess some other evolutionary highly conserved biological functions, such as immune defence, tissue forming and regeneration. These properties are now clinically used by the virtue of platelet gel preparations. Technically, the clinical precursor of platelet gel has been fibrin glue that was composed of two separate solutions - fibrinogen and thrombin. When mixed together, these agents were similar to the last stages of the clotting cascade in forming a fibrin clot. Fibrinogen has been obtained from pooled allogeneic single-donor plasma units and from the autologous blood of the patients and has been usually isolated by the process of cryoprecipitation. The additional thrombin component has been generally derived from commercial bovine sources. Some investigators have even added calcium chloride and/or antifibrinolytics (i.e., aminocaproic acid, aprotinin) to their preparations in order to enhance the clot formation. Although fibrin glue has been used in a variety of surgical procedures for the prevention of bleeding, it has been especially useful in heparinized patients undergoing cardiovascular procedures requiring extracorporeal circulation, as it does not need an intact hemostatic system to be effective. Fibrin glue has also been evaluated in the presealing of woven or knitted Dacron vascular grafts. The major drawback to its use has been attributed to the potential risk of transmitted serological disease from pooled and single-donor blood donors, so the patient's own blood was considered to be the safest preparation to prepare fibrin glue. Overall, fibrin glue is a useful adjunct to other methods to control bleeding in selected surgical patients (Thompson et al., 1988; Lee & Kang, 2011).

Autologous platelet rich plasma, which is by the activation of platelets converted to platelet gel (PG) was developed in the early 1970s as a by-product of multicomponent apheresis. The first published paper relating to the use of platelet-fibrinogen-thrombin mixture as a "corneal adhesive" is dated from 1975 (Rosenthal et al., 1975). In 1979, a report was published about platelet gel "gel foam" used to obtain sutureless nerve anastomosis (Fischer, 1979). Another publication in 1980, described the role of fibrin to drive fibroblast migration and collagen deposition and leading to granulation tissue formation (Brandstedt et al., 1980).

Articles published in the 1990s provided numerous insights into the basic mechanisms of the physiologic process of healing after injury had occurred. Some of the results provided by these papers were that fibroblasts are recruited at the injury site by soluble factors; that the platelet releasate contains a high level of chemotactic factors that recruit fibroblasts in a strictly dose-dependent manner, that the fibroblasts migrate along a fibrin-rich matrix; and that fibronectin is necessary for such migration to occur since it provides a conduit for transmigration. In their migration, fibroblasts adhere to the fibronectin fibres through alpha5beta1 and alpha5beta3 receptors. Proteases are necessary for fibroblast transmigration through the fibrin-rich matrix (the role of metalloproteases was depicted some years later). Interestingly, the fibrin/ fibronectin fibres retain growth factors released from the activated platelets within the injured site (Greiling & Clark, 1997).

In 1996, the physiological relationship between mesenchymal cells, fibrin and growth factors was shown to result in the establishment of granulation tissue. It was demonstrated that the formation of granulation tissue is strongly enhanced by the platelet releasate. The platelet releasate provides continuous stimulation for mesenchyme-derived cells to interact with fibrin, thus accelerating the development of granulation tissue (McClain et al., 1996). It is now confirmed that the human platelet lysate can induce the expansion of human mesenchymal stem cells *in vitro*, which will by omitting the animal derived albumin, change the cell cultivation approaches for regenerative medicine.

One of the first disciplines that readily accepted the new regenerative capacity of the PRP/PG was the maxillofacial surgery (Schallmoser et al., 2007). PRP was introduced to the maxillofacial community by Whitman et al. as an alternative to the fibrin glue in 1997 (Whitman et al., 1997). Marx et al. showed that by combining PRP with autologous bone in mandibular continuity defects resulted in significantly faster radiographic maturation and a histomorphometrically denser bone regeneration (Marx et al., 1998).

In 2000 - 2010 authors observed various healing effects of PG, for instance that chronic lower extremity ulcers (diabetic, post-traumatic, vascular) reepithelialized faster with the course of twice-daily wound treatment with platelet releasate as compared with similar wounds treated with a placebo. The supernatant or releasate of the platelet gel was able to promote dose-dependent proliferation and changes in gene expression as well as in metabolic activities related to protein synthesis (Junger et al., 2000; Valencia et al., 2001; Fang & Galiano, 2008; Bernuzzi et al., 2010; Ficarelli et al., 2008; Chen et al., 2010). The platelet concentrate was used for healing skin, soft tissue wounds, fractures, surgical injuries, diabetic ulcers, dental, oral and maxillofacial settings (Altman et al., 2001; Margolis et al., 2001; Anderson & Baker, 2003; Castro, 2004; Belli et al., 2005; Burkus, 2005; Carreon et al., 2005; Sclafani et al., 2005; Trowbridge et al., 2005; Brown et al., 2006; Driver et al., 2006; Savarino et al., 2006; Rozman & Bolta, 2007; Borzini & Mazzucco 2007; Smrke et al., 2007; Cieslik-Bielecka et al., 2008;).

Novel strategies of platelet gel application in cardiology were under investigation in the year 2004. Studies evaluated the use of platelet gel in the case of myocardial injury, in order to promote remodelling through the regeneration of myocytes, the induction of angiogenesis and the restoration of a normal extracellular matrix composition (Mogan & Larson, 2004; Balbo et al., 2010;).

PG/PRP was further intensively studied. Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord veins and grown in appropriate conditions *in vitro*. The platelet gel-released supernatant was able to induce proliferation and to stimulate motility and the invasiveness of HUVECs. Higher concentrations of PG induced a reversion of the stimulatory processes. It became clear that different concentrations of the platelet growth factors do not have the same efficacy in inducing these processes: excessively high concentrations can have an inhibitory effect on the processes. Further studies are necessary to reach a better understanding of these complex biological processes (Rughetti et al., 2008). PG treatment in 2008 revealed a unique capacity of articulating a pro-inflammatory and pro-angiogenic cytokine profile in human peripheral blood mononuclear cells, co-cultured with PG, which may partially explain the clinical success of PG application in a wide range of diseases (Naldini et al., 2008).

A 1-year retrospective review of a cardiac surgery patient in 2008 showed that the treatment group with PG significantly shortened the duration of intensive care and decreased the total length of stays as well as decreased postoperative blood loss, compared with the control group. In addition, the treatment group had no reported incisional wound infections (Englert et al., 2008; Gunaydin et al., 2008).

## 2. Platelet structure and physiology

Platelets are not "real" cells but rather non-nuclear cellular fragments derived from the megakaryocytes in the bone marrow through controlled cellular fragmentation, followed by their release into circulation (Jurk & Kehrel, 2005). A healthy adult aged between 30 and 70, weighing 70 kg, has in total about 1,300-1,500 grams of platelets (thrombocytes); 40% of them in the spinal bone marrow, 25% in the ribs and sternum, 15% in the pelvis, 10% in the skull, and 10% in other bones. Progenitor cells of the thrombocytic lineage develop in the same way as other immature cells of bloodlines in the bone marrow, originating from the common hematopoietic stem cell. When immature cells differentiate into directed stem cells they are morphologically recognizable as the progenitor cells of thrombocytic lineage. The progenitor cell is the megakaryoblast, with 21-50 mm diameter, which develops from the common myeloid progenitor named CFU-GEMM. The megakaryoblast gives rise to the promegakaryocyte and then the megakaryocyte - the largest cell in the bone marrow with 70-100 mm in diameter. Several growth factors are involved in the platelet differentiation, among which the thrombopoietin plays the most decisive role, inducing thrombopoiesis in a negative loop fashion by binding to the thrombocytes when they are present in sufficient numbers, thus decreasing their production from the HSCs in the bone marrow (See Figure 1).

There are 1/3 immature and 2/3 mature elements of megakaryocyte lineage in the bone marrow. Thrombocytes are formed by the breaking off of small fragments of the

megakaryocyte cytoplasm. Each megakaryocyte gives rise to 2,000 to 4,000 thrombocytes. The development of the megakaryocyte from the megakaryoblast takes 4-5 days, and the life-span of a thrombocyte is 7-10 days. The number of thrombocytes in the peripheral blood is  $150-350 \times 10^9/L$ . If functionally classified, platelets consist of various molecules: membrane proteins 3%, signalling proteins 24%, cytoskeletal 15%, vesicular 6%, extracellular 4%, mitochondrial 7%, protein processing 22%, metabolic enzymes 6%, unknown 4%, miscellaneous 5.5% and nuclear proteins 3.5% (Garcia et al., 2004).

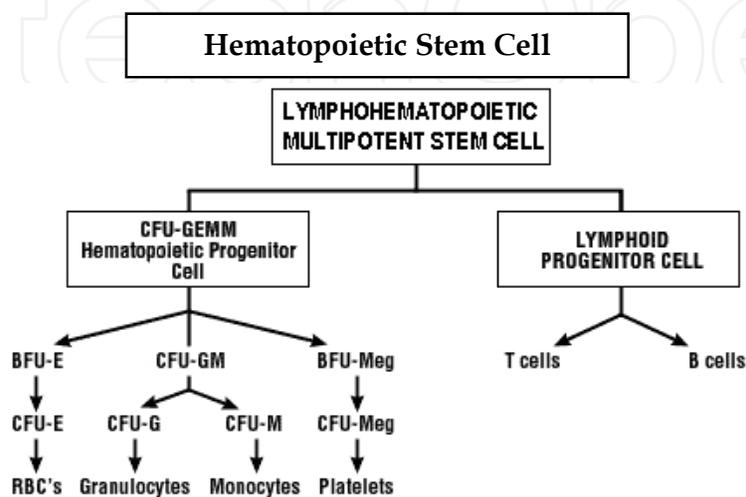


Fig. 1. Thrombopoiesis in the bone marrow

As mentioned previously, platelets are primarily specialized secretory cells that release the contents of their intracellular granules in response to activation. Beside their well known function in haemostasis where they prevent blood loss at the sites of vascular injury, they also release substances that promote tissue repair and influence the reactivity of vascular and blood cells in angiogenesis and inflammation (Jurk & Kehrel, 2005). At the site of the injury, platelets release an arsenal of potent inflammatory and mitogenic substances which are involved in all aspects of the wound healing process. Based on these facts, platelet releasate in the form of activated platelet gel has been extensively used for the topical *in loco* therapy of various clinical conditions (Borzini & Mazzucco, 2007). The purpose of this chapter is to review the current laboratory and clinical findings related to the therapy of these diseases with the use of platelet gel derived growth factors.

### 3. Platelet activation

Platelets are normally circulating in the blood in their resting form (Gresele et al., 2002). Non-activated platelets contain a complete array of presynthesized protein molecules important for platelet function, among which the high presence of cytoskeletal proteins, signalling proteins, membrane proteins, protein processing proteins and cytoskeleton regulatory proteins is noted (Garcia et al., 2004; Anitua et al., 2004). These molecules are synthesized already in the megakaryocytes and packaged into the granules. Three major storage compartments in platelets are alpha granules, dense granules and lysosomes. The majority of the substances are contained in alpha granules (Rendu & Brohard-Bohn, 2001). (See Tab. 1).

Category	Term	Biological activities
<b>Adhesive proteins</b>	VWf + propeptide, Fg, Fn, Vn, TSP-1, laminin-8	Cell contact interactions, clotting, extracellular matrix composition
<b>Clotting factors and associated proteins</b>	Factor V/Va, factor XI, multimerin, gas6, protein S, high-molecular weight chininogen, antithrombin, tissue factor pathway inhibitor (TFPI)	Thrombin production and its regulation, angiogenesis
<b>Fibrinolytic factors and associated proteins</b>	Plasminogen, PAI-I, u-PA, osteonectin, $\alpha$ 2-antiplasmin, histidine rich glycoprotein, TAFI, $\alpha$ 2-macroglobulin	Plasmin production and vascular remodelling
<b>Proteases and anti-proteases</b>	Tissue inhibitor of metalloprotease-4 (TIMP-4), matrix metalloproteinases MMP-1,-2,-3, -4, -9, platelet inhibitor of FIX, protease nexin-2, C1 inhibitor, $\alpha$ 1-antitripsin	Potential of aggregation, angiogenesis, vascular modelling, regulation of coagulation, regulation of cellular behaviour
<b>Growth factors, cytokines and chemokines</b>	PDGF, TGF $\beta$ 1 and 2, EGF, IGF-1, VEGF (A and C), bFGF and FGF-2, hepatocyte GF, RANTES, IL-8, MIP-1 $\alpha$ , growth regulated oncogene- $\alpha$ , ENA-78, MCP-3, angiopoietin-1, Il-1 $\beta$ , IGF BP-3, neutrophile chemo active protein	Chemotaxis, cell proliferation and differentiation, angiogenesis
<b>Basic proteins and others</b>	PF4, $\beta$ -thromboglobulin, platelet basic protein, connective tissue activating peptide III, neutrophile activating peptide-2, endostatins	Regulation of angiogenesis, vascular modelling, cellular interactions. Endostatin is an endogenous inhibitor of angiogenesis and the growth of both primary tumors and metastasis.
<b>Anti-microbial proteins</b>	Thrombocidins	Bactericidal and fungicidal properties
<b>Others</b>	-chondroitin 4-sulfate, albumin, immunoglobulins	Diverse
<b>Membrane glycoproteins</b>	$\alpha$ IIb $\beta$ 3, $\alpha$ $\beta$ 3, GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonists, CD40L, tissue factor, P-selectin	Platelet aggregation and adhesion, endocytosis of proteins, inflammation, thrombin generation, platelet-leukocyte interactions

Table 1. The contents of the platelet alpha-granules

When adhered to exposed endothelium or activated by agonists, platelet change their shape and secrete the contents of the granules (including ADP, fibrinogen and serotonin), which is followed by the platelet aggregation. The initiation of the signalling event within the platelet leads to the reorganization of the platelet cytoskeleton, which is seen as an extremely rapid shape change (See Figure 2).



Legend: Resting platelets are smooth and disc shaped (left). Activated platelets have an irregular shape with many protruding pseudopodia (right).

Fig. 2. Platelets in their resting form and at the beginning of the activation.

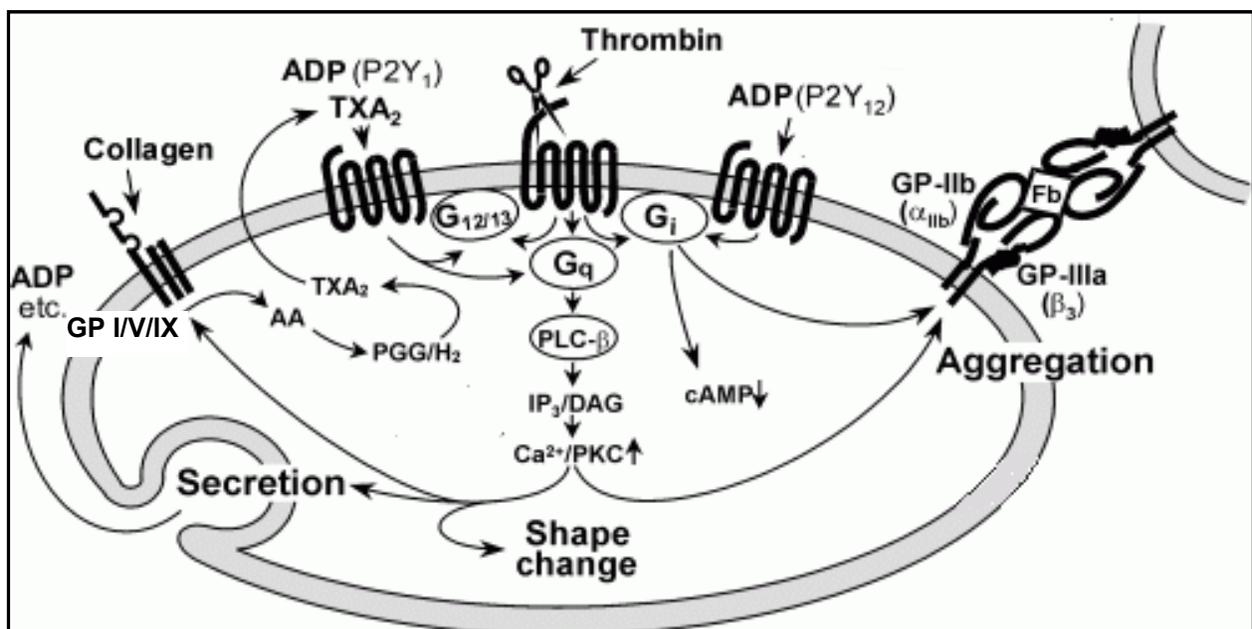


Fig. 3. Schematic representation of intracellular events during platelet activation - "outside in" and "inside out" signalling

Initiation of the signalling event within the platelet leads to the reorganization of the platelet cytoskeleton, which is seen as a shape change. Aggregation of platelets is mediated by molecules of fibrinogen or vWf, which connect platelets by bridging complexes of glycoprotein IIb/IIIa (integrin  $\alpha_{IIb}\beta_3$ ) on adjacent platelets, forming a platelet aggregate. In order to bind the fibrinogen and vWf, GP-IIb/IIIa initially has to be converted from a low affinity/avidity state to a high affinity/avidity state by a process described as "inside-out signalling" that is initiated during and after platelet activation. 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 (See Figure 3.). Under these conditions there is also activation of the coagulation cascade; therefore thrombin is formed, which also markedly stimulates the platelet activation in a positive feedback loop fashion (Gresele et al., 2002).

#### 4. Platelet receptors and antigens

It has been proposed that the original function of the mammalian platelets resembled the function of primitive haemocytes in certain lower organisms such as caterpillars, in which they mostly act as a defence against foreign organisms by adhering and aggregating to foreign bodies. This is probably the reason why human platelets also possess many features of classical inflammatory cells. As such, they can undergo chemotaxis, release potent inflammatory mediators such as platelet factor PF4 and histamine, they can phagocytose foreign particles, interact with neutrophils, stimulate basophiles, excrete a number of mediators, cytokines, growth factors and other proteins and even interact with bacteria and parasites. Platelet membranes also bear some immune molecules, such as MHC Class I molecules, Fc receptors and complement binding molecules. The platelet membrane also incorporates ABO and some other polysaccharide blood group Antigens, which are very important in blood transfusion (Rožman, 2002).

The membrane of the resting platelet can modify considerably after the activation following adhesion. This stimulus causes numerous intracellular molecules that are a part of the lysosomes, granules and other cellular compartments, to appear on the platelet surface. These molecules are therefore considered as markers of platelet activation. A short description of more than 45 distinctive platelet membrane structures found on resting platelets is given in Table 2. They are usually divided into five groups: adhesion molecules, immune molecules, receptors, blood group antigens and some other molecules.

An extensive review of all molecules found on platelets and their precursors is given in references (Kishimoto et al., 1997). The CD designations of all integral molecules of the platelet membrane, as agreed upon at the 9<sup>th</sup> HLDA (Human Leukocyte Differentiation Antigens) workshop in 2010., can also be found on the internet addresses: <http://hcdm.org>.

Many antigenic systems that are typical for erythrocytes and leukocytes, are also present on platelet membranes, including ABO and HLA antigens. Some others are exclusively found on platelets and their precursors. They are called human platelet alloantigens (HPA) and located on the platelet glycoproteins GPIa/IIa, GPIIb/IIIa and GPI/V/IX as a polymorphic part of the aminoacid chain. These polymorphisms are usually caused by a single nucleotide and the consequent aminoacid substitutions. The polymorphisms that cause alloimmunisation following transfusion and pregnancy were classified into the immunogenetic system of HPA (human platelet alloantigens) (Santoso & Kiefel, 1998). (see Table 3. and EMBL EBI immune polymorphysm database at the <http://www.ebi.ac.uk>).

For transfusion and transplantation, ABO, HPA and HLA antigens are important due to their capability of inducing an immune response in antigen- negative recipients. This could play a role in the allogeneic platelet gel settings, but surprisingly we could not find any immune consequences of the allogeneic platelet gel use in observed patients, treated topically with allogeneic platelets, as will be discussed later.

Group	SUBGROUP	Members	Characteristics and function
1. ADHESION MOLECULES	Integrins	CD41 (Integrin $\alpha$ IIb subunit, GPIIb)	Associates with GPIIIa into GPIIb/IIIa complex. The complex binds fibrinogen, vWf, fibronectin, vitronectin, thrombospondin
		CD61 (Integrin $\beta$ 3 subunit)	GPIIIa; associates with CD41 (into the GPIIb) or CD51 (into the vitronectin receptor)
		CD49a (Integrin $\alpha$ 1 subunit)	Associates with CD29 ( $\beta$ 1 subunit) into VLA-1 (GPIIa), binds to collagen and laminin
		CD49b (integrin $\alpha$ 2 subunit)	GPIa or VLA-2 $\alpha$ , associates with CD29 (GPIIa), binds collagen, laminin,
		CD49e (integrin $\alpha$ 5 subunit)	$\alpha$ subunit of VLA-5 (vitronectin receptor), GPIc' subunit, of GPIc'/IIa, binds fibronectin
		CD49f (integrin $\alpha$ 6 subunit)	VLA-6 $\alpha$ subunit, GPIc, associates with CD29 ( $\beta$ 1) into VLA-6 (CD49f/CD29), binds laminin
		CD29 (integrin $\beta$ 1 subunit)	Associates with CD49a in VLA-1 integrin (GPIIa) on platelets
		CD51 (integrin $\alpha$ V subunit)	Associates with CD61 into the vitronectin receptor. A receptor for RGD-containing proteins (fibrinogen, vWf, fibronectin, laminin, thrombospondin).
	Selectins	CD62 (P-selectin)	Binds sialyl Lewis <sup>x</sup> , mediates the interaction of platelets with neutrophils, monocytes and a rolling interaction of neutrophils on endothelium
	Leucine-rich proteins (GPIb-IX-V complex)	CD42b $\alpha$ (GPIb $\alpha$ )	Binds to the von Willebrand factor, receptor for thrombin
		CD42b $\beta$ (CD42c) (GPIb $\beta$ )	Subunits of the GPIb-IX-V complex, bind to the von Willebrand factor
		CD42d (GPV)	
		CD42a (GPIX)	
	Ig - superfamily	CD54 (ICAM-1)	Interactions with integrins, receptor for rhinovirus
		CD31 (PECAM-1)	Interactions with integrins, inhibiting neutrophile migration
	CD44 variants	CD44 isoforms	Adhesion to the extracellular matrix
	TM4 superfamily	CD9	Tetra spanning membrane protein, regulation of integrin function
CD165 (AD2, gp37, A108)		Adhesion molecule, thymocytes/ thymic epithelium	
2. IMMUNE MOLECULES	MHC class I.	HLA-A, B, C	Immune recognition
	Fc receptors	CD32 (Fc $\gamma$ RII)	Low affinity Fc receptor for aggregated Ig/immune complexes; activation
	C receptors	C1q-, C2-, C4-receptors	Complement receptors
	Regulation of complement activation	CD55 - DAF (decay acc. factor)	Blocking and deactivating components of complement and inhibition of lysis
		CD59 - MIRL	
C8bp (C8-binding protein)			
	CD46 - MCP (membr. cof. prot.)		

3. RECEPTORS	Receptors for primary agonists	Medium affinity thrombin rec.	Mediate activation by agonistic ligands through different mechanisms
		High affinity thrombin rec.	
		Epinephrine receptor	
		TX A2 - thromboxane receptor	
		PAF receptor	
		ADP receptor	
		PAF receptor	
		Serotonin receptor	
	Vasopressin receptor		
	Inhibitory receptors	β2 adrenoreceptor	Inhibit platelet activation
		Adenosine receptor	
		Prostaglandin D2 (PGD2) rec.	
		Prostaglandin I2 (PGI2) rec.	
Multipurpose receptors	CD36 (GPIV)	Adhesion receptor for collagen and thrombospondin, receptor for Plasmodium falciparum, macrophage scavenger receptor	
	Other receptors	CD151 (PETA-3)	Activates platelets upon stimulation with MAbs
CD110 (TPO R, MPL)		Thrombopoietin receptor, megakaryocyte proliferation and differentiation	
CD23 (FcεRII)		C-type lectin, low affinity receptor for IgE	
4. BLOOD GROUP ANTIGENS	AB0	A,B,H	Oligosaccharides
	Le	Le <sup>a</sup> , Le <sup>b</sup>	
	Ii	I <sub>i</sub>	
	P	P	
5. OTHER MOLECULES	Constitutive glycosphingolipid	CDw17 (lactosyl ceramide)	Activation, release of granules
	Oligosaccharides	CD60	Oligosaccharide, present on gangliosides
	α-granules proteins	GMP-33	Translocated to the cell surface after activation
	Lyzosomes proteins	CD107a,b (Lyzosomes-associated membrane proteins LAMP-1&2)	Liposomal membrane protein translocated to the cell surface after activation
	TM4 superfamily - other members	CD63 (Granulophysin, LIMP)	Tetra spanning membrane protein, liposomal membrane protein translocated to the cell surface after activation
		CD82	Tetra spanning membrane protein, suppresses metastasis, transduce signals in B, T cells and monocytes
GPI-linked proteins	CD109	Platelet activation factor, GR56, Gov <sup>a/b</sup> alloantigen on platelets	

Table 2. Molecules on the non-activated platelet membrane

System	Antigen	Phenotype frequency*	Glycoprotein	Nucleotide Change*	Aminoacid change	CD
HPA-1	HPA-1a	97.9%	GPIIIa	T <sup>176</sup>	Leucine <sup>33</sup>	CD61
	HPA-1b	28.8%		C <sup>176</sup>	Proline <sup>33</sup>	
HPA-2	HPA-2a	>99.9%	GPIb $\alpha$	C <sup>482</sup>	Threonine <sup>145</sup>	CD42b
	HPA-2b	13.2%		T <sup>482</sup>	Methionine <sup>145</sup>	
HPA-3	HPA-3a	80.95%	GPIIb	T <sup>2621</sup>	Isoleucine <sup>843</sup>	CD41
	HPA-3b	69.8%		G <sup>2621</sup>	Serine <sup>843</sup>	
HPA-4	HPA-4a	>99.9%	GPIIIa	G <sup>506</sup>	Arginine <sup>143</sup>	CD61
	HPA-4b	<0.1%		A <sup>506</sup>	Glutamine <sup>143</sup>	
HPA-5	HPA-5a	99.0%	GPIa	G <sup>1600</sup>	Glutamic acid <sup>505</sup>	CD49b
	HPA-5b	19.7%		A <sup>1600</sup>	Lysine <sup>505</sup>	
	HPA-6bw	0.7%	GPIIIa	G <sup>1544</sup> A <sup>1544</sup>	Arginine <sup>489</sup> Glutamine <sup>489</sup>	CD61
	HPA-7bw	0.2%	GPIIIa	C <sup>1297</sup> G <sup>1297</sup>	Proline <sup>407</sup> Alanine <sup>407</sup>	CD61
	HPA-8bw	<0.01%	GPIIIa	C <sup>1984</sup> T <sup>1984</sup>	Arginine <sup>636</sup> Cysteine <sup>636</sup>	CD61
	HPA-9bw	0.6%	GPIIb	G <sup>2602</sup> A <sup>2602</sup>	Valine <sup>837</sup> Methionine <sup>837</sup>	CD41
	HPA10bw	<1.6%	GPIIIa	G <sup>263</sup> A <sup>263</sup>	Arginine <sup>62</sup> Glutamine <sup>62</sup>	CD61
	HPA11bw	<0.25%	GPIIIa	G <sup>1976</sup> A <sup>1976</sup>	Arginine <sup>633</sup> Histidine <sup>633</sup>	CD61
	HPA-12bw	0.4%	GPIb $\beta$	G <sup>119</sup> A <sup>119</sup>	Glycine <sup>15</sup> Glutamic acid <sup>15</sup>	CD42c
	HPA-13bw	0.25%	GPIa	C <sup>2483</sup> T <sup>2483</sup>	Threonine <sup>799</sup> Methionine <sup>799</sup>	CD49b
	HPA-14bw	<0.17%	GPIIIa	AAG <sup>1909-1911</sup> deletion	Lysine <sup>611</sup> deletion	CD61
HPA-15	HPA-15a	81%	CD109	C <sup>2108</sup>	Serine <sup>703</sup>	CD109
	HPA-15b	74%		A <sup>2108</sup>	Tyrosine <sup>703</sup>	
	HPA-16bw	<0.1%	GPIIIa	C <sup>497</sup> T <sup>497</sup>	Threonine <sup>140</sup> Isoleucine <sup>140</sup>	CD61
	HPA-17bw	<0.1%	GPIIb/IIIa	C <sup>662</sup> T <sup>662</sup>	Threonine <sup>195</sup> Methionine <sup>195</sup>	CD61
	HPA-18bw	<0.1%	GPIa	G <sup>2235</sup> T <sup>2235</sup>	Glutamine <sup>716</sup> Histidine <sup>716</sup>	CD49b
	HPA-19bw	<0.1%	GPIIIa	A <sup>487</sup> C <sup>487</sup>	Lysine <sup>137</sup> Glutamine <sup>137</sup>	CD61
	HPA-20bw	<0.1%	GPIIb	C <sup>1949</sup> T <sup>1949</sup>	Threonine <sup>619</sup> Methionine <sup>619</sup>	CD41
	HPA-21bw	<0.1%	GPIIIa	G <sup>1960</sup> A <sup>1960</sup>	Glutamic acid <sup>628</sup> Lysine <sup>628</sup>	CD61

\*Nucleotide change - numbers are given in relation to the reference sequence in the NCBI database

Table 3. Human platelet alloantigens (HPA) (Modified from Metcalfe et al. 2003 and IPD HPA database [http://www.ebi.ac.uk/ipd/hpa/freqs\\_1.html](http://www.ebi.ac.uk/ipd/hpa/freqs_1.html))

## 5. Platelet growth factors

As already stated, intact platelets contain alpha-granules and other granular bodies in which important mediator molecules are stored, and are released from the platelet following activation. Among these are the growth factors (GFs), which when released from activated platelets, exhibit an extensive tissue forming ability, such as the initiation and modulation of wound healing in both soft and hard tissues (Anitua et al., 2004; Harrison & Cramer, 1993; Lacoste et al., 2003; Weibrich et al., 2002). (See Table 1. and Table 4.)

<b>IL-1<math>\beta</math>, Interleukin 1beta</b>	<ul style="list-style-type: none"> <li>stimulates lymphocyte proliferation</li> <li>influences collagenase activity</li> </ul>
<b>IL-8, Interleukin 8</b>	<ul style="list-style-type: none"> <li>chemotactic for all known types of migratory immune cells</li> <li>specifically activates neutrophil granulocytes</li> <li>mitogen for epidermal cells</li> </ul>
<b>EGF-<math>\beta</math>, Epidermal growth factor (beta-Urogastron)</b>	<ul style="list-style-type: none"> <li>stimulates re-epithelization, angiogenesis, and collagenase activity</li> </ul>
<b>bFGF/ FGF-2, Fibroblast growth factor (basic)</b>	<ul style="list-style-type: none"> <li>stimulates angiogenesis, endothelial cell proliferation, collagen synthesis, wound contraction, matrix synthesis, epithelization, keratinocyte growth factor production</li> </ul>
<b>aFGF/ FGF-1, Fibroblast growth factor (acidic)</b>	<ul style="list-style-type: none"> <li>most potent GF identified thus far for skin keratinocytes playing role in tissue repair following skin injuries.</li> </ul>
<b>KGF/ FGF-7 Keratinocyte growth factor</b>	<ul style="list-style-type: none"> <li>growth and development of progenitors of granulocytes and macrophages, stimulates myeloblasts and monoblasts and triggers their irreversible differentiation, synergises with EPO in the proliferation of erythroid and megakaryocytic progenitor cells, in combination with another colony stimulating factor, M-CSF, synergically suppresses the generation of macrophage-containing cell colonies</li> <li>for some types of blast cells acute myeloid leukemia acts as an autocrine mediator of growth.</li> <li>a strong chemoattractant for neutrophils, enhances microbicidal activity, oxidative metabolism, and phagocytic activity of neutrophils and macrophages, improves their cytotoxicity</li> </ul>
<b>GM-SCF/ CSF<math>\alpha</math>, granulocyte/macrophage colony-stimulating factor alpha</b>	<ul style="list-style-type: none"> <li>stimulates both the proliferation and the differentiated function in osteoblasts.</li> </ul>
<b>IGF-1 Insulin-like growth factor</b>	<ul style="list-style-type: none"> <li>a growth factor for normal fibroblasts, promotes the synthesis of collagenase and prostaglandin E2 in fibroblasts, may function also as an autocrine growth modulator for human chronic lymphocytic leukemia cells in vivo, an autocrine growth modulator for neuroblastoma cells, the autocrine growth-promoting activity is inhibited by IL4.</li> </ul>
<b>TNF- <math>\alpha</math> Tumor necrosis factor alpha</b>	<ul style="list-style-type: none"> <li>Besides its immune functions, TNF is a potent promoter of angiogenesis in vivo. TNF-alpha is a growth factor for human fibroblasts, where it promotes the synthesis of collagenase and prostaglandin E2</li> </ul>

	<ul style="list-style-type: none"> <li>• promotes the proliferation of astroglial cells and microglial cells and therefore may be involved in pathological processes such as astrogliosis and demyelination.</li> </ul>
<p><b>PDGF A</b> Platelet derived growth factor A</p>	<ul style="list-style-type: none"> <li>• PDGF isoforms are potent mitogens for connective tissue cells, including dermal fibroblasts, arterial smooth muscle cells, chondrocytes and some epithelial and endothelial cells</li> <li>• chemotactic and mitogenic for fibroblasts and smooth muscle cells, neutrophils and mononuclear cells</li> <li>• activates TGF-<math>\beta</math>, stimulates neutrophils and macrophages, stimulates chemotaxis, mitogenesis of fibroblasts and smooth muscle cells, collagen synthesis and collagenase activity, angiogenesis</li> <li>• a major mitogen for connective tissue cells and certain other cell types</li> <li>• stimulates cell growth, but also changes cell shape and motility; induces the reorganization of the actin filament system and stimulates chemotaxis, i.e., a directed cell movement toward a gradient of PDGF</li> </ul>
<p><b>PDGF B</b> Platelet derived growth factor B</p>	<ul style="list-style-type: none"> <li>• regulates the proliferation and the differentiation of multiple cell types. TGF found in platelets is subdivided into TGF <math>\alpha</math> and <math>\beta</math>, which are the more generic connective tissue growth factors involved with matrix formation influencing osteoblasts to lay down bone matrix through the process of osteogenesis.</li> <li>• TGF<math>\beta</math>1 and TGF<math>\beta</math>2 activate fibroblasts, endothelial and osteoprogenitor cells, chondroprogenitor cells and mesenchymal stem cells. A chondroprogenitor cell will further differentiate and produce the matrix for cartilage. A mesenchymal stem cell stimulated to mitose provides wound healing cells</li> </ul>
<p><b>TGF-<math>\alpha</math>,</b> Transforming growth factor alpha</p>	<ul style="list-style-type: none"> <li>• stimulates mesenchymal, epithelial, and endothelial cell growth, endothelial chemotaxis</li> </ul>
<p><b>TGF-<math>\beta</math>1,</b> Transforming growth factor <math>\beta</math>1</p>	<ul style="list-style-type: none"> <li>• stimulates monocytes to secrete FGF, PDGF, TNF- <math>\alpha</math> , Interleukin-1</li> <li>• stimulates fibroblast chemotaxis and proliferation</li> <li>• stimulates collagen synthesis</li> <li>• decrease dermal scarring</li> </ul>
<p><b>VEGF/ VEP</b> Vascular endothelial growth factor</p>	<ul style="list-style-type: none"> <li>• highly specific mitogen for vascular endothelial cells</li> <li>• influences vascular permeability and is a strong angiogenic protein and probably also plays a role in neovascularisation under physiological conditions</li> <li>• in endothelial cells, VEGF induces the synthesis of von Willebrand factor.</li> <li>• a potent chemoattractant for monocytes and thus has procoagulatory activities.</li> <li>• in microvascular endothelial cells induces the synthesis of plasminogen activator and plasminogen activator inhibitor type 1.</li> <li>• induces the synthesis of the metalloproteinase, interstitial collagenase, which degrades interstitial collagen type 1, collagen type 2, and collagen type 3 under normal physiological conditions.</li> </ul>

Table 4. Platelet growth factors ( Modified from <http://www.copewithcytokines.de/cope.cgi>)

Growth factors modulate the functional activities of individual cells and tissues, mediate interactions between cells directly and regulate processes taking place in the extracellular environment in autocrine, paracrine, iuxtacrine or retrocrine fashion. GFs work by binding to specific cell surface receptors and can target cells in a number of recognized ways or modes. Release of these substances into the blood stream allows them to get to distant targets (endocrine mode), diffuse over short distances to affect other cells (juxtacrine mode), and to influence neighbouring cells (paracrine mode). Growth factors can even act on the cell in which they are produced (autocrine mode). These different modes are all likely to be operative during tissue repair.

The most important GFs released by the platelets are PDGF (the platelet derived growth factor), TGF- $\alpha$  &  $\beta$  (transforming growth factor alpha & beta), EGF (epidermal growth factor), FGF (fibroblast growth factor), IGF (insulin growth factor), PDEGF (platelet derived epidermal growth factor), PDAF (platelet derived angiogenesis factor), IL-8 (interleukin-8), TNF- $\alpha$  (tumour necrosis factor alpha), CTGR (connective tissue growth factor), GM-CSF (granulocyte macrophage colony stimulating factor), KGF (keratinocyte growth factor), and Ang-2 (angiopoetin) (Frechette et al., 2005; Borzini & Mazzucco, 2005; Westerhuis et al., 2005; Everts et al., 2006) (see Table 4.). Beside these GFs, there are at least 60 different biologically active substances in the platelets that are involved in tissue repair mechanisms such as chemotaxis, cell proliferation and differentiation, angiogenesis, extracellular matrix deposition, immune modulation, antimicrobial activity, and remodelling (Borzini & Mazzucco, 2007). All these functions have been demonstrated in numerous *in vitro* (Phillips et al., 1994; Dankert, 1995; Brill et al., 2004; Cenni et al., 2005; Kark et al., 2006) and *in vivo* experiments (Debus et al., 2001; Kevy et al., 2004).

## 6. Therapeutic platelet products

Even the topically applied fibrin glue itself improves the haemostasis, reduces blood loss, and tightens the sealing of sutures due to fibrin formation, providing a stabilized matrix for ingrowing fibroblasts and therefore promoting the support of the granulation tissue, revascularization and re-epithelization. Further inclusion of growth factor containing platelets into this concept of tissue regeneration provided and increased tissue repair and regeneration. Various types of platelet products, such as platelet rich plasma, platelet-leukocyte gel and platelet gel, derived from autologous or allogeneic peripheral blood, have been tested for tissue repair.

### 6.1 Platelet-rich plasma

Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline. Usual product of blood banking is the allogeneic platelet concentrate or platelet-rich plasma, obtained from random blood donors. Autologous platelet rich plasma preparations involve the procurement of 50-100ml of blood drawn from the patient at the point of care. The blood is processed using PRP devices yielding 5-10ml of concentrated PRP (3 to 5X baseline levels). PRP serves as a growth factor vehicle and has both mitogenic and chemotactic properties. It contains a high level of platelets and a full complement of clotting and growth factors (Lacci & Dardik, 2010). The PRP is then activated and applied to the wound site or mixed with bone material for

implantation. This procedure is usually performed in under an hour by a well trained and qualified technician.

### 6.2 Platelet gel

Autologous platelet-gel (PG) is the result of harvesting one's own cells (platelets) that are concentrated by means of centrifugation and exposed to an agonist, which induces activation resulting in the release of intrinsic substances that are applied to the target area where they accelerate wound healing. Autologous PG is attractive because it concentrates a large number of biologically active substances, which are primarily proteins that participate in a complex series of mechanisms involved in inflammation and wound healing (Everts et al., 2006; Stammers et al., 2009; Tschon et al., 2011; Mehta & Watson, 2008). Lately, the possible use of allogeneic PG with similar results was demonstrated in our study (Smrke et al. 2007).

### 6.3 Platelet-leukocyte gel

Platelet-leukocyte gel contains high concentrations of platelets and leukocytes. As leukocytes play an important role in the innate host-defence, PLG might have antimicrobial properties. A unit of whole blood is centrifuged to obtain platelet-leukocyte-rich plasma (P-LRP). The PLG is then formed by mixing P-LRP with a thrombin-calcium chloride preparation. Similar to PG, the treatment with PLG provides a source of concentrated platelets, with granules that contain growth factors. In addition the high content of non-activated leukocytes, present in the PLG, promotes anti-microbial activity at the wound site through the destruction of bacteria and foreign materials and the removal of damaged tissue (Everts et al, 2006b; Everts et al. 2008).

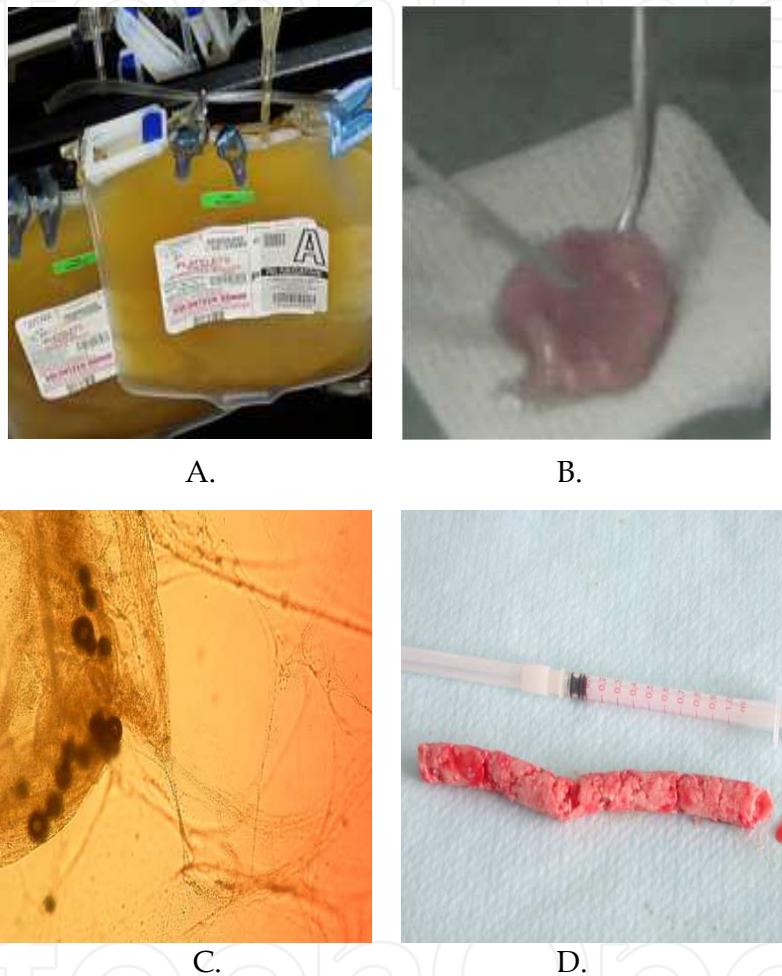
Platelet gel can be nowadays prepared using a range of commercially available instruments from a number of manufactures. There are no uniform manufacturing standards for platelet gel in existence, which has disturbed the classical blood banking. The existing literature adds controversies to the use of PLT concentrates. When talking about platelets and their products, a great number of variables have to be considered. These variables are mainly related to the PRP preparation methods, the type of activators used, intra- and inter-species variability, types of pathology to be treated, the ways and times of administration and the association of PRP or PG with other treatments. The improved knowledge on the variables affecting therapeutic efficacy will surely help in addressing the best combination of factors implied in the different steps of PLT concentrate preparation and use (Mazzucco et al., 2009).

## 7. Activation of PRP and the formation of the gel

The typical source of platelet GFs is platelet-rich plasma (PRP) - Figure 4A. Platelet gel (PG) is formed after the PRP is exposed to a strong coagulation stimuli, which is usually the addition of thrombin and calcium (to counteract the anticoagulant citrate if plasma is citrated) (Mazzucco et al., 2004; De Somer et al., 2006) or some other fibrinogen-digestion based activator, such as batroxobine from the viper *Bothrops atrox* snake venom (Carr et al., 2003; Mazzucco et al., 2008).

Consequently, platelet activation and a coagulation cascade follow in a positive loop fashion, which finally results in the formation of a thrombus-like gelatinous substance -

platelet gel. (See Figures 4B, 4C). In the gel, activated platelets are trapped in a fibrin network and they release their active substances, which slowly diffuse into the surroundings. The final result – platelet gel – can be shaped according to the needs, 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. It can be used as a vehicle for the stem cells and ground cancellous bone transplant, allowing the formation and shaping of grafts with determined shapes (See Fig. 4D).



*Legend: A. Platelet rich plasma; B. Platelet gel; C: Microscopical view of the platelet gel. Inverted microscope Nikon Eclipse 300, 10x enlarged.; D: Graft with the shape used for mandibular defects, containing PG, stem cells and cancellous bone*

Fig. 4. Platelet gel

At the beginning autologous PRP/platelet gel was developed as a by-product of multicomponent apheresis (Rosenthal et al, 1975). Later several systems became available to prepare PRP and from this, the platelet gel. These systems produce two- to six-fold platelet - enriched concentrations. Very few data have been reported on the kinetics of growth factor release from PRP-gels. The bioavailability of growth factors in tissue healing depends on the amount of growth factors stored in the platelets but a portion of these is lost during platelet manipulation. In one report, three commercially available devices (Fibrinet, RegenPRP-Kit, Plateltex) and one manual procedure (home-made) were evaluated with reference to the

resulting platelet concentration, growth factor content and the kinetics of growth factor release from the gel. Similar methods for platelet gel preparation have revealed different performances concerning the growth factor recovery and the kinetics of its release from the gel. It is unclear whether these noticeable differences are important for clinical management (Mazzucco et al., 2009).

Some research groups have successfully engineered kits for platelet gel formation, such as REGENLAB® and PLATELTEX®, which include sodium carboxymethylated cellulose fibres (Hydrofibre™) as a biocomposite-scaffold, entrapping human platelets in PRP. The PLATELTEX® activation kit, popular in Europe, ensures very rapid gelation and the production of platelet and fibrin gel for acute and chronic ulcer treatment. This reduces spillage of the gel during topical application. The new generation of PRP activation, provides for excellent platelet recovery, collection efficiency and PDGF-AB availability. Gelation of the PRP is induced by the enzyme batroxobin, which does not exert pharmacological activity after topical gel administration. In Europe, clinical trials have been authorised using PRP-gel preparation including activation with batroxobin. PRP-gel has not been considered a somatic cell therapy product.

Platelets treated with thrombin become activated and they release their growth factors quickly. Furthermore, thrombin-platelet interaction is a physiological mechanism that hastens the clot-retraction rate. On the contrary, platelets treated with batroxobin do not become activated; they are passively entrapped within the fibrin network, and their growth factor release occurs slowly. In these conditions, the clot retraction takes longer to occur. According to these differences between thrombin and batroxobin, it is expected that batroxobin-induced PRP activation will tailor the slow release of the platelet content, thus providing longer in loco availability of trophic factors. In selected clinical conditions, this durable anabolic factor availability might be preferable to a quick thrombin-induced growth factor release (Mazzucco et al, 2008).

An alternative means of PRP activation was documented in 2007, when PRP was pulsed with one 300ns pulse with an electric field of 30kV/cm, platelets aggregated and a platelet gel was produced (Zhang et al., 2008).

The lack of effective delivery and the efficient targeting specificity limits clinical applications of platelet gel. In 2008, heparin possessing PDGF binding domain was crosslinked to the collagen-based demineralized bone matrix (DBM) for the delivery of human PDGF. In *in vitro* experiments, heparin improves the binding of PDGF to collagen. The *in vitro* activity assay indicates that the collagen-heparin-PDGF (CH-PDGF) complex promotes human fibroblasts to proliferate on collagen gel. In addition, CH-PDGF stimulates cells to migrate into DBM scaffolds after implantation. The histological analysis shows that CH-PDGF promotes the vascularization of the implants. In summary, heparin-DBM/PDGF could prevent the diffusion of PDGF, prolong its activity, and promote the cellularization and vascularization of the scaffold (Sun et al., 2009).

Recent findings on multiple biological properties of human umbilical cord blood (UCB) and its high level of viral safety prompted some authors to investigate the characteristics of its platelets and the possibility of producing PG from cord blood. UCB-derived PG releases high levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor-BB (PDGF-BB), substantial amounts of fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and transforming growth factor-beta 1 (TGFbeta1), and minimal amounts of PDGF-AB. These findings suggest that UCB-derived PG can be a preferable tool for tissue engineering applications where high levels of VEGF and PDGF may be desirable (Parazzi et al., 2010).

## 8. The results of recent clinical research

The results of clinical research indicate that platelet-derived growth factors act in synergy with plasma-derived factors to activate a complex network of autocrine functions that modulate and enhance healing. Therefore, autologous platelet rich plasma (PRP) in the form of activated platelet gel and recombinant morphogenic proteins have been used for healing all kinds of tissues with various results.

### 8.1 Chronic wounds

When the fibroblasts were sealed in the wound with fibrin to which platelet releasate was added, a cellular infiltrate that had characteristics of granulation tissue invaded the wound space. Platelet releasate appeared to be an additional requirement for the early induction of granulation tissue and for enhanced granulation tissue accumulation. It is now widely accepted that the correct strategy to promote the wound-healing cascade is to prepare an autologous PRP/platelet gel that contains growth factors, and administer it directly to sites of surgical interventions or injuries.

Patients suffering from diabetes mellitus, are seventeen times more likely to develop gangrene and five out of six major limb amputations occur in diabetic patients. In the United States, the diabetic foot problem accounts for 20% of all diabetic hospital admissions and 50% of all non-traumatic amputations. Ulcers occur in 15% of diabetics, and 6-20% of all hospitalized diabetic patients have foot ulcers. The topical use of platelet rich plasma may play an important role in the initiation of the repair process of chronic wounds. Many patients can be treated at home without difficulty, requiring only periodic outpatient examination (Steed et al., 1992; Millington and Norris, 2000; Borzini, 2006). Although some of the earlier studies resulted in poor outcomes (Reutter et al., 1999), newer studies tend to produce much better results.

### 8.2 Bone

Platelet gel is very effective in formation of new bone when applied together with cancellous bone containing stem cells for treatment of non healing of long bone fractures (Smrke et al., 2007). Platelet gel enhanced the bone formation contrary to the using of plasma alone as measured by quantity of incorporated bone both *in vitro* and *in vivo* (Geuze et al., 2009). In an article presented in 2010, 115 patients with finger amputations or wounds were treated with platelet gel. Loss of bone tissue represented an obstacle to the total tissue recovery, but the aesthetic results were satisfactory in nearly all cases, the recovery of soft tissue in all patients ranged from 80 to 100% (Balbo et al., 2010). However, platelet gel was ineffective for the spinal fusions where it failed to enhance fusion rate when added to autograft in patients undergoing instrumented posterolateral spinal fusion so several authors do not recommend its use to supplement autologous bone grafting during instrumented posterolateral spinal fusion (Castro, 2004; Burkus, 2005; De Somer et al., 2006; Savarino et al., 2006).

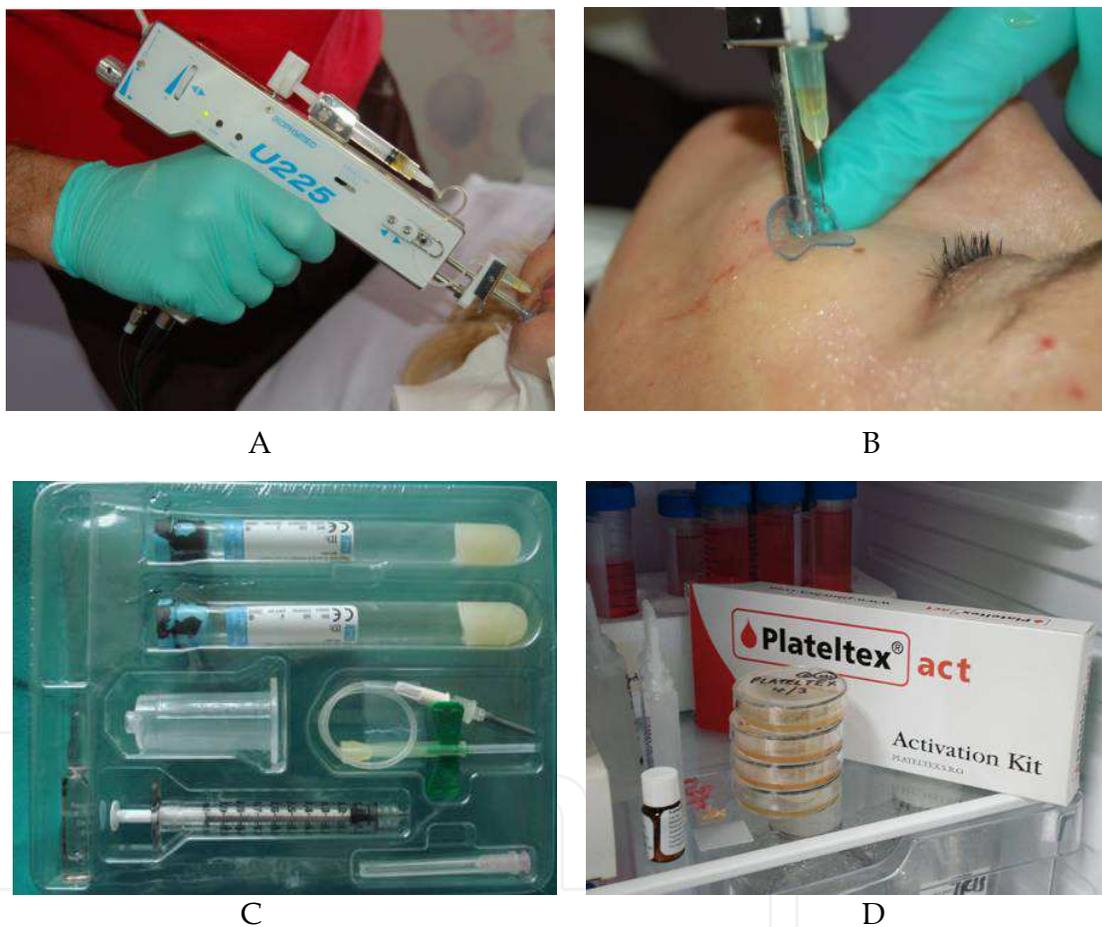
### 8.3 Tendons and ligaments

Tendons and ligaments naturally have poor blood supply. Damage most often occurs in the knee, ankle, shoulder, elbow, wrist, biceps, calf, hamstrings and Achilles tendons. The use of platelet gel has been generally well tolerated by the patients and it resulted in significant increase of healing parameters. Platelet injection therapy has changed the way orthopedic specialists treat sports injury patients. Usually PRP is injected into the damaged area using

minimally invasive protocols that involve ultrasonically guided platelet rich plasma (PRP) injections. (Everts et al. 2007; Everts et al.,2008; Gardner et al., 2006).

#### 8.4 Aesthetic surgery

There is especially extensive marketing for the use of PRP in aesthetic surgery and lifting (Bhanot & Alex, 2002; Powell et al., 2001). Private organisations tend to offer this therapy throughout the world. They offer PRP delivery by various instruments into the skin tissues, rendering good aesthetic effects (see Figure 5). The indications include facelifts, browlifts, the creation of flaps, blepharoplasty, mammoplasties, and abdominoplasties. PRP enhanced grafts generally had a higher graft acceptance and better graft retention than traditional fat grafting techniques, along with decreased swelling and bruising in the donor sites (Sclafani et al., 2005; Brown et al, 2006).



*Legend: A: The instrument Mesogun for delivering PRP (U225) as marketed by company Boland; B: The needle depth can be accurately set for precise delivery; C: An example of kit for PRP preparation and application from Omnimed (Pty), Ltd., JHB, South Africa; D: An example of platelet gel activation kit PLATELTEXT® (<http://www.bolandcell.co.za/platelet.htm>)*

Fig. 5. Use of autologous platelet-rich plasma (PRP).

#### 8.5 Cardiovascular

Wound infection is a devastating complication after cardiovascular surgery. This can lead to surgical reoperation, prolonged intensive care unit stay, and increased mortality promoting

the raising the cost of cardiac surgery while decreasing the patient quality of life. The antimicrobial action of white blood cells and platelets, both found in the platelet gel, may reduce the potential for infection to develop during cardiac surgery. Various protocols have documented such an improved healing of surgical wound in cardiac surgery (Mogan and Larson, 2004; Trowbridge et al., 2005; Englert et al., 2008; Gunyaqdin et al., 2008)

### **8.6 Oral and craniofacial surgery**

As mentioned, the oral surgeons were the leading community in the use of platelet gel for transplantation of artificial and natural grafts. Platelet gel reached a huge popularity in maxillofacial settings where it is considered standard treatment of bone defects, artificial endosseal implants, oral reconstructions, maxillary sinus augmentation, etc. (Marx et al., 1998; Belli et al., 2005; Pomerantz and Dutton, 2005; Mendez et al., 2006).

### **8.7 Other applications**

Platelet gel has been successfully used for ocular diseases, such as for enhanced healing of corneal surface and ocular surgery (Koffler, 2006; Liu et al., 2006; Lee & Kang, 2011).

Lately, the studies of PG use addressed some unexpected fields of medicine. For instance, PG was used as a safe and effective tool in the management of mucosal skin lesions related to the graft-versus-host disease GVHD. Six patients with multiple lesions involving dermis, subcutaneous or oral mucosa and related to GVHD underwent PLT gel as local therapy. After the second PLT gel application, the pain disappeared in all cases and the granulation tissue was observed in the four patients, five of six patients showed a complete response, while one patient with a partial response died early from multiorgan failure (Picardi et al., 2010).

Platelet gel mixed with centrifuged fat tissue has been successfully applied for the patients affected by Parry - Romberg Syndrome (progressive hemifacial atrophy and volumetric deficit), which is an uncommon degenerative and poorly understood condition. It is characterized by a slow and progressive atrophy affecting one side of the face. The atrophy progresses slowly for several years and becomes stable. After stabilization of the disease, plastic surgery of autologous fat grafts can be performed. Study suggested a therapeutic plan comprised of two sequential treatments: acquisition of platelet gel from a small volume of blood (9 ml) followed by the Coleman technique for reconstructing the three-dimensional projection of the face contour, restoring the superficial density of the facial tissues. The results obtained prove the efficacy of these two treatments combined, and the satisfaction of the patient confirms the quality of the results. (Cervelli & Gentile, 2009).

Recently it is surprisingly becoming clear that platelets have a definite role in the cancer, which is probably due to their pro-angiogenesis stimulation and regulation of new blood vessel growth through numerous stimulators and inhibitors of angiogenesis, such as endostatin and VEGF, by several pathways. Cancer cells probably preferentially stimulate platelets to secrete their pro-angiogenic payload, which is counteracted by the anti-platelet agent aspirin that inhibits the platelet-mediated angiogenesis (Elisabeth et al., 2011).

## **9. Pros and cons of platelet gel therapies**

The common advantages of the PG driven healing can be summarized as follows: there seems to be a complete absence of keloid formation, as far as pathologic hyperplasia, metaplasia or dyschromia of the healed lesion (Pomerantz et al., 2005, Lundblad & White, 2005). Other advantages are comfort and pain diminution (Pomerantz et al., 2005, Mendez et

al., 2006), less oedema and ecchymosis (Powell et al., 2001; Mendez et al., 2006; Brown et al., 2006). Particularly valuable advantages are reported through literature: reduced mortality after cardiac surgery (Trowbridge et al., 2005), reduced infection rate after cardiac and orthopaedic surgery (Gilsanz et al., 2001; Salvadè et al., 2010), reduced transfusion supply and hospital stay after orthopaedic surgery (Gardner et al., 2006), reduced amputation rate in diabetic foot (Knighton et al., 1990). PRP therapy became very popular in the USA, which was due to the fact that it attracted enormous attention after the Super Bowl finals in 2009, when the athletes promoted it on TV (Donald, 2011).

Drawbacks of PG therapies are far less pronounced. It is interesting that besides the considerable variability of the clinical studies, PG therapy had practically no or only minute ill effects when compared to standard therapies. Owing to the high heterogeneity of the products, a need for standardization is a commonly shared sentiment. Heterogeneous production generates heterogeneous products which, in turn, have distinct biological properties and healing capacities (Zimmermann et al., 2003; Eppley et al., 2006; Fried et al., 2006).

Considering that heterogeneity involves products, clinical conditions, tissues to be cared for, treatment protocols and probably many other minor variables, authors strongly support the project for the establishment of a clinical database in order to collect enough data for multivariate analysis of the clinical results (Kantor & Margolis, 2000).

## 10. The dilemma of allogeneic vs. autologous platelet gel

In the majority of clinical applications autologous platelets were used for the platelet gel formation. As mentioned previously, autologous PG products are prone to enormous variability, resulting in scientific scepticism and disbelief regarding the actual value of this method. In our study in 2007, we used instead allogeneic PRP. We reported a case of a 50-year-old type II diabetic male with a comminuted fracture of the tibia and delayed union after insufficient initial osteosynthesis with a resulting pseudarthrosis, who was treated operatively by using a graft composed of allogeneic platelet gel mixed with autologous cancellous bone. Due to a history of diabetes, allogeneic instead of autologous platelets that were ABO and RhD matched, leukocyte depleted, irradiated and activated by human thrombin were used. No side effects were observed and no immune reactions such as platelet- or HLA-class I antibodies were detected (Smrke et al., 2007).

We explored several advantages of allogeneic platelets, as compared to the classical autologous setting. Allogeneic platelet units from the blood bank are available in larger quantities, they are safe and affordable, they are highly standardized in terms of platelet, residual leukocyte and red blood cell content, the centrifugal forces used for their isolation, the temperature of the centrifugation, techniques of separation and processing and the composition of the preservative solution, as mandated by international standards in USA and Europe. In addition, they are constantly resuspended on agitators in a standard manner (Brecher, 2005; Council of Europe, 2011). On the other hand, autologous platelet preparations are subject to enormous variability, which hinders serious clinical studies.

The extensive use of allogeneic platelets could, beside the regenerative medicine, influence the blood transfusion policies in future. The platelet units have a short shelf life of 3 – 7 days and are therefore prone to outdating, regardless of the best practices performed. In 2001 in USA, at least 1,234,000 out of a total of 12,898,000 produced platelet units (9.5%) were outdated and discarded (Sullivan et al, 2007). In another study, expiry rates of platelet units were different in various hospitals, ranging between 2% and 13.8% (Novis et al, 2002). It is

clear from these figures that a considerable amount of platelet units are routinely discarded, which could be reversed by the utilization of frozen units in regenerative purposes, such as platelet gel preparation.

Besides, the use of platelet gel could have an important impact on economy of medicine. Let us focus only at the therapy of chronic wound with the platelet gel. Ulcer secondary to venous hypertension and venous insufficiency is the most common chronic wound with 57-80% incidence of all chronic wounds. Average incidence is 15-30 in 100.000 persons and they account for nearly 70% of all leg ulcers (Margolis et al., 2001). All these conditions are more prevalent in elderly population, which represents 20% of the total, a figure that is likely to increase to 25% by 2020. In addition, the increased occurrence and longevity of these ulcers are further compounded by the detrimental effects ageing has on the skin and the wound healing process. Chronic wounds significantly impair the quality of life of more than 9 million of people worldwide and account for a disproportionate share of healthcare expenditures: it is estimated that yearly treatment cost for health care systems averages 9 – 12 k€ per each patient. Even with increasing knowledge and the development of more sophisticated interventions, many clinicians encounter wounds that are hard-to-heal where, despite best efforts, wound healing is prolonged or never achieved (Moffatt, 2008). Indeed, chronic and hard-to-heal wounds are a challenge to the health care professionals. Thus, in the frame of progressively ageing population, the efficient therapy for chronic wounds and tissue reparation promises improving life quality and combating rising healthcare costs. Currently available and practiced therapies such as wound dressings, pentoxifylline, laser and intermittent pneumatic compression treatments applied in concomitance with compressive bandages have shown rather disappointing overall outcomes except some encouraging results obtained by combination of compression and bilayer of artificial skin (Palfreyman et al., 2006).

## 11. Conclusion

Platelets are intriguing cellular particles that have, in the course of human and animal evolution, beside their specialisation into the haemostasis, kept some other abilities of their predecessors - universal blood cells - the abilities of immune defence and tissue forming. For the purpose of tissue forming, platelets can also be used therapeutically. Autologous or allogeneic blood can be used for this purpose and centrifuged to produce a platelet concentrate, which is then further *in vitro* activated to form a thrombus-like gelatinous substance - the platelet gel. Many *in-vitro* studies have established that platelet-derived growth factors accelerate proliferation of an array of cells involved in soft and bony tissue regeneration. These effects have also been evaluated and confirmed by numerous *in vivo*, both in animal and human clinical studies. Consequently, we can say that the platelet-derived products currently represent a valuable therapeutic modality, offering opportunities for various applications in regenerative medicine and tissue engineering.

## 12. Acknowledgment

The authors acknowledge the kind assistance of Petra Pavlovčič in preparing the manuscript.

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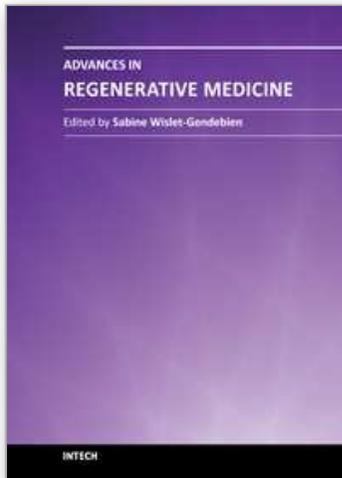
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## **Advances in Regenerative Medicine**

Edited by Dr Sabine Wislet-Gendebien

ISBN 978-953-307-732-1

Hard cover, 404 pages

**Publisher** InTech

**Published online** 21, November, 2011

**Published in print edition** November, 2011

Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Primož Rožman, Danijela Semenič and Dragica Maja Smrke (2011). The Role of Platelet Gel in Regenerative Medicine, *Advances in Regenerative Medicine*, Dr Sabine Wislet-Gendebien (Ed.), ISBN: 978-953-307-732-1, InTech, Available from: <http://www.intechopen.com/books/advances-in-regenerative-medicine/the-role-of-platelet-gel-in-regenerative-medicine>

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University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
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### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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