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DENTAL MEDICINE REVIEW ARTICLE

MASTER IN DENTAL MEDICINE

**PLATELET-RICH PLASMA IN THE TISSUE
HEALING PROCESS OF PATIENTS WITH
BLEEDING DISORDERS**

Joana Filipa Monteiro Moutinho

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PATIENTS WITH BLEEDING DISORDERS**

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“The mind that opens to a new idea,
never returns to its original size.”

Albert Einstein

Acknowledgments

The limited space of this section, surely will not allow me to thank, as it I should, to all the people who, throughout my Master in Dentistry helped me, directly or indirectly, to fulfill my goals and accomplish this academic preparation. Thus, I leave a few words of deep sense of recognized and meaning.

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List of abbreviations

Abbreviation	Meaning
PRP	Platelet-rich plasma
PPP	Platelet-poor plasma
ECM	Extracellular matrix
PDGF	Platelet Derivated Growth Factor
FGF	Basic Fibroblastic Growth Factor
EGF	Epidermal Growth Factor
TGF- α / TGF- β	Transforming Growth Factor α / β
KGF	Keratinocyte Growth Factor
HGF	Hepatocyte Growth Factor
TNF	Tumor Necrosis Factor
VEGF	Vacular Endothelial Growth Factor
IGF	Insulin-like Growth Factor
PDAF	Platelet-derived Angiogenesis Factor
PDEGF	Platelet Derived Epidermal Growth Factor
PF-4	Platelet Factor 4
Ca ²⁺	Calcium ion
PT	Prothrombin time
PTT	Partial thromboplastin time
aPTT	Activated partial thromboplastin time
vWD	Von Willebrand Disease
GT	Glanzmann's Thrombasthenia
INR	International Normalized Ratio
PRF	Platelet-rich fibrin

Abstract

Introduction: The process of tissue healing in the oral cavity involves a series of as well orchestrated cell-cell interactions. For the healing to occur properly it is necessary that immediately after the injury, and with the disruption of the vasculature, be established the formation of the clot with fibrin formation, platelet aggregation and release of many growth factors into the tissue. Based on this concept, comes the platelet rich plasma (PRP), a supplement that is rich in growth factors and which allows their liberation in order to assist and improve the tissue healing. Patients with hemostatic impairment, undergoing oral surgery, are at risk of further complications such as excessive bleeding and difficulties in healing and are therefore adequate candidates to the use of PRP.

Objectives: Understand the clinical applications and therapeutic relevance of the use of PRP in the processes of tissue healing in patients with bleeding disorders and know the clinical protocol of PRP for this type of patients.

Materials and Methods: A literature search was performed in the database "Pubmed ®" and "Google Scholar", through scientific articles.

Discussion: PRP is used in the functional recovery of tissues, which play an important role in accelerating the mechanisms of repair and tissue regeneration. This is obtained by low speed centrifugation of a blood sample, preferentially removed from the own patient, and four types of formulations can be obtained. The PRP has multiple applications in dentistry and is mainly used in bone grafts, alveolar surgical repair after tooth extraction, treatment of intrabony periodontal defects and implant placement. This is now also been applied, successfully, in patients with bleeding disorders for improving post-operative healing conditions and faster recovery and regeneration of oral tissues and control bleeding immediately after surgery.

Conclusions: Despite the evidence that PRP helps in healing, hemorrhage control and repair of oral tissues process, there are few studies that demonstrate its effectiveness in patients with bleeding disorders.

Key words: platelet-rich plasma, PRP applications, bleeding disorders, prevention of bleeding, coagulation problems, oral surgery, dental medicine.

Resumo

Introdução: O processo de cicatrização tecidual na cavidade oral envolve uma série de interações célula-célula bem orquestradas. Para que a cicatrização ocorra de forma adequada é necessário que logo após a lesão, e com a interrupção da vasculatura, se verifique a formação do coágulo com formação de fibrina, agregação de plaquetas e liberação de vários fatores de crescimento para os tecidos. Tendo por base este conceito, surge o plasma rico em plaquetas (PRP), um complemento rico em factores de crescimento e que permite a sua liberação de forma a facilitar o processo de reparação tecidual. Pacientes com comprometimento hemostático, que se submetam a cirurgias orais, correm o risco de complicações adicionais como sangramento excessivo e dificuldades na cicatrização, sendo por isso considerada a utilização do PRP.

Objetivos: Compreender as aplicações clínicas e relevância terapêutica da utilização do PRP nos processos de cicatrização de tecidos, em pacientes com coagulopatias e saber o protocolo clínico de formulação do PRP para este tipo de pacientes.

Material e Métodos: A pesquisa bibliográfica foi realizada na base de dados "Pubmed ®" e "Google Scholar", através de artigos científicos.

Desenvolvimento: O PRP é usado na recuperação funcional dos tecidos, desempenhando um papel importante na aceleração dos mecanismos de reparação e regeneração tecidual. Este é obtido pela centrifugação a baixa velocidade de uma amostra de sangue, preferencialmente retirada do próprio paciente, podendo ser obtidos 4 tipos de formulações. O PRP tem múltiplas aplicações e na Medicina Dentária é usado principalmente em enxertos ósseos, reparo cirúrgico alveolar após extração dentária, tratamento de defeitos periodontais infra-ósseos e na colocação de implantes. O PRP está agora a ser também aplicado, e com sucesso, em pacientes com coagulopatias para melhorar as condições pós-operatórias de cicatrização e de forma a acelerar a recuperação e regeneração dos tecidos orais e controlar a hemorragia imediatamente após a cirurgia.

Conclusões: Apesar de estar provado que o PRP ajuda na cicatrização, controlo de hemorragia e processo de reparação dos tecidos orais, ainda há poucos estudos que evidenciam essa eficácia nos pacientes com distúrbios hemorrágicos.

Palavras-chave: Plasma rico em plaquetas, aplicações do PRP, coagulopatias, prevenção do sangramento, problemas de coagulação, cirurgia oral, medicina dentária.

Introduction

Wound healing in general can be defined as the physiology by which the body replaces and restores function to damaged tissues; a process to a state of soundness any injury ¹.

Within the oral cavity, the gingiva is lined by stratified squamous epithelium that is a boundary between the external environment and underlying connective tissue. Gingival wounds or surgical procedures, such as tooth extractions, periodontal or implant surgery or bone grafts, involve disruption of this barrier function ².

The process of tissue healing in the oral cavity consists of a seamless and coordinated chain of cellular and molecular events that interact in order to assure the restructuring and constitution of the tissues. This event is a dynamic process that implicates a series of well-orchestrated cell-cell interactions between epithelial cells, gingival fibroblasts, osteoblasts and periodontal ligament fibroblasts ^{3, 4, 5}. For healing to occur properly and in order to guarantee the restoration of tissue, it is necessary that immediately after the injury, and disruption of the vasculature, there is clot formation with fibrin formation, platelet aggregation and release of some growth factors into the tissue ^{3,4,5}.

The wound healing process (*Figure 1*) is classically demarcated by series of continuous, sometimes overlapping, events and can be divided into three major phases: inflammation, proliferation and remodeling. The first occurs immediately after injury and may continue for up to 6 days and is characterized by hemostasis, constriction of the blood supply, release of growth factors, platelet aggregation and degranulation, clot formation resulting in fibrin and migration of phagocytic leukocytes that secretes chemicals which kills and removes foreign substances and microorganisms of the wound. The second (5 days to 3 weeks) is characterized by the formation of the extracellular matrix (ECM) and mainly involves the migration and proliferation of three cell classes: fibroblasts, endothelial cells and keratinocytes. These cells proliferate in response to growth factors and cytokines (released from macrophages, platelets, and mesenchymal cells, or that were stored in the fibrin clot). In addition to chemotactically drawing fibroblasts into the wound, there are many growth factors that induce cellular activation and

proliferation (Table 1). Then, proliferating cells respond by releasing collagen and glycosaminoglycans (mainly hyaluronic acid, chondroitin-4-sulphate, dermatan sulphate, and heparin sulphate). The combination of the secreted products forms the new ECM, which is determinant for the organization of granulation tissue that eventually fills the wound. The formation of new blood vessels (angiogenesis) accompanies fibroblast proliferation and allows nutrients and healing factors to enter the wound space. In the third and final phase (from 3 weeks to 2 years) change occurs in the pattern of collagen organization and its main type, occurring replacement of collagen type III by collagen type I, increasing the tensile strength of the scar tissue because of the amplification of cross-links between monomers of this substance ^{6,7}.

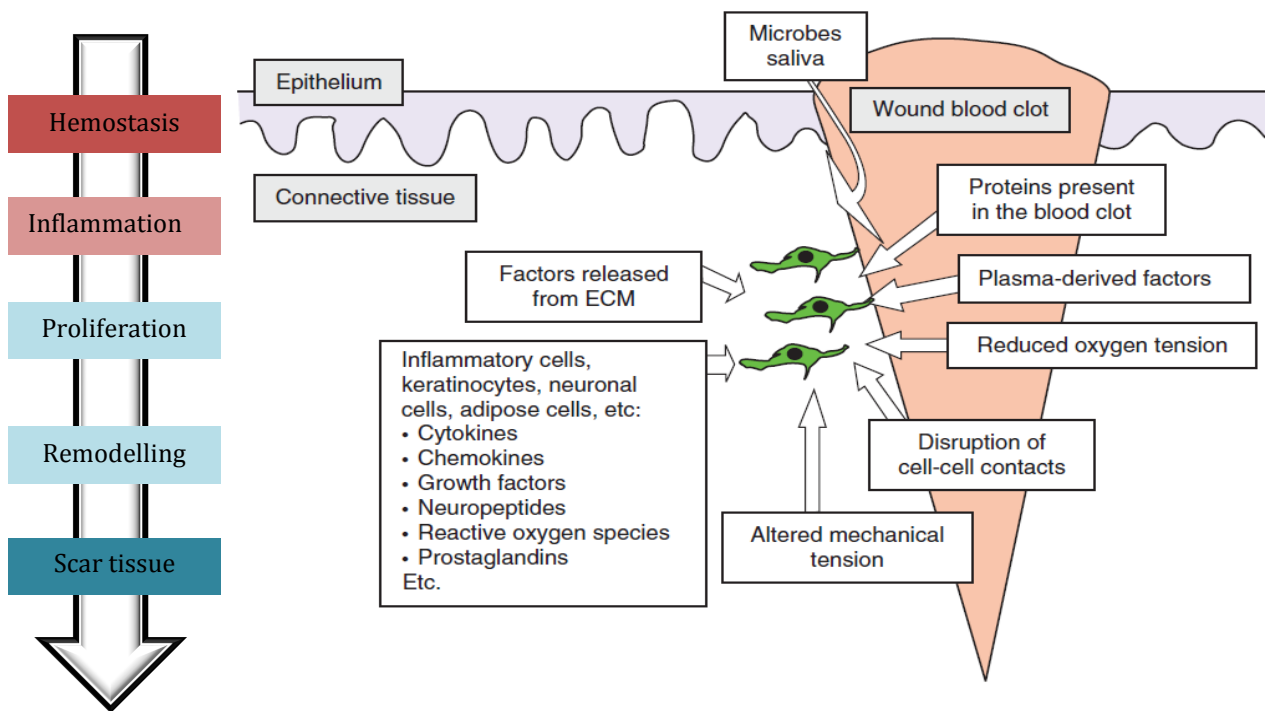


Figure 1: Main phases and factors of the wound healing process (adapted from Larjava H. 2012) ⁸

Epithelial proliferation	EGF, TGF- α , KGF, HGF
Monocyte chemotaxis	PDGF, FGF, TGF- β
Fibroblast migration	PDGF, FGF, TGF- β
Fibroblast proliferation	PDGF, EGF, FGF, TNF
Angiogenesis	VEGF, Angiopoietin, FGF
Collagen synthesis	TGF- β , PDGF
Collagenase secretion	PDGF, FGF, EGF, TNF; TGF- β inhibits

Table 1: Growth Factors and Cytokines Affecting Various Steps in Wound Healing (adapted from Kumar V. 2007) ⁹

A valuable complement which is now occasionally used in functional recovery of tissues and that plays an important role in the acceleration of the mechanisms of tissue regeneration and repair is the Platelet-rich plasma (PRP) ^{3, 4, 10}. PRP is applied in many surgical fields, such as, orthopaedics, ophthalmology, head and neck surgery, otolaryngology, cardiovascular surgery, maxillofacial surgery and healing therapies of bone, muscle, tendon and cartilage injuries^{10, 11, 12}.

PRP is a rich source of growth factors, used in many techniques in dental and oral surgery to stimulate the healing process ^{3, 10, 13}. It is defined as a high concentration of autologous platelets in a small volume of plasma, that mimics the natural ways of wound healing ^{4, 10, 14}. Platelets contribute to hemostasis by preventing blood loss at sites of vascular injury, and they contain a large number of growth factors and cytokines that have a key role in bone regeneration and soft-tissue maturation ¹⁵.

Drago L. *et al* (2013) investigated the antimicrobial properties of pure PRP and its effects on surgeries in the oral cavity, and he concluded that PRP is very useful in the reduction of postoperative infections, because it inhibits the growth of *Enterococcus faecalis*, *Candida albicans*, *Streptococcus agalactiae* and *Streptococcus oralis* ¹⁶.

Wound healing may be changed by a variety of influences, frequently reducing the quality or adequacy of the reparative process. This is the case of patients with bleeding disorders ⁹. Patients with compromised hemostasis, after oral surgery procedures have risks of additional complications, such as uncontrolled and excessive bleeding and difficulty in healing and is therefore considered the use of PRP ^{17, 18}.

Thus, it becomes essential understand how the use of platelet-rich plasma can influence and help in the healing process of these patients in order to ensure a better and faster recovery and greater treatment success.

Accordingly, this paper aims to understand the clinical applications and therapeutic relevance of the use of platelet rich plasma in the processes of tissue healing in patients with coagulopathies and know the clinical protocol of PRP for this type of patients, particularly the question of the use of autologous blood or blood from other patients with blood compatibility.

Material and Methods

For this paper, a review of scientific literature was conducted about the concept of platelet-rich plasma and the results of its application in patients with bleeding disorders. As a result, a literature search was performed in the database "Pubmed ®" and "Google Scholar", through scientific articles and specialty papers.

The following keywords were used: "*platelet-rich plasma*", "*PRP applications*", "*bleeding disorders*", "*prevention of bleeding*", "*coagulation problems*", "*and oral surgery*", "*dental medicine*".

In the survey no restriction was used due to the difficulty in fully access to all articles and the insufficiency of content, however, after a first analysis, the articles selection was limited to english and portuguese language and to the last 10 years.

After reading the abstracts and a brief analysis of the full article, the articles that presented studies in animals and articles that reference studies in anticoagulated patients, were excluded.

Thus, 46 articles were included in this work, relating to reliable and scientific clinical studies and were also used 3 books.

Discussion

PRP Generalities and Overview

Platelet-rich plasma is a volume of autologous plasma that has a high concentration of platelets, above baseline, and it has provided an advance in the acceleration and stimulation of bone and soft tissue healing, because it is a rich source of growth factors ^{3, 4, 10}. PRP is a platelet concentration with at least 1.000.000/1 L in a 5 mL volume of plasma, when normal human platelet counts in the blood range from 150.000/1 L to 350.000/1 L ¹⁰.

Platelets participate actively in the healing process of wounds. They are the first component that is present at the site of trauma and exhibit anti-inflammatory and regenerative properties. Once activated, platelets release growth factors present in alpha granules, which have an important role in the healing process. These growth factors are a group of polypeptides, which have a significant action at various stages of tissue repair, acting as regulators and stimulators of cellular processes of mitogenesis, chemotaxis, differentiation and metabolism. These factors include a number of proteins, commonly known as platelet derived growth factors ¹³. These growth factors present in PRP include PDGF (PDGF- $\alpha\alpha$, PDGF- $\beta\beta$ e PDGF- $\alpha\beta$), TGF- β (TGF- β 1 e TGF- β 2), IGF (IGF-1 e IGF-2), Vascular endothelial growth factor (A and C), FGF-2, PDEGF, HGF, PDAF, PF-4, as depicted in *Table II* ^{3, 4, 10, 11, 13, 19, 20}.

Growth factor	Source cells	Target	Biological functions
PDGF	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, macrophages/neutrophils	Stimulates chemotaxis/mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion/ collagen synthesis; stimulates macrophage/neutrophil chemotaxis
TGF-β	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells, endothelial cells, epithelial cells, preosteoblasts	Stimulates/inhibits endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis/collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis
PDEGF	Platelets, macrophages, monocytes	Fibroblasts, endothelial cells, epithelial cells	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis

PDAF	Platelets, endothelial cells	Endothelial cells	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells by direct or indirect actions; several cytokines and growth factors up-regulate PDAF, including IGF-1, TGF alpha and beta, PDGF, bFGF, PDEGF, and IL-1 beta
IGF-1	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondrocytes	Stimulates cartilage growth, bone matrix formation, and replication of preosteoblasts and osteoblasts; acts as an autocrine and paracrine factor; in combination with PDGF can enhance the rate and quality of wound healing
PF-4	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts; potent antiheparin agent

Table II: Summary of Growth Factors Released from Platelets. (Adapted from Sánchez A. 2003) ¹⁹

Because of its individualities, PRP is used in many procedures in dental and oral surgery such as ablative surgical procedures, bone grafts, mandibular reconstruction and repair of the alveolar cleft, treatment of periodontal plastic surgery and infrabony periodontal defects, even as procedures related to the placement of osseointegrated implants ^{10, 13}.

The advantages of these clinical uses of PRP are the contribution to accelerate postoperative wound healing and tissue repair and rapid revascularization and re-epithelialization. Because it is autologous, it eliminates concerns about immunogenic reactions and infectious diseases transmission ^{3, 10, 13, 15, 19}.

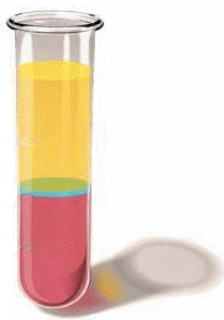
The single disadvantage of PRP is their preparation, the cost versus outcome benefit. The cost of the PRP-processing system for the clinician, and the disposable kits, compared to the uncertain success, may not justify their purchase. The cost for the patient to pay for this treatment is also high and the fact to be subjected to a venipuncture and blood drawing procedure is also a limitation ¹⁰.

PRP Preparation and formulation

Firstly, blood collection from patient is performed in tubes containing sodium citrate as anticoagulant, because this element does not modify the platelet membrane receptors and consequently preserves the platelets' integrity. Successively, centrifugation is achieved in a specifically designed centrifuge that has parameters to maximize the production of platelets and keep the plasma leucocyte free ^{4, 13}.

The device must use a double centrifugation method (*Figure 3*). The first centrifugation step (termed the hard spin) separates the red blood cells from the plasma. This plasma contains the white blood cells, the platelets, and the clotting factors. The second step (named soft spin) separates the white blood cells and the platelets together with a few red blood cells from the plasma. This last spin separates PRP from the PPP, free from the obstruction provided by a large number of red blood cells and produces the factual PRP ^{13, 19, 21}.

To attempt the production of PRP with a single spin would not produce a true PRP. A single spin in the production of PRP would not produce a true PRP, but instead, a mixture of PRP and PPP, which have low platelet counts. Regardless of the rate of centrifugation or the time of centrifugation, only one spin cannot adequately obtain a concentrate of platelets, because the red blood cells will interfere with the fine separation of the platelets ²⁰.



After centrifugation 3 typical layers can be obtained: an upper yellowish layer (plasma), which contains a gradient of platelets, a thin intermediate white layer containing leucocytes (*buffy coat*), and a lower dense layer containing the red cells (like is demonstrated in *Figure 2*) ^{4, 12}.

Figure 2: Blood physiology after centrifugation.

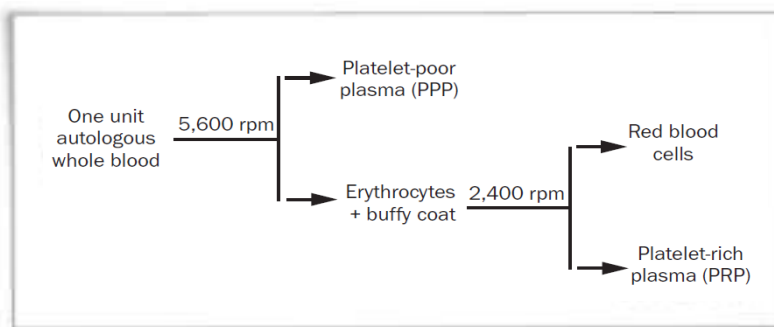


Figure 3: Centrifugations carried out in the PRP preparation (Sánchez A. 2003) ¹⁹.

Some available systems and PRP preparation methods use bovine thrombin as anticoagulant to activate the clotting mechanism and to induce platelet activation, however, some studies have associated the use of bovine thrombin with development of antibodies to human clotting factors V, XI, and thrombin, resulting in a risk of potentially life-threatening coagulopathies ^{19, 21, 22}.

From the patient's blood, four different formulations with therapeutic potential can be obtained: a 3-dimensional scaffold, the liquid formulation, the supernatant and the elastic and dense autologous fibrin membrane (*Figure 4*) ⁴. Accordingly, PRP may be mixed into a bone graft, sprayed on a soft tissue surface, layered in as the graft is placed, or used as a biological membrane ^{4, 15}.



Figure 4: Different types of PRP presentations and formulations. Adapted from Anitua E, et al. 2012 (Without author permission) ⁴

Normal Hemostasis

Under normal conditions, hemostasis (or the arrest of bleeding) occurs through 2 independent, but linked processes: the coagulation cascade and the platelet activation pathway²³.

The first step in hemostasis process is the immediate constriction of damaged vessels, which temporarily reduces the flow and pressure within the vessel, aiding in the formation of a platelet plug. Then, there is a mechanical blockage of the orifice by the platelet plug. The platelets bind to exposed collagen and become activated by releasing cytokines in the area around the lesion. Other platelet factors reinforce local vasoconstriction and further activate platelets, which are grouped with each other. Subsequently, the exposed collagen and the factors present in tissues, initiate a series of reactions known as the coagulation cascade (Figure 5)²³.

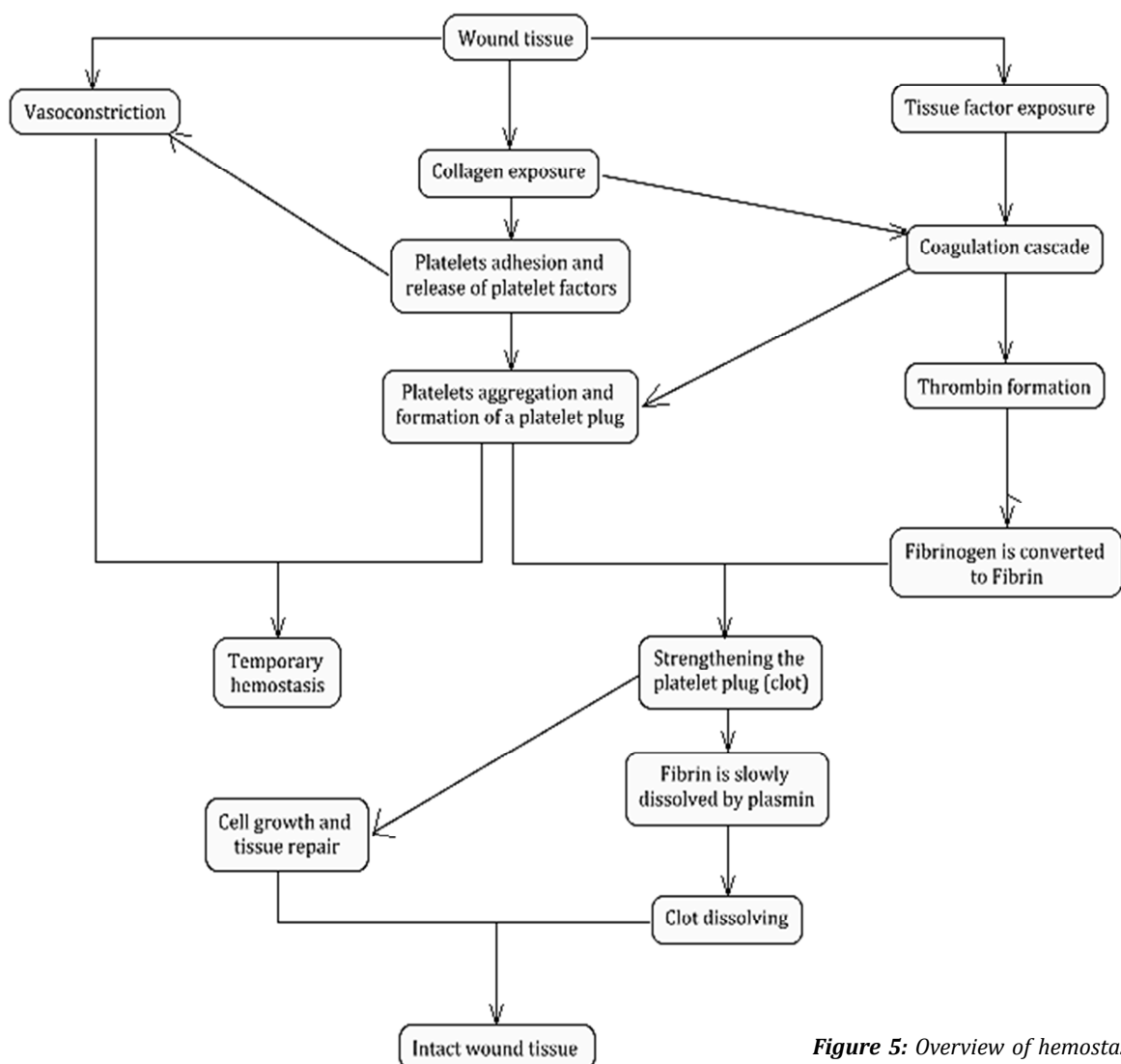


Figure 5: Overview of hemostasis and tissue repair.

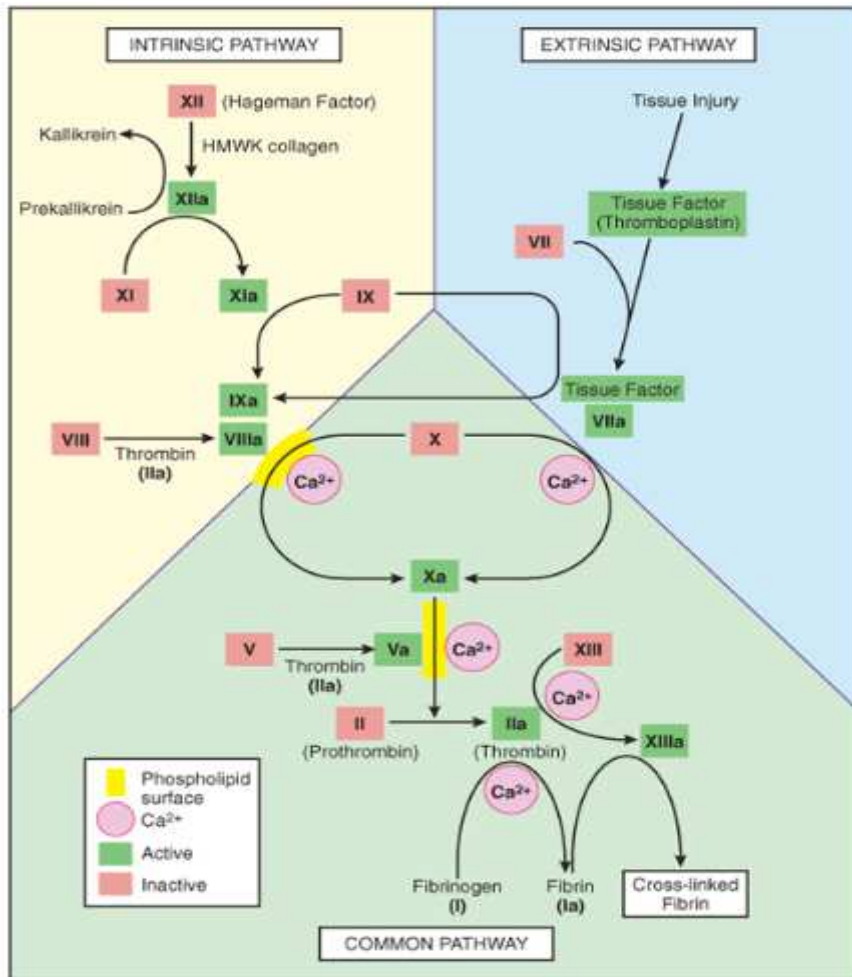


Figure 6: Coagulation cascade: the intrinsic, extrinsic and common pathways (Kumar V. 2007) ⁹

The coagulation cascade (Figure 6) is a sequential series of enzymatic reactions that ends with the formation of a network of elastic fibers which stabilizes and strengthens the platelet plug and turns it into hemostatic plug; it is the conversion of a soluble plasma protein (fibrinogen) into an insoluble polymer (fibrin). Some chemical factors involved also contribute to adhesion and aggregation of platelets in the damaged region. Coagulation is divided into two pathways: the intrinsic pathway (dependent on contact activation by a negatively-charged surface, and involving coagulation factors XII, XI, IX, VIII and V), and the extrinsic pathway (dependent on tissue-factor being exposed to the circulation, and involving tissue factor and factor VII), that converge on a common pathway to activate factor X, leading to conversion of prothrombin (factor II) to thrombin (factor IIa) and culminating in the conversion of fibrinogen to fibrin, which becomes part of the clot. At each step, an enzyme converting an inactive precursor into an active enzyme often with the aid of Ca^{2+} , membrane phospholipids or additional factors ^{9, 24}.

Pathologic Hemostasis

Bleeding disorders

Patients with blood coagulation disorders have more predispositions to excessive bleeding or thrombosis, and because of that, they become a serious challenge in the dental practice ²⁵.

Bleeding disorders can be classified as coagulation factor deficiencies, platelet disorders, vascular disorders or fibrinolytic defects (*Table III*). The most common are haemophilia A, haemophilia B and von Willebrand's disease ²⁶.

Coagulation factor deficiencies	<u>Congenital</u> Haemophilia A and B Von Willebrand's disease Other factor deficiencies (rare) <u>Acquired</u> Liver disease Vitamin K deficiency, warfarin use Disseminated intravascular coagulation
Platelet disorders	Quantitative disorder (thrombocytopenia) <u>Immune-mediated</u> Idiopathic Drug-induced Collagen vascular disease Sarcoidosis <u>Non-immune-mediated</u> Disseminated intravascular coagulation Microangiopathic hemolytic anemia Leukemia Myelofibrosis Qualitative disorder <u>Congenital</u> Glanzmann thrombasthenia von Willebrand's disease <u>Acquired</u> Drug-induced Liver disease Alcoholism
Vascular disorders	Scurvy Purpura Hereditary hemorrhagic telangiectasia Cushing syndrome Ehlers-Danlos syndrome
Fibrinolytic defects	Streptokinase therapy Disseminated intravascular coagulation

Table III: Common bleeding disorder (Gupta A, et al. 2007) ²⁶.

Haemophilia A (factor VIII deficiency) is the most common hereditary coagulation disorder ²⁵, associated with serious bleeding and accounts for about 85% of haemophilia patients. It has an incidence of 1:5.000 in the male population. It is a sex-linked disorder. This X-linked recessive disorder is caused by a reduced amount of factor VIII. Approximately 30% of cases are caused by new genetic mutations and therefore do not have a family history ²⁷.

Can be classified as severe (less than 1% of normal factor VIII activity), moderate (1-5% of normal activity), or mild (5-25% of normal activity) ²⁵ and prolonged bleeding following a dental extraction can sometimes be the first sign of mild disease. Haemophilia is usually asymptomatic, although bleeding following minor trauma can be excessive ²⁷.

Factor VIII assay is required for diagnosis. These patients will have a normal bleeding time, normal platelet count and normal prothrombin time (PT), but a prolonged partial thromboplastin time (PTT) ²⁷.

Haemophilia B (factor IX deficiency) is much less common than haemophilia A; however, the clinical picture is very similar, with a prolongation of activated Partial Thromboplastin Time (aPTT). Some patients present an abnormal factor IX that slightly prolongs PT ²⁵. Is inherited as X-linked recessive trait and results in reduced levels of factor IX. Account for approximately 15% of haemophilia patients. It has an incidence of 1:15.000 in the male population. This disorder is categorised as mild (factor IX levels >5%), moderate (1 -5%), or severe (<1 %) ²⁷.

There are 3 types of haemophilia B ²⁸:

- Type 1 - Dominant Symptoms (tend to be mild)
- Type 2 - Dominant Symptoms (tend to be mild)
- Type 3 - Recessive Symptoms (tend to be severe)

Von Willebrand's disease is a hereditary coagulation disorder characterized by a deficient or abnormal plasma protein known as the Von Willebrand Factor (vWF), whose physiological role is to stabilize Factor VIII and mediate platelet adherence. Thus, Factor VIII levels are low ^{17, 25}. Increased easy bruising, epistaxis and significant oral surgical bleeding are the most common manifestations of the disease ¹⁷. The bleeding associated with the disease is of variable intensity.

The disorder is classified as follows:

Type 1	Quantitative alterations of Von Willebrand factor: vWF concentration is below normal, the bleeding time and aPTT are prolonged, and F VIII concentrations are low ²⁵ . Partial deficiency (80% of those with vWD) ²³ .
Type 2	Qualitative alterations of Von Willebrand factor: vWF concentration is not below normal, but the factor is unable to stabilize F VIII and aggregate platelets, and thus bleeding time and aPTT are prolonged, and F VIII concentrations are low ^{23, 25} .
A	Decreased function; absent medium and high-molecular-weight multimers ²³
B	Increased affinity for platelet GPIb; decreased high-molecular-weight multimers ²³
M	Decreased function; variable multimer pattern ²³
N	Decreased affinity for FVIII
Type 3	Severe deficiency of the total protein complex ^{17, 23} ; autosomal recessive

Table IV: Classification of von Willebrand disease (Adapted from Israels S, et al. 2006)²³.

Glanzmann thrombasthenia (GT) is a rare autosomal recessive bleeding disorder. Platelets from these patients fail to aggregate due to quantitative or qualitative defects of the integrin, $\alpha\text{IIb}\beta\text{3}$. In normal haemostasis, $\alpha\text{IIb}\beta\text{3}$ on activated platelets binds the adhesive proteins which form the protein bridges that link platelets during aggregation. Although GT platelets are able to adhere via other receptors, such as GPIb/IX/V complex, platelet spreading is defective in the absence of functional glycoprotein IIb/IIIa ($\alpha\text{IIb}\beta\text{3}$). As a consequence, the damaged vessel wall cannot be efficiently sealed, resulting in a bleeding phenotype. The platelet count and morphology are normal, the bleeding time is prolonged, and *in vitro* platelet aggregation is defective in response to various physiological platelet agonists²⁹.

Platelet-rich plasma in Oral Surgery

Many studies have evaluated the use of PRP in the field of Dentistry, especially in small bone grafts in the alveolar region for future dental implants and in periodontal and maxillofacial surgery¹³.

Alissa *et al.* (2010) conducted a pilot study on the effect of PRP on the healing of the hard and soft tissues of extraction sockets and concluded that PRP may have additional benefits in reducing healing complications, because soft tissue healing was significantly improved in patients treated with PRP compared with patients that were not treated with PRP. Moreover, patients untreated with PRP experienced complications (dry sockets and alveolar osteitis), which were considered to be borderline statistically significant³⁰.

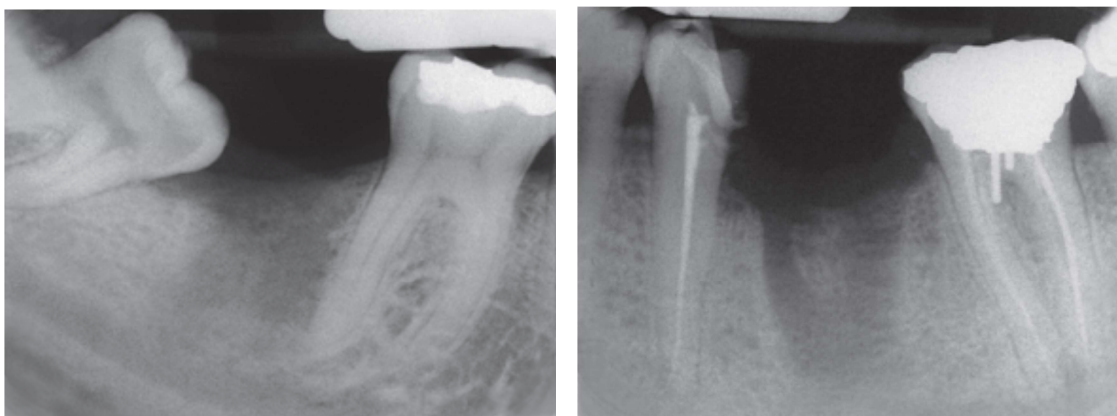


Figure 7 – Periapical radiographs of PRP use (a) and control sockets, with no PRP use (b) at 12 weeks after tooth extraction. Adapted from: Alissa R, *et al.* (2010) (Without author permission)³⁰.

The results of the combination of PRP with bone grafts have demonstrated more rapid consolidation and a mineralization of the graft in 50% of the time required, and an increase from 15 to 30% of the density of the trabecular bone and the efficiency of growth factors released by platelets occurs from the beginning of the surgery, contributing to bone formation from the first stage, determining the acceleration of tissue repair and bone producing a higher density¹³.

Dusse *et al.* (2008) and Sánchez *et al.* (2003) cited a clinical study from Anitua, where twenty patients undergoing dental extraction prior to implant placement were evaluated. Anitua found that the alveolar sockets treated with PRP showed greater buccal-lingual bone thickness and better epithelialization than the group that did not receive PRP (control group). This author reported the use of PRP in other 250 patients with evidence of clinical success^{13,19}.

The same authors also mentioned Marx and Garg that suggested the use of PRP in cases with less chance of success in osseointegration and bone grafts, like in edentulism (severely resorbed jaw), in patients with osteoporosis, and in cases of dental disease with subsequent changes in nearby tissues ^{13,19}.

Although there are still controversies about the benefits of PRP in bone regeneration, Daif (2012) reported that the direct application of PRP along the fracture lines can be regarded as a promising method for improving bone regeneration in mandibular fractures ³¹.

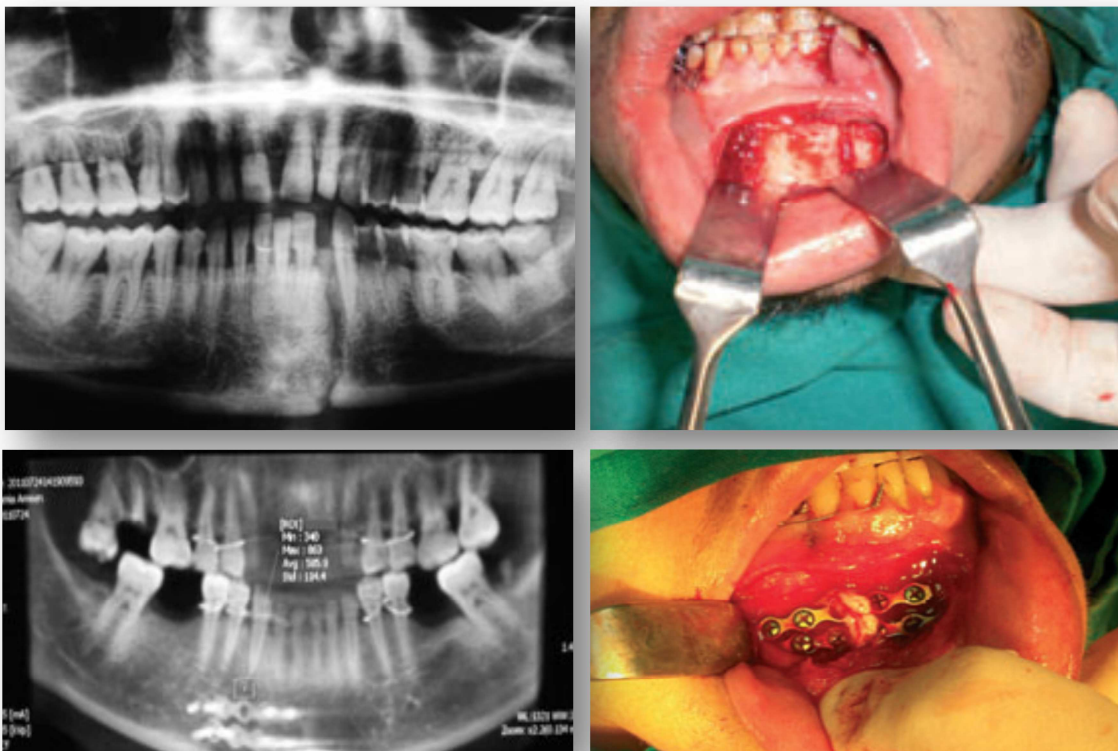


Figure 8 – Fracture line of the mandible treated with PRP. Adapted from: Daif *et al.* (2012) (Without author permission) ³¹.

Sánchez *et al.* (2003) also considered scientific evidence the use of PRP in combination with bone grafts in reconstructive surgery, noting the unanimous opinion of acceleration and improve of quality of the regenerated bone ¹⁹.

A clinical study on immediate implant placement in severely resorbed maxillae using xenogenous and PRP products revealed an undoubted success ¹³.

Dusse *et al.* (2008) mentioned Wojtowick *et al.*, that observed the effects of the combination of PRP with xenogenous material, suggested that genetic mechanisms influence the organization of the trabecular pattern of the

regenerated bone tissue, probably under the influence of growth factors released by platelets¹³.

This author also avowed that a higher platelet count in PRP, after double centrifugation of the sample, reinforces the idea that different laboratory procedures for obtaining PRP may interfere decisively in the success of this preparation, minimizing the beneficial effect expected¹³.

Albanese *et al.* (2013) cited Gentile *et al.* study, where they emphasized the efficacy of the PRP treatment, post-operative patient's satisfaction and advantages of faster healing on 15 cases, comprising reconstructive surgery of the jaw, oral implantology and post-extraction alveolar bone regeneration¹⁰.

Por and co-workers (2009) showed in a meta-analysis about the use of tissue sealants in face-lifts that exists a strong trends towards the reduction of wound drainage and postoperative ecchymosis among patients who had used tissue sealants, such as PRP³².

Jadhav *et al.* (2013), presented 3 cases, where compare the clinical and radiographic treatment outcome of revascularization with and without PRP in immature, non-vital anterior teeth and found that PRP supplement improve the outcome of revascularization³³.

Sammartino *et al.* (2005) showed that PRP is effective to induce bone regeneration and acceleration for the treatment of periodontal defects on the distal root of the second molar, after the surgical removal of an impacted mandibular third molar, with good results both clinically and histologically. Due to its content of fibrin, PRP allows stabilizing the blood coagulation, thereby promoting the regeneration of bone defects, particularly in the early stages³⁴.

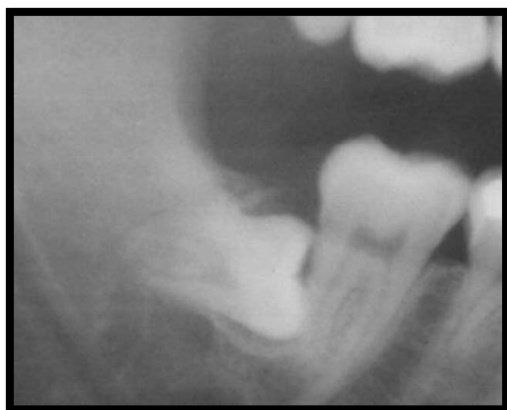


Figure 9 – Pre-operative X-ray showing the inferior third molar deeply impacted. Adapted from: Sammartino *et al.* (2005) (Without author permission)⁴⁵.

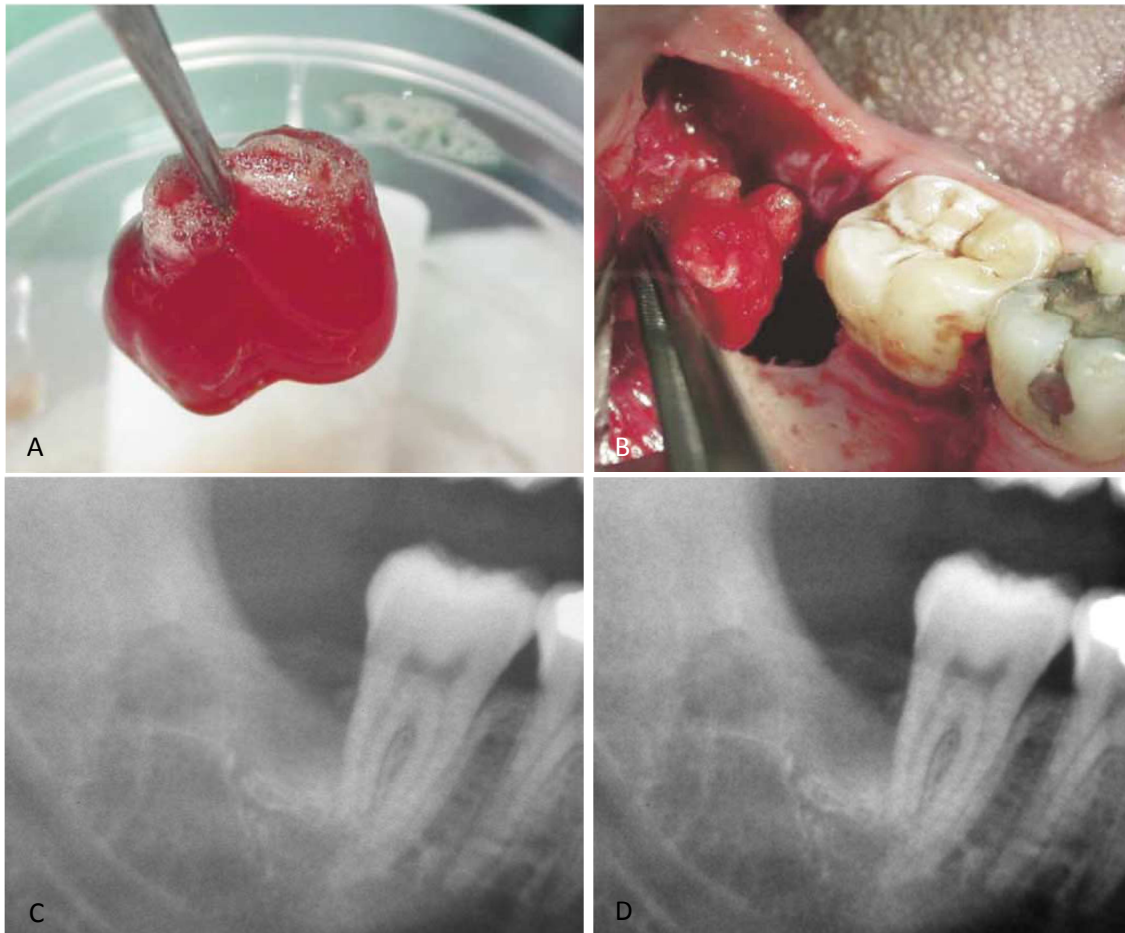


Figure 10 – (A) Preparation of the PRP gel. (B) The PRP gel is inserted into the post-extractive bone defect. (C) X-ray showing bone regeneration at 12 weeks from the intervention. (D) X-ray showing the conserved osseous level 18 weeks after the intervention. Adapted from: Sammartino et al. (2005) (Without author permission) ³⁴.

Sánchez et al (2011), showed significant benefits and a success in over 90% of patients in a study, where it was used PRP mixed with autologous bone graft for the closure of palatal fistulas by local mucoperiosteal flaps ³⁵.

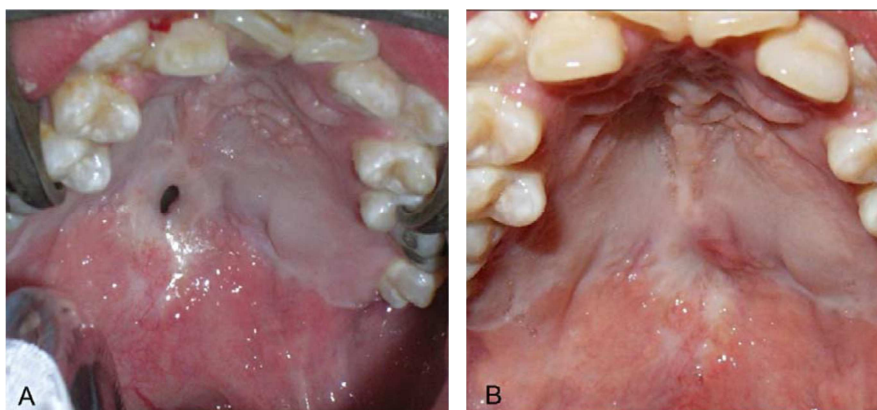


Figure 11 – (A) Nasopalatine fistula (Pre-operative). (B) Post-operative after 4 months from the surgery. Adapted from: Sánchez et al. (2011) (Without author permission) ³⁵.

Even though numerous studies confirm the PRP efficiency in the bone repair acceleration, Choi *et al.* (2004) in their research does not show any benefit in its use or even report the faster bone healing³⁶.

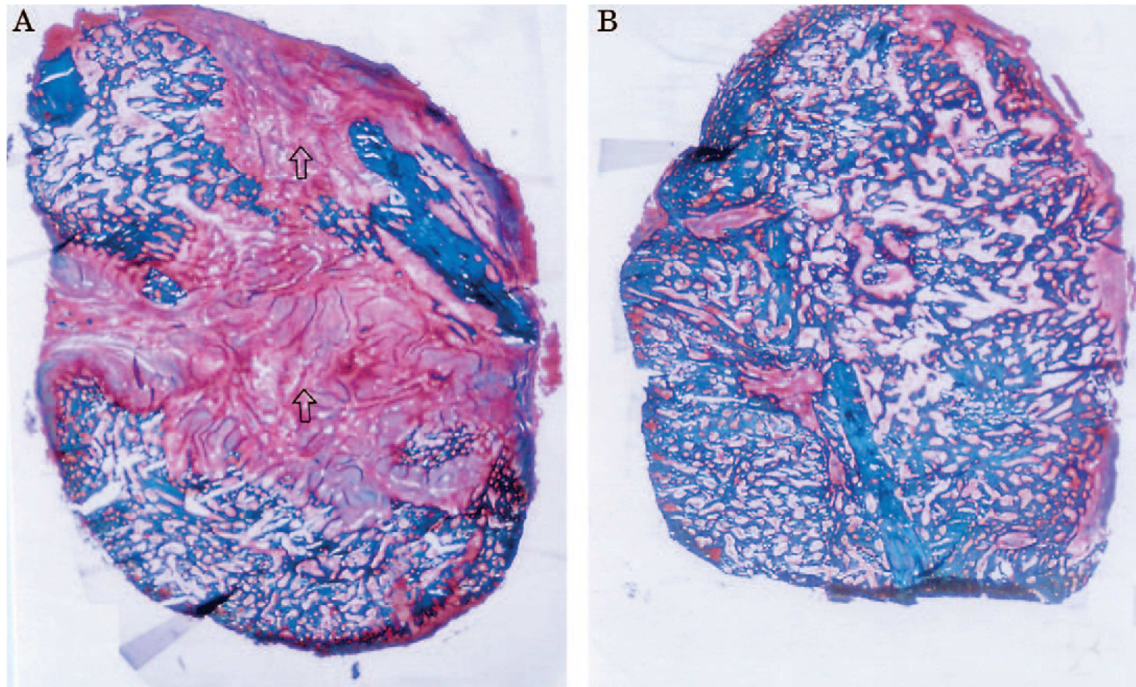


Figure 12 – Frontal sections through the middle of defects. Large islands of connective tissue (arrowed) were seen throughout the defect in the PRP group (A), whereas the non-PRP group (B) showed extensive bone formation throughout the defect. Adapted from: Choi *et al.* (2004) (Without author permission)³⁶.

Likewise, Albanese *et al.* (2013) mentioned Esposito, that said that treatments using PRP in sinus lift procedures did not seem to improve the clinical outcome and quoted a review of Khairy *et al.*, that showed that enrichment with PRP did not significantly improve bone density in sinus which had been augmented with autogenous bone with PRP, compared to those without PRP¹⁰.

Arenaz-Bua *et al.* (2010), in a prospective study about the efficacy of PRP in promoting bone regeneration after third molar extraction, have not observed that PRP accelerates bone formation in post-extraction sockets, but established that PRP mixed with other biomaterials simplifies the manipulation of the graft and for that reason could be useful as a biological carrier in mandibular bone reconstruction³⁷.

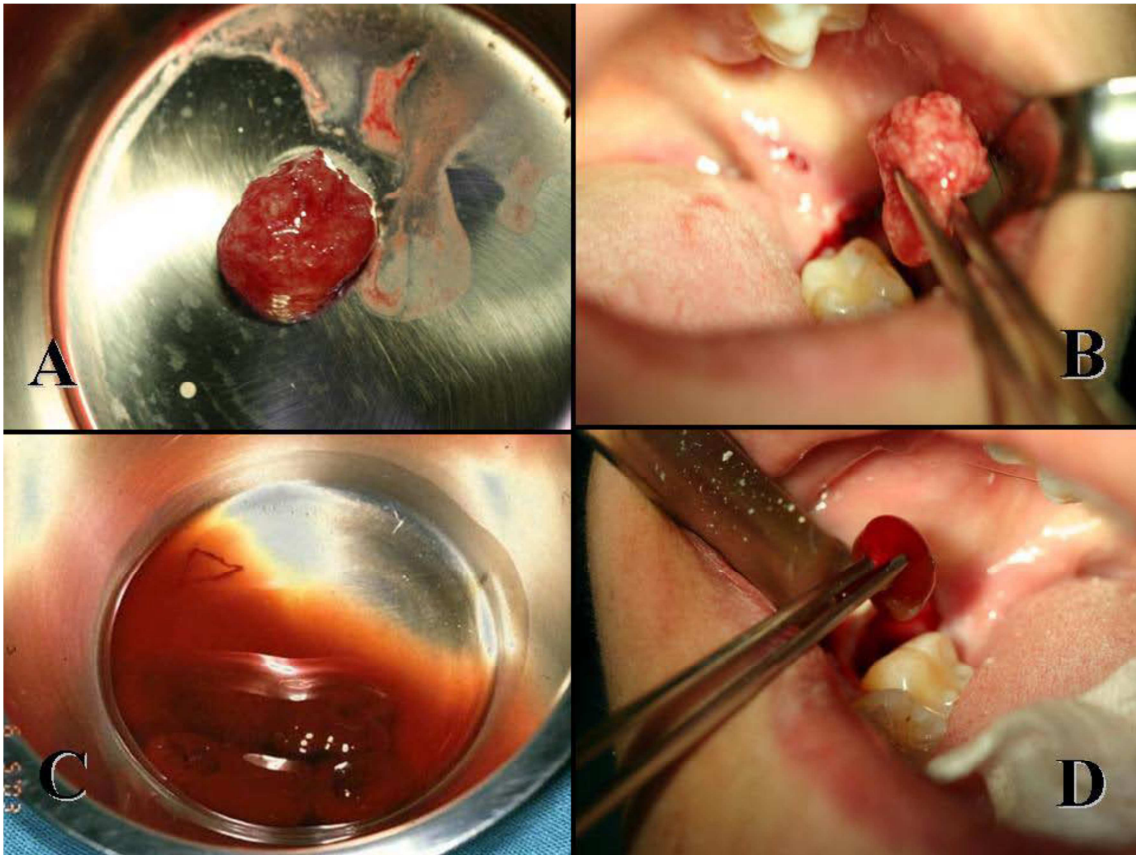


Figure 13 – A mixture of autologous bone collected by filtration during the osteotomy and platelet-rich plasma is obtained (A) and applied in the postextraction socket (B). Note that PRP facilitates the manipulation of the particulate bone graft. The rest of the platelet-rich plasma obtained by the same method as above (C) is applied alone in the socket of the control side (D). Adapted from: Arenaz-Bua et al (2010) (Without author permission) ³⁷.

Anitua *et al.* (2008), in a study of BTI implants used in conjunction with PRP, with up to 5 years of follow-up, reported a 99.2% overall implant survival rates for 5.787 implants placed in 1.060 patients. The implants failures occurred in one half of the first year of loading, and in 70% of patients that had presented chronic or aggressive periodontitis ³⁸.

Posteriorly, Fabbro *et al.* (2009) studied the combination of PRP and immediate post-extraction implant placement and found excellent clinical results and high success rate in preservation of hard and soft tissues ³⁹.

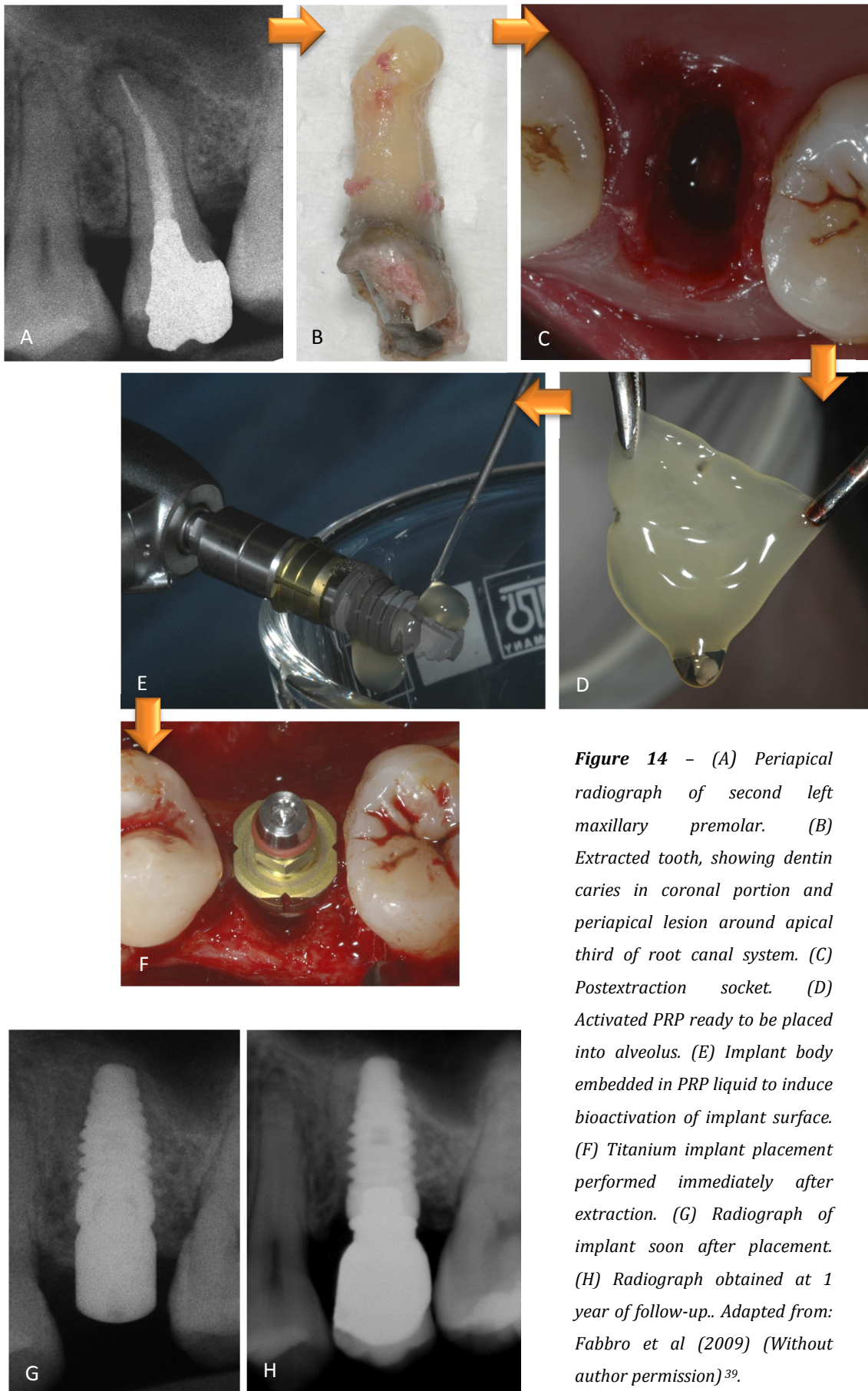


Figure 14 - (A) Periapical radiograph of second left maxillary premolar. (B) Extracted tooth, showing dentin caries in coronal portion and periapical lesion around apical third of root canal system. (C) Postextraction socket. (D) Activated PRP ready to be placed into alveolus. (E) Implant body embedded in PRP liquid to induce bioactivation of implant surface. (F) Titanium implant placement performed immediately after extraction. (G) Radiograph of implant soon after placement. (H) Radiograph obtained at 1 year of follow-up.. Adapted from: Fabbro et al (2009) (Without author permission)³⁹.

Platelet-rich plasma in patients with bleeding disorders

The incidence of postoperative bleeding after minor oral surgical procedures in patients with appropriate haemostasis is given with values between 0.2% and 3.3%, while in chronically anticoagulated patients ranges between 8.6% and 32.1%¹⁸.

Use of autologous platelet-rich plasma has been reported in many situations in dentistry and oral surgery and implantology, for the stimulation and acceleration of tissue healing and bone regeneration⁴⁰.

So what about using PRP in patients with bleeding disorders?

There are a small number of studies and patients that can answer this question but Nurden *et al* (2011) published a clinical study where they use autologous platelet-rich clots for preventing local injury bleeding in patients with bleeding disorders⁴⁰.

Four patients with a moderate-to-severe inherited bleeding syndrome were enrolled in the study (only three of them for doing oral surgeries) and all of them received 50mg/Kg of an antifibrinolytic agent, tranexamic acid (Exacyl, Sanofi-Aventis, Paris,France), for four consecutive days starting the day before surgery. The clots were prepared from the patient's own platelet-rich plasma after the addition of Ca²⁺ and placed on the injured site immediately after surgery⁴⁰.

The first patient (a 60-year old woman), diagnosed with VWD type 2B at the time of study, had a platelet count of 47 000 platelets μL^{-1} , with giant platelets and some small aggregates. It was performed a single extraction of the tooth 36 with alveolectomy under local anaesthesia (the cartridge also contained epinephrine)⁴⁰.

The second patient (a 22- year old woman) also diagnosed with VWD, had a platelet count of 98 000 platelets μL^{-1} , with giant platelets. She was submitted to extraction of three wisdom teeth (positions 18, 28 and 38) under general anaesthesia⁴⁰.

The other patient is a 60-year old man, with type 1 GT, with no platelet aggregation, and less than 5% expression of the $\alpha\text{IIb}\beta\text{3}$ integrin on his platelets. His platelet count was normal, but platelet aggregation was absent. Extraction of two premolars and one molar was performed under local anaesthesia during his first visit and two molars were extracted during a second visit, two weeks later⁴⁰.

Paracetamol was given for pain management for all patients and non-steroidal anti-inflammatory drugs were avoided ⁴⁰.

For all patients, the platelet-rich clot formed normally; the autologous clots formed after 20–30 minutes and were carefully placed into the cavity immediately after tooth extraction and held in place by closing and suturing the lesion ⁴⁰.

In the first patient no bleeding was observed at the site of her lesion following the application of her platelet-rich clot. Wound healing and bone formation occurred normally in the following weeks. For the second patient, the untoward bleeding was prevented and no vWF or platelet transfusion was required. In the last one, after tooth removal with local anaesthesia, some bleeding occurred at the points of needle puncture and during extraction, but as soon as the clots were placed in the dental cavity, bleeding stopped and did not restart during the following days ⁴⁰.

Management of patients with bleeding disorders in dental procedures

Hemorrhagic diseases have a major impact on the proper evaluation of medical and dental needs and individuality of each patient, so before any treatment, especially if it is a surgical procedure, the clinical history should be well-detailed and patients should be consulted about any spontaneous bleeding, episodes of previous unusual bleeding after injuries or surgeries, and easy or recurrent bruising ^{25,26}.

The management and treatment of these patients depends on the severity of the bleeding disorder condition and the level of invasion of the planned dental procedure. The main objective is to decrease and minimize the patient's risk by restoring the hemostatic system to acceptable levels and sustain hemostasis by local and adjunctive methods ²⁶.

So the dentist should take into consideration the following aspects ²⁵:

- The type of bleeding disorder involved;
- The greatest dental treatment according to the disease;
- Evaluate whether the standard treatment must be modified due to the requirements of dental treatment;
- Evaluate which medication the patient is taking;
- Evaluate the contraindicated medications;
- Assess the half-life of the transfused factor and plan the procedure according to the values obtained;
- Assess of whether conversion to heparin with INR control is required if the patient is receiving oral anticoagulation.

To know which systemic therapy is the best suited for dental procedures, one should consult the patient's hematologist, because decisions about the type and the need for replacement of coagulation factor depend on the specific hemostatic diagnosis, the severity of bleeding and the type of dental procedure which will be performed (more or less invasive). *Table V* shows the replace systemic therapy options for some patients. More severe disorders will require measures to temporarily increase the platelet count or improve its function ²³.

Bleeding disorder	Pre-treatment for extraction or nerve block	Post-extraction treatment for all bleeding disorders
vWD, Type 1	Desmopressin, 0.3 µg/kg (maximum dose 20 µg) IV over 20–30 minutes or subcutaneously	Antifibrinolytic agents (e.g., tranexamic acid, 25 mg/kg, 3 times a day for 3–5 days Soft diet for 7 days Within 24 hours, assess need for repeat treatment
vWD, Types 2A and 2M	Desmopressin, as above, if tested response or Humate-P, 50 IU of vWF:RCoF/kg	
vWD, Types 2B and 3	Humate-P, 50 IU of vWF:RCoF/kg	
Haemophilia A (mild)	Desmopressin, as above, if tested response is adequate	
Haemophilia A (moderate or severe)	Recombinant Factor VIII concentrate, 20–25 IU/kg Re-evaluate post-procedure	
Haemophilia B (mild, moderate or severe)	Recombinant Factor IX concentrate, 40–60 IU/kg Re-evaluate post-procedure	

Table V: Management of patients with coagulation factor deficiencies, who require dental extraction or complex procedures (Adapted from Israels S, et al. 2006)²⁴.

The INR (International Normalized Ratio) is recommended to be monitored and measure of the extrinsic pathway of coagulation in the 24 hours before the dental procedure. It is calculated using the ratio of the prothrombin time (PT) of the patient and the mean PT of a plasma with normal reference values, corrected by the International Sensitivity Index (ISI) of the reagent used, which correlates the sensitivity of commercial thromboplastins with a reference one. It can be obtained by the formula $INR = (PTR)^{ISI}$, where the PTR is the PT of the patient divided by the plasma with the reference value^{41, 42}.

To Aframian D. *et al* (2007), patients who have an unstable INR or INR >3.5 should be referred to their physician or hematologist for dose adjustment before invasive dental procedures⁴¹ and Pototski M. *et al* (2007) consider that patients with INR above 4.0, shall not be subject to any surgical procedure without consulting the clinician responsible for the maintenance of their therapeutic⁴³.

In a study, Bajkin B. *et al* (2009) demonstrated that minor surgical procedures such as tooth extractions, are safe to perform in hipocoagulated patients when the INR<4.0, using local hemostatic measures⁴⁴. Flap surgeries, implant placement and apicoectomies are not recommended in patients with an INR between 3.0-4.0⁴⁵.

Dental management of patients with bleeding disorders using PRP

For patients with bleeding disorders, the surgical procedures are always a source of difficulties, due to the risk of prolonged bleeding, such as dental extractions. Thus, the new technique based on the use of autologous platelet-rich clots (PRP), which act as both a physical barrier to blood loss as a source of growth factors promotes wound healing and reduces inflammation, because platelets activated, which contain large quantities of stored protein, stimulate cell proliferation and differentiation ⁴⁰.

If necessary, the patient's hematologist should be consulted before planning the surgery, and patients with severe disease should be treated in specialized centers. Local hemostatic agents and techniques, such as pressure and surgical sutures, can be used individually or in combination. It is necessary to be careful with the use of vasoconstrictors, because of the risk of rebound vasodilatation, which may increase late bleeding risk. The INR should be measured before some surgical procedures, and the normal range is between 2.0 and 3.0 ²⁶.

PRP has the inconvenience that must be done freshly soon after blood extraction ⁴⁶. Marx (2004) believed that true PRP is always autologous and is not homologous. The use of lyophilized donor platelets, homologous platelets, is not viable and does not secrete bioactive growth factors. The platelets are also antigenic due to their abundance of cell membranes and, therefore, anti-platelet antibodies could develop from this reaction product and initiate second set reactions ⁴⁷.

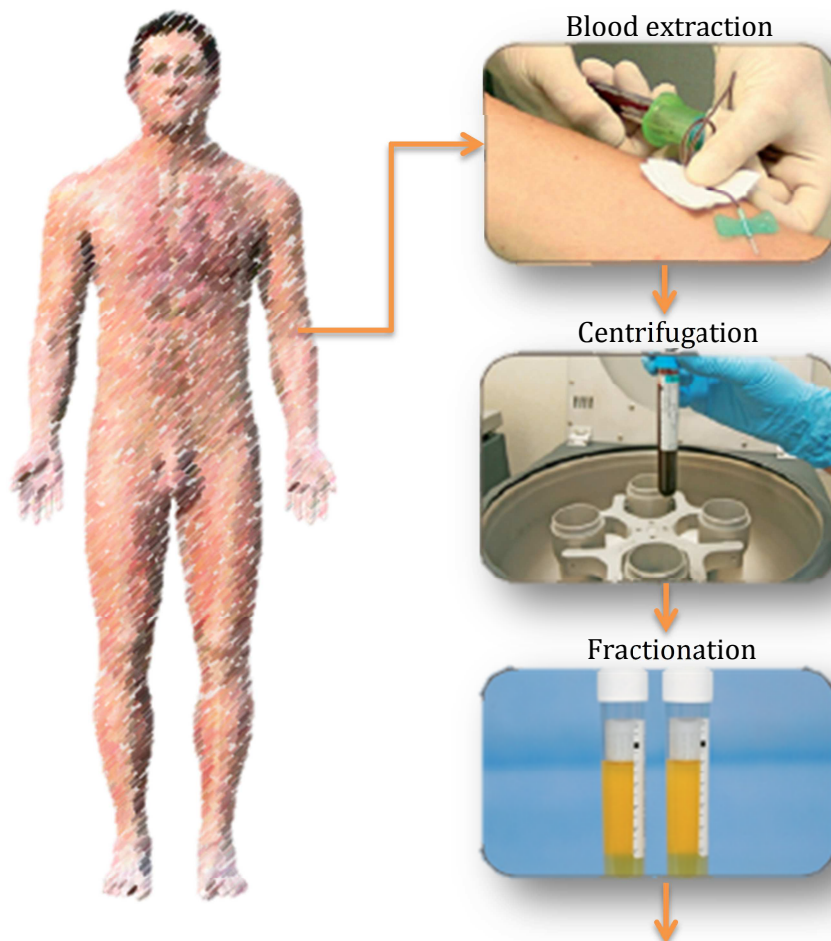
However, a study performed by Vila V. *et al* (2013), about the influence of frozen plasma on the thrombin generation assay (TGA - the platelet absence causes a significant decrease in TGA), and using patients with severe haemophilia and with severe bleeding phenotype, showed that in PRP there were no differences in the results obtained from fresh or frozen samples. This result is in agreement with a previous report, which showed that freezing does not influence either the endogenous thrombin potential or the thrombin peak in PRP from healthy individuals. As PRP freezing did not affect the TGA, this fact opens the possibility of using frozen PRP to facilitate the clinical procedures in the workplace ⁴⁶.

With all the information, it may be speculated and set a protocol of care and management of patients with coagulation disorders, using PRP as an aid in

controlling bleeding after minimally invasive dental surgeries, like in dental extractions (*Scheme 1*).

Experimental Protocol (*Scheme 1*)

If necessary, and in agreement with the patient's haematologist, it can be recommended the antifibrinolytic agent tranexamic acid ^{40, 48} (given orally 500mg, 3 times a day for 7 days). Local anesthesia can be administered with vasoconstrictor (example: infiltration of 2% lidocaine with 1:80.000 epinephrine) ⁴⁸.



PRP formulations can be used in oral surgery procedures

Local measures for haemorrhage, include minimally traumatic surgical technique; sectioning of difficult teeth, limiting the total number of dental extractions in one appointment; use of local haemostatic agents, use of sutures and use of local pressure with gauze packs can be performed ⁴⁸.

Conclusions

Platelet-rich Plasma is a technique that has been proposed and used for several practises in dental and oral surgery.

Many clinical cases have been reported in literature with high success rates, and with better results, when PRP is used to control postoperative bleeding and improve and accelerate the healing process and tissue repair.

To establish the real benefit to the use of such preparations in dentistry and in other clinical areas, highly standardized and reliable methods for obtaining PRP should be used and developed by qualified professionals. Not only the number of platelet preparations should be observed, but also qualitative aspects, that is, the function of platelets. A considerable number of platelets, but with compromised viability, certainly will not provide the desired effect, because the growth factors are crucial to the success of preparation, and may have already been eliminated as a result of improper procedures.

The prospects of using PRP, as well as the need for optimization of their preparation procedure, open new opportunities for professionals, especially in the field of hematology. In this work it was described a standardized and simple protocol that can be used for minor surgeries in Dentistry, for patients with bleeding disorders, using PRP to improve the quality and accomplishment of the treatments. The benefits include not only decreased bleeding, but also added security that will encourage patients to seek early help for painful local procedures.

The PRP is defined as produced from autologous blood, however, in bleeding disorders, platelet function is modified (either by deficiencies in clotting factors or lack of adherence of platelets to subendothelium), so the question that arises is: PRP should be used from the patient own blood or from a donor with blood compatibility? In the only study ever conducted and published, referenced in this work, PRP was obtained from patient's own blood, nevertheless, studies on the properties and benefits of using PRP fresh/frozen (using a donor) showed no significant differences. Then, it can be concluded that both types of obtaining PRP (either autologous blood or compatible donor) can be used in such patients, not forgetting that own blood has its advantages, especially in immunological reactions

and communicable diseases. However, the small number of studies and patients contributed to a lack of more true and correct statistical evidences.

A new generation of platelet concentrates with fibrin, PRF (Platelet-rich Fibrin), have been described in some studies to have higher success rate of healing compared to PRP results, because for their preparation, that requires neither anticoagulant nor bovine thrombin, or any other gelling agent. The absence of anticoagulant activates rapidly, most of the platelets from the blood sample in contact with the tube wall and starts the coagulation cascade. The fibrinogen is transformed into fibrin by circulating thrombin and a fibrin clot is formed, between acellular plasma and red blood cells in the tube ⁴⁹.

Notwithstanding, for patients with bleeding disorders, it is assumed that PRP is better, because due to coagulation and platelet adhesion disorders, the use of gelling agents, will promote more platelet counts in the end of the procedure of obtaining PRP.

As a result it is required further studies, with larger sample sizes, to support the use of PRP in Dentistry current practice.

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ANNEXES

DECLARAÇÃO

Monografia de Investigação/Relatório de Atividade Clínica

Declaro que o presente trabalho, no âmbito da Monografia de Investigação/Relatório de Atividade Clínica, integrado no MIMD, da FMDUP, é da minha autoria e todas as fontes foram devidamente referenciadas.

29 / 05 / 2014

Joana Moutinho
O / A investigador(a)

