AUTOLOGOUS PLATELET CONCENTRATES

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LIST OF ABBREVIATIONS

ACD-A	Anticoagulant citrate dextrose-A
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ABBM	Anorganic bovine bone mineral
ADM	Acellular dermal matrix
A-PRF	Advanced platelet-rich fibrin
BMP	Bone morphogenic proteins
BMMC	Bone marrow mononuclear cell
BMA	Bone marrow aspirate
b-TCP	beta Tricalcium phosphate
Ca	Calcium
$Cacl_2$	Calcium chloride
CAL	Clinical attachment level
CFU-M	Colony forming unit-megakaryocyte
c-PRP	Concentrated platelet rich plasma
CPD	Citrate Phosphate Dextrose
CAF	Coronally advanced flap
CPT	Coronally positioned tunnel
CTG	Connective tissue graft
DFDBA	Demineralized freeze-dried bone allograft
EGF	Epithelial growth factor
ePTFE	expanded polytetrafluoroethylene
FGF	Fibroblast growth factor
FS	Fibrin Sealants
FDBA	Freeze-dried bone allograft
GFs	Growth factors
GTH	Gingival thickness
GTR	Guided tissue regeneration
HIV	Human immunodeficiency virus

HA	Hydroxyapatite
HCP	Human cultured periosteum
IBDs	Intrabony defects
IGF-1	Insulin-like growth factor -1
I-PRF	Injectable platelet-rich fibrin
KTC	Keratinized tissue change
LXA4	Lipoxin A4
LLLT	Low-level laser therapy
L-PRF	Leukocyte and platelet-rich fibrin
MCAF	Modified coronally advanced flap
MF	Metformin
NSAIDS	Non-steroidal anti-inflammatory drugs
n-CS	Nanocrystalline Calcium Sulfate
OFD	Open flap debridement
PC	Platelet concentrate
PF	Platelet factor
PG	Platelet gel
PRP	Platelet rich plasma
PRF	Platelet rich fibrin
PPP	Platelet poor plasma
PDGF	Platelet-derived growth factor
PDAF	Platelet-derived angiogenesis factor
PAF	Platelet activating factor
PD	Probing depth
PGF	Platelet growth factor
RBC	Red blood cell
SDF-1α	Stromal Derived Factor 1 alpha
SCTG	Subepithelial connective tissue graft
SEM	Scanning electron microscope
TGF	Transforming growth factor
TT	Tissue thickness

T-PRF	Titanium-prepared platelet-rich fibrin
VISTA	Vestibular incision sub-periosteal tunnel access
VCR	Volumetric computed radiography
vWF	von Willebrand factor
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cell
5-HT	5-hydroxytryptamine /serotonin

INTRODUCTION

Periodontitis is an inflammatory process of bacterial origin affecting the periodontal tissues and provoking the destruction of the supporting soft and hard tissues around teeth. Conventional periodontal therapy includes both non-surgical treatment and surgical approaches following which, histologic analysis has revealed that periodontal healing occurs with the repair rather than regeneration. During the repair process, long junctional epithelium exists between the treated root surface and the alveolar bone. In recent times, the major goal of periodontal therapy is to arrest the progression of periodontal disease followed by regeneration of lost tissues.[1,2]

Various surgical treatment protocols have been introduced to induce/support periodontal regeneration and they include the use of root conditioning agents, bone graft materials, Guided Tissue Regeneration, Guided Bone Regeneration procedures. In 1974 the regenerative potential of platelets was first introduced and the role of growth factors present in platelets was described by Ross et al. Moreover, Platelets are the reservoirs of growth factors and cytokines which play a key role in the regeneration of the bone and maturation of the soft tissue.[3,4,5]

The preparation and use of Platelet Concentrate such as PRP, PRF, and PPP, a concentrated suspension of growth factors found in platelets is a recent novel method in tissue engineering and are applied in various dental fields.[6, 7]

In this textbook, an attempt has been made to review the concepts regarding the two main types of autologous platelet concentrates, namely PRP and PRF with their detailed explanation on the composition, different types, preparation method and different devices used, potential benefits, limitations, clinical implications, special clinical uses, recent advances and related studies were done using these in the treatment of various periodontal therapies.

HISTORY

In 1974, Ross et al. identified platelet-derived growth factor as a serum growth factor for fibroblasts, smooth muscle cells and glial cells and established the regenerative potential of platelets and in 1975 CJ. Oon and JR. Hobbs used Continuous Flow Blood Separator Machine for the selective removal or exchange of either packed red blood cells, leucocyte-rich or platelet-rich layers or plasma. However, the clinical use of activated PRP was rarely reported in the 1980s though it was discovered earlier[8,9]

The first clinical application was used in 1987 by Ferrari et al in open-heart surgery to avoid excessive transfusion of homologous blood products. The autologous PRP has been safely used and documented for over 20 years in many fields including maxillofacial surgery, aesthetic plastic surgery, treatment of soft-tissue ulcers and upto regenerative surgery. In 1990, Gibble and Ness introduced autologous fibrin gel (fibrin sealant or fibrin glue), a biomaterial with hemostatic and adhesive properties.[9,10]

PRP was first introduced by Whitman et al in 1997 in which enhanced wound healing was observed with resultant release of growth factors. However, the popularity of PRP increased in 1998 as Marx et al, showed that a combination of PRP with autogenous bone in mandibular continuity defects resulted in significantly faster radiographic maturation and histomorphometrically denser bone. It certainly seemed as though a new age in bone grafting had begun.[11]

Landesberg et al in 1998 suggested alternative methods of activating PRP for its use in dentistry. In 1999 Anitua E using plasmapheresis reported preliminary clinical evidence of the beneficial effect of the use of platelet-rich plasma in bone regeneration.[9,12]

Platelet-rich plasma (PRP), the first-generation platelet concentrates showed positive results. However, the complexity of PRP preparation protocol and the risk of cross-infection due to the use of bovine thrombin led to the development of a newer generation of autologous platelet concentrates- platelet-rich fibrin also called Choukroun's platelet-rich fibrin.[9]

PRF was developed in France by Joseph Choukroun et al. in 2001 and they found improved bone healing when used around implants. Platelet cytokines, growth factors and cells are entrapped in a fibrin matrix which is released after a certain time that can serve as a resorbable membrane.[7]

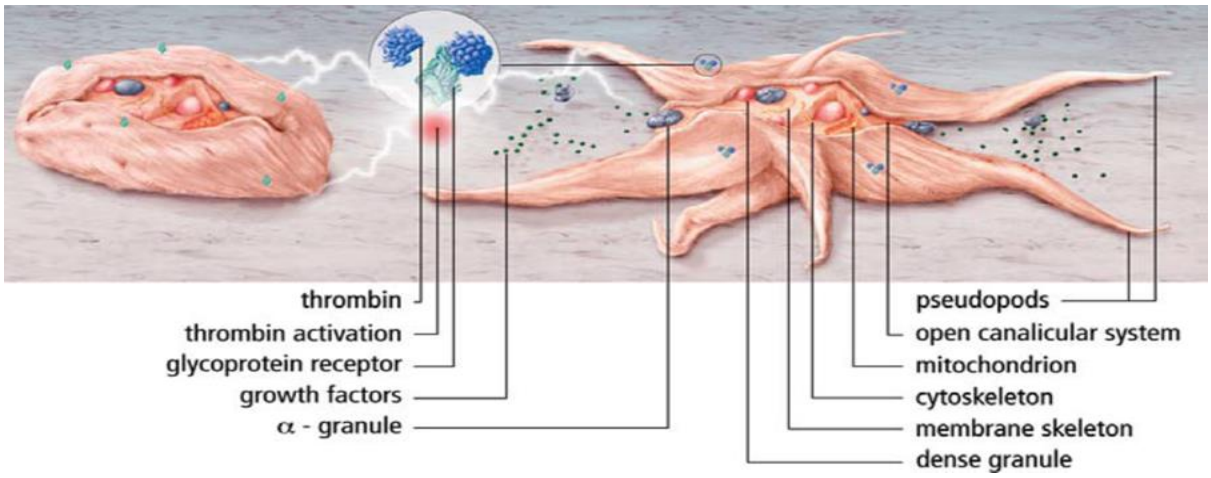
Sanchez et al.in 2003 have elaborated on the potential risks associated with the use of PRP. The findings of Wiltfang et al. in 2005 - from a series of clinical trials are encouraging, in that they showed improved properties of PRF as compared with PRP.[13]

PHYSIOLOGY OF PLATELETS

Platelets or thrombocytes are the formed elements of blood. Platelets are colourless, non-nucleated and moderately refractive bodies. These formed elements of blood are considered to be fragments of cytoplasm. Platelets are 2.5 μ (2 to 4 μ) in diameter and 7.5 cu μ (7 to 8 cu μ) by volume. Normal platelet count in adults is 2,50,000/cu mm of blood (2,00,000 to 4,00,000/cu mm) and in infants ranges between 1,50,000 to 2,00,000/cu mm and reaches normal level by 3rd month after birth. Both males and females have no significant difference in platelet count. During menstruation there will be reduced platelet count in females. The platelet count increases in high altitude and after taking food.

Normally, platelets appear in several shapes, viz. spherical or rod-shaped and become oval or disk-shaped when inactivated. Sometimes, it may take dumbbell shape, comma shape, cigar shape or any other unusual shape. Inactivated platelets are without processes or filopodia and the activated platelets develop processes or filopodia.

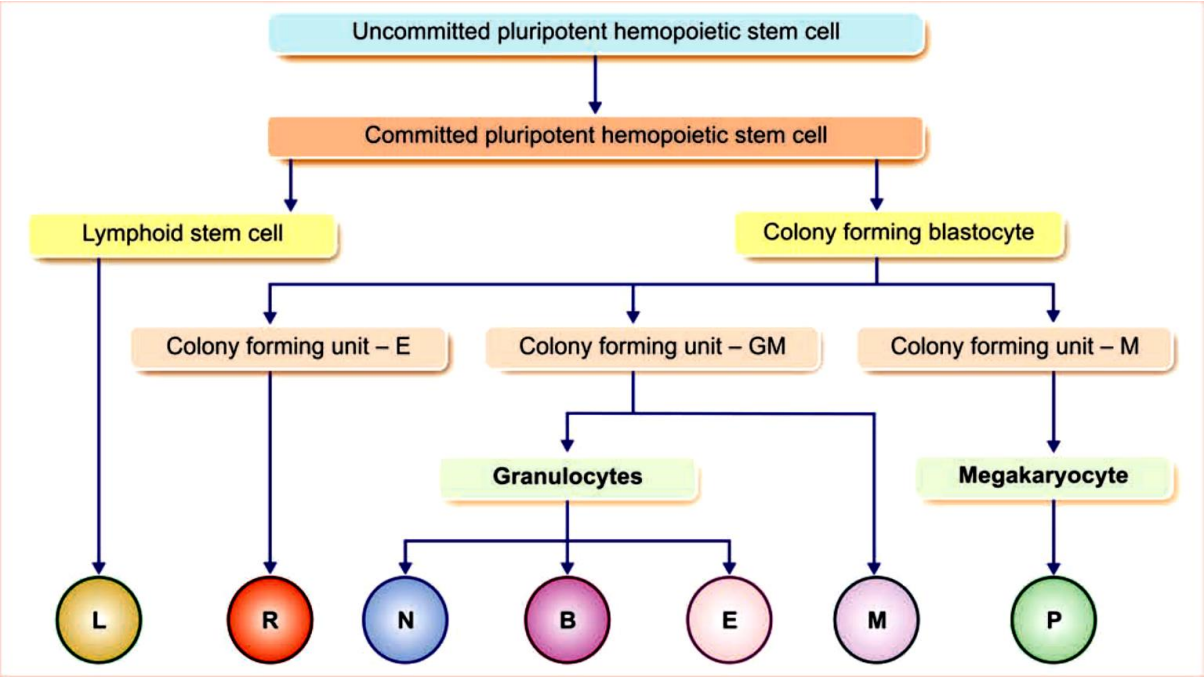
DIAGRAM SHOWING RESTING AND ACTIVATED PLATELET



The colony forming unit-megakaryocyte (CFU-M) is formed by pluripotent stem cells. This develops into megakaryocytes and cytoplasm of which form pseudopodium. A portion of pseudopodium is detached to form a platelet, which enters the circulation. Colony-stimulating factors and thrombopoietin play a role in the production of platelets. Colony-stimulating factors are secreted by monocytes and T lymphocytes. Thrombopoietin is a glycoprotein like erythropoietin. It is secreted by liver and kidneys. The average lifespan of platelets is 10 days

ranges between 8- 11 days. Platelets are destroyed by the tissue macrophage system in spleen. So, splenomegaly (enlargement of the spleen) decreases platelet count and splenectomy (removal of the spleen) increases the platelet count.

FLOW CHART FOR THE FORMATION OF PLATELETS



Stem cells. L = Lymphocyte, R = Red blood cell, N = Neutrophil, B = Basophil,

E = Eosinophil, M = Monocyte, P = Platelet.

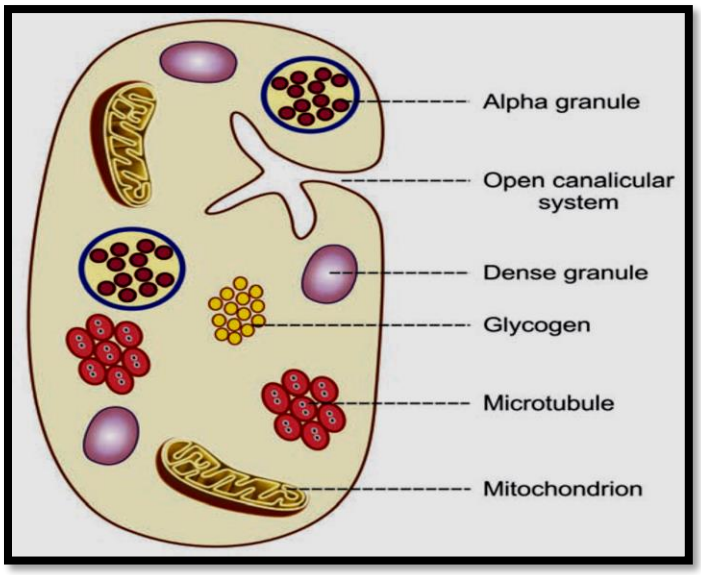
MORPHOLOGY OF THE PLATELETS:

Platelet is constituted by three zones, namely:

1) Peripheral zone (stimulus receptor-transmitter region)

2) Sol-gel zone (cytoskeleton)

3) Organelle zone (metabolic & secretory region)



PLATELET UNDER ELECTRON MICROSCOPE

1) Peripheral zone (stimulus receptor-transmitter region)

It is divided into 3 parts

a) Outer glycocalyx layer:

Glycoproteins present in the outer glycocalyx layer prevent the adherence of platelets to normal endothelium instead enhance the adherence of platelets to damaged endothelium of ruptured blood vessels. In addition, the receptors for adenosine diphosphate (ADP) and thrombin are formed by glycoproteins.

b) Inner lipoprotein layer:

Phospholipids that accelerate the clotting reactions form the inner lipoprotein layer and also form the precursors of thromboxane A2 and other prostaglandin-related substances.

c) Open canalicular system- allows the movement of granules from interior to exterior.

2) Sol-gel zone (cytoskeleton):

Mainly contains contractile proteins namely:

a) Actin & Myosin: Contractile proteins are responsible for the contraction of platelets and for their movements. Actin is the major constituent of actin filaments. Each actin molecule (also known as F-actin) is the polymer of a small protein known as G-actin. About 300 to 400 actin molecules are present in each actin filament and the molecular weight of each molecule is 42,000. The actin molecules in the actin filament form a double-helical pattern. The myosin head gets attached to the active site present on each F actin.

Myosin transports substances by moving micro filaments. Myosin's are classified into 18 types according to the amino acid sequence. However, myosin II and V are functionally significant and also Myosin V participates in the transport of vesicles.

b) Thrombosthenin: Third contractile protein, which is responsible for clot retraction.

3) Organelle zone (metabolic & secretory region):

The cytoplasm of platelets contains the cellular organelles, Golgi apparatus, endoplasmic reticulum, mitochondria, micro-vessels and granules. In addition chemical substances such as proteins, enzymes, hormonal substances, etc are also found in cytoplasm.

A) Dense tubular system: mainly has 2 actions

i) Prostaglandin synthesis

ii) Calcium secretion

B) Granules present in the cytoplasm of platelets are of two types:

i) Alpha granules and

ii) Dense granules.

i) Alpha granules contain:

1. Clotting factors – namely Factor I -fibrinogen, factor V-Labile factor (Proaccelerin or accelerator globulin) and factor XIII- Fibrin-stabilizing factor (Fibrinase). Coagulation of blood occurs through a series of reactions due to the activation of many clotting factors.

2. Platelet-derived growth factor (PDGF): it activates cells of mesenchymal origin which are among the first cells to reach at the wound site. Strayhorn et al suggested that PDGF might act mostly on osteoblastic cell proliferation, exerting most of its effects during the early phases of wound healing. It also stimulates chemotaxis, proliferation, and new gene expression in monocytes-macrophages and fibroblasts in vitro, these cell types are essential for the repair of the tissues.[7]

3. Vascular endothelial growth factor (VEGF): It is a major angiogenic growth factor. It acts on endothelial cells, being produced by numerous cell types, including vascular smooth muscle cells (VSMC), fibroblasts etc. initiating blood vessel formation.[7]

4. Fibroblast growth factor (FGF): it stimulates osteoblast proliferation, has chemotactic effects towards human osteoblasts, increased the expression of osteocalcin and enhances wound healing.[14]

5. Endostatin: is a naturally occurring 20 kDa C-terminal derived from type XVIII collagen and is important as an anti-angiogenic agent, similar to angiostatin and thrombospondin which act by inhibiting and interfering the pro-angiogenic action of growth factors such as FGF and VEGF.

6. Thrombospondin: Inhibits angiogenesis (formation of new blood vessels from pre-existing vessels).

ii) Dense granules contains:

1. Nucleotides: namely ADP and ATP- are the intermediates formed in carbohydrate metabolism are high-energy phosphates. In this most important high-energy phosphate compound is adenosine triphosphate (ATP). This ubiquitous molecule is the energy store house of the body.

On hydrolysis to adenosine diphosphate (ADP), it liberates energy directly to such processes for muscle contraction, active transport, and the synthesis of many chemical compounds.[14]

2. Phospholipid and Calcium: factor X is fully activated by the phospholipids from aggregated platelets and calcium.

3. Lysosomes – Lysosomes are the membrane-bound vesicular organelles found throughout the cytoplasm and are formed by Golgi apparatus. The enzymes which are synthesized in rough endoplasmic reticulum are processed and stored in the form of small vesicles in the Golgi apparatus. Then, these vesicles are broken up from Golgi apparatus and become the lysosomes. Lysosomes are also called 'garbage system' of the cell because of their degradation activity. About 50 different acid hydroxylase enzymes are present in the lysosomes, through which lysosomes execute their functions.

C) Hormones namely:

1. Adrenaline: Adrenaline causes constriction of blood vessels through alpha receptors. It also causes dilatation of blood vessels through β2 receptors.

2. Serotonin (5-hydroxytryptamine; 5-HT): is present in the highest concentration in blood platelets and is formed by hydroxylation and decarboxylation of the essential amino acid tryptophan. Platelet aggregation and smooth muscle contraction are mediated by 5-HT2A receptors.

3. Histamine: mainly produces acute hypersensitivity reactions by causing inducing vascular and tissue responses.

D) Enzymes:

1. Adenosine triphosphatase /ATPase
2. Enzymes necessary for the synthesis of Prostaglandins

E) Proteins:

1. von Willebrand factor: Responsible for adherence of platelets and regulation of plasma level of factor VIII.
2. Fibrin stabilizing factor: helps in clotting.
3. Platelet derived growth factor (PDGF): Causes aggregation of platelets during the injury of blood vessels, resulting in prevention of excess loss of blood.
4. Platelet activating factor (PAF): Causes aggregation of platelets during the injury of blood vessels, resulting in the prevention of excess loss of blood.
5. Vitronectin (serum spreading factor): Promotes adhesion of platelets and spreading of tissue cells in culture.
6. Thrombospondin: Inhibits angiogenesis (formation of new blood vessels from pre-existing vessels).

F) Chemical & inorganic substances:

Platelets also contain chemical and inorganic substances like glycogen, blood group antigens, copper, magnesium and iron.[13]

FUNCTIONS OF PLATELETS:

- The healing of hard and soft tissue is mediated by a wide range of intra and extracellular events that are regulated by signaling proteins. Although the knowledge about these molecules remains incomplete, Platelets have been found to play a crucial role in hemostasis also in wound healing.
- They contain granules that are spherical or oval structures with diameter ranging from 200 to 500 nm each enclosed by a unit membrane, which contains proteins vital for wound healing, they are
  - platelet-derived growth factor (PDGF),
  - transforming growth factor (TGF β), and
  - insulin-like growth factor (IGF-I).

- The fusion of the granules with the platelet cell membrane causes secretion of the active proteins which subsequently bind to the transmembrane receptors of the target cells. Once bound, intracellular signal proteins are activated. This results in the expression of a gene sequence that directs cellular proliferation, collagen synthesis, osteoid production, and so on .[14,15]

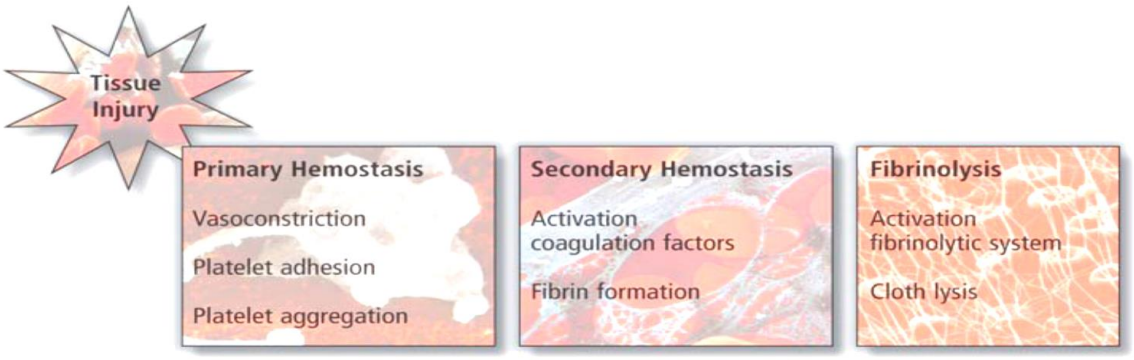
Growth factors released from platelets and their biologic actions

Growth factor	Source cells	Action
PDGF	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	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	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	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
PDAF	Platelets, 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	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	Chemoattractant for neutrophils and fibroblasts; potent antiheparin agent

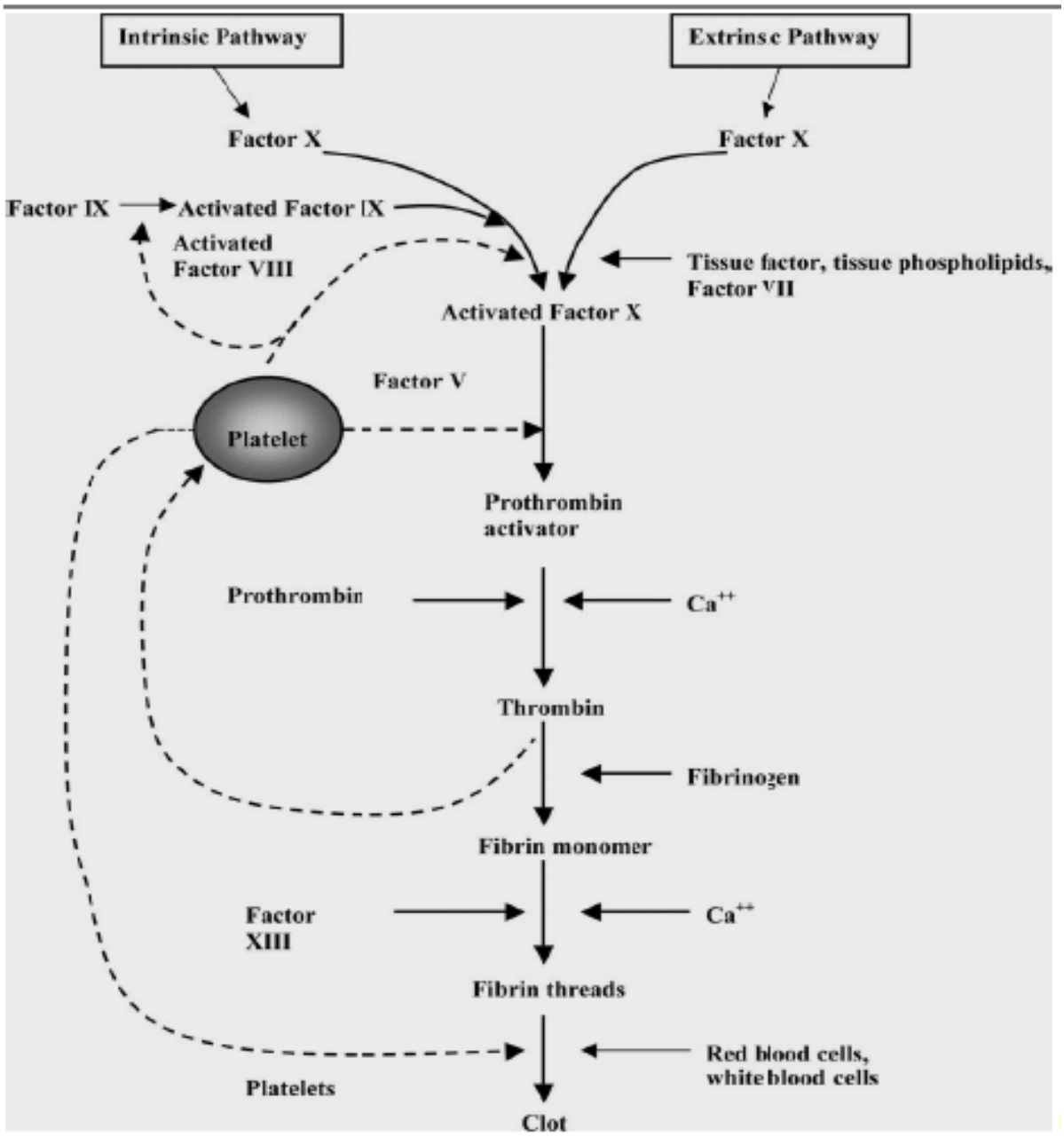
Platelets in Hemostasis:

- Hemostasis is a result of balanced interaction of platelets, vasculature, plasma clotting proteins and low molecular weight substances. After any kind of injury (e.g. by surgical trauma), the most important initial reactions leading to immediate blood coagulation are mainly mediated by platelets and blood vessel wall changes. During surgery, damaged blood vessel walls expose sub-endothelial collagen, binding of von Willebrand factor present in the plasma, subsequently changing the structure so that the platelets can adhere to the blood vessel wall. The platelet adhesion acts via the glycoprotein Ib and IIb/IIIa receptors, which are present in the platelet membrane. After this event platelets get activated and aggregate at the site of injury. The platelet cytoskeleton changes from discoid to a spherical shape with protruding pseudopods which spread over injured tissues to form platelet aggregation.[16]
- After aggregation, the granules containing serotonin are released via the canalicular system which assists in tissue vasoconstriction. The release of granular contents from other platelets is mediated by Adenosine diphosphate (ADP) thus forming a hemostatic plug. Many other agents can cause platelet aggregation and also activate phospholipase A2 present in the platelet membrane. Subsequently, membrane phospholipids release arachidonic acid, which is converted into thromboxane A2 leading to platelet aggregation and platelet growth factor (PGF) release. Independent of thromboxane and ADP, another mechanism that causes platelet aggregation and platelet granule release, is induced by the presence of thrombin. Ultimately, the formed platelet plug stops blood loss from damaged vessels.
- Primary hemostasis is by the formation of a platelet plug following which secondary hemostasis is achieved with the activation of coagulation factors and the formation of a fibrin network that stabilizes the platelet plug. However, the activated leucocytes release cytokines at the affected area which in turn activates the fibrinolytic system leading ultimately to clot lysis.
- Platelet-derived growth factors are released from α-granules at the wound site following injury, repair of tissues takes place with the formation of new connective tissue and re-vascularization. The temporary formation of fibrin plug at the wound site prevents the entry of micro-organisms.[16]

THE DIFFERENT CASCADE STAGES IN HEMOSTASIS AFTER TISSUE INJURY



- Blood clotting is initiated by one of two pathways, namely, the intrinsic and extrinsic pathways. The damage or alteration to the blood itself initiates the intrinsic pathway whereas the extrinsic pathway is initiated by contact of blood with factors that are extraneous to the blood (e.g., damaged tissue). Both pathways involve a cascaded reaction sequence whereby inactive factors become activated leading to clot formation. Although both pathways begin differently, they converge and share many of the later steps in the reaction series. Moreover, calcium ion is required for the reaction to proceed to completion.
- Platelets generate fibrin threads and are part of the final clot composition, which consists of a fibrin mesh and red and white blood cells interposed within. The clot retracts within 20 minutes to 1 hour by the contraction of the actin-myosin fibers thereby leading to further closure of the vessel. Local vasoconstriction in response to the release of thromboxane and serotonin from the platelet aggregate also aids hemostasis.[17]



Schematic diagram of the role of platelets in clot formation

Platelets in wound healing:

- Platelets play an important role in wound healing initiating the body's response to injury. Following the surgical incision, the wound healing process immediately begins.[18]

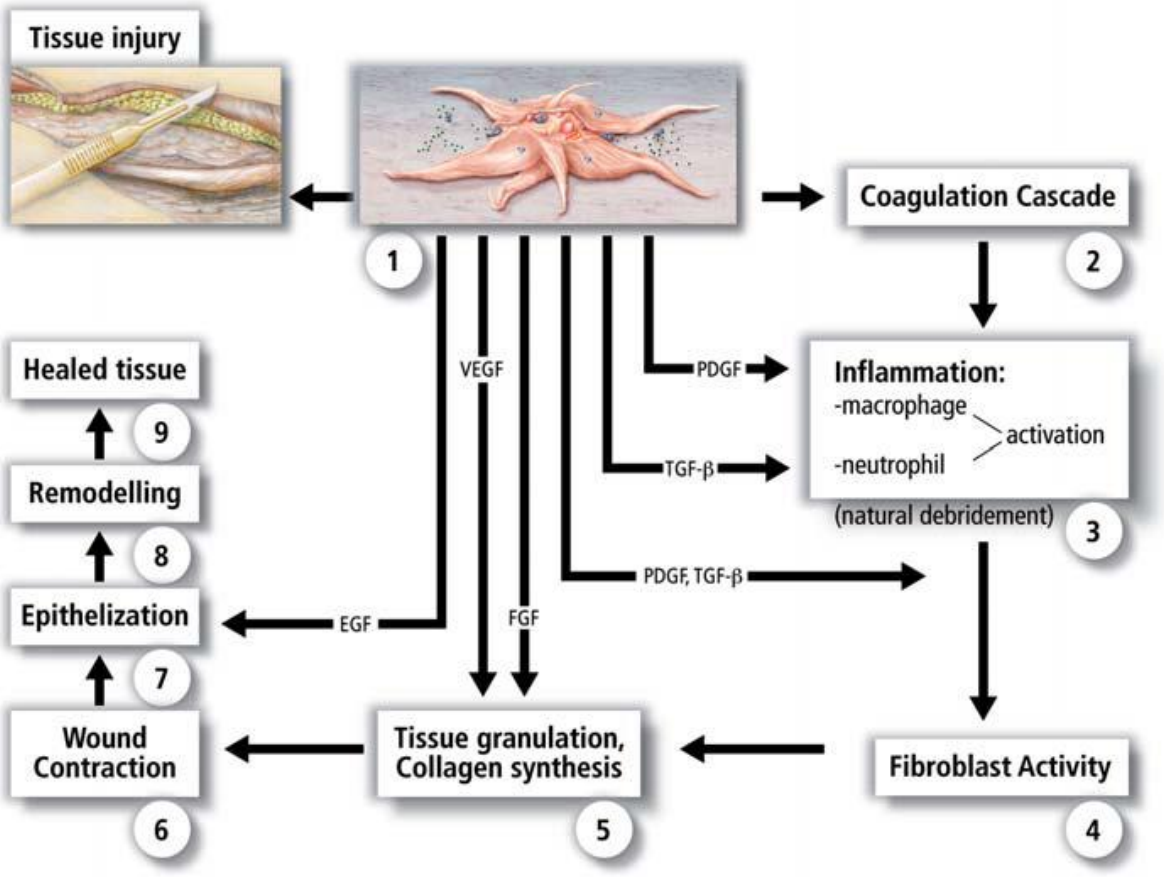
There are 4 phases of wound healing:

- The hemostatic phase (clot formation)
- The inflammatory phase (clean-up and recruitment),
- The proliferative phase (regeneration), and

    - The tissue remodeling phase.
- Although these phases overlap significantly during the healing process. Platelets and fibrin play an active role in the formation of the clot. The clotting cascade is activated with the interaction of platelet membranes and receptors with damaged vascular endothelium. The exposed collagen triggers changes in the platelet membrane. The once disc-shaped platelet changes to many long pseudopodia and binding sites with platelet membrane receptors GPIIb and GPIIIa becoming activated. Some of these receptors have an affinity for fibrinogen and some for the von Willebrand factor (vWF) protein thereby allowing activated platelets to remain at the site of injury. This stage is known as adhesion and is followed by platelet aggregation (a platelet plug of activated platelets). Fibrinogen is converted to fibrin strands forming a 3- dimensional mesh that entraps more platelets. These sticky platelet membranes bind to the fibrin strands adding further structural integrity to the clot. The clot acts as a hemostatic barrier that halts bleeding and prevents foreign bodies from entering the site. It is important to note that the red blood cells provide no function to the clot. The body will send macrophages to the clot to remove the red cells and the released hemoglobin.
- In the inflammation phase of healing, white cells infiltrate the wound site and activate platelets to release growth factors (GF). Monocytes become phagocytic and are referred to as macrophages. Together with neutrophils, they can destroy bacteria, remove red cells and release hemoglobin, and digest injured tissue and other foreign materials. Cytokines, released from both white cells and platelets will attract neutrophils and fibroblasts and numerous GF are released from the platelets.
- Growth factors are needed to start the proliferation phase and are critical for any wound to heal and are involved in every phase of wound healing. They are contained in the alpha granules of platelets as well as other cells such as macrophages and endothelium. Platelet GF is responsible for the early migration of cells to the injury site and the triggering of mitosis of these cells once at the site. Specific platelet-isolated growth factors include platelet-derived growth factor (PDGF), transforming growth factors-beta (TGF-beta), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF).
- The proliferative phase is responsible for restoring vascular integrity, replacing lost or damaged tissue, and resurfacing the wound. Growth factors released from platelets and contained within the clot send out signals to trigger cell division. Platelet-derived growth

factors initiate connective tissue healing, bone generation and repair, increase mitogenesis of fibroblasts, stimulate angiogenesis in the wound bed, and activate macrophages. The newly created blood vessels and blood flow bring necessary nutrients and oxygen for optimal healing.

- Transforming growth factors beta promotes cell mitosis and differentiation for connective tissue and bone. This growth factor acts on stem cells, osteoblast precursors, and fibroblasts. Vascular endothelial growth factor stimulates angiogenesis and related vascular permeability enhancing activities specific for endothelial cells. This GF is chemo attractive for osteoblasts. Epithelial growth factor induces (EGF) epithelial development and promotes angiogenesis.
- In the remodeling phase, collagen is continually produced and broken down. Inflammatory cells regress and the maturation of the scar may last up to 2 years. Several growth factors regulate the remodeling process. Restoration of the wound site to a mature scar depends on the perfect balance of collagen degradation and synthesis.[18]



Schematic illustration of the role of platelet-derived growth factors (the numbers indicate the sequence of the wound healing actions) during the different stages of the wound healing process. (EGF: epidermal growth factor; FGF: fibroblast growth factor; PDGF: platelet-derived growth factor; TGF-β: transforming growth factor-beta, VEGF: vascular endothelial growth factor).[16]

Platelets in bone healing:

- Bone is defined as a biological tissue composed of dynamically active cells which are integrated into a rigid framework. Bone cells consist of osteoblasts, osteoclasts, osteocytes, osteoprogenitor cells and hematopoietic components.
- During the bone healing process, there is a balance between bone deposition, resorption, and remodelling. This is influenced by numerous biochemical, biomechanical, cellular, and pathological mechanisms. During bone healing, mature bone-forming osteoblasts secrete growth factors that are also present in platelets. Osteoclasts in contrast are bone-resorbing cells, controlled by hormonal and cellular mechanisms.
- Under normal conditions, there will be a balance in the activity of osteoblasts and osteoclasts. In fracture, platelets act as an exogenous source of growth factors stimulating the activity of bone cells, based on their particular relevance to bone growth. Bone-fracture healing also incorporates all three stages of inflammation, proliferative, repair, and remodelling.
- Platelet release PDGF, TGF-β, and EGF to the injury site and the richest source of TGF-β is found in platelets, bone, and cartilage. Both the isoforms, TGF-β1 and TGF-β2, are present in the platelets. TGF-β1 has the greatest potential for bone repair since both chondrocytes and osteoblasts are enriched with receptors for TGF-β1. TGF-β may contribute to bone healing at all stages. It has been demonstrated that a combination of platelet growth factors TGF-β, FGF, and EGF causes differentiation and proliferation of osteoblasts to osteogenic cells. Similarly, proliferation was increased by the mitogenic action of PDGF in mesenchymal stem cells when TGF-β and EGF were added.[16]

FIBRIN SEALANTS (FS):

Fibrin sealants or fibrin tissue adhesives or 'fibrin glues' are human plasma derivatives that mimic the final stages of blood coagulation, forming a fibrin clot. They are used for topical hemostasis and tissue sealing and as melting agents for particulate bone substitutes.

The risk of cross-infection for commercial adhesives led to the development of autologous fibrin sealants from the patient's plasma. However, their fabrication resulted in less reproducible or less satisfactory rheological properties.[16]

Types of Fibrin Sealants, (FS)

(1) Homologous (commercial) FS
(2) Autologous FS:

(1) Homologous (commercial) Fibrin Sealants -FS: these are available as freeze-dried two-component preparations which have to be mixed before using

• A fibrinogen/fibronectin/factor XIII concentrate dissolved in an antifibrotic solution (usual aprotinin).
• Thrombin concentrate dissolved in dilute calcium chloride.[16]

Mixing the two components imitate the last stage of the coagulation cascade resulting in a fibrin clot independent of the patient's coagulation pathway. The fibrinogen component contains factor XIII, and the thrombin component contains calcium (Ca2+) ions. Factor XIII, activated by thrombin in the presence of Ca2+ ions, catalyzes cross-linking between the fibrin molecules, resulting in a crosslinked insoluble fibrin matrix. Homologous fibrinogen concentrates are prepared from Cohn fraction I or plasma cryoprecipitate.

These products are heat-treated to reduce the risk of disease transmission but not completely. Therefore, the commercially available adhesives constitute an infinitely small risk of disease transmission

(2) Autologous Fibrin Sealants- FS: Due to the risk of transmitting infectious agents, fibrin sealants were prepared from the patient's own whole plasma and Fibrin polymerization is always initiated with bovine thrombin.[16]

PREPARATION OF FIBRIN SEALANTS:

Fibrin glue is formed by polymerizing fibrinogen with thrombin and calcium FG can be prepared from either pooled blood (blood from several donors) or single donor blood. In the case of single donor blood, it can be autogenous or allogenic. In Europe, pooled commercial sources of FG are available. Purification involves treatment for viral deactivation using a solvent/detergent or dry heat6. In the US, the FDA has not approved any pooled commercial source and, therefore, single donor blood that has been tested for viral contamination or an autogenous source is most commonly used.

Fibrinogen has been isolated from whole blood using centrifugation in combination with cryoprecipitation, ethanol, ammonium sulphate or poly (ethylene glycol) (PEG) precipitation. Concentrated solutions of fibrinogen when resolubilized and mixed with thrombin are used as an FG or a fibrin adhesive (FA) to adhere tissues together or as a fibrin sealant (FS) to close tissue defects in many phases of surgery. Important considerations in the preparation of fibrin products include the time required for gelation, the toxicity of added chemicals, and the complexity of the preparational process.[20]

Fibrinogen precipitation:

Precipitation of fibrinogen is accomplished after the cellular elements of blood are removed by centrifugation. Typically, a 10% v/v sodium citrate solution (10% v/v sodium citrate in distilled water) is added to prevent coagulation of blood before the separation of the plasma from the cellular elements. Separation is achieved by centrifuging the blood at 600g for 10 to 20 min7-I". Fibrinogen precipitation is then employed to concentrate fibrinogen for fibrin sealant preparation.

Cryoprecipitation:

Cryoprecipitation is the 'gold standard for making fibrin sealant, and it has been described many times in the literature. The problem is that there are numerous variables involved in glue preparation, including freezing time and temperature, thawing time and temperature and

the number of freeze-thaw cycles. Precipitation of fibrinogen from plasma by a freeze-thaw cycle has also been reported. Initial blood volume ranges from 9 to 250ml. After centrifugation, the volume is reduced by about 50% following which the plasma is frozen at temperatures between -20°C and -80°C. The freezing time ranged from 1 hour to greater than 24 hours. Thawing times of between 12 h and overnight at about 4°C were reported. Fibrinogen is collected by centrifugation at forces between 1000 and 6500g for periods of 5 to 15min. Reconstitution of the pellet in 0.5 to l ml of the supernatant was reported. A calcium chloride solution (40mM) containing between 500 and 1000 units per ml of thrombin and 1000 units per ml of aprotinin was formulated to mix with the fibrinogen concentrate.

According to Spot & z et al., the fibrinogen-rich concentrate can be stored at -30°C for up to 5 years or in a blood bank refrigerator for up to 5 days. Howard et a reported that thawed cryoprecipitate stored at temperatures between 1 and 6°C is maintained essentially unchanged in most bags for 1 month. The addition of equal amounts of fibrinogen and thrombin solution or a 1:2 ratio of fibrinogen and thrombin solutions is used to make FG or FS. The fibrinogen solution prepared in this manner typically has a concentration of between 21.6 and 40.0 mg /ml. The gelling time has been demonstrated to be as short as 1 to 3 min. The use of twice frozen and thawed plasma to prepare the cryoprecipitate was reported by Saltz et a1. The plasma was frozen at less than -32°C and stored at less than -18°C for 18h. Frozen plasma was thawed at 4°C in a circulating water bath for 1 h, then kneaded to mix it, and finally removed after 2 h. It was then stored at -18°C for at least 18 h, thawed again in the 4°C bath and then centrifuged at 0°C at 4200rpm for 12 min. The concentrated fibrinogen solution was mixed with CaCl2(40mM) and thrombin (250 units ml).

The ‘gold standard’ for the preparation of either pooled or donor blood remains cryoprecipitation since large amounts of concentrated fibrinogen solutions can be made. Due to variation in the freeze-thaw cycle, concentration of thrombin, calcium and sodium ions and antifibrinolytic agents such as aprotinin, it is difficult to compare the gelling times of different products. Therefore, there is a great need to introduce a standard protocol for forming the cryoprecipitate. In addition, the preparation is time-consuming, with small amounts of blood. Hence cryoprecipitation is more suitable for processing pooled or donor blood. In some cases. after cryoprecipitation, the concentrated fibrinogen does not truly re-dissolve, thereby generating a glue with dispersed particles.

PROPERTIES OF FIBRIN SEALANTS:

1) Fibrin sealant promotes new attachment and bone regrowth when placed over the denuded root surface.

2) Fibrin sealant promotes regeneration by three mechanisms; osteoblasts proliferation, clot stabilization and presence of fibronectin and growth factor.

3) Bosch et al. have shown that fibrin sealant produced an early enhancement of bone repair in rabbits. The physical properties of the bony callus seemed to be enhanced by its use.

4) Isogai et al. reported that fibrin clots support growth, adhesion, migration, and differentiation of osteoblasts in vitro.

5) In addition FS contains several factors which can promote regeneration such as fibrinogen, fibronectin, Factor XIII and platelet-derived growth factor (PDGF). Fibrin and factor XIII is known to promote fibroblast adhesion and multiplication. Fibronectin enhances wound healing by promoting new attachment by the growth of fibroblast and their attachment to root surfaces and forms covalent linkages with fibrin and collagen. PDGF is a polypeptide, which enhances fibroblast reduplication and helps in periodontal regeneration and repair. Hence, the FS may act like osteoconductive materials for bone healing.

6) Fibrin sealant has been a vehicle for the delivery of mesenchymal stem cells and growth factors and BMPs for bone regeneration.

7) Fibrin glues may have antibacterial properties as an undefined mechanism, evidenced by studies on skin grafts in infected sites. This property would be beneficial as resorbable membranes have the drawback of getting exposed, which could lead to infection and delayed wound healing.20

CONCEPT OF FIBRIN SEALANTS USE IN PERIODONTAL SURGERY:

Periodontal wound healing is a well-orchestrated process and more complex than dermal or mucosal healing. Healing is often complicated by anatomical location, microbial plaque, pH, temperature variations and tissue destruction. Hence, a minimally invasive procedure and a

tension-free primary wound closure can facilitate postsurgical periodontal healing along with the use of biocompatible wound closure material.

Suturing has certain shortcomings like postoperative infection resulting in compromised wound healing, acute inflammation, allergic reaction, wound dehiscence and late complications including local abscesses, sinus formation and scarring. Furthermore, it is time-consumingtime-consuming, requires skill and an additional patient visit for removal. Lack of wound stability with the use of silk sutures has also been reported. Fibrin-based tissue adhesives for sutureless wound closure have gained increased acceptance in periodontal surgery. Fibrin sealant (FS)is a biologically derived tissue adhesive for securing flaps.[20]

CONCEPT OF FIBRIN SEALANTS USE IN GTR PROCEDURE:

Fibrin sealant made the membrane much easier to handle than suturing.22 The basic concept of conventional periodontal regenerative treatment is the reduction or elimination of tissue inflammation, correction of defects or anatomical problems caused by the disease process, and regeneration of lost periodontal tissues by preventing the formation of the long junctional epithelium. This can be achieved by guided tissue regeneration (GTR) as it has a “space maintenance effect”. The rationale of GTR is to place a barrier membrane over the debrided periodontal defect to prevent down the growth of epithelial cells and allow periodontal ligament and alveolar bone cells to repopulate the isolated space. Biodegradable collagen membranes, both synthetic and natural are popular and have been used by several authors. The results have not been consistent with the use of this membrane, some have shown regeneration while others have found no difference when compared against open flap debridement. The different results observed could be due to a lack in the expression of the biological potential for regeneration by the barrier membranes. However, the drawbacks of the GTR membrane are early exposure, infection and resorption of barrier membrane which influences the healing process.

The use of fibrin sealant provides stability and maintains space underneath the membrane and additionally increases the overall regenerative and healing potential. Earlier studies have shown enhanced regeneration and early wound healing with use of fibrin sealant. Hence, FS could be used to enhance and overcome the drawbacks of barrier membranes for the treatment of deep intrabony defects.

Fibrin sealant allowed; cell exclusion, space maintenance tissue integration, regeneration and easy handling. Cell exclusion property requires the membrane to separate the gingival flap from the coagulum in the wound space. Fibrin sealant promotes clot formation and increases clot strength by forming cross-links with fibrin which further prevents its proteolytic cleavage. Early clot adhesion to the root surface promotes periodontal regeneration and serves as a barrier to the apical migration of junctional epithelium. The next property is space maintenance; the membrane should withstand masticatory forces, flap tissue tension and prevent the collapse of soft tissues or reduction of the wound space. The clot formed from FS between the membrane and root surface provides space for bone regeneration and also gives stability to the overlying membrane. FS provided high compatibility and good mechanical strength to the barrier membrane. The third essential property is tissue integration. Tissue integration property ensures wound stabilization and inhibition of epithelial migration, resulting in a gain of attachment level. In our report reduction in probing was achieved with the use of FS. Lastly, the membrane should be easy to handle and manipulate allowing the clinician to conduct the surgical procedure without undue difficulty

Indications

- When aesthetics are critical.
- When patients need to travel long for suture removal.
- Hemostasis.
- To seal the gingival margin after soft tissue augmentation.

Clinical Applications in Dentistry

- Treatment of intrabony defects.
- Alveolar ridge augmentation.
- Treatment of gingival recession.
- Bone regeneration around dental implants.18
- Sinus floor augmentation.
- Treatment of extraction wounds.

Limitations:

- Varied Composition and the characteristics of the sealants (commercial, homologous, autologous).
- Autologous fibrin sealants are weak and have less resistance to physical stress than commercial sealants.
- The potency of fibrin sealants for soft tissues are well known, yet their contribution to bone surgery and periodontal surgery remains controversial.
- Fibrin glue requires either predonation or costly processing of autologous blood or the use of homologous blood products which may be associated with a risk of viral transmission.[18]

PLATELET-RICH PLASMA/ PRP:

First-generation platelet concentrates:

The use of autologous products with high platelet concentrations such as Platelet-rich plasma (PRP), Platelet concentrates (PC) and platelet gels developed to combine the fibrin sealant properties with growth factor effects of platelets—providing an ideal growth factor delivery system at the site of injury. The scientific rationale behind the use of these preparations lies in the fact that growth factors (GFs) are known to play a crucial role in hard and soft tissue repair mechanisms. These GFs exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, regeneration and cell proliferation .[18]

Platelet-rich plasma (PRP) has been a breakthrough in the stimulation and acceleration of bone and soft tissue healing. It represents relatively new biotechnology that is part of the growing interest in tissue engineering and cellular therapy today. [12]

WHAT IS PRP?

Platelet-rich plasma is just that; it is a volume of autologous plasma that has a platelet concentration above baseline. Normal platelet counts in blood range between 150,000/µl and 350,000/µl and average about 200,000/µl. Because the scientific proof of bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/µl, it is this concentration of platelets in a 5-ml volume of plasma that is the working definition of PRP today. Lesser concentrations cannot be relied upon to enhance wound healing, and greater concentrations have not yet been shown to further enhance wound healing. [22]

WHAT IS PRP ABOUT RECOMBINANT GROWTH FACTORS?

Because PRP is developed from autologous blood, it is inherently safe and is free from transmissible diseases such as HIV and hepatitis. Within PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area.[22]

The seven known growth factors in PRP are:

- Platelet-derived growth factor as – PDGF aa, PDGF bb, PDGF ab,
- Transforming growth factor-beta- TGF-β 1, TGF-β,
- Vascular endothelial growth factor -VEGF, and
- Epithelial growth factor - EGF.

These are native growth factors in their biologically determined ratios. This is what distinguishes PRP from recombinant growth factors.

Recombinant growth factors: are pure human growth factors, but they are not native growth factors. Human cells such as platelets do not synthesize them. Instead, they are synthesized usually by a culture of Chinese hamster ovarian cells that have a human gene inserted into their nucleus through a bacterial plasmid vector. Recombinant growth factors are single growth factors and are delivered in high doses within either a synthetic carrier or a carrier derived from processed animal proteins.

PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration such as is seen in osteoconduction, wound epithelialization, and osseointegration. PRP, however, contains only the same concentrations of these cell adhesion molecules as does a normal blood clot (200 μg- 400 μg/ml). Therefore, PRP is not fibrin glue. Platelet Rich Plasma is also not osteoinductive. It cannot induce new bone formation de novo. Only the bone morphogenetic proteins (BMPs) are known to induce bone de novo. However, the prolonged length of time required by recombinant BMP to produce de novo new bone formation and its immature osteoid nature suggests an opportunity for PRP to accelerate BMP activity in the future.[22]

TERMINOLOGY:

There has already been some mistaken terminology related to PRP. Some have advanced the term "platelet concentrate." This is not correct because a platelet concentrate is a solid composition of platelets without plasma, which would therefore not clot. The clinically useful

product is a concentration of platelets in a small volume of plasma and is, therefore, a "platelet-rich plasma."

Some have advanced the term "platelet gel." This is also incorrect because PRP is nothing more than a human blood clot with increased platelet numbers. The clot by its cell adhesion molecules has additional biologic activity, whereas a gel does not.

Still, others have reversed the term platelet-rich plasma into plasma rich in platelets, plasma very rich in platelets, and even plasma very rich in platelets. [22]

PROCESSING PRP:

Ideal requisites of properly processed PRP:

1. Any PRP device should process a concentration of at least 1,000,000 platelets/μl in a 5-ml volume,
2. Process viable undamaged platelets, and
3. Process PRP in a sterile fashion and be pyrogen-free.
4. Liability, consent, and licensing must be discussed because both patient and auxiliary staff safety issues are pertinent.
5. The FDA clearance is important.

It should be noted that "sterile" and "pyrogen-free" are not the same. Sterile means the absence of microorganisms. Pyrogen-free means the absence of any microorganism products or foreign body particles that might produce a fever. Therefore, the PRP device must use only certified pyrogen-free disposable materials.[22]

GENERAL CONSIDERATIONS IN PRP PROCESSING TECHNIQUE:

- To truly concentrate platelets from autologous blood, the device must use a double centrifugation technique.
- The first spin (called the hard spin) will separate the red blood cells from the plasma, which contains the platelets, the white blood cells, and the clotting factors.

- The second spin (called the soft spin) finely separates the platelets and white blood cells together with a few red blood cells from the plasma. This soft spin produces the PRP and separates it from the platelet-poor plasma (PPP) free from the obstruction provided by a large number of red blood cells.
- To attempt PRP with a single spin would not produce a true PRP. Instead, it would produce a mixture of PRP and PPP and have disappointingly low platelet counts.
- Regardless of the rate of centrifugation or the time of centrifugation, a single spin cannot adequately concentrate platelets, because the red blood cells will interfere with the fine separation of the platelets.
- This is germane to those who may use a laboratory centrifuge to develop PRP or may purchase a device that is merely a modification of a laboratory centrifuge. Such centrifuges are designed for diagnostic purposes-not PRP development. They may not produce a sufficient platelet yield, they may damage platelets, they may not use pyrogen-free test tubes, and they are not FDA cleared. Therefore, they should not be used.
- The FDA clearance is indeed important. Although the patient is protected from transmissible diseases because of the autologous nature of PRP, the practitioner and the auxiliary staff are not. Devices that leak blood or have the potential to malfunction from centrifuge misbalance, or design characteristics intended for diagnostic blood work, are real health, medical, and legal risk.
- Practitioners are recommended to look to devices that have simple FDA clearance to process PRP from autologous whole blood.
- A further FDA clearance to mix PRP with autologous grafts and bone substitutes is the advanced security of some devices.
- No dental practitioner or medical practitioner is licensed to infuse or re-infuse blood or blood products systemically in an office setting.
- However, it is within the licensure of each to apply blood products topically in the office as is done with PRP.
- Office devices that produce PRP use only 45 ml to 60 ml of blood, which is insignificantly related to a normal 4- to 5-L blood volume. There is no reason to re-infuse the blood that is not used, and it would be risky to do so.[22]

Preparation of PRP:

PRP is pre-operatively prepared from a unit of autologous whole blood using Extra-corporeal blood processing techniques.

PRP can be prepared by two techniques either through

1. Standard blood banking techniques, or through
2. Point-of-care devices -including
    a) Blood cell savers/separators
    b) Table-top devices.

1.Standard blood banking technique:

The preparation of PRP by blood banks, through discontinuous plasmapheresis methods, should be limited because of higher production costs and delayed availability of PRP, when compared to bedside devices. Furthermore, blood bank prepared PRP is out of reach of the clinician and demands a highly controlled logistic system to avoid product mismatch before application to the patient.

2. Point-of-care devices

Two different point-of-care blood centrifugation machines were introduced to the market recently that achieve optimal blood separation for the production of PRP.

a. With cell savers/separators, larger pre-donation blood volumes (250 mL to more than 500 mL of whole blood) can be obtained, resulting in a PRP volume ranging from 20 m to more than 50 ml.

b. Table-top centrifuges have been used to manufacture smaller volumes of PRP from lesser amounts of whole blood (50mL-150 ml).

The choice for either system is mainly dependent on the type of surgical procedure and the anticipated need for the amount of Platelet Gel (PG). It seems reasonable that cell savers are used when both wound blood cell salvage and PG application are indicated. By contrast, table-top devices are used when only small amounts of PG are required during minimal blood loss surgical procedures.[19]

Device Name	Manufacturer	Characteristics	Flow	Bowl size (mL)
Brat 2	Cobe Cardiovascular Inc Arvada, CO, USA	Baylor bowl	Discontinuous	55,125,175 225,240
Compact A Electa	Sorin Group Mirandola, Italy	Latham bowl	Discontinuous	55,125,175, 225
Fresenius CATS	Fresenius Kabi AG Bad Homburg Germany	Separation chamber	Continuous	N/A
Haemonetics CS 5 Plus	Haemonetics Corporation Braintree, MS, USA	Latham bowl	Discontinuous	70,125,225
Sequestra 1000	Medtronic Inc. Minneapolis, MN, USA	Latham bowl	Discontinuous	125, 225

Table 1. *Overview of currently available cell saver/separator devices*

Device Name	Manufacturer	Characteristics	Components	PRP Volume	RPM
Angel™	Sorin Group Mirandola, Italy	Variable chamber disk	RBC,PPP, PRP	5-18 mL	Max 4000
Genesis CS™	Emcyte Corporation, Ft. Myers, FL, USA	Concave Aspiration Disc	BMC, PPP, PRP	4-10 mL	2400
GPS II™	Biomet Warsaw, IN, USA	Container + buoy	PPP, PRP	5-6 mL	3200
Magellan™	Medtronic Inc Minneapolis, MN USA	Chamber	RBC, PPP, PRP	1-8 mL	Max 4000
Secquire™	PPAI Medical Fort Myers, FL, USA	Container	RBC, PPP, PRP	7 mL	3500
Symphony II™	dePuy Inc Raynham, MS, USA	Two chambers	PPP, PRP	7 mL	Fixed two step
Vivostat™	Vivolution A/S Birkeroed, Denmark	Preparation chamber	PRF, FS	5 -7 mL	N/A

Table 2. *Overview of currently available table top platelet rich plasma devices (BMC: bone marrow concentrate; FS: fibrin sealant; N/A: not applicable; PPP: platelet poor plasma; PRF: platelet rich fibrin; PRP: platelet rich plasma; RBC: red blood cells).*

1.General-purpose cell separators

2.Platelet-concentrating cell separators

General-purpose cell separators:

It requires large quantities of blood (450 ml) and generally requires to be operated in a hospital setting. Blood is drawn into a collection bag containing citrate-phosphate-dextrose anticoagulant. It is first centrifuged at 5,600 rpm to separate RBCs from platelet-poor plasma (PPP) and PRP. The centrifugation speed is then reduced to 2,400 rpm to get a final separation of about 30 ml of PRP from the RBCs. With this technique, the remaining PPP and RBCs can be returned to the patient's circulation or can be discarded. The ELMD-500 (Medtronic Electromedical, Auto Transfusion System, Parker, CO, USA) cell separator is widely used for this technique.

Platelet-concentrating cell separators:

It requires a small quantity of blood and can be prepared by using certain equipment in a dental clinic setup. Currently, two such systems are approved by FDA and commercially available: Smart PreP (Harvest Technologies, Plymouth, MA, USA) and the Platelet Concentrate Collection System (PCCS; 3i Implant Innovations, Inc, West Palm Beach, FL, USA). Several studies have been performed to compare the efficacy of these systems (7-9). A study conducted by Marx et al indicated that of all of the devices tested these 2 FDA-cleared PRP devices produced the greatest platelet concentrates and most important, the release of the therapeutic level of bioactive growth factors.

The preparation and processing of PRP are quite similar in most of the platelet-concentrating systems although the anticoagulant used and the speed and duration of centrifugation may differ with different systems.[10]

1.Venous blood is drawn into a tube containing an anticoagulant to avoid platelet activation and degranulation.

2.The first centrifugation is called "soft spin", which allows blood separation into three layers, namely bottom-most RBC layer (55% of total volume), topmost acellular plasma layer called PPP (40% of total volume), and an intermediate PRP layer (5% of total volume) called the "buffy coat".

3.Using a sterile syringe, the operator transfers PPP, PRP and some RBCs into another tube without an anticoagulant.

4.This tube will now undergo second centrifugation, which is longer and faster than the first, called "hard spin". This allows the platelets (PRP) to settle at the bottom of the tube with very few RBCs, which explains the red tinge of the final PRP preparation. The acellular plasma, PPP (80% of the volume), is found at the top.

5.Most of the PPP is removed with a syringe and discarded, and the remaining PRP is shaken well.

6.This PRP is then mixed with bovine thrombin and calcium chloride at the time of application. This results in gelling of the platelet concentrate. Calcium chloride nullifies the effect of the citrate anticoagulant used, and thrombin helps in activating the fibrinogen, which is converted to fibrin and cross-linked. [10]

PREPARATION OF PRP WAS ACCORDING TO THE "GONSHOR" PROPOSED GUIDELINES- 2002

i)Two blood collection tubes:

1. Blue top 10 mL vacutainers containing 1.5 mL of 3.2% sodium citrate solution.
2. Red top 10 mL vacutainers- not containing any anticoagulant

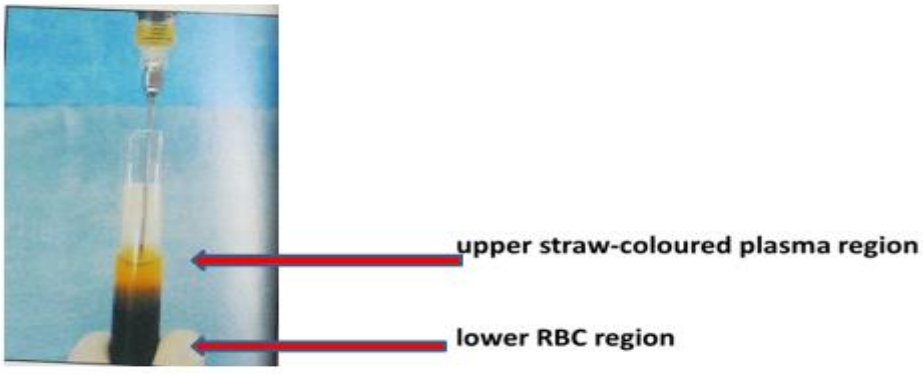
ii)63mm and 76mm blunt needles

iii)Bovine thrombin – 5,000units

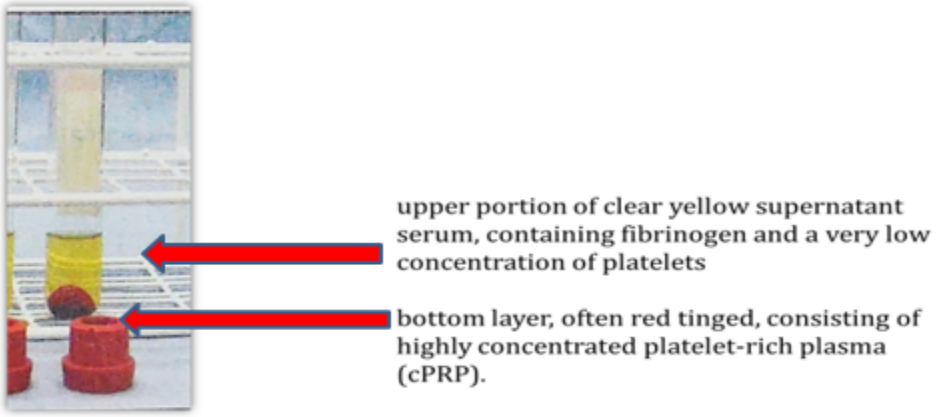
iv)10% CaCl2

	Rpm	G - Force	duration
First spin / soft spin	1,300	160	10 min
Second spin / hard spin	2,000	400	10 min

1.After first spin



2.After the second spin



PRP ACTIVATION

- Alpha granules of the non-activated platelets in the PRP contain PGF, and are thus non-functional, since they are not released or in contact with the tissue. To initiate the release of these growth factors, platelets must be activated. Thrombin, the most potent platelet activator, will induce immediate PGF release from the PRP in a dose-dependent fashion. In the USA, commercially available thrombin, derived from bovine plasma is used as a

'gold standard', despite the fact that bovine thrombin has been associated some years ago with the development of antibodies to clotting factors V, XI, and thrombin, which had occasionally led to life-threatening coagulopathies. Alternatively, PRP can be activated by autologous thrombin, produced with commercially available thrombin production kits, which either use autologous whole blood sequestered PPP or PRP (Table 3).

- Recently, Tsay et al. reported that the use of a synthetic peptide that mimics thrombin known as peptide-6 SFLLRN (TRAP). Activation with TRAP results in a more sustained release of the PGF with less PG retraction and higher PDGF-AB and TFG-β concentrations.
- The mechanism of this sustained-release phenomenon is unclear, but it may possibly be useful in the development and maturation of platelet enriched bone grafts and also in tissue healing.
- Mixing PRP with thrombin and calcium chloride, to antagonize the anticoagulative effect of the citrate present in the pre-donation blood bag, will result in the activation of the platelet concentrate with the development of the viscous PG solution. Thereafter, the PG can be exogenously applied with a syringe or as a solid clotted jelly mass applied to soft tissues, bone or synthetic bone.
- From a surgical point of view, an "ideal" PG procedure is often defined as a procedure forming a platelet coagulum within 10 seconds. However, the formation of the coagulum is merely a function of the activated fibrinogen concentration, rather than the number of platelets.[19]

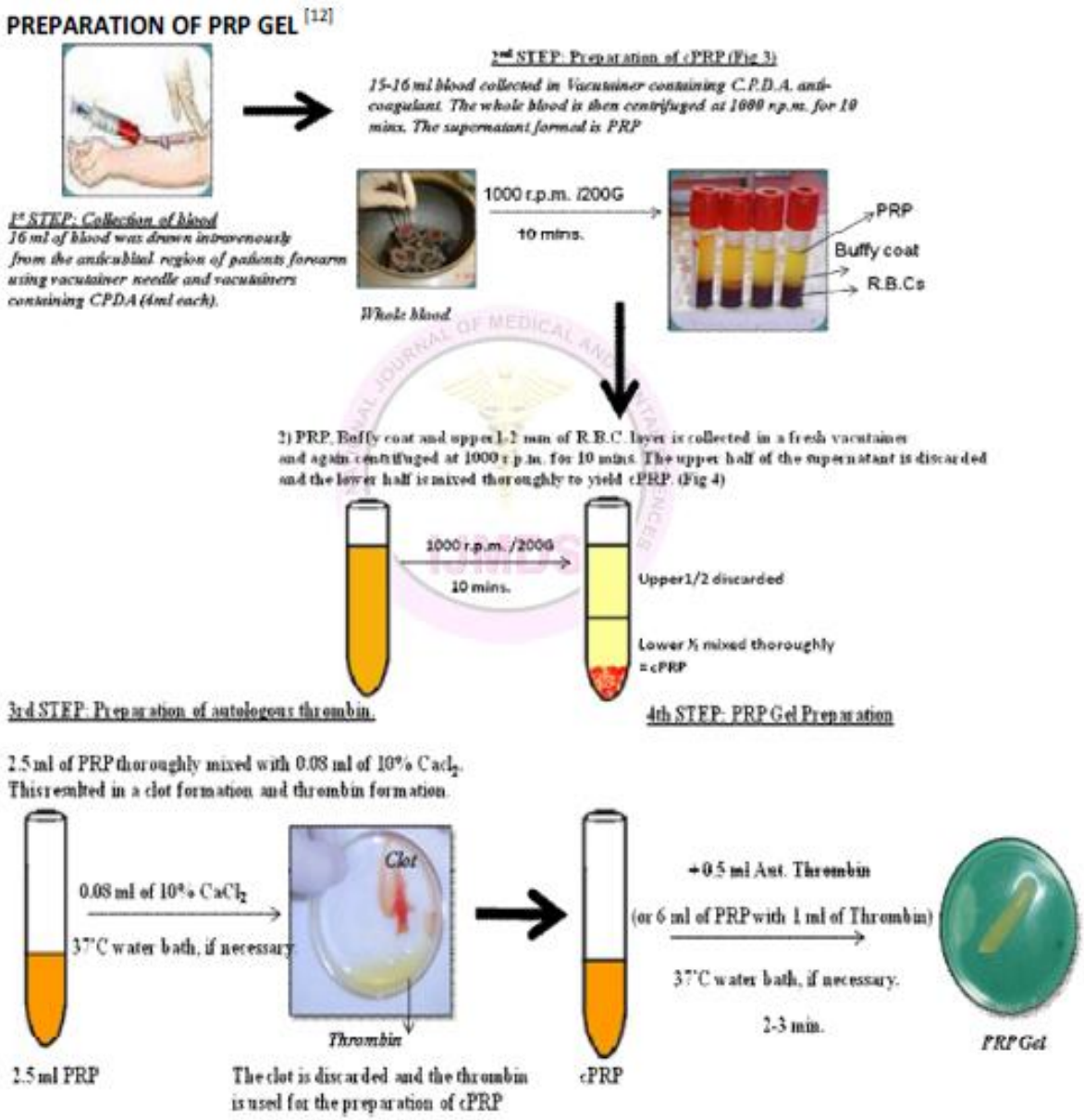
Autologous thrombin kit Manufacturer	Required volume. Product	Thrombin volume	Activator Reagent	Thrombin Activity	Ratio AT:PRP
ActivAT™ Sorin Group, Mirandola Italy	12 mL PPP	5 – 6 mL	ethanol 17%, glass beads calcium chloride 10%	40-90 IU	1:10
Magellan™ Medtronic, Minneapolis, MN, USA	3 mL WB	2,5 mL	glass fiber calcium chloride 10%	10 – 15 IU	1:4
Petri dish Catharina Hospital	variable PPP/PRP	variable	glass Petri dish calcium chloride 10%	10 – 15 IU	1:4
Thrombin Assessing Device™ Thermogenesis, Rancho Cordova, CA, USA	9,5 – 10,5 mL PPP	8 mL	ethanol 18,8%, ceramic beads calcium chloride 10%	40 – 50 IU	1:3

Table 3. *Autologous Thrombin Processing Kits. The ratio AT:PRP refers to the manufacturer's proposed ratio for mixing PRP with thrombin to produce PG. (AT: autologous thrombin; IU: international units; PG: platelet gel; PPP: platelet poor plasma; PRP: platelet rich plasma; WB: whole blood).*

WHICH ANTICOAGULANT TO USE?

There are several choices of anticoagulants the clinician can use. However, only two support the metabolic needs of platelets and the viable separation of platelets in an undamaged manner.

- Anticoagulant citrate dextrose-A (ACD-A) is preferred and will best support platelet viability. The citrate binds calcium to create anticoagulation. The dextrose, buffers, and other ingredients support platelet metabolism. ACD-A is the anticoagulant used to store viable platelets for platelet transfusions from blood banks.
- Citrate Phosphate Dextrose (CPD) is also useful for PRP development. It is similar to ACD-A but has fewer supportive ingredients and, therefore, is 10% less effective in maintaining platelet viability.



CLINICAL DEVELOPMENT AND USE OF PRP

PRP is best developed from autogenous whole blood shortly before or at the very beginning of the surgical procedure. This is because platelets will collect at the surgical site to initiate clotting and healing. This will reduce the whole blood platelet count somewhat. In addition, during surgery intravenous fluid will dilute whole blood, further reducing platelet numbers. Once developed, PRP is stable and remains sterile in the anticoagulated state for 8 hours. Therefore, with longer surgeries, PRP is just as effective and sterile as it would be if used immediately.

However, the PRP must be separated from the PPP soon after centrifugation because the concentrated platelets will slowly diffuse into the PPP over time and would reduce the platelet count of the PRP preparation.[22]

PRECAUTIONARY MEASURES DURING CLINICAL APPLICATIONS OF PRP

PRP may be mixed into a bone graft, layered in as the graft is placed, sprayed on a soft tissue surface, applied on top of graft, or used as a biologic membrane.

However, clotting of the PRP should be done only at the time of use. Clotting activates platelets, which begin secreting their growth factors immediately. Within 10 minutes they secrete 70% of their stored growth factors and close to 100% within the first hour. They then synthesize additional amounts of growth factors for about 8 days until they are depleted and die. Therefore, clinicians should only clot (activate) PRP when they are ready to use it and not in advance. Clinicians should also critically assess publications, which may claim to study PRP but are actually studying growth factor depleted clots or supernatants. Complete PRP is both a fresh clot and a supernatant.

This knowledge is germane to those who have advanced the concept of developing PRP from clotted blood or to companies that have promoted "serum separator tubes." The serum is not plasma and contains almost no platelets. It is impossible to develop PRP from clotted whole blood. Because the two functional roles of platelets in nature are the initiation of healing and hemostasis, platelets become part of the physical blood clot and, therefore, the serum is devoid of platelets. PRP can only be developed from anticoagulated blood.

GROWTH FACTORS, PRP, AND CANCER

Because growth factors stimulate cellular proliferation, some have advanced a concern that the recombinant BMP's and PRP might stimulate cancers. Actually, no growth factor can provoke cancer. All growth factors act on cell membranes, not the cell nucleus. Growth factors activate an internal cytoplasmic signal protein, which promotes a normal gene expression, not an abnormal gene expression. Growth factors are not mutagens, unlike true carcinogens such as radiation, tobacco anthracene tars, UV light, etc. Instead, growth factors are normal body

proteins. The security specifically related to PRP and cancer is that PRP is nothing more than the same blood clot that would be in any normal wound, except it contains a greater number of platelets.

Peptide growth factors are present in platelet-rich plasma (PRP).

Name	Cytogenetic location	Biologic activities
Transforming growth factor, beta-I; TGFB1	19q13.2	Controls proliferation, differentiation, and other functions in many cell types
Platelet-derived growth factor, alpha polypeptide; PDGFA	7p22.3	Potent mitogen for connective tissue cells and exerts its function by interacting with related receptor tyrosine kinases
Platelet-derived growth factor, beta polypeptide; PDGFB	22q13.1	Promotes cellular proliferation and inhibits apoptosis
Platelet-derived growth factor C; PDGFC	4q32.1	Increases motility in mesenchymal cells, fibroblasts, smooth muscle cells, capillary endothelial cells, and neurons
Platelet-derived growth factor D; PDGFD	11q22.3	Involved in developmental and physiologic processes, as well as in cancer, fibrotic diseases, and arteriosclerosis
Insulin-like growth factor I; IGF1	12q23.2	Mediates many of the growth-promoting effects of growth hormone
Fibroblast growth factor I; FGF1	5q31.3	Induces liver gene expression, angiogenesis and fibroblast proliferation
Epidermal growth factor; EGF	4q25	Induces differentiation of specific cells, is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin
Vascular endothelial growth factor A; VEGFA	6p21.1	Is a mitogen primarily for vascular endothelial cells, induces angiogenesis
Vascular endothelial growth factor B; VEGFB	11q13.1	Is a regulator of blood vessel physiology, with a role in endothelial targeting of lipids to peripheral tissues
Vascular endothelial growth factor C; VEGFC	4q34.3	Angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessels

Includes, name, cytogenetic location, and biologic activities of platelet growth factors. Furthermore, PRP content other proteins like interleukin-8, macrophage inflammatory protein-1 alpha, and platelet factor-4.

ADVANTAGES OF PLATELET GEL AND PRP OVER FIBRIN SEALANTS

• Safe autogenous preparation, free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever and Creutzfeld–Jacob disease (mad cow disease).

• Convenient for the patient since blood is collected in the immediate preoperative period.

• More patients are eligible for this procedure because the criteria of blood bank donation do not have to be met; this would include children up to age 6, weights upto 25 kg, the elderly, those whose medical condition would preclude the blood bank from drawing a unit of whole blood.

• Presence of platelets brings cytokines and growth factors to the site of surgery in a manner that would not occur with fibrin glue.

CLINICAL APPLICATIONS OF PRP:

1) Split Thickness Skin Graft Donor Sites:

2) Sinus Lift Procedures:

3) Ridge Augmentation Procedures:

4) Periodontal surgery:

i) Vertical intrabony defects
ii) Furcation defects
iii) Root coverage procedures

5) PRP Added to Commercial Membranes:

6) Extraction sockets:

7) Alveolar Bone Grafting:

8) Implant surgeries:

9) Distraction osteogenesis:

10) PRP in patients with anti-coagulants:

1) Split Thickness Skin Graft Donor Sites: PRP has demonstrated efficacy in the healing of split-thickness skin graft (STSG) donor sites. The revascularization is quickly enhanced by the angiogenic activity of PDGF and TGFß.

2) Sinus Lift Procedures: PRP also improves the handling of particulate graft apart from enhancing the osteogenesis of graft

3) Ridge Augmentation Procedures: Both vertical and horizontal ridge augmentation procedures are benefited from PRP.

If either a cortical-cancellous block or a strictly cancellous marrow graft is used, the PRP is incorporated into and on the surface of the graft.

4) Periodontal surgery

i) Vertical intrabony defects

ii) Furcation defects

iii) Root coverage procedures

A study was conducted in which the freeze-dried cortical bone allograft was grafted into wide three wall, two wall, and one wall combination furcation defects. The authors concluded that out of 97 defects treated, 23 manifested complete bone regenerations, 30 showed greater than 50%, 24 less than 50% osseous repair and 12 defects failed to demonstrate any regeneration, of which nine were furcation involvement.

5) PRP Added to Commercial Membranes: Commercial Membranes such as Collatape®, Resolute®, or Osseoquest®, have a texture that will absorb the "activated" PRP gel. This will allow the clinician to apply growth factors to longer-lasting membranes so as to gain the benefit of each.

6) Extraction sockets: A study on 117 patients was done in which platelet-rich plasma was placed in the extraction sockets after third molar surgery. The result showed a decreased rate of alveolar osteitis in sockets treated with PRP. The PRP treated sockets also showed better hemostasis, faster soft tissue flap healing, and decreased swelling post-operatively. One-month postoperative radiographs showed subjectively more dense bone fill and radioopacity in the PRP treated sockets.

7) Alveolar Bone Grafting: In a study on 7 Cleft lip and palate patients, the cleft alveolus was grafted with autologous iliac cancellous bone incorporated with platelet-rich plasma (PRP). The bone regenerates at the cleft site was quantitatively evaluated using 3-dimensional computed tomography scans at 5 or 6 months postoperatively. The results showed a higher volume ratio of regenerated bone to the alveolar cleft in cases treated with PRP than in controls.

8) Implant surgeries: In a case presented by Thor A et al in the year 2002, particulate autogenous bone, platelet gel, and a titanium mesh were used for alveolar bone reconstruction of the anterior maxilla prior to implant placement. After 4.5 months of healing the mesh was removed and titanium implants were placed. The results showed that the healing was uneventful, and the anterior maxilla had increased in height and width during the initial healing. All implants became integrated and supported a fixed dental bridge for over 3 years with no dramatic dimensional changes of the graft. It was concluded that the autogenous growth factors in the gel possibly contributed to the positive outcome.

9) Distraction osteogenesis: Robiony M, Polini F,Costaf, Poloti M evaluated a new method on restoring severe atrophic mandible using platelet-rich plasma (PRP) during distraction osteogenesis. During the surgery, a mixture of autologous iliac bone graft and an autologous platelet concentrate filled the distraction gap. This mixture constituted an autologous bone-platelet gel that was used to create a useful bony scaffold for distraction regenerate. After a latency period of 15 days, a distraction run of 0.5 mm/d, and a 60-day period of consolidation, the distraction device was removed and implants were placed simultaneously. The results showed that in all the treated patients, planned distraction height was achieved with a considerable enhancement of bony regeneration, and in all cases, it was possible to place implants at a planned time. The study concluded that the combination of these recent and innovative regenerative methods seems to be effective in restoring the severely atrophic mandible

10). PRP in patients with anti-coagulants: Antonio Della Valle et al in their study had put PRP gel in the extraction socket in 40 patients on anti-coagulant drugs (suspended 36 hrs prior to the extraction). The results showed that only 2 patients reported hemorrhagic complications (5%). Sixteen patients (40%) had mild bleeding that was easy to control with haemostatic topical agents. The remaining 22 patients (55%) presented with adequate hemostasis. Thus, it was concluded that oral surgery in cardiac patients under oral anticoagulant therapy might be facilitated with PRP gel. This biological and therapeutic improvement can simplify systemic management and help avoid hemorrhagic and/or thromboembolic complications.[14]

The positive effects of PRP as reported in the literature are:

1.“Jump-starts” the cascade of osteogenesis in a bone graft.

2.Promotes early consolidation of the graft

3.Speeds up mineralization of the graft.

4.Improves trabecular bone density.

5.Allows placement of implants into the graft at an earlier time.

6.Provides earlier availability of growth factors and BMP.

7.Enhances osteoconduction.[14]

Contraindications:

1) Absolute Contraindications:

- Platelet dysfunction syndrome.
- Critical thrombocytopenia.
- Hemodynamic instability.
- Septicaemia.
- Local infection at the site of the procedure.
- Patient was unwilling to accept risks.6

2) Relative Contraindications:

- Consistent use of NSAIDs within 48 hours of the procedure.
- Corticosteroid injection at a treatment site within 1 month.
- Systematic use of corticosteroids within 2 weeks.
- Tobacco use.
- Recent fever or illness.
- Cancer-especially hematopoietic or bone.
- HGB < 10 g/dl.
- Platelet count < 105 / ul.6

The Efficacy of an Autologous Platelet Concentrate (APC):

- Depends upon the concentration of the released components at the site of application. For the maximum biologic effect, the optimal platelet concentration of an APC is 1.5 – 3.0 million platelets per microliter.
- Is directly related to the composition of the white blood cell concentration: primarily mononuclear vs. granulocyte. Clinically relevant APCs contain CD34+ cells (markers for mesenchymal stem cells) and their homing agent (SDF-1!), and they have characteristics similar to the requirements of the American Association of Blood Banks for transfusable platelets.25

Test tube systems, Lab centrifuges and many other so-called "PRP" systems fail to achieve the threshold of platelet concentration and white blood cell composition required

AABB and FDA/CBER Guidelines for Transfusion Therapy

- While no system is completely closed, the SmartPReP 2 System was designed to follow AABB guidelines for cell separation. Instead of using luer connectors that can be easily contaminated and cannot be disinfected, SmartPReP 2 disposables incorporate re-sealable injection ports that can be aseptically disinfected with alcohol prior to needle entry. The SmartPReP 2 is the closest system to a closed system on the market today.

Sterility Testing of PRP:

Harvest Technologies meet state-of-the-art requirements for sterility testing. Prepared with the SmartPReP 2 System, PRP aliquots were incubated, cultured and sub-cultured over 18 days aerobically and anaerobically. All cultures were negative.

Harvest Technologies has documented sterility of the concentrated platelet product when following the manufacturer's instructions for use.[25]

SmartPReP Performance[1]		
	Whole Blood	**SmartPReP PRP**
Platelets	250 x 10^3 µL	1,500 x 10^3 µL
SDF-1α pg/ml	1,000	2,663
PDGF-AB ng/ml	30	398
TGF-ß1 ng/ml	43	319
VEGF pg/ml	55	600
WBC Count	5.7 x 10^3 µL	20.1 x 10^3 µL
Mononuclear	37.5%	75.1%
Granulocyte	62.5%	24.5%
$CD34^+$ (Total Cells Delivered)		171,571 (64% Yld.)

1) Summary of the RCTs using PRP in periodontal surgery:

Authors	Year of publication	Number of patients	Treatment	Follow-up (wks)	Main results	Effect of PRP
Pradeep et al.	2009	20	Treatment of furcation defects	24	No complete closure of furcation defects	weak
Menezes et al.	2012	60	Treatment of infrabony defects	48-192	Positive effect of PRP used with other graft materials in infrabony defects but not when used alone	weak
Saini et al.	2011	20	Treatment of infrabony defects	12-24-36	Positive effect of PRP used with other graft materials in infrabony defects	moderate
Bharadwaj et al.	2011	10	Treatment of infrabony defects	24	Significant improvement in PD, CAL and bone radio-density	strong
Ozdemir et al.	2012	14	Treatment of infrabony defects	24	Positive effect of PRP used with other graft materials in infrabony defects but not when used alone	weak
Harnack et al.	2009	22	Treatment of infrabony defects	24	No improvement in PPD and CAL derived from the adjunt of PRP to other graft material	weak
Rodrigues et al.	2011		Treatment of infrabony defects	12-24-36	Better clinical results for PRP used with other graft materials in infrabony defects than with PRP used on its own	weak
Dori et al.	2008	26	Treatment of infrabony defects	48	No adjunctive benefit with the use of PRP	weak
Dori et al.	2009	30	Treatment of infrabony defects	48	No adjunctive benefit with the use of PRP	weak
Piemontese et al.	2008	60	Treatment of infrabony defects	48	No adjunctive benefit with the use of PRP	weak
Keceli et al.	2008	40	Root coverage	6-36-48	No adjunctive benefit with the use of PRP	weak

2) Summary of the RCTs, using PRP in soft/bone tissue surgery and implant surgery

Authors	Year of publication	Number of patients	Treatment	Follow-up (wks)	Main results	Effect of PRP
Anitua et al.	2006	295	Implantology	8	Improvement in implant prognosis	strong
Anand et al.	2012	11	Implantology	12-24-36-48	Improved early bone apposition around the implant	strong
Gentile et al.	2010	15	Reconstructive surgery of the jaw	2-4-12-24	Efficacy of PRP treatment in terms of patient satisfaction and low-morbidity	strong
Wojtowicz et al.	2007	16	Augmentation of mandibular bone	12	PRP is more effective than bone marrow, containing CD34+ cells	strong
Daif	2012	24	Bone regeneration of mandibular fractures	1-12-24	Direct application of the PRP along the fracture lines may enhance bone regeneration in mandibular fractures	strong
Khairy et al.	2012	15	Sinus lift	12-24	PRP- enriched bone grafts were associated with superior bone density at 6 months post grafting	strong
Poeschl et al.	2012	14	Sinus lift	28	Increased new bone formation when PRP was used	strong
Cabbar et al.	2011	10	Sinus lift	28	No statistically significant differences were observed	weak

PLATELET-RICH FIBRIN/PRF

SECOND GENERATION PLATELET CONCENTRATES:

Platelet-rich fibrin (PRF) was first developed in France by Choukroun et al. in 2001. This second-generation platelet concentrate eliminates the risk associated with the use of bovine thrombin.[18]

Properties of PRF

(1) The biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycemic chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on healing processes

(2) PRF is not only a platelet concentrate but also an immune node able to stimulate defense mechanisms. It is likely that the significant inflammatory regulation noted on surgical sites treated with PRF is the outcome of retro control effects from cytokines trapped in the fibrin network and released during the remodeling of this initial matrix.

(3) Role of fibrin matrix of PRF:

• Fibrin meshwork in PRF differs from that in PRP. In PRP, there are bilateral junctions resulting in a rigid network that does not honor the cytokine enmeshment and cellular migration. The increased thrombin required for rapid setting of the PRP leads to a rigid polymerized material.

• Fibrin and wound coverage: Fibrin matrix guide the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts[18]

• Role of Fibrin in Angiogenesis. Entrapment of cytokines in the 3-dimensional architecture of the fibrin matrix results in their sustained release which is monumental in the initiation of angiogenesis. The cytokines responsible for this action include the FGF, VEGF, angiopoietin, and PDGF within the fibrin gel. It is the rigidity of the fibrin matrix that is instrumental in the process of angiogenesis in response to FGF and VEGF stimulation. Increased expression of $\alpha v\beta 3$ integrin in response to fibrin allows the binding of endothelial cells to fibrin itself, fibronectin, and vitronectin.[17]

• Fibrin-Assisted Immune Response. -: Fibrin constitutes natural support to immunity. Increased expression of CD11c/CD18 receptor on endothelial cells by fibrin aids in enhanced adhesion to endothelial cells and fibrinogen, and transmigration of neutrophils. Fibrin and fibronectin also modulate wound colonization by the macrophages.

• Effect of Fibrin on Mesenchymal Stem Cells-: Fibrin matrix acts as a scaffold for the undifferentiated mesenchymal cells that facilitate the differentiation of these cells thus aiding in tissue regeneration.[3]

• Effect of Fibrin on Osseous Tissue-: Direct interaction between fibrin and the osseous tissue lacks significant documentation. However, bone morphogenic proteins enmeshed in the fibrin matrix have the ability to be released consistently highlighting the angiogenic, hemostatic, and osteoconductive properties. Fibrin is accredited as a support matrix for BMP. BMPs enmeshed in fibrin are progressively released and are able to induce bone. Consistent release of VEGF, FGF and PDGF helps in angiogenesis. Hemostasis is achieved through the ability of fibrin clots to trap circulating stem cells, allowing vascular and tissue restoration.[17]

Difference between first- and second-generation platelet concentrates:

First generation—PRP	**Second generation—PRF**
1)Use of bovine thrombin and calcium chloride(anticoagulants)	1) No anticoagulants used
2)Sudden fibrin polymerization-depending on the amount of surgical additives (thrombin and calcium chloride)	2) Slow natural polymerization on contact with glass particles of the test tube results in physiologic thrombin concentration
3) 3-D organization of a fibrin network-condensed tetra molecular or bilateral junctions constituted with strong thrombin concentrations, allows the thickening of fibrin polymers: this leads to a rigid network, not very favourable to cytokine enmeshment and cellular migration	3) 3-D network-connected trimolecular or equilateral junctions-allows the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration

4) The 3-D structure provides great resistance of such a gel, appropriate to firmly seal biologic tissues 5) Favorable healing due to slow polymerization 6) More efficient cell migration and Proliferation 7) PRF has supportive effect on immune system 8) PRF helps in haemostasis[26]	4) The 3-D structure gives elasticity and flexibility to the PRF membrane [18]

WHY PLATELET-RICH FIBRIN OVER PLATELET-RICH PLASMA?

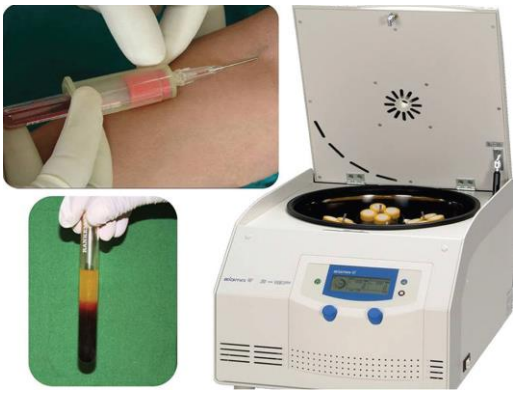
Conversion of fibrinogen to fibrin takes place slowly with small quantities of physiologically available thrombin present in the blood sample itself. Thus, a physiologic architecture, which is very favorable to the healing process, is obtained due to slow polymerization. The fibrin network generated here is very similar to a natural one, and leads to more efficient cell migration and proliferation, and thus cicatrization.

Slow polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and organic chains in the fibrin meshes. This result would imply that PRF, unlike the other platelet concentrates would be able to release cytokines during the fibrin matrix remodelling. Such a mechanism might explain the clinically observed healing properties of PRF. And also, PRF has a supportive effect on the immune system.

Studies showed PRP has limited potential to stimulate bone regeneration as it releases growth factors quickly, just before the cell outgrowth from the surrounding tissue.

It is also been demonstrated that bovine thrombin which is used for PRP preparation may have toxic effects on the body cells.[26]

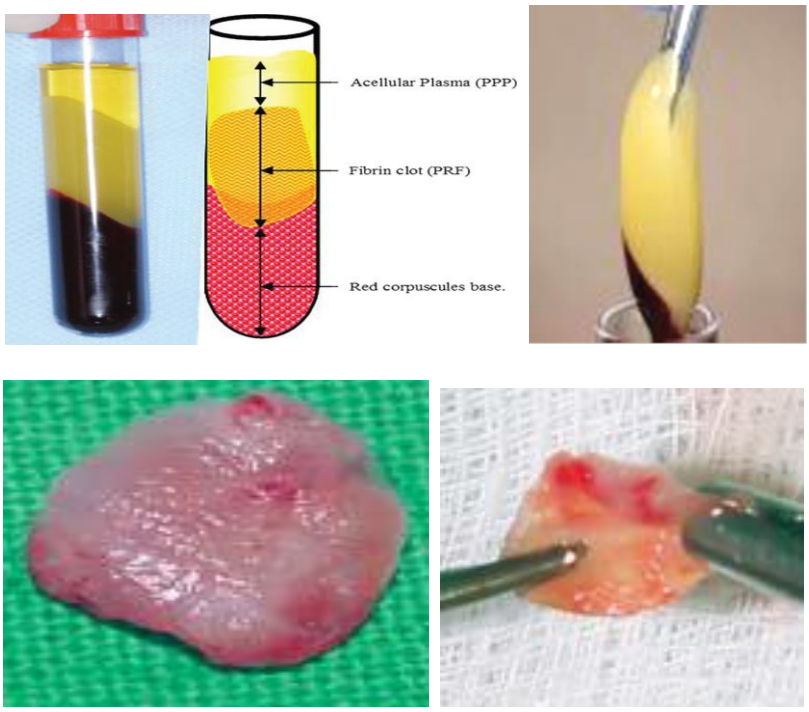
PREPARATION OF PRF



The protocol for PRF preparation is very simple and simulates that of PRP. It includes a collection of whole venous blood (around 5 ml) in a sterile vacutainer tube (6 ml) without anticoagulant and the vacutainer tube is then placed in a centrifugal machine at 3,000 revolutions per minute (rpm) for 10 min, after which it settles into the following three layers: Upper straw-colored acellular plasma, the red-colored lower fraction containing red blood cells (RBCs), and the middle fraction containing the fibrin clot .[12]

The upper straw-colored layer is then removed and the middle fraction is collected, 2 mm below the lower dividing line, which is the PRF. The mechanism involved in this is; the fibrinogen concentrated in the upper part of the tube combines with circulating thrombin due to centrifugation to form fibrin. A fibrin clot is then formed in the middle between the red corpuscles at the bottom and acellular plasma at the top. The middle part is platelets trapped massively in fibrin meshes. The success of this technique entirely depends on the time gap between the blood collection and its transfer to the centrifuge and it should be done in less time.

The blood sample without anticoagulant starts to coagulate almost immediately upon contact with the glass, and it decreases the time of centrifugation to concentrate fibrinogen. Following proper protocol and quick handling is the only way to obtain a clinically usable PRF clot charged with serum and platelets. Resistant autologous fibrin membranes may be available by driving out the fluids trapped in the fibrin matrix.[12]



Clinical Implications of PRF

• In sinus lift procedures.

• Socket preservation.

• PRF membrane has been used for gingival recession coverage with coronally advanced or lateral pedicle flap for multiple and single recession respectively. PRF acts both as a healing and interposition biomaterial.

• Filling of the cystic cavity.

• In the treatment of combined periodontic endodontic lesion/furcation defect [11]

Advantages of PRF over PRP

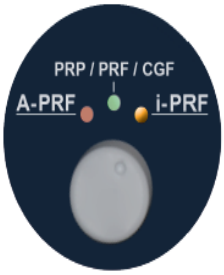
• No need for the addition of bovine thrombin or other anticoagulants so it is completely safe.

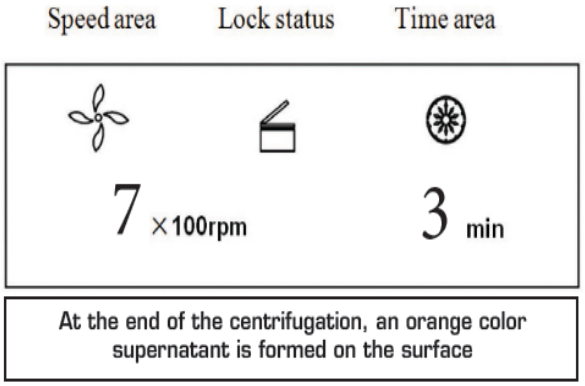
• Standard production protocol.[18]

Limitations of PRF Technology

(1) Only a limited volume of PRF can be used. Because it is obtained from an autologous blood sample, the quantities produced are low. This fact limits the systematic utilization of PRF for general surgery.

(2) PRF tissue banks are unfeasible. The fibrin matrix contains all the circulating immune cells and all the highly antigenic plasmatic molecules. That is why PRF membranes are totally specific to the donor and cannot constitute an allogenic graft tissue[18]





1. Use the i-PRF9 tubes (orange).
2. Turn the switch on position 3: i-PRF, the orange LED lights on.
The machine is ready for the use of i-PRF. The settings are preset and locked.
Details: 7 x 100 rpm / 3 minutes for the i-PRF.
To start centrifugation, press ***START/STOP*** button
At the end of centrifugation the lid will open automatically
3. At the end of the spin, an orange supernatant will form on the surface.
4. Penetrate the cap with a 21G needle (green) mounted on a syringe.
5. Place the bevel of the needle in the middle of the i-PRF supernatant, against the wall of the tube (better visibility).
6. Aspirate until the level of the red blood cells raises up to the needle bevel.
7. Remove the needle maintaining suction.
8. i-PRF remains liquid for about 10 -12 minutes, then it will clot. The injection will have to be done before the end of these 10-12 minutes.

RECENT ADVANCES IN AUTOLOGOUS PLATELET CONCENTRATE

1.Titanium-prepared platelet-rich fibrin (T-PRF):

This method is based on the hypothesis that titanium may be more effective in activating platelets than the silica activators used with glass tubes in Choukroun's leukocyte and platelet-rich fibrin (L-PRF) method. The silica particles in the tube, although dense enough to sediment with the red blood cells, are small enough for a fraction to remain colloidally suspended in the buffy coat, fibrin, and platelet-poor plasma layers; therefore, these particles might reach the patient when the product is used for treatment.

Following these discussions, a modified initial L-PRF method was developed by changing the structure of the tubes and using a more biocompatible material, titanium. This method was tried to eliminate the speculations about the potential negative effects of silica from dry glass or glass-coated plastic tubes.

Based on the above concept, a study was done by Mustafa Tunal Jet al in 2014 aimed to define the structural characteristics of T-PRF and compare it with L-PRF. Blood samples were collected from ten healthy male volunteers. Nine milliliters was transferred to a dry glass tube, and 9 ml was transferred to a titanium tube. Half of each clot (i.e., the blood that was clotted using T-PRF or L-PRF) was processed with a scanning electron microscope (SEM) and the other half of each clot was processed for fluorescence microscopy analysis and light microscopy analysis. The T-PRF samples seemed to have a highly organized network with continuous integrity compared to the other L-PRF samples. Histomorphometric analysis showed that the T-PRF fibrin network covers a larger area than the L-PRF fibrin network; also fibrin seemed thicker in the T-PRF samples. Thus, the author concluded that platelet activation by titanium seems to offer some high characteristics to T-PRF.[69]

2.Advanced platelet-rich fibrin:(A-PRF)

i)Concept of A-PRF

Many recent studies have shown the interest and potential of white cells in the inflammatory cascade, as a corollary, a prominent action in the early days of stimulation of Osseo-progenitor cells and vessels growth. It was, therefore, natural to try to capture the whole amount of white cells (especially the monocytes) in the PRF, to make it more active in stimulating bone grafts by the production of BMPs and VEGF, but also to turn to a more rapid transformation of monocytes into macrophages to increase the effect bone stimulation.

ii)The *in vivo* test of A-PRF shows a faster vascularization after 2 weeks than with classic PRF (around x 2.5 times.) The clinical results show more efficiency in soft and hard tissue healing. Probably, the presence of stems cells and endothelial cells is also a factor of more fast vascularization.[70]

iii)Indications:

1) Oral surgery: implantology, bone grafts, sinus lifts, soft tissue surgery, socket preservation

2) Orthopaedics, regenerative medicine

3) Dermatology, Aesthetics (surgery & medicine)

iv)Methodology

The equipment's used

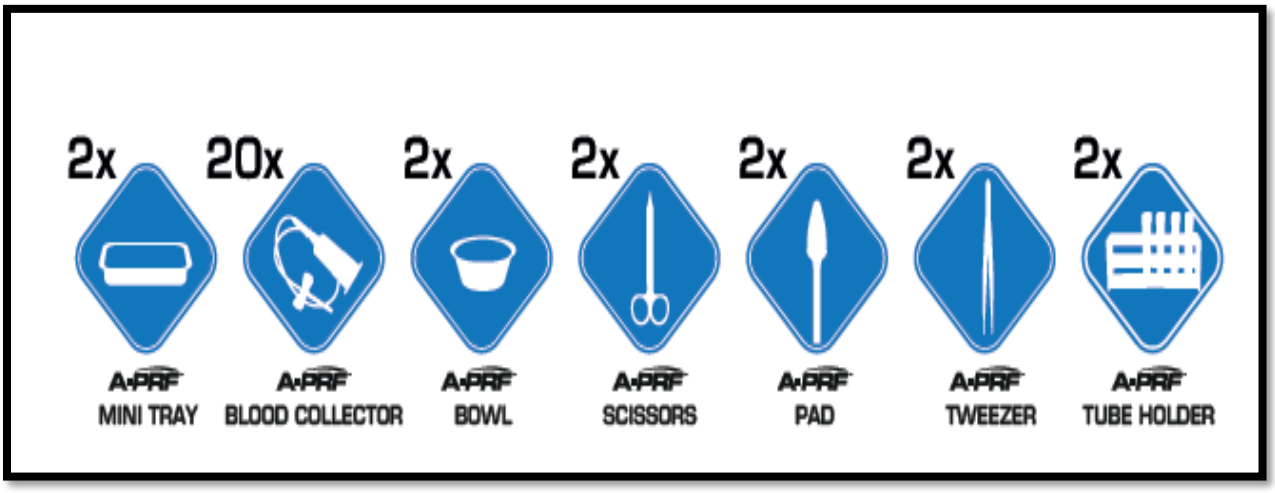
1)The new centrifuge DUO

It has a «switch» button that affords to select various settings modes for the A-PRF and i-PRF. The values of these settings are present and locked except for PRP / PRF / CGF. When the knob is turned on the desired mode of operation, the corresponding LED light will be on and the machine is ready to use. To start the centrifugation the start button should be pressed.

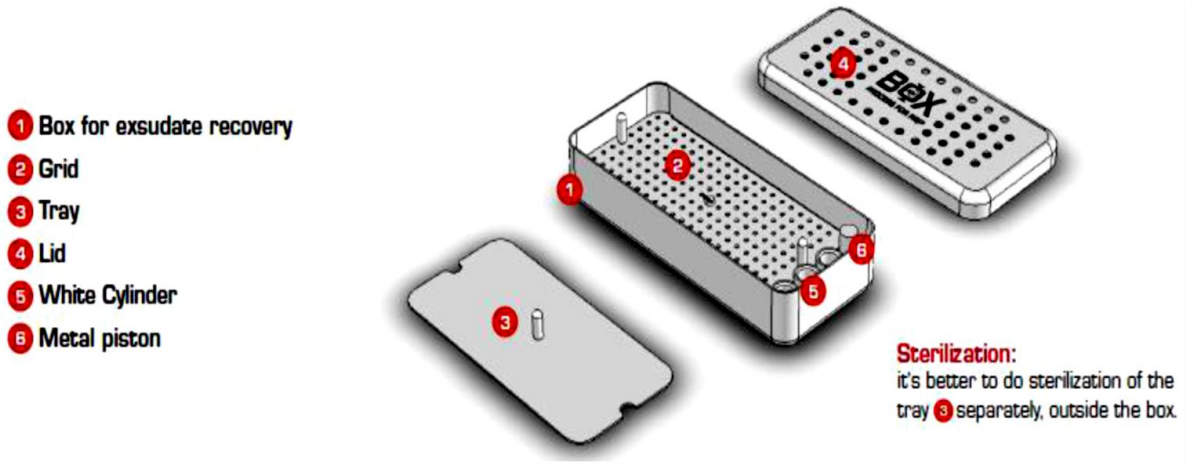
2)A-PRF red tubes + are

- Class IIa medical device.
- Glass tubes without anticoagulants or additives

3)Other instruments



4)PRF BOX:



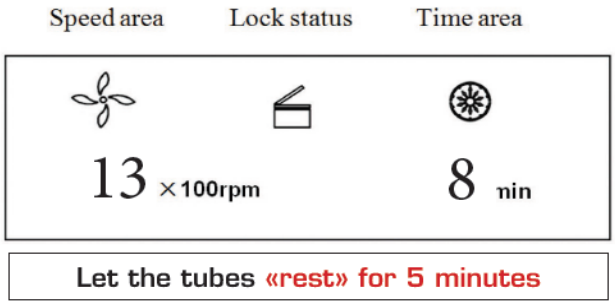
Put the clots after separation on the grid. Cover with the tray and then put the lid. The membranes will be ready for use after 2 min. The membranes remain intact (without any dehydration and with a constant thickness. The exudate collected in the bottom is very rich in proteins (Fibronectin and Vitronectin).[70]

Uses of PRF- BOX:

1. To get membranes of constant thickness and always hydrated
2. To remain them intact for 2 or 3 hours (no dehydration)
3. To recover the exudate (in the BOX): rich in proteins: Vitronectin and Fibronectin
4. To produce ‘‘plugs’’ of PRF, for socket extraction filling (in the white cylinders, with the piston)

v)Settings for the preparation of A-PRF:

Turn the switch on the left to position 1: A-PRF



1. Press the **ON/OFF** button: on position **ON** at the back of the machine
2. The lid opens automatically
3. Take off the rubber
4. Close the lid
5. Turn the switch on the left to position 1: A-PRF, the red LED lights on.

Your machine is ready for use. The settings are preset and locked.
Details : (13) x 100 rpm / (8) minutes

6. To start the spin, press the ***START*** button
7. At the end of the spin, the lid opens automatically

⚠ **Caution! Starting the spin without removing the rubber may destroy the motor.**

A study was done by Ghanaati S, protocols for standard platelet-rich fibrin (S-PRF) (2700 rpm, 12 minutes) and advanced platelet-rich fibrin (A-PRF) (1500 rpm, 14 minutes) were compared to establish by histological cell detection and histomorphometric measurement of cell distribution, the effects of the centrifugal force (speed and time) on the distribution of cells relevant for wound healing and tissue regeneration. Immunohistochemistry for monocytes, T and B -lymphocytes, neutrophilic granulocytes, CD34-positive stem cells, and platelets was performed on clots produced from four different human donors. Platelets were detected throughout the clot in both groups, although in the A-PRF group, more platelets were found in the distal part, away from the buffy coat (BC). T- and B-lymphocytes, stem cells, and monocytes were detected in the surroundings of the BC in both groups.

Decreasing the rpm while increasing the centrifugation time in the A-PRF group gave an enhanced presence of neutrophilic granulocytes in the distal part of the clot. In the S-PRF group, neutrophils were found mostly at the red blood cell (RBC)-BC interface. Neutrophilic granulocytes contribute to monocyte differentiation into macrophages. Accordingly, a higher presence of these cells might be able to influence the differentiation of host macrophages and

macrophages within the clot after implantation. Thus, A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors. The relevance and feasibility of this tissue-engineering concept have to be proven through *in vivo* studies.[71]

3)Injectable platelet-rich fibrin :(I-PRF)

i) Concept of I-PRF

Numerous scientific publications describing the action of the white cells on vascularization and wound healing were published these recent years. With the unanimous conclusion that platelet concentrates enriched leukocytes are more effective on tissue and bone healing. The research on the A-PRF (PRF enriched leukocytes) and clinical outcomes confirm absolutely this scientific position. However, the use of platelet concentrates in «liquid» and not coagulated remains an important indication in various medical and dental applications.

The research was led by Dr. Joseph Choukroun, the inventor of the PRF technique with the collaboration of two laboratories - FORM in Frankfurt, Germany and Research Lab Clarion in Clarion, USA) focused to obtain a liquid «blood concentrate» enriched in white blood cells but also platelet enriched to increase the healing properties while retaining the principle of centrifugation «without anticoagulants» or «no additives».[70]

ii)Indications:

- Oral surgery: implantology, bone grafts, sinus lifts, soft tissue surgery, socket preservation

- Orthopaedics, regenerative medicine

- Dermatology, Aesthetics (surgery & medicine)

iii)Methodology

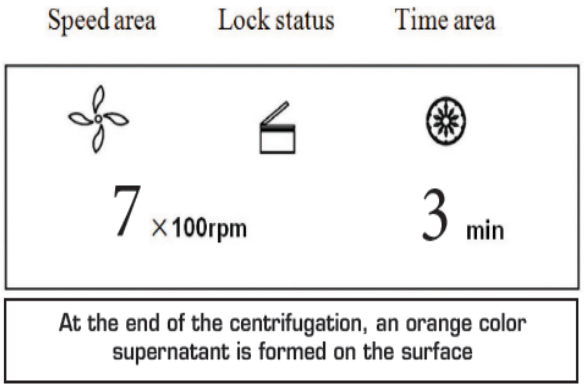
The equipment’s used

1) The new centrifuge DUO

2)I-PRF orange tubes 9

- Class IIa medical device.
- Tubes without anticoagulant or additives

iv)Settings for the preparation of I-PRF:



1. Use the i-PRF9 tubes (orange).
2. Turn the switch on position 3: i-PRF, the orange LED lights on.

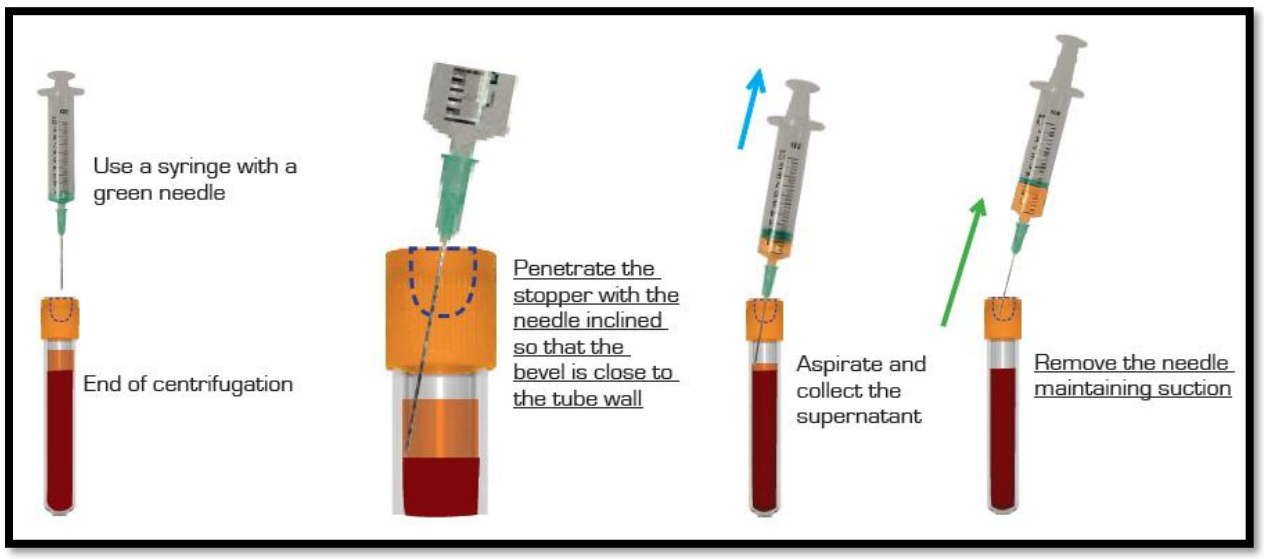
The machine is ready for the use of i-PRF. The settings are preset and locked.

Details: 7 x 100 rpm / 3 minutes for the i-PRF.

To start centrifugation, press ***START/STOP*** button

At the end of centrifugation the lid will open automatically

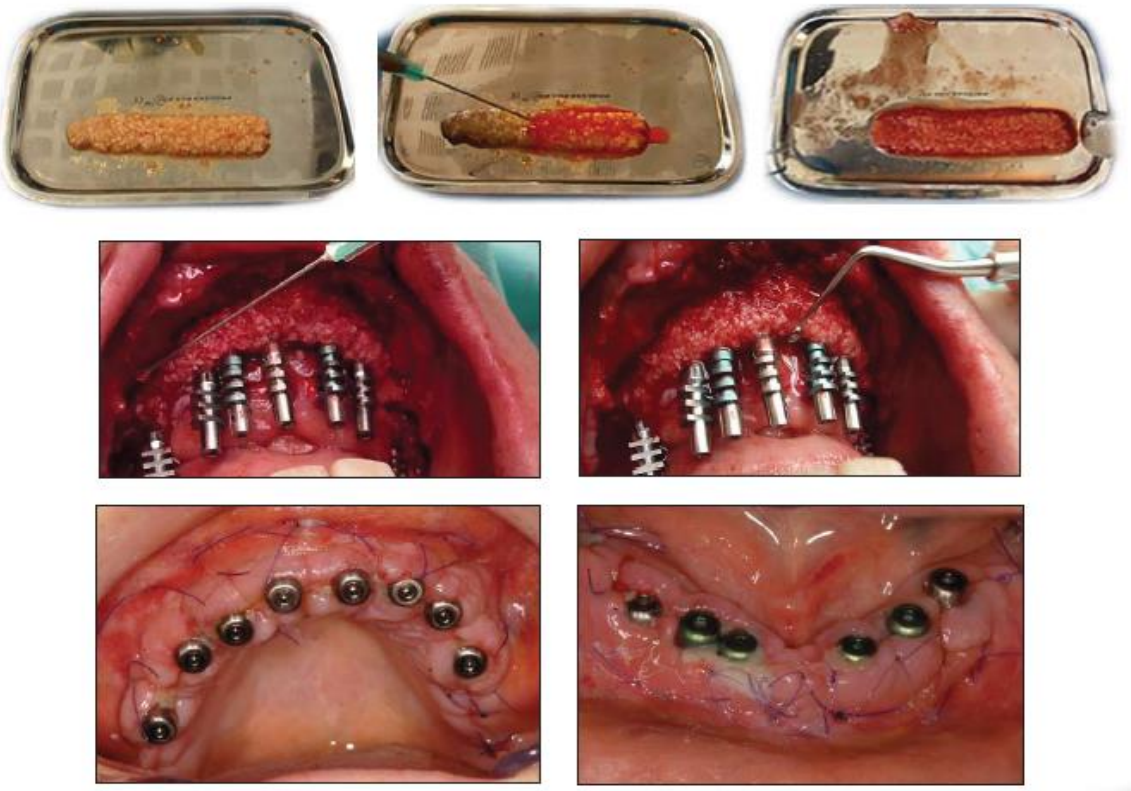
3. At the end of the spin, an orange supernatant will form on the surface.
4. Penetrate the cap with a 21G needle (green) mounted on a syringe.
5. Place the bevel of the needle in the middle of the i-PRF supernatant, against the wall of the tube (better visibility).
6. Aspirate until the level of the red blood cells raises up to the needle bevel.
7. Remove the needle maintaining suction.
8. i-PRF remains liquid for about 10 -12 minutes, then it will clot. The injection will have to be done before the end of these 10-12 minutes.



v)Clinical applications of I-PRF

- The blood for i-PRF must be drawn just before injection. It cannot be prepared in advance.
- The i-PRF can be injected:

1. Into the soft tissues
2. In the bone graft: the granules are mixed with the A-PRF (as usual), and poor the i-PRF drop by drop to avoid an overflow. Wait a few seconds and go on until the complete coagulation of the biomaterials (in less than a minute) is obtained. If it is injected too fast, the i-PRF will overflow from the bone graft.
3. Into the sinus, after the filling. The granules can be fixed and coagulated.
4. The i-PRF can be used to coagulate the biomaterials before application. This is the steak technique: Use the same technique of dropwise preparation.[70]



STUDIES ON PRP

1) A study was conducted by Garg and Arun K in 2000 to evaluate the effect of Platelet-Rich Plasma to Enhance Pre-Prosthetic Bone Grafts using the freeze-dried cortical bone allograft which was grafted into wide three wall, two wall, and one wall combination furcation defects. The authors concluded that out of 97 defects treated, 23 defects showed complete bone regeneration, 30 defects showed greater than 50%, 24 defects showed less than 50% osseous repair and 12 defects failed to demonstrate any regeneration, of which nine were furcation involvement. Thus, they concluded that PRP, when combined with FDBA, resulted in significant clinical and radiographical improvement in human periodontal endosseous defects at 6 months postoperatively.[30]

2) A study on 117 patients was done by Michael Tichler in 2002 in which platelet-rich plasma was placed in the extraction sockets after third molar surgery. IOPARs were used to evaluate the bone density & alveolar bone level after the 1st, 2nd and 7th day and 3rd & 6th month post-operatively. The result showed a decreased rate of alveolar osteitis in sockets treated with PRP and also showed better hemostasis, faster soft tissue flap healing, and decreased swelling postoperatively. Furthermore, one-month postoperative radiographs showed subjectively more dense bone fill and radioopacity in the PRP treated sockets.[31]

3) In a case report presented by Thor A et al in the year 2002, particulated autogenous bone, platelet gel, and a titanium mesh were used for alveolar bone reconstruction of the anterior maxilla before implant placement. Cortico-cancellous bone from the iliac crest was mixed with a preparation of autogenous platelet gel (platelet-rich plasma, thrombin, and calcium chloride) and placed against a titanium mesh fixed to the bone of the palate in a patient with severe resorption of the anterior maxilla. After 4.5 months of healing the mesh was removed and titanium implants were placed. A prolonged healing period of 8 months was allowed before healing abutments were placed and a fixed dental bridge was fabricated. The results showed that the healing was uneventful, and the anterior maxilla had increased in height and width during the initial healing. All the implants became integrated and supported a fixed dental bridge for over 3 years with no dramatic dimensional changes of the graft. It was concluded that the autogenous growth factors in the platelet gel possibly contributed to the positive outcome.[32]

4) Robiony M et al in 2002 evaluated a new method of restoring severe atrophic mandible using platelet-rich plasma (PRP) during distraction osteogenesis. During the surgery, a mixture of autologous iliac bone graft and an autologous platelet concentrate filled the distraction gap. This mixture constituted an autologous bone-platelet gel that was used to create a useful bony scaffold for distraction regenerate. After a latency period of 15 days, a distraction run of 0.5 mm/day, and 60 days of consolidation, the distraction device was removed and implants were placed simultaneously. The results showed that in all the treated patients, planned distraction height was achieved with a considerable enhancement of bony regeneration, and in all cases, it was possible to place implants at a planned time. The study concluded that the combination of the mixture of autologous iliac bone graft and an autologous platelet concentrate seems to be effective in restoring the severely atrophic mandible.[33]

5) A study was conducted by Antonio Della Valle et al in 2003 to evaluate the prevention of postoperative bleeding in anticoagulated patients undergoing oral surgery with the use of platelet-rich plasma. PRP gel was placed in the extraction socket in 40 patients on anti-coagulant drugs (suspended 36 hrs before the extraction). The results showed that only 2 patients reported hemorrhagic complications (5%). Sixteen patients (40%) had mild bleeding that was easy to control with haemostatic topical agents. The remaining 22 patients (55%) presented with adequate hemostasis. Thus they concluded that, oral surgery in cardiac patients under oral anticoagulant therapy might be facilitated with PRP gel. This biological and therapeutical improvement can simplify systemic management and help avoid hemorrhagic and/or thromboembolic complications.[34]

6) A study was done by Tomoki Oyama in 2004 to evaluate the efficacy of Platelet-Rich plasma in Alveolar bone grafting on 7 Cleft lip and palate patients. The cleft alveolus was grafted with autologous iliac cancellous bone incorporated with platelet-rich plasma (PRP). The bone regenerates at the cleft site were quantitatively evaluated using 3-dimensional computed tomography scans at 5 or 6 months postoperatively. The results showed a higher volume ratio of regenerated bone to an alveolar cleft in cases treated with PRP than in controls.[35]

7) Hesham El-Sharkawy et al in 2007, conducted an *invitro* study to analyze the growth factors in PRP and to study the effects of PRP on monocyte cytokine release and lipoxin A4 (LXA4)

generation. PRP was prepared from healthy donors and growth factors like PDGF-AB, PDGF-BB, TGF-b1, IGF-I, FGF-b, EGF, VEGF, IL-12 and normal T-cell expressed and secreted

RANTES levels were evaluated by enzyme-linked immunosorbent assay and bead-based multiplexing. The authors concluded that PRP is a rich source of growth factors and promoted significant changes in monocyte-mediated proinflammatory cytokine/chemokine release. LXA4 was increased in PRP, suggesting that PRP may suppress cytokine release, limit inflammation, and, thereby, promote tissue regeneration.[36]

8) A clinical study was done by Ferenc Dori et al in 2007 to evaluate the effect of PRP on the healing of deep intrabony defects treated with anorganic bovine bone mineral (ABBM) and GTR using a non-resorbable expanded polytetrafluoroethylene (ePTFE) membrane. Twenty patients with a total of 48 intrabony defects were randomly treated with a combination of either PRP +ABBM+ GTR (test group) or ABBM+ GTR (control group). Clinical parameters were evaluated at baseline and 1 year after treatment. No differences in any of the studied parameters were observed at baseline between the two groups. They concluded that at 1 year after regenerative therapy in periodontal intrabony defects, optimal clinical results were obtained with ABBM + GTR with a non-resorbable barrier, with or without the addition of PRP.[37]

9) Fabricia Ferreira Suaid et al in 2008 conducted a study to histometrically evaluate the healing process of gingival recessions treated with platelet-rich plasma (PRP) in combination with a subepithelial connective tissue graft (SCTG) and to compare it to that obtained with SCTG alone. Six mongrel dogs with 5-7 mm contralateral gingival recessions were randomly assigned to the test group (SCTG + PRP) or the control group (SCTG). Dogs were sacrificed 45 days after the surgeries, and the blocks containing the experimental specimens were processed for histologic analysis. The authors found that the combination of PRP with SCTG was more effective in promoting new cementum formation than the graft alone in the treatment of gingival recession.[38]

10) Duretti Fufa et al in 2008 conducted a study to investigate the use of Type I soluble collagen as an alternative to bovine thrombin as a PRP clot activator. The samples of PRP were obtained from human donors, induced platelet activation and gelation using either bovine thrombin or Type I collagen. Clot retraction was determined by measuring clot diameters over time. The release of PDGF-AB, TGF-β1 and VEGF from both types of clots was measured over 10 days

using ELISA. They concluded that the use of Type I collagen to activate clotting of PRP may be a safe and effective alternative to bovine thrombin. The use of collagen results in less clot retraction and equal release of PDGF-AB and VEGF.[39]

11) Ferenc Dori et al in 2008 conducted a study to clinically evaluate the effect of PRP on the healing of deep intrabony defects treated with beta-tricalcium phosphate (b-TCP) and GTR using a non-bioresorbable expanded polytetrafluoroethylene membrane. Twenty-eight subjects with advanced chronic periodontal disease and displaying one intrabony defect were treated randomly with a combination of PRP + b-TCP + GTR or b-TCP + GTR. Plaque index, gingival index, bleeding on probing, probing depth (PD), gingival recession, and clinical attachment level (CAL) were evaluated at baseline and 1 year after treatment. Clinical attachment level (CAL) was the primary outcome variable. The authors concluded that 1 year after surgery, both therapies resulted in significant PD reductions and CAL gains.[40]

12) Kanoko Yamamiya et al in 2008 conducted a controlled clinical and radiological study to compare the response of human cultured periosteum (HCP) sheets in combination with platelet-rich plasma (PRP) and porous hydroxyapatite (HA) granules to a mixture of PRP and HA in the treatment of human infrabony periodontal defects. Thirty infrabony osseous defects in 30 healthy were randomly assigned to the test group (HCP sheets combined with PRP and HA) and the control group (PRP with HA). They concluded that a 12-month postsurgical comparison of PRP with HA, treatment with a combination of HCP sheets, PRP, and HA led to a significantly more favorable clinical improvement in infrabony periodontal defects. A factor likely contributing to these favorable clinical results is the presence of osteogenic cells in the HCP sheets, which provided greater regeneration potential.[41]

13) A randomized, controlled, blinded clinical pilot study was done by Neal Shepherd et al in 2009 to compare the percentage of recession defect coverage obtained with a coronally positioned tunnel (CPT) plus an acellular dermal matrix allograft (ADM) to that of a CPT + ADM + platelet-rich plasma (CPT+ADM+PRP) 4 months post-surgically. Eighteen patients with Miller Class I or II recession of 3 mm at one site were treated and followed for 4 months. Nine patients were treated with CPT +ADM (control group) and nine patients with CPT +ADM +PRP (test group). The authors concluded that CPT+ADM +PRP produced defect coverage of about

90%, whereas the CPT+ADM produced only 70% defect coverage. This difference was not statistically significant, but it may be clinically significant.[42]

14) An animal study was conducted by Charles A. Powell et al in 2009 to test the effect of PRP on flap strength at various post-surgical time points in twelve Yucatan minipigs with four sites per animal which were treated with PRP+OFD (test group) and open flap debridement alone (control group). The flap strength in each quadrant was tested by attaching to a loop of 3-0 silk suture through the tissue; the force required to separate the flap from the tooth/bone interface was recorded for each site. The authors concluded that PRP did not seem to contribute to greater flap strength at any post-surgical time point, nor was it associated with any histologic differences in wound healing in this Yucatan minipig model. The time points were chosen for observation post-surgery, as well as the variability in the PRP platelet count, may have contributed to the lack of positive findings.[43]

15) A Pilot Study was done by Ferenc Dori et al in 2009 to clinically compare the healing of intrabony defects treated with either a combination of an anorganic bovine bone mineral (ABBM) + PRP with ABBM alone. Thirty patients with advanced chronic periodontal disease and displaying one intrabony defect were randomly treated with PRP + ABBM (test group) and ABBM alone (control group). The authors concluded that at 1 year after regenerative surgery significant PD reductions and CAL gains were found in both the groups and the use of PRP failed to improve the results obtained with ABBM alone.[44]

16) A study was done by Luciana Reichert da Silva et al in 2011 to evaluate the effect of PPP, calcium chloride–activated PRP (PRP/Ca), calcium chloride and thrombin-activated PRP (PRP/Thr/Ca), and bone marrow mononuclear cells and PRP/Ca (BMMCs/PRP/Ca) on the healing of replanted dog teeth. After 30 minutes of extraction, teeth were replanted with 1) no material (control); 2) PPP; 3) PRP/Ca; 4) PRP/Thr/Ca; or 5) BMMCs/PRP/Ca. Histologic, histomorphometric, and immunohistochemical analysis was done 120 days after replantation. The authors concluded that platelets activated with thrombin play an important role in the healing of tissues after tooth replantation. [45]

17) A randomized, controlled, masked, clinical and histological trial was done by Mehmet Akif Eskan et al in 2014 to determine if PRP combined with a rapidly resorbing cancellous allograft would enhance the regenerative result compared with an allograft without PRP. A total of 28

patients, 14 were treated with cancellous allograft (CAN group) and the other 14 received a cancellous allograft mixed with PRP (PRP group). All 28 grafted sites were covered with a resorbable polylactide membrane. The author concluded that PRP enhanced bone regeneration and resulted in increased horizontal bone gain and percentage of vital bone.[46]

18) A histomorphometrical animal study was done by Maria J.H. Nagata et al in 2014 to analyze the influence of platelet-rich plasma (PRP), low-level laser therapy (LLLT), or their combination on the healing of periodontal fenestration defects (PFDs) in rats. PFDs were surgically created in the mandibles of 80 rats. The animals were randomly divided into four groups: 1) C (control) and 2) PRP, defects were filled with a blood clot or PRP, respectively; 3) LLLT and 4) PRP/LLLT. Animals were euthanized at either 10 or 30 days post-surgery. The authors concluded that LLLT, PRP, or their combination all promoted new cementum formation with a functional periodontal ligament. The combination PRP/LLLT did not show additional positive effects compared to the use of either therapy alone.[47]

19) An animal study was done by Maria J.H. Nagata et al in 2014 to evaluate the influence of platelet-rich plasma derived from bone marrow aspirate (PRP-BMA) on the healing of periodontal fenestration defects. Periodontal fenestration defects were surgically created in the mandibles of 40 rats. The animals were randomly divided into two groups, the control group in which defects were filled with a blood clot and the test group PRP-BMA, in which defects were filled with PRP-BMA. Animals were euthanized at either 10 or 30 days post-surgery. Histologic, histometric, and immunohistochemical analyses were performed.

The authors concluded that PRP-BMA promoted new cementum formation with a functional periodontal ligament when applied at experimental periodontal fenestration defects.[48]

20) A study was done by Li-Chiu Yang et al in 2015 to evaluate the antimicrobial activities of platelet-rich plasma (PRP) and related plasma preparations against periodontal disease-associated bacteria. Four distinct plasma fractions were extracted in the formulation used commonly in dentistry and were tested for their antibacterial properties against three periodontal bacteria: Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Fusobacterium nucleatum. The minimum inhibitor concentration of each plasma preparation was determined. PRP interfered with P. gingivalis and A. actinomycetemcomitans attachment and enhanced exfoliation of attached P. gingivalis but had no influences on F. nucleatum bacterial

adherence. Thus, they concluded that PRP expressed antibacterial properties, which may be attributed to platelets possessing additional antimicrobial molecules. The application of PRP on periodontal surgical sites is advisable because of its regenerative potential and its antibacterial effects.[49]

21) Anitua E et al in 2015 conducted an *invitro* study to evaluate for the first time, whether different protocols of ozone treatment of plasma rich in growth factors (PRGF) alter the biological properties and outcomes of this autologous platelet-rich plasma. Human plasma rich in growth factors was treated with ozone using one of the following protocols: a continuous-flow method; or a syringe method in which constant volumes of ozone and PRGF were mixed. In both cases, ozone was added before, during and after the addition of calcium chloride. Three ozone concentrations, of the therapeutic range 20, 40 and 80 lg/ mL, were tested. Fibrin clot properties, growth factor content and the proliferative effect on primary osteoblasts and gingival fibroblasts were evaluated. The authors found that ozone dose and the way that ozone combines with PRGF may alter the biological potential and therapeutic outcomes of PRGF.[50]

STUDIES ON PRF

1) A histological study was done by Choukroun et al in 2006 to evaluate the potential of PRF effects on freeze-dried bone allograft (FDBA) in sinus floor bone regeneration and augmentations (maturation). Out of nine sites, six sites were treated with FDBA and PRF (test group), and three sites were treated with FDBA without PRF (control group). The test group was evaluated after 4 months and the control group was evaluated after 8 months and bone specimens from the augmented region during the implant insertion procedure were harvested and evaluated histologically. They found that sinus floor bone augmentation with FDBA and PRF combination leads to a reduction of healing time before implant placement and from the histological point of view healing time could be reduced to 4 months (shorter healing period 4 months instead of 8 months).[51]

2) Dohan DM et al in 2006 conducted an *invitro* retrospective analysis for the understanding of fibrin technologies and the evaluation of the biochemical properties of three generations of surgical additives, respectively fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF. Initial analyses revealed that slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes. This result would imply that PRF, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling; such a mechanism might explain the clinically observed healing properties of PRF. The authors concluded that a slower release of growth factors from PRF than PRP and also better healing properties with PRF.[52]

3) A radiological and histological case series study done by Mazor et al in 2009, to evaluate sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material. Twenty-five sinus elevations with simultaneous implantation were performed on 20 patients with Choukroun's PRF. The radiological examination was performed presurgically and 6-month post-surgically with a panoramic x-ray and three-dimensional volumetric computed radiography (VCR). They concluded that the use of PRF as the sole filling material during a simultaneous sinus lift and implantation, stabilized a high volume of natural regeneration of bone in the sub-sinus cavity and also PRF is a simple and inexpensive biomaterial and its systematic use during a sinus lift seems like an acceptable option, particularly for the protection of the schneiderian membrane.[53]

4) Simonpieri et al in 2009 conducted a study to validate the use of PRF membranes in the complex maxillary reconstruction protocols along with freeze-dried bone allograft (FDBA) and 0.5% metronidazole solution in about 20 patients and followed-up for 1-5 years and finally 184 dental implants were placed and they found no implant or graft loss in a case series. They found that PRF membranes protect the surgical site; promotes soft tissue healing; and when its fragments mix with graft material, it functions as a "biological connector" between the different elements of graft and acts as a matrix that supports neo-angiogenesis, the capture of stem cells, and migration of osteoprogenitor cells to the centre of graft.[54]

5) Anil kumar et al in 2009 conducted a study on PRF membrane in a potential novel root coverage approach. In a 19-year-old male, root coverage was accomplished using a laterally displaced flap technique with platelet-rich fibrin (PRF) membrane on the labial surfaces of the mandibular anterior teeth. There were no postoperative complications and healing was satisfactory. Complete coverage was achieved six months after the procedure, with excellent tissue contour and color. They concluded that PRF membrane can be a potential novel root coverage approach for treating gingival recession in mandibular anterior teeth using combined laterally positioned flap technique and PRF membrane.[55]

6) Aroca et al in 2009 conducted a 6-month randomized controlled clinical trial to compare the effect of modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions. Twenty subjects, presenting three adjacent Miller Class I or II multiple gingival recessions of similar extent on both sides of the mouth, were enrolled in the study. Probing depth (PD), recession width, clinical attachment level (CAL), keratinized gingival width, and gingival/ mucosal thickness (GTH) were measured at baseline and 6 months post-surgery. They concluded that addition of PRF membrane positioned under the MCAF (modified coronally advanced flap) provided inferior root coverage, but an additional gain in gingival/mucosal thickness (GTH) at 6 months compared to conventional therapy.[56]

7) A double-masked split-mouth randomized study by Anuj Sharma and A.R. Pradeep in 2011 was designed to evaluate the effectiveness of autologous PRF in the treatment of mandibular degree II furcation defects compared with open flap debridement (OFD). Eighteen patients with 36 mandibular degrees II furcation defects were treated either with autologous PRF+OFD (test

group) or OFD alone (control group). Nine months postoperatively clinical and radiographic parameters showed statistically significant improvement at the sites treated with PRF+OFD compared to those with OFD alone. They concluded that significant improvement with autologous PRF implies its role as a regenerative material in the treatment of furcation defects.[57]

8) A comparative study was done by Abhishek Singh et al in 2012 to evaluate the efficacy of autologous platelet-rich fibrin in soft tissue healing and bone regeneration in mandibular third molar extraction sockets. Twenty patients requiring extraction of bilateral mandibular third molar were included. Following extraction, platelet-rich fibrin (PRF) was placed in one extraction socket, the other socket was studied as the control site with no PRF. The results showed that trabecular bone formation had started earlier in PRF site compared to the control site and evaluation of bone density by radiological assessment calculated after 3 months at the PRF site was comparatively higher than the control site. They concluded that autologous PRF is biocompatible and has significantly improved soft tissue healing, bone regeneration and increase in bone density in extraction sockets.[58]

9) A randomized controlled clinical trial was done by Pradeep et al in 2012 to evaluate the clinical and radiographic effectiveness of autologous PRF and PRP in the treatment of 3-wall intrabony defects in chronic periodontitis. A total of 54 systemically healthy patients with ninety 3-wall intrabony defects were treated with either autologous PRF with open-flap debridement or autologous PRP with open-flap debridement or open-flap debridement alone. They concluded that 9 months postoperatively there was similar PD reduction, CAL gain, and bone fill at both sites treated with PRF or PRP when compared to conventional open-flap debridement alone and also PRF is less time consuming, less technique sensitive, when compared to PRP.[59]

10) Gülnihal Eren and Gül Atilla in 2012 conducted a case study to evaluate the comparison of the clinical effectiveness of two combined techniques, the coronally advanced flap + platelet-rich fibrin (CAF+PRF- test group) and Subepithelial connective tissue graft + coronally advanced flap (CAF+SCTG- control group) in the treatment of bilateral gingival recessions in the maxillary cuspids of a 23-year-old female patient. Clinical periodontal parameters were recorded and clinical photographs were taken at baseline; 1, 3, and 6 months; and 1 year. The results reported improved root coverage amount, gingival thickness, and keratinized tissue width in both groups. They concluded that CAF+PRF presents an alternative to CAF+SCTG in the treatment

of gingival recessions. The PRF method is practical and simple to perform. Additionally, PRF seems to be superior to SCTG since it eliminates the requirement of a donor site. [60]

11) A clinical and radiographical study by A R Pradeep et al in 2012 aimed at the comparison of the effectiveness of autologous PRF Vs PRF+HA in the treatment of intrabony defects in chronic periodontitis subjects. Ninety intrabony defects were treated either with autologous PRF with open flap debridement (OFD) or PRF+HA with OFD or OFD alone. Mean PD reduction, mean CAL gains and mean percentage of bone fill was greater in PRF and PRF+HA groups than control group 9 month post-operatively. Thus, they concluded that treatment of intrabony defects with PRF results in significant improvements in clinical parameters compared with baseline. Hydroxyapatite when added to PRF increases the regenerative effects observed with PRF in the treatment of human three-wall intrabony defects.[61]

12) Chhaya Bansal and Vipin Bharti conducted a clinical and radiographical split-mouth study in 2013 to clinically evaluate and compare the efficacy of autologous PRF combined with demineralized freeze-dried bone allograft (DFDBA)(test group) to DFDBA alone (control group) in the treatment of periodontal intrabony defects. Ten patients having bilateral intrabony defects with a clinical probing depth of at least 6 mm were enrolled. They concluded that a combination of PRF with DFDBA demonstrated better results in probing pocket depth reduction and clinical attachment level gain as compared to DFDBA alone in the treatment of periodontal intrabony defects.[62]

13) A case report by Jayakumar N D et al in 2014 aims to investigate the clinical and radiological (bone fill) effectiveness of autologous PRF along with the use of xenogenic bone mineral (OSSEOGRAF TM) in the treatment of intra bony defects. A 25-year-old male with an intrabony defect extending up to apical third of right maxillary central incisor (number 11) with a probing depth of 8 mm, with no history of pain and tender on percussion. Minced PRF was mixed with xenograft (OSSEOGRAF TM) and was applied to the defect walls and root surfaces. After 6 months of follow-up there was a reduction in pocket depth, gain in clinical attachment and radiographs revealed improved bone fill in the intrabony defect. They concluded that the positive clinical impact of the additional application of PRF with xenogenic graft material in the treatment of periodontal intrabony defect.[63]

14) A case series by Shyam Prasad Aravindaksha et al in 2014 evaluates the use of platelet-rich fibrin (PRF) membrane as a palatal bandage to cover donor sites. Five patients requiring augmentation of KT were included and the palatal donor sites of four of these patients were covered with PRF membranes as a palatal bandage. The donor site of the fifth patient was allowed to heal conventionally without PRF membrane to evaluate the difference in healing. The healing was evaluated visually by a hydrogen peroxide test on days 12, 13, 18, 19, 24, 25, 30, and 31. They concluded that superior healing was observed at the PRF membrane sites supports its use in accelerating soft-tissue healing. PRF membrane as a palatal bandage is an efficacious approach to protect the raw wound area of a palatal donor site to reduce healing time and patient discomfort.[64]

15) A case series by Geeti Gupta et al in 2014 describes the use of vestibular incision sub-periosteal tunnel access (VISTA) technique in combination with platelet-rich fibrin (PRF) membrane in the treatment of gingival recession defects. Four patients presenting with either maxillary or mandibular Class I or Class II type multiple recession defects were included in this study. Six months postoperatively, there was 97.22% root coverage, with a significant gain in CAL of 2.28 mm. Mean gingival tissue thickness and keratinized tissue width were also increased significantly. The tissue at the site appeared healthy, with no visible signs of inflammation. They concluded that the use of PRF membrane along with VISTA technique allows the clinician to successfully treat multiple recession defects with optimal esthetic results and excellent soft-tissue biotype.[65]

16) Ziv Mazor and Sachin Mamidwar in 2015 presented a case report to compare the effect of Nanocrystalline Calcium Sulfate Bone Graft alone or in combination platelet-rich fibrin in two different Bilateral Sinus-Augmentation procedures. A 70-year-old female patient requiring a full maxillary rehabilitation was included in the study. The osteotome closed approach was used on the right side, which was grafted with nCS bone graft and the lateral window sinus elevation approach was used on the left side, which was grafted with nCS in combination with platelet-rich fibrin. Implants were placed simultaneously in both sites. A 2-year follow-up showed satisfactory results with good implant stability. They concluded that nCS can be successfully used alone and in combination with PRF for sinus augmentation by either the osteotome or lateral window technique and nCS may be a viable graft material to incorporate into sinus elevation surgery.[66]

17) Metformin (MF), a member of the biguanide group, has been shown to facilitate osteoblast differentiation and thus may exhibit a favorable effect on alveolar bone. Thus based on this concept A.R. Pradeep et al in 2015 designed a randomized controlled clinical trial to evaluate the efficacy of open-flap debridement (OFD) combined with PRF, 1% MF gel, and PRF + 1% MF gel in the treatment of intrabony defects (IBDs) in patients with chronic periodontitis (CP). One hundred twenty patients with single defects were categorized into four treatment groups: OFD alone, OFD with PRF, OFD with 1% MF, and OFD with PRF plus 1% MF. Clinical and radiographic parameters were recorded at baseline (before surgery) and 9 months postoperatively. The authors found that the PRF + 1% MF group showed greater improvements in clinical parameters, with greater percentage radiographic defect depth reduction compared to MF, PRF, or OFD alone in the treatment of IBDs in patients with CP.[67]

18) Huseyin Gencay Kecel et al in 2015 conducted a clinical trial to evaluate the effectiveness of coronally advanced flap (CAF)+connective tissue graft (CTG)+PRF in Miller I and II recession treatment by comparing with CAF+CTG. Forty patients were surgically treated either with CAF+CTG+PRF (test group) or CAF+CTG (control group). Clinical parameters and radiological parameters were recorded at baseline, 3 months (PS1) and 6 months (PS2) post-surgery. Root coverage (RC), complete RC (CRC), attachment gain (AG), and keratinized tissue change (KTC) were also calculated. According to the results, the authors concluded that PRF did not develop the outcomes of CAF+CTG treatment except increasing the tissue thickness (TT). However, it is not sufficient to advocate the true clinical effect of PRF on recession treatment with CAF+CTG and needs to be elucidated with further trials.[68]

SUMMARY

Platelets are the primary mechanism for hemostasis. They circulate in the bodies looking for exposed endothelium. They then aggregate to the site of injury and further platelet degranulation occurs. The release of various growth factors can also aid in the healing process and the Platelet gel mimics the final stages in the clotting. Platelets are activated either by adhesion to the molecules that are exposed on an injured endothelium, such as von Willebrand Factor (vWF), collagen, fibronectin, and laminin, or by physiologic agonists such as thrombin, ADP, collagen, thromboxane A2, epinephrine, and platelet-activating factors.[14]

Platelet-rich plasma (PRP) was used as a method of introducing concentrated growth factors platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor 1 (IGF-1) to the surgical site, thereby enriching the natural blood clot to hasten wound healing and stimulate bone regeneration. A natural human blood clot consists of 95% red blood cells (RBCs), 5% platelets, less than 1% white blood cells (WBCs), and numerous amounts of fibrin strands. A PRP blood clot, on the other hand, contains 4% RBCs, 95% platelets, and 1% WBCs.[20]

PRP has been used clinically in humans since the 1970s for its healing properties attributed to autologous GF and secretory proteins that may enhance the healing process on a cellular level. The PRP preparation protocol requires the collection of blood with anticoagulant, centrifugation in two steps, and induced polymerization of the platelet concentrate using calcium chloride and bovine thrombin. Depending on the device and technique used, PRP can contain variable amounts of plasma, erythrocytes, white blood cells, and platelets. The platelet concentration should be increased above baseline or whole blood concentration. It is generally agreed upon that PRP should have a minimum of 5 times the number of platelets compared to baseline values for whole blood to be considered “platelet-rich”. PRP has been used in conjunction with different grafting materials in bone augmentation procedures.[21]

PRF represents a new revolutionary step in the platelet gel therapeutic concept. Unlike other platelet concentrates, this technique does not require any gelifying agent, but not more than centrifugation of the natural blood without additives. Choukroun et al., developed the PRF in 2001 in France and the production protocol of PRF attempted to accumulate platelets and

released cytokines in a fibrin clot. The platelets and leukocyte cytokines are an important part of the role-play of this biomaterial, but the fibrin matrix supporting them is very helpful in constituting the determining elements responsible for the real therapeutic potential of PRF. Cytokines are immediately used and destroyed in a healing wound. The harmony between cytokines and their supporting fibrin matrix has much more unique importance than any other component.1 Newer advances such as A- PRF, i- PRF, t- PRF, CGF, Sticky bone concept, etc have been reported in fewer cases but no long-term or controlled trials have been done to prove their advantage over conventional PRP and PRF. So, long-term studies with more sample size are required to arrive at a definite conclusion.

CONCLUSION

Periodontal regeneration is unpredictable with any regenerative therapy currently used and to overcome this unpredictability, the use of specific biomaterials/ biologicals has shown strong potential in improving clinical results in periodontal defects. One such biomaterial is autologous platelet concentrates which are the new application in the field of periodontal regeneration and developing areas for clinicians and researchers. It is the storage vehicle for growth factors, especially PDGF and TGF-b. Although the GFs and the mechanism involved are still poorly understood, the ease of applying in the dental clinic and its beneficial outcomes, including reduction of bleeding and rapid healing, hold promise for further procedures. Most importantly, these APC products eliminate concerns about immunogenic reactions and disease transmission.[3]

Animal studies and recently published human trials with smaller sample sizes have demonstrated successful results. However, the effectiveness of APC in regenerative procedures should be evaluated in studies that involve a large number of subjects. Moreover, the use of APC in randomized control trials has to be encouraged.

It is now necessary to look further into platelet and inflammatory features of the biomaterial autologous platelet concentrate. Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol.[12]

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