# From Hematology to Tissue Engineering: Current Status and Projection of Platelet Concentrates and their Derivatives*

De la hematología a la ingeniería tisular: actualidad y proyección de los concentrados plaquetarios y sus derivados

Da hematologia à engenharia de tecidos: situação atual e projeção dos concentrados de plaquetas e seus derivados

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## Abstract:

**Background:** In the last decade, tissue engineering, cell therapy, and research advances in hematological sciences have become tools for regenerative dentistry, using platelet concentrates as the cornerstone. The results are promising, but it is necessary to consolidate the existing evidence on their real relevance and clinical impact. **Purpose:** To conduct an integrative review of literature to present scientific evidence on platelet concentrates, their use, characteristics, benefits, and usefulness for tissue engineering. **Methods:** The search for information was performed in the integrated search engine EBSCOhost, including studies published from 2010 to early 2020. **Results:** Most of the publications present the evolution of platelet concentrates with evaluations of their *in vitro* and *in vivo* use, clinically proving their regenerative potential in soft tissue and bone repair. Marked differences exist between platelet-rich plasma and platelet-rich fibrin. Conclusion: There is controversy and indiscriminate use of the terminology referring to platelet concentrates; however, they are not only a source of growth factors, but also a living biomaterial, based on fibrin, and its positioning as a surgical material is increasingly evident to become a tissue-oriented solution, by optimizing regeneration and healing, and an important tool of low economic profile in tissue engineering.
**Keywords:** dentistry, growth factors, guided regeneration, hemostasis, oral medicine, platelet concentrates, platelet-rich fibrin, platelet-rich plasma, regenerative dentistry, tissue engineering, tissue regeneration.

## Resumen:

**Antecedentes:** En la última década, la ingeniería tisular, la terapia celular y los avances investigativos en ciencias hematológicas se han convertido en herramientas para la odontología regenerativa, al utilizar los concentrados plaquetarios como piedra angular. Los resultados son prometedores, ante lo cual es necesario consolidar la evidencia existente sobre su real relevancia e impacto clínico. **Objetivo:** Realizar una revisión integradora de literatura para presentar evidencia científica referente a concentrados plaquetarios, su uso, características, beneficios y utilidad para la ingeniería tisular. **Métodos:** La búsqueda de información se realizó en el buscador EBSCO host, de estudios publicados entre 2010 y principios de 2020. **Resultados:** La mayoría de las publicaciones presenta la evolución de los concentrados plaquetarios, evalúan su uso *in vitro* e *in vivo* y comprueban clínicamente su potencial regenerativo en la reparación de tejidos blandos y óseos. Se encontraron marcadas diferencias entre, el plasma rico en plaquetas y la fibrina rica en plaquetas. **Conclusión:** Existe controversia y uso indiscriminado de la terminología de concentrados plaquetarios; sin embargo, ellos no sólo son una fuente de factores de crecimiento, sino también un material vivo, con base en la fibrina, cuyo posicionamiento como material quirúrgico es cada vez más evidente. Se torna en una solución de orientación tisular al optimizar la regeneración y la cicatrización, y una importante herramienta de bajo costo en la ingeniería tisular.
**Palabras clave:** concentrados plaquetarios, factores de crecimiento, fibrina rica en plaquetas, hemostasia, ingeniería tisular, medicina oral, odontología, odontología regenerativa, plasma rico en plaquetas, regeneración guiada, regeneración tisular.

## Resumo:

**Antecedentes:** Na última década, a engenharia de tecidos, a terapia celular e os avanços da pesquisa em ciências hematológicas tornaram-se ferramentas para a odontologia regenerativa, usando concentrados de plaquetas como pedra angular. Os resultados são promissores, mas é necessário consolidar as evidências existentes sobre sua real relevância e impacto clínico. **Objetivo:** Realizar

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uma revisão integradora da literatura para apresentar evidências científicas sobre concentrados de plaquetas, seu uso, características, benefícios e utilidade para a engenharia de tecidos. **Métodos:** A busca de informações foi realizada no buscador integrado Escolhos, incluindo estudos publicados de 2010 ao início de 2020. **Resultados:** A maioria das publicações apresenta a evolução dos concentrados de plaquetas com avaliações de seu uso *in vitro* e *in vivo*, comprovando clinicamente suo potencial regenerativo em tecidos moles e reparação óssea. Existem diferenças marcantes entre o plasma rico em plaquetas e a fi brina rica em plaquetas. **Conclusão:** Há controvérsia e uso indiscriminado da terminologia referente aos concentrados de plaquetas; no entanto, não são apenas uma fonte de fatores de crescimento, mas também um material vivo, à base de fibrina, e seu posicionamento como material cirúrgico é cada vez mais evidente para se tornar uma solução tecidual, otimizando a regeneração e a cicatrização, e uma importante ferramenta de baixo perfil econômico na engenharia de tecidos.
**Palavras-chave:** concentrados de plaquetas, engenharia de tecidos, fatores de crescimento, fibrina rica em plaquetas, hemostasia, medicina oral, odontologia, odontologia regenerativa, plasma rico em plaquetas, regeneração de tecidos, regeneração guiada.

# INTRODUCTION

Never before as in the last two decades, hematology and, especially, hemostasis and coagulation science in a synergistic collaborative work with tissue engineering, have been so linked to regenerative dentistry. This trend leads us to think that they build a single path to become particularly important collaborative sciences, in which dentistry needs repair and regeneration and obtains from hematology the functionality of hemostasis. This is what different researchers who have obtained satisfactory results in the engineering used for tissue regeneration have wanted to verify (1-4) and that promote the repair and regeneration of tissue defects, as well as the regulation, modulation, and acceleration of inflammation and cicatrization.

The history of autogenous platelet concentrates (PC) began for science in approximately 1954 with one of the first published articles entitled "Evidence of an Antagonist to Factor VI in Platelet-Rich Human Plasma" (5). In that article, Kingsley spoke of platelet-rich plasma (PRP) referring to autologous PCs. However, other researchers claim that studies began in the 1970s with fibrin glue (6-9). The truth is that PCs have been used in dental practice for more than three decades as a regenerative tool capable of releasing supraphysiological doses of growth factors that are responsible for inducing tissue regeneration derived from autologous sources (10). With its maximum development in the last 20 years, PCs have had more supporters than detractors and more studies have shown their great advantages and contributions to tissue engineering.

The regenerative potential of platelets, the foundation of PCs, is attributed to growth factors and cytokines as they are responsible, among other processes, for increased collagen production, cell proliferation, angiogenesis, cell migration, and induction of cell differentiation (11). These benefits can be enhanced by using them in the patient's body and at the time when their physiological action is required, as is the case of periodontal defects, intraosseous defects, situations in which sinus elevation is required, alveolar preservation after extraction, and gingival recession, to name a few (12).

Due to the above, the purpose of this integrative review, the result of the compilation and analysis of existing scientific research, was to present current evidence of the benefits of PCs in the last decade and verify the efficiency of their characteristics and development for tissue engineering.

# MATERIALS AND METHODS

The information search for the integrative literature review was conducted in the integrated search engine EBSCOhost, mainly in the index databases: PubMed, Scopus, Embase, and PROSPERO without language restrictions. Areas of interest were the use of plasma and platelet concentrates in dentistry, regenerative dentistry, tissue regeneration, and tissue engineering. Only studies in English and Spanish and two studies in Portuguese were included. The search was limited to the uses and investigations of PCs in the dental area in clinical studies in humans and in animal models published from 2010 to April 2021. The following keywords

with their Boolean connectors were used: "platelet concentrates" OR " platelet concentrates" AND "PRF (platelet-rich fibrin)," AND "PRP (platelet-rich plasma)," AND "tissue engineering in dentistry." Of 3,558 titles, 71 were selected (37 reviews, 19 case reports, 9 *in vitro* studies, 3 animal studies, and 3 consensus). The main selection criterion was the relevance and the authors of the topic. Appendices 1-3 summarize the articles used to conducts this literature report.

# RESULTS AND DISCUSSION

## History

There are investigations of the use of PCs since 1940 that are attributed to Young and Medawa (9,13). Although some authors speak of the beginnings in the 1950s (14,15), most refer to the 1970s as the beginning of the application of PCs in dentistry (11,14,16-18) with the fibrin glue. The first PC protocols were described by the so-called autologous platelet-derived wound healing factors (PDWHF) and published in 1986 (13). In the 1990s, PCs and everything related to them were called PRP and it was in the new millennium that platelet-rich fibrin (PRF) and all its derivatives were created. Between 2009 and 2010, a wide variety of PCs was presented, which were called PRP to refer to the generic term used in transfusion medicine: platelet-rich plasma. This last denomination was too general for the qualification of the various products developed (19), so Dohan-Ehrenfest, *et al.* (19–22) classified the different platelet derivatives or PCs into four families, depending on their leukocyte content and fibrin architecture. Figure 1 shows the trajectory and evolution of PCs from 1954 to 2021.

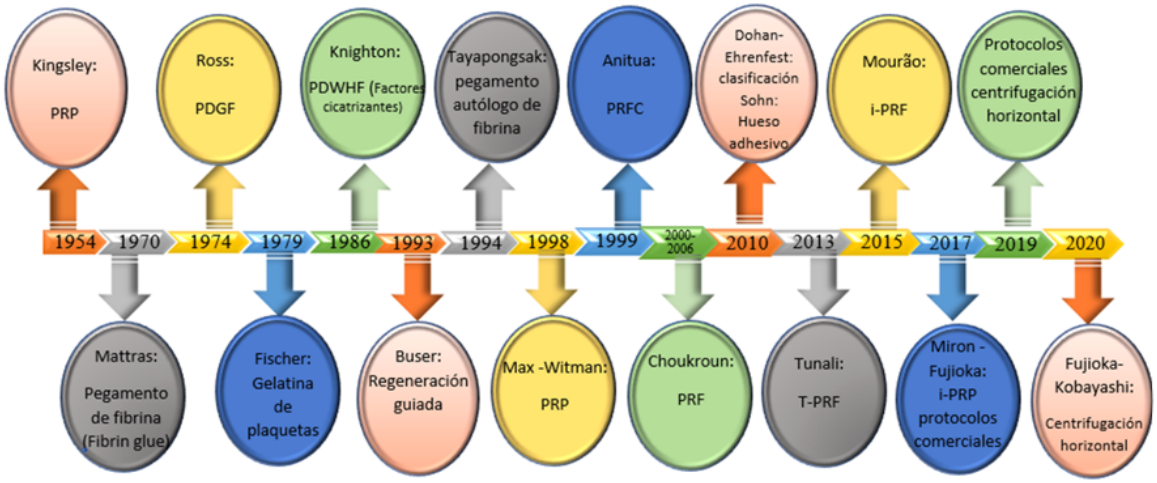


FIGURE 1
Trajectory and evolution of PCs from 1954 to 2020. Authors and creators of different technical proposals are on the list
Source: the authors.

## What Are and What Are PCs Used For in Tissue Engineering?

According to Gilberto Sammartino, autologous PCs are a concentrated suspension of growth factors and cytokines found in platelets, which function as bioactive surgical additives, and are applied locally to induce wound healing (6,11,19). PCs are increasingly used for the regeneration and reconstruction of bone and connective tissues in the periodontal and maxillofacial fields (16). Tissue engineering has produced a change

in the restorative paradigm and proposes regenerative dentistry that allows the restoration of biological functions that synthetic materials cannot achieve (23,24).

The mechanism of action of PCs is the same as that of autologous healing, but with greater and repotentialized healing. The healing cascade begins with clot formation (hemostasis), which is followed by inflammation, the proliferative stage, and maturation and remodeling. PCs catalyze the proliferation of phagocytic cells that help defibrillate the injury. In turn, white blood cells and platelets release important cellular mediators such as growth factors (GFs) and cytokines that begin the healing process (2,14,18). GFs are polypeptides whose mission is to facilitate and enhance intercellular communication at the molecular level that stimulates cell migration, proliferation, and differentiation and are the main growth-regulating molecules for cells *in vivo* and in culture (2,25). On the other hand, these molecules, by promoting angiogenesis, mitosis, chemotaxis, and stem cell proliferation, help increase bone and soft tissue regeneration (2,18,26). Table 1 shows the most important GFs and cytokines present in PCs (2,15,16,27). Proteins such as coagulation factors and others secreted by platelets ensure initial hemostasis. After 24 to 48 hours, the proliferative phase takes over, by virtue of the presence of the inflammatory mixture of chemokines created during the inflammatory phase, to then give way to the boosted proliferation of fibroblasts, leukocytes, macrophages, and mesenchymal stem cells that begin to lay the first foundations of the new tissue. In this way, GF and other bioactive molecules form one of the essential components of the tissue engineering approach, in combination with an appropriate scaffolding and a population of stem or progenitor cells (24,25).

TABLE 1
Main Growth Factors and Cytokines Present in PCs

| Bioactive Molecules | Cytokines | Acronym | Biological Function |
|---|---|---|---|
| Coagulation Factors and Other Procoagulants | Von Willebrand Factor | vWF | Hemostasis |
| | Fibrinogen | F1 | Platelet adhesion |
| | Fibronectin | FN | Cell adhesion |
| | Vitronectin | VTN | Cell anchor |
| | Thrombospondin 1 | TSP-1 | Vascular stability |
| | Laminin-8 | LAMA-8 | |
| | V Factor / activated V | F V / Va | |
| | Plasmatic Thromboplastin | F XI | |
| | Multimerin (≠ vWF) | MMRN | |
| | Protein S | Pro S1 | |
| | High molecular weight kininogen | HMWK | |
| | Antithrombin III | AT-III | |
| | Platelet factor 4 | PF4 | |
| Growth Factors | Platelet-Derived Growth Factor | PDGF | Stimulate cell growth, proliferation, healing, and differentiation through regulation of cellular and inhibitory processes of osteoclasts with extracellular matrix synthesis and remodeling |
| | Transforming Growth Factor-Beta | TGF-beta | |
| | Insulin-Like Growth Factor-I And 2(IGF-BP3) | IGF-I-2 | |
| | Vascular Endothelial Growth Factor | VEGF | |
| | Epithelial Growth Factor | EGF | |
| | Hepatocyte Growth Factor Chemokine | HGF | |
| | Granulocyte/Macrophage-Colony Stimulating Factor | GM-CSF | |
| | Bone Morphogenetic Protein | BMP | |
| Chemokines | CXCL5 | ENA78 | Platelet Aggregation |
| | CCL5 Chemokine 5 | RANTES | |
| | CCL7 Chemokine 7 | MCP-3 | |
| | CXCL2 Neurotrophines BDNF/ Beta-NGF | GRO/ NT-3 | |
| | Platelet Factor 4 | PF4 | |
| | Thromboglobulin Isoform of CXCL7-Beta | β-TG - NAP-2 | |
| | Matrix Metallopeptidases | MMP1-2-9 | |
| | Stromal Cell-Derived Factor 1 CXCL12 | SDF-1 | |
| | Metalloproteinases | TIMP | |
| | Metallopeptidase with Thrombospondin Type 1 Motif, 13 | ADAMTS | |
| Angiogenic Factors | Granulocyte Colony Stimulating Factor | GCSF | Fundamental role in the formation of blood vessels |
| | Vascular Endothelial Growth Factor | VEGF | |
| | Fibroblast Growth Factor | FGF2-4-6 | |
| Proinflammatory Cytokines | Interleukin-1 Beta | IL-1beta | Promote and regulate systemic inflammation and cell activation |
| | Interleukin-6 | IL-6 | |
| | Interleukin-4 | IL-4 | |
| | Tumor Necrosis Factor Alpha | TNF-alfa | |
| | Macrophage Inflammatory Protein | MIP 1 | |
| | Interleukine-8 ( Neutrophil Chemotactic Factor ) | IL8 | |
| | Amphotericin | HMGB1 | |
| Others | Serotonin | (5-HT) | Influence biological aspects of wound healing (vasoconstriction) |
| | Histamine | H | |
| | Dopamine | DA | |
| | Adenosine | n.a. | |
| | Calcium | Ca | |

Source: Table translated and modified from Tomoyuki Kawase (2015) and updated from Andia and Maffulli (2018) (2,15,16,27 ).

## Classification of PCs

As mentioned above, the four families of platelet concentrates proposed by Dohan-Ehrenfest are pure platelet-rich plasma (P-PRP), leukocyte-platelet-rich plasma (L-PRP), pure platelet-rich fibrin (PRF), pure platelet-rich fibrin (PRF), and leukocyte- and platelet-rich fibrin (L-PRF) (15,21,28). Currently, based on this classification, there are mainly two PCs (PRF and PRP), each with its by-products (table 2).

TABLE 2
Main Characteristics of PRF y PRP (15,21,28)

| Acronym | Platelet Concentrate | Cell Content and Characteristics |
|---|---|---|
| **Platelet-Rich Fibrin (PRF)** | | |
| PRF (P-PRF) | Pure Platelet-Rich Fibrin | Solid fibrin mesh |
| L-PRF® | Leukocyte- and Platelet-Rich Fibrin | Leukocytes and a highly dense network of fibrin |
| A-PRF® | Advanced-Modified PRF | Slower and longer spin speed |
| A-PRF+® | Advanced PRF + | Less speed with the same centrifugation time |
| i-PRF® | Injectable Platelet-Rich Fibrin | Liquid preparation |
| i-PRF-yellow | Injectable Platelet-Rich Fibrin | Upper layer |
| i-PRF-red | Injectable Platelet-Rich Fibrin | Lower layer |
| T-PRF® | Titanium-Platelet-Rich Fibrin | Titanium tubes – thicker and more extensive fibrin network |
| L-PRF and SATE | Platelet-Rich Fibrin Lysate | PRF incubated at 37 °C in a CO2 atmosphere and exudate rich in growth factors is used |
| Fibrin glue ® | Fibrin Glues or Sealants | Polymerizing fibrinogen with thrombin and calcium |
| Stick Bone | L-PRF plug or block | L-PRF with traditional bone grafts |
| PP | Platelet Patch | PRP and PRF combined |
| CGF | Growth Factor Concentrate | Fibrin matrix that is larger, denser and richer in growth factors than PRF |
| PRGF® | Preparation rich in Growth Factors | Fibrin matrix that is larger, denser and richer in growth factors than PRF |
| **Platelet-Rich Plasm (PRP)** | | |
| PRP | Platelet-Rich Plasm | Liquid plasm activated with calcium |
| P-PRP | Platelet-Rich Plasm-pure<br>Leukocyte-poor and Platelet Rich platelet-rich plasma | Leukocyte-poor platelet-rich plasma |
| L-PRP® | Leukocyte-Rich and Platelet Rich platelet-rich plasma | Leukocytes and a fibrin network after activation |
| PDGF® | Platelet-Derived Growth Factor | Plasm |
| PRGF® | Growth Factor-Rich Plasm | Plasm |
| CPRP | Concentrated Platelet-Rich Plasm | Factory produced (e.g., Baxter Healthcare's Tisseel) |

Source: the authors.

## Platelet-Rich Plasm (PRP)

PRP is a first-generation autologous platelet concentrate that contains a high concentration of platelets in a small volume of plasma. Its concentration ranges from 2 to 8.5 times the normal level (29). It also contains a minimal amount of natural fibrinogen and is obtained from whole blood anticoagulated with sodium citrate. It also requires calcium chloride (CaCl) and bovine thrombin to activate (initiate platelet degranulation) and initiate the coagulation process. For some authors, the presence of bovine thrombin is one of its disadvantages (21,23,30–32).

Platelets begin to actively secrete bioactive molecules within 10 minutes of clot formation. They complete the secretion of more than 95% of the pre-synthesized growth factors in one hour. Alpha granules release growth factors between one and four days after platelet activation but sustain their stimulation of the proliferative phase for approximately 10 days after release. It has been shown that, in addition to growth factors, the granular tissue of PRP-treated wounds contains intra- and extra-platelet components that also contribute to tissue regeneration. When the direct influence of platelets begins to decrease, the macrophages that arrive through the vascular stream are stimulated by the platelets and assume responsibility for the regulation of healing by secreting their own factors (14,18,28,29,34).

The group of Anitua, *et al*. (34) have been the great promoters of the PRP preparation. They established the simplest and most popular technique for its preparation (2). Certainly, it should be considered that modifications in the protocol can change the platelet concentration factor (30) (Table 2).

## Uses of PRP

There are numerous applications of PRP, not only in regenerative dentistry but also in maxillofacial surgery, ulcer treatment, spinal surgery, orthopedic surgery, and aesthetic medicine (13,16,22).

### *Periodontics*

PRP is used as an adjuvant to regenerative therapy (22,35). In this sense, Okuda-Kawase, *et al*. (36) show an increase in DNA synthesis during the periodontal regeneration process in patients treated with PRP. When evaluating the mitogenic activity through the incorporation of 5-bromodeoxyuridine, these researchers found a significantly higher cell count in the periodontium of patients treated with PRP in relation to a control group (36).

### *Implantology*

When preparing the maxillary bone for implant placement, there is greater buccolingual/palatal bone width, greater bone density, and faster tissue coverage in the alveoli where PRP is used (35). Another comparative study showed that the addition of PRP with beta-tricalcium phosphate (beta-TCP) results in less bone loss around implants (38). These findings reaffirm the multiple publications by Anitua (34) in which bone regeneration, osseointegration, and soft tissue gain are confirmed when using PRP alone or combined with other substances such as bone substitutes. The properties of PRP have also been evidenced in the management of gingival recession defects, alone or in combination with different guided bone regeneration techniques and biomaterials (2).

### *Maxillofacial and Reconstructive Surgery*

PRP is used to accelerate bone healing and maturation which improves alveolar healing (17,22,23). PRP improves bone regeneration and leads to platelets acting as local regulators of the healing process by increasing the microcirculation of the gingival mucosa surrounding the wound (35)

### *Maxillary Floor Elevation*

An example is a clinical case published in Mexico (29) that was evaluated for seven years, in which maxillary sinus floor elevation was performed using plasma rich in growth factors (GFRP), absorbable hydroxyapatite, and bone allograft as graft materials, and two implants were placed simultaneously. That study concluded that using GFRP makes it possible to simplify the technique by compacting the subantral graft, in sinus elevation, which improves consistency, facilitates handling, and increases the amount of graft. In that study, the radiological evaluation showed optimal results, because, in addition to reducing the total treatment time, it was effective in improving soft tissue healing and as was the purpose, increasing bone volume in the posterior maxillary area to implant placement. Other reports in the literature show a high success rate when using this technique and make it a predictable, effective, and safe procedure (29).

### *Healing of Mucous Tissue After Extraction*

A study by Bonilla, *et al*. (33) shows the effective healing action on mucosal tissue after third molar extraction by comparing the healing action between mucosal tissue treated with PRP and control tissue. When performing the extraction and observing the improvement in the postoperative period of the patients, they concluded that using PRP, due to the high content of growth factors, helps to accelerate the healing process, prevents the tissues from becoming inflamed, and reduces postoperative pain (33). Acosta, *et al*. (38) also confirmed these findings, in an *in vitro* study that evaluated the proliferation and cell viability of fibroblasts and osteoblasts of the periodontal ligament stimulated with PRP. They found statistically significant differences when PRP was used in cell cultures. Additional evidence shows that the rate of alveolar osteitis is lower in bone sites treated with GFRP (18).

### *Antimicrobial Action*

CieVlik-Bielecka, *et al*. (39), using the Kirby-Bauer disk diffusion method, found that L-PRP inhibited the growth of *Staphylococcus aureus* and was active against *Enterococcus faecalis* and *Pseudomonas aeruginosa*. They also found that a high concentration of thrombin, which, as an activator, increases the potency of the antimicrobial action of L-PRP (39). Hartshorne and Gluckman (40) explain the antimicrobial activity to the diverse populations of leukocytes and stem cells present in PRP. On the other hand, PRP can provide early protection against bacterial contamination (16) during surgical procedures, but it is cautioned that, although the antibacterial action of PRP is favorable, it should not be overestimated or considered comparable in efficacy with antibiotic therapy, according to Kawase (16). After reviewing various studies, PRP has also inhibited the growth of bacteria such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella* sp.

## Platelet Rich Fibrin (FRP) or Fibrin Rich Plasma (FRP)

PRF is a fibrin matrix with platelets, cytokines, and GF, which acts as a biodegradable structure not requiring additives. The enhanced fibrin structure protects developing tissues and controls the gradual release of GF as the membrane degrades (2). Actually, PRF is a second-generation technology (4,16,17,21,25,41-43) based on PRP but, by preserving growth factors in a three-dimensional matrix, it can exert its effects days or weeks after surgery (26). PRF also contains fibronectin and vitronectin that promote angiogenesis. Within minutes after phlebotomy and contact with the collection tube material, the absence of anticoagulant allows most of the platelets contained in the collected blood to be activated to trigger the coagulation cascade. Fibrinogen is concentrated until the effect of thrombin circulation transforms it into a three-dimensional network capable of trapping molecules and cells (fibrin network) that also has the characteristic of acting as a barrier membrane in bone regeneration procedures and guided tissue regeneration, that is, GBR and GTR (6,24,25,41,43).

Specifically, PRF is a three-dimensional structure composed of fine, flexible, mature, and dense polymerized fibrin strands. In the structure, the red blood cell zone is trapped in a relatively immature fibrin network (17). At the junction of the red corpuscular zone (RBC) and the fibrin body, lymphocytes with an irregular surface are observed microscopically along with numerous platelet aggregates. Groups of large and dense platelets are observed in the area of the buffy coat, indicating that they are in a state of aggregation and coagulation. In the remainder of the fibrin body, a thick mature fibrin strand is seen running parallel to each other. Platelets are concentrated in the first millimeter after the red part and steadily decrease with increasing distance from the red end (17). Figure 2 shows the structural characteristics and distribution of the PRF components.

The histological analysis performed by Gutiérrez, *et al.* (44) showed that there is no homogeneous platelet distribution throughout the FRP membrane. In the upper part, which is called platelet poor fibrin (PPF), there is a lower platelet concentration than in the lower BC (white blood cell layer) area. In the RBC, layers of polymorphonuclear cells and erythrocytes are observed. Regarding fibrin, it is homogeneous in most of the FRP, except in the upper zone that is a bit loose. In the same study, scanning electron microscopy (SEM) analyses confirmed that the structure of the fibrin network and the cellular content are different in the three zones of the membrane, the concentration of platelets is higher in zone BC, and it is diminished in the PPF zone. Likewise, fibrin in zone BC is denser (29,33,36,44).

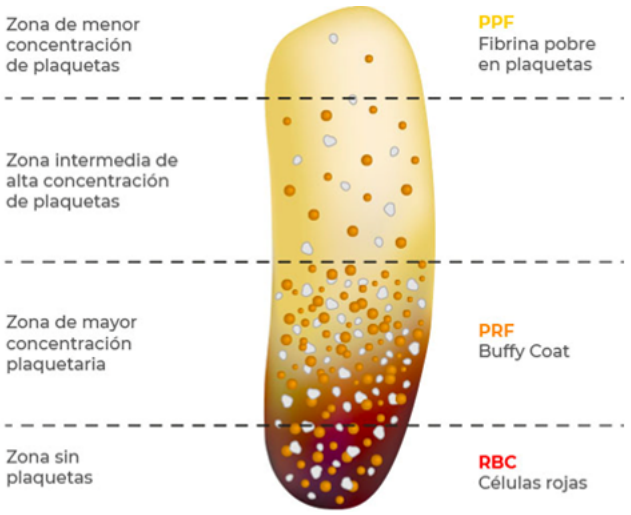


FIGURE 2
Structural Characteristics and Distribution of PRF Components
Figure adapted by the authors (29,33,36,44)

PRF provides a temporary matrix for the injured tissue until remodeling is complete. It is important to note that the preparation and formation of the fibrin network must be tightly controlled as it influences the final properties of the product (24,45-47).

In 2020, Thanasrisuebwong, *et al.* (46) evidenced the physical and biological properties of i-PRF compared to PRP. They used techniques such as SEM and ELISA and analyzed viscoelastic properties using the rotational thromboelastometry technique (ROTEM®) (46). To demonstrate the effects of C-PRF on angiogenesis, tissue growth studies in chicken eggs have counted the number of vessels formed during the incubation period and have compared it to baseline (day 0). Researchers found the formation of new capillary blood vessels, cross-linked with each other to generate networks, as well as the C-PRF content containing PDGF isoforms at higher levels, which significantly stimulated cell proliferation and neovascularization (36).

Apoorv Goel, *et al.* (43), also in 2020, concluded in a study that PRF membranes in combination with their exudates result in a high cell-proliferation rate. This basically translates into great therapeutic and wound healing potential that is not affected by a patient's periodontal condition.

PRF is used in combination with bone grafts (bovine porous bone mineral), nanocrystalline hydroxyapatite, and freeze-dried demineralized bone allograft (DFDBA), or pharmacologic agents, such as metformin gel. PRF has been found to be more effective in improving clinical and radiographic parameters in patients whose grafts were treated with PRF (1,6,25). When compared to PRP, PRF preparations tend to have a higher leukocyte count, due to improvements in preparation techniques (14,25).

Since its creation in 2001 by Choukroun, *et al.* (11,17,21,28,31,48), several modifications have been made to the conventional protocol to prepare PRF: Advanced PRF (A-PRF®) which is PRF collected in glass tubes and centrifuged at a slower speed; Injectable PRF (i-PRF®), which has the minimum centrifugation time and its application or injection is immediate; Lysed PRF (PRF LySATE®), which is obtained from PRF that is

incubated at 37 °C in a humidified atmosphere of 5% CO2 / 95% air; and Titanium PRF (T-PRF®), which comes from the PRF that is collected in medical-grade titanium tubing. All variations of PRF use different centrifugation conditions, with the basic structural design, and use different collect tube materials (11,49).

Over the last decade, PRF has gained tremendous momentum for being used in a wide variety of dental and medical procedures (6,10,17,50,51). The ability of PRF to stimulate regeneration in a wide range of tissues has been repeatedly found. PRF has been shown to rapidly stimulate tissue healing by significantly increasing the recruitment and proliferation of a variety of cells, including endothelial cells, gingival fibroblasts, chondrocytes, and osteoblasts. In this way, it promotes tissue repair and angiogenesis at the injury site (4,16,17,20–22,25,41,51).

## Main Uses of PRF in Clinical Dentistry as a Membrane

In surgery, PRF is used as a resorbable membrane for guided bone regeneration (GBR) that includes alveolar ridge augmentation (11,37,51) and prevents undesirable cell migration into bone defects. PRF also provides a space for osteogenic and angiogenic cell adhesion, allowing the underlying blood clot to mineralize (16,52). This membrane also protects open wounds from the oral environment when sutures cannot bridge the mucosal margins and has shown favorable clinical results in the treatment of intra-bone periodontal defects (11,51-53).

In 2010, Gassling, *et al.* (1) showed the superiority of PRF over collagen (Bio-Gides®) as a scaffold for periosteal cell proliferation in periosteal tissue engineering. Other clinical studies with promising results used PRF membranes as sole graft material to seek augmentation of the maxillary sinus floor (11). Since PRF does not require the use of other biological materials to cover an exposed flap, it offers the additional advantage of not creating a risk of infection, if exposed to the oral cavity. It has been proven that, within a three-month healing period, the fibrinous matrix transforms into new tissue by regenerating bone in the soft-tissue-lined cavity. Another randomized clinical trial, reported in the systematic review by Miron, *et al.* (53), published in 2017, showed that the exclusive use of PRF (studies by Inchingolo*, et al.* [54]), before implant placement, could minimize dimensional changes after extraction and improve osteogenesis (53). In all the cases of said study, the authors observed a satisfactory implant-prosthetic rehabilitation, according to the Albrektsson criteria (54). By applying these criteria, a success rate of 85% at 5 years of observation and 80% at 10 years of observation is expected to classify the implant at the minimum levels of success expected.

The concept of socket preservation was implemented to minimize bone resorption after tooth extraction and to preserve alveolar bone using PRF. A clinical study with 117 patients, after dental extraction, showed the benefit of PRF in preventing alveolar osteitis and improving hemostasis (15). A PRF membrane can also be used to improve wound healing in immunocompromised patients and as an adjuvant in patients on anticoagulant therapy (11).

## PRF in Oral and Maxillofacial Surgery and Dental Implantology

In most clinical studies in implantology, the use of PRF focuses on improving clinical outcomes, for example, in sinus lifts, using PRF as the sole grafting material simultaneously with implant placement, or using a combination of PRF and bone allograft (FDBA) prior to implant placement. Other focus areas of clinical research are implantology with the use of PRF in preservation of the alveolar ridge, healing of the tissues around the implant, and reduction of mobility, due to the rapid healing of the tissues surrounding the implant and efficiency of osteogenesis, which improves its stability. *In vitro* studies have shown gene expression (PRF-induced) of early and late markers of osteogenesis, bone marrow stimulation, and soft tissue healing (13,27,40,49,55,56).

PRF can also be used as a filling material, especially in multiple extractions, to preserve the height of the alveolar ridge. In such cases, PRF acts as a super blood clot for neovascularization and acceleration of tissue regeneration (4,53). This fact was also shown by Cortese, *et al*. (56), in 10 elderly patients, by promoting neo-angiogenesis. They concluded that the most relevant advantages of using PRF are the healing and regenerative properties of the bone and its complete resorption after surgery, which leads to avoiding a second surgery, an especially important fact in patients with advanced age (56,57).

Hartshorne and Gluckman (40) showed in extensive studies, results of improvement in the clinical performance of paranasal sinus lifts (11). PRF in combination with bone graft materials has been used in several direct and indirect sinus lift techniques, including bone graft sinus floor lift, osteotome-mediated sinus floor lift, and osteotome-mediated sinus floor lift, minimally invasive antral membrane balloon techniques (11). Other studies in which PRF was used alone as a graft material for sinus lift led to the conclusion that PRF significantly promoted bone healing with particularly good bone gains, between the sinus floor and the upper part of the alveolar crest, at 6 months, 1 year, and 6 years, preservation of the implant was obtained (53,58).

In cases of wide cavities and lesions where primary closure is difficult, a PRF membrane can be used as a lining and protection membrane that promotes re-epithelialization of the site and accelerates the union of the wound margins. The resistance and elasticity of the PRF membrane make it easy to suture. As a membrane for GBR, the dense matrix architecture of PRF covers, protects, and stabilizes the bone graft material and the operative site. In addition, PRF can be used to treat peri-implant bone defects and to regenerate bone and soft tissue in immediate implants (11).

## PRF in Periodontics

Currently, most clinical studies in periodontics focus on improving clinical outcomes with treatment of intra-osseous periodontal pockets, furcation defects, gingival recession defects, healing of connective tissue graft sites in the palate (13,58), oral lesions such as ulcer or open sore, and periapical lesions (29). A case study by Hernández Tejeda in Mexico (29), with a potential root coverage approach (11,29), reported the use of a combination of PRF gel, hydroxyapatite graft, and membrane-guided tissue regeneration to treat an open sore injury. PRF can be used to cover localized gingival recession in mandibular anterior teeth by the combined technique of laterally placed flap and PRF membrane, to stimulate osteogenic differentiation of dental pulp cells (11). The relevance of PRF in periodontal regeneration is attributed mainly to platelet-derived growth factor (PDGF) and transforming growth factor (TGF). For its part, the production of osteoprotegerin (OPG) causes the proliferation of osteoblasts (11,35,37).

In 2020, a study with 23 dogs showed evidence of the use of PRF in periodontal healing after open flap debridement (OFD) in canine periodontitis. In the OFD plus PRF group, a considerably decreased inflammatory score was observed, when compared to the OFD group alone or the control group. Also, collagen accumulation improved in the OFD + PRF group at a later time, when compared to the baseline. The application of PRF also significantly reduced the expression of inflammatory cytokines (TNFA and IL1B) and promoted the expression of genes related to the production of collagen (COL1A1, COL3A1, and TIMP1) and growth factors (PDGFB, TGFB1, and VEGFA) (59). .

As has been shown, the mandibular third molar can cause periodontal defects in the distal root of the second molar. Gasparro, *et al.* (60) conducted a split-mouth randomized clinical trial to treat periodontal pockets in the distal mandibular second molar after surgical extraction of the impacted third molar with application of L-PRF®. After six months, sites treated with L-PRF® showed better results in terms of CAL clinical attachment level gain and depth reduction when compared to sites that did not have L-PRF® applied (60). In a panel discussion in 2020, while celebrating 20 years of continued use of biologic products in medicine and reviewing existing evidence, it was concluded that periodontal regeneration must show

evidence of new bone, cementum, and periodontal ligament on the surface of previously diseased tooth root. This should be verified histologically because clinical findings can be difficult to identify and are not very objective (57). In different periodontal procedures, the elastic consistency of the PRF membrane also allows a hole to be drilled in the membrane to hang a healing abutment prior to flap suture (40).

## PRF in Intrabony Defect Regeneration

Randomized clinical trials have shown that the use of PRF leads to periodontal repair, with statistically confirmed superior results for intrabony defects than when PRF is not used. When using PRF, repotentiation and increased effective performance of biomaterials such as bone grafts or collagen barrier membranes are shown, in addition to periodontal regeneration of intrabony defects (53,61). A case described by Miron, *et al.* (53) showed results of the potential for tissue repair using PRF in furcation defects. It also showed that PRF-enriched palatal dressing significantly accelerated the healing of a palatal wound.

Recently, Miron, *et al.* (51) performed a systematic review and meta-analysis to conclude that open flap debridement in conjunction with PRF showed significantly higher values for probing depth, clinical periodontal attachment level, and bone formation gain. Such findings were confirmed with radiographic recording that showed the regenerative properties of PRF. They concluded that the combination of PRF with bone grafts or small biomolecules may offer important clinical advantages.

## PRF to Cover Roots with Gingival Recessions

PRF has also been widely used as a bioactive matrix in numerous studies for the coverage of roots with gingival recessions. The use of coronally advanced flap (CAF) was compared with CAF + PRF. PRF was found to induce a significant increase in root coverage (4,6,18,41,53).

## PRF in Endodontics

The combination of PRF membrane, as a matrix, and mineral trioxide, in apexification procedures, shows to be an effective alternative to create artificial barriers at the end of the root and induce faster periapical healing, in cases of large periapical lesions, by using a membrane can prevent extrusion. PRF has also been used to fill bone defects after periapical surgeries, such as root-end resection (resorption). PRF is an ideal scaffold for the revascularization of immature permanent teeth with necrotic pulps (11,13,63). These procedures, according to Hartshorne and Gluckman (40,58), are biologically designed to restore the function of a damaged pulp by stimulating dental pulp stem cells and progenitor cells present in the root canal under conditions that are favorable for their differentiation.

## Sohn's Sticky Bone

Sohn's sticky bone is a combination of I-PRF and graft particles, which allows the bone graft to be easily shaped and manipulated, to obtain a good consistency and to reduce leaching of the graft, since it is firmly encapsulated in the fibrin matrix (17.37). Sticky bone has the advantage of releasing growth factors at the recipient site, which would otherwise be missing from a regular bone graft. It has the potential of converting any osteoconductive graft into an osteoprotective one (due to the presence of platelets and growth factors), which would result in faster and more efficient bone formation (17). Sticky bone retains much of its shape during the healing process, with success primarily in alveolar crest and sinus augmentation (14,17,37,55).

Implants placed in cavities with CP are more stable and experience less resorption than implants placed in untreated bone. The greatest reported advantage of combining PRF with a bone graft material is decreased overall healing time and better handling of the graft material (53).

## PRF Antimicrobial Effects

A split-mouth design study showed that, by placing PRF in third molar extraction sockets, an almost 10-fold decrease in osteomyelitis infections can be expected (53), due to the increase in white blood cells. The different types of leukocytes are concentrated in the fibrin matrix, of these, the monocytes infiltrate the injury and differentiate into macrophages, which produce collagenases and provide antimicrobial properties to "clean" the wound (40). In addition, macrophages have been shown to be key elements in osteogenesis, both during bone modeling and remodeling, and in association with bone biomaterials (16). Jasmin, *et al.* (63) evaluated the *in vitro* antimicrobial effects of i-PRF against pathogenic oral biofilm producing staphylococcal bacteria isolated from patients with dental and oral abscesses. In that study, the antibacterial activity of i-PRF was determined using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) techniques. i-PRF showed bactericidal activity against both biofilm-producing and non-biofilm-producing bacteria and it was concluded that i-PRF could be a potential antimicrobial peptide to combat postoperative infections caused by biofilm-producing staphylococcal microorganisms (63).

Another study by Khorshidi, *et al.* (64) compared the antimicrobial, mechanical, and histological characteristics of the L-PRF membrane before and after the addition of silver nanoparticles (SNPs). Results showed a marked improvement in the mechanical properties and antibacterial activity of L-PRF after the addition of such particles. Only growth of *Klebsiella pneumoniae*, and no other bacteria, was found at 24 hours (65). For its part, in the clinical trial by Daugela, *et al.* (65) cited Tang's findings in the article, "Antimicrobial Peptides of Human Platelets," shows how activated platelets and activated leukocytes release antimicrobial peptides, among which are platelet factor 4, RANTES, connective tissue activating peptide III, platelet basic protein, thymosin beta-4, fibrinopeptide B and fibrinopeptide A, which together with fibrinogen degradation products, are bioactive substances that have been shown to be potent antimicrobials against *Escherichia coli* and *Staphylococcus aureus* (66).

## PRF in Pain Treatment

Several studies mention the additional advantage of PRF to manage pain and reduce postoperative discomfort in different procedures (3,13,24,27,33,39,52). In this sense, two more studies conducted by Daugela, *et al.* (65) and Canellas, *et al.* (67) evaluated the influence of L-PRF and its impacts on wound healing after extraction of the mandibular third molar (MTM), in addition to the benefits obtained in the patient's postoperative discomfort and the incidence of alveolar osteitis. In all cases, a decrease in pain was reported in the pain scale scores (VAS) (65,67). The use of PRF in alveoloplastic extractions has significantly reduced post-surgery pain and caused fewer functional limitations (e.g., pronunciation of words, sense of taste), improving the patients' quality of life after use. Although these are subjective aspects, say the authors, the decrease in pain intensity and post-surgery analgesic consumption were also described by other studies (3) that were showing a healing time that was considerably shorter when using the PRF application after dental extractions (3).

## PRP Compared to PRF

Due to availability and profitability, PRF has obtained more relevance, regarding uses in dentistry, in relation to its predecessor, PRP (50). PRF membranes have no contraindications; they can be used in all types of patients, especially in patients with systemic conditions whose healing is compromised (e.g., diabetics and smokers) and PRP cannot be used in anticoagulated patients (50). Several studies claim that PRF accelerates tissue growth even more, compared to PRP (14,16,25,27,38) (Table 3).

He, et al. (68) compared the effect of PRF and PRP on cell proliferation and osteoblast differentiation in an animal model with rats and quantified the values of PDGF-AB and TGF-Beta1. The researchers found that PRP releases large amounts of these factors during the first day, while PRF releases TGF-Beta1 at day 14 and PDGF-AB at day 7, corroborated by Sunil, *et al.* (18), and Miron, *et al.* (42) who reviewed several studies referring to it.

TABLE 3
PRF Compared to PRP

| PRF | PRP |
|---|---|
| Developed by Choukroun, Dohan, *et al.* | Developed by Anitua, *et al.* |
| Containing a greater number of platelets, leukocytes, cytokines and a very representative amount of fibrin, fibronectin and vitronectin | Decreased number of platelets, leukocytes, growth factors, and other cytokines |
| Three-dimensional/trimolecular structure | Tetramolecular structure |
| Its low thrombin concentration determines a more flexible structure capable of favoring cytokine trapping and cell migration. Their spatial arrangement serves as a substrate for platelets to chemotactically attract circulating stem cells. | The bilateral junctions that are formed due to the high concentrations of thrombin determine a mesh with a very rigid structure. |
| The exact content and architecture of the membrane are known. | The exact content and architecture of the membrane are unknown. |
| The strong architecture of fibrin allows it to be used as a true membrane or tissue. | It is used as a temporary fibrin layer added at the surgical site. |
| The release time of growth factors and membrane proteins is greater than 10 days and can reach several weeks. | The release time of growth factors is shorter; its matrix soon disappears. |
| Solid biomaterial. | It is a transient pharmaceutical adjuvant. |
| Simple collection technique. | Varias fases para su obtención. |
| More standardization studies. | Less standardization studies. |
| No additives are used, which makes it a strictly autogenous technique. | Requires the use of anticoagulants as additives. |

Source: the authors

The fact that there are some differences between these platelet concentrates does not prevent them from being used jointly and complementarily, as shown by Tsai, *et al.* (68). Those researchers in a study with induced wounds in pigs used mixtures of both PRP and i-PRF to make a platelet patch (PP) and promote wound repair and regeneration. Results indicated that all wounds showed significant size reduction (69).

## Commercial Systems of CP Preparations

There are registered commercial techniques to prepare platelet concentrates with predefined times and fixed regulations for centrifugation. There are also CP already prepared industrially with trademark registration and patenting. Table 4 shows the best known commercial products and systems (10,14,16,18,19,27,45,50,58,70).

TABLE 4
Techniques and Trademarks of CP Preparations (10,14,16,18,19,27,445,50,58,70)

| Brand | Manufacturer |
|---|---|
| LW PRP | LW Scientific, Inc. |
| Angel PRP | Whole blood processing system Angel (AWBPS), Sorin Group, Mirandola, Italia |
| Plateltex PRP | Plateltex, Prague Czechia |
| Regen PRP | Regen Lab, Mollens, Switzerland |
| SmartPReP PRP | Harvest Corp, Plymouth, MA, USA |
| Gravitational Platelet Separation System GPS PRP | Biomet Biologic, Varsovia, IN, USA |
| Magellan PRP (Magellan APS) Autologous platelet separator | Medtronic, Minneapolis, MN, USA |
| Anitua's PRGF | BTI BioTechnology Institute, Vitoria, Spain |
| Intra-Spin ™ (intra-Lock) | Ehrenfest David M. Dohan© BioHorizon |
| A-PRF Duo ™ | PRF de Choukroun. France |
| Fibrinet PRFM | Cascade Medical, Wayne, NJ, USA |
| SephylCascade PRF | Medical enterprises, LLC, Wayne, USA |
| Vivostat PRF. Autologous Platelet Cascade System | Musculoskeletal Transplant Foundation, NJ, USA |
| Salvin regenerative | Salvin Dental |
| PRGF-endoret® (SCLP) (Growth Factor-Rich Plasma) | BTI, Vitoria-Gasteiz, Spain. PRP Proteal, Barcelona, Spain |

Source: the authors.

In 2014, an evaluation of 7 commercial PRP systems was performed in which platelets, white blood cells (WBC), red blood cells (RBC), and growth factors were quantified. The study concluded that differences in the purification method used to produce PRP can lead to notable differences. Other differences occurred in the amount of whole blood required, final yield, cellular components, and growth factor concentrations (70). Knowledge of these differences can lead to commercial kits or systems being used depending on the required clinical purposes and making better decisions.

A comparative study of commercial PRF systems, conducted by Dohan Ehrenfest, *et al.* (45), with L-PRF (Intra-Spin, Intra-Lock), A-PRF 12 (Advanced PRF, Process), LW-UPD8 (LW Scientific) and Salvin1310 (Salvin Dental). The study quantified growth factors (by ELISA technique) and found that PRF, when analyzed by light microscopy, shows relatively similar characteristics in all L-PRF centrifugation kits. The authors state that there are differences in the macroscopic structure, which led them to conclude that the characteristics of the centrifuge and the centrifugation protocols have a significant impact on cells, growth factors, and L-PRF architecture (46).

## Limitations of Use and Implementation of CPs

Certainly, there are no drawbacks that discourage the use of PC, but due to the lack of diffusion that studies deserve, detractors of PC emphasize their limitations. These limitations can be attributed to flaws in the preparation protocols, due to lack of standardization, of which the variability of the results between one patient and another depends in part on. These variabilities ultimately make it difficult to predict and reproduce results.

It is necessary to treat PC as a surgical biomaterial in which asepsis and rigor are controlled in all manufacturing steps. If these requirements are not conducted properly, they could become a critical aspect for the results and efficacy of clinical and surgical procedures (28,44,62,63).

Another limitation occurs when the amount of blood collected is not sufficient, when large amounts of PC are necessary, since, as they are autologous products, their quantity is limited. It must be considered that

PRF membranes should be used within a short time after preparation, since they tend to shrink as a result of dehydration (11). Currently, there is little information on the restrictions that exist for the use of PC, in patients with coagulation disorders or who use drugs that affect blood coagulation (i.e., heparin, warfarin, or platelet inhibitors) (50).

# CONCLUSIONS

CP, especially PRF, have become a tissue-targeted solution in surgical situations where protection, stimulation of healing, and regeneration are critical, and where the prognosis for tissue repair is poor, or is potentially compromised. These facts make CP a novel tool for tissue engineering (2,50). However, further research and standardization of procedures is needed to support the beneficial effects of these biologics.

Platelet concentration levels in PC range from less than 2 to 8.5 times the normal level, obtaining the same regenerative benefit in each case (17). It can be said that CP can function as an initiator and enhancer of autologous healing, having synergistic effects as regenerative material, serving in dentistry as a support for conventional surgical processes that improve results in clinical and radiographic parameters. It is evident that the use of CP as a biomaterial reduces postoperative incompatibility (due to its regenerative, antimicrobial, and pain-reducing activity).

The basic structure of the matrix and cellular composition of the different PCs is the same. What changes is the physical presentation and, in some cases, the additives and added particles.

The clinical effects of CP depend on the intrinsic, versatile, and adaptive characteristics of the patient's blood and on the standardization and validation of the protocols used for its preparation.

Those derived from recombinant sources (manufactured in mammalian cells or bacteria) introduce high supraphysiological concentrations in the regeneration zones and are developed industrially. They are approved by the US Food and Drug Administration and are marketed at high prices. The different CPs generate growth factors in a natural, dosed form, and are manufactured exclusively from autologous sources. They allow to reduce costs considerably and lead to a comparable benefit in the desired clinical results.

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# APPENDIX 1

Studies on PRF

| Author(s) | Title | Purpose | Conclusions |
|---|---|---|---|
| Gassling, *et al.* Germany (2019)(1) | Platelet-rich fibrin membranes as scaffolds for periosteal tissue (Case study) | Make a comparison of PRF with Bio-Gide collagen membrane commonly used as a scaffold in periosteal tissue engineering. | PRF appears to be superior to collagen (Bio-Gide) as a scaffold for human periosteal cell proliferation. PRF membranes are suitable for culture of periosteal cells used in bone tissue engineering. |
| Dimofte, *et al.* Rumania (2016)(3) | Quality of Life after using of Platelet-rich fibrin (PRF) in patients with alveoloplastic extraction (Case study). | To evaluate the effects of PRF on the healing of post-extraction wounds in the case of plastic socket extractions, reducing the pain and discomfort of the patient in the immediate postoperative period, until the removal of suture threads. | The use of PRF in alveoloplastic extractions has significantly reduced post-surgery pain and has also reduced functional limitations (pronunciation of words, sense of taste). |
| Zumarán, *et al.* Chile (2018)(4) | The 3 R's for Platelet-Rich Fibrin: A "Super" Tri-Dimensional Biomaterial for Contemporary Naturally-Guided Oro-Maxillo-Facial Soft and Hard Tissue Repair, Reconstruction and Regeneration (Review). | To provide a practical update on the application of PRFs during oral surgery procedures in randomized, controlled clinical trials in humans. | PRF is an advanced and original tool in regenerative dentistry, presenting a solid and presumably profitable alternative as a biomaterial for the repair and regeneration of oro-maxillofacial tissues (soft and hard). However, preparation protocols continue to be a source of confusion and need to be revised and standardized. |

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| Umesh, *et al.* India (2017)(6) | Platelet-rich Fibrin: A Paradigm in Periodontal Therapy – A Systematic Review (Systematic review). | Collect in vitro, animal, and clinical studies using the PubMed electronic database from January 2006 to August 2016 highlighting PRF for soft and hard tissue regeneration and/or wound healing. | Studies have confirmed that PRF is a therapeutic regenerative biomaterial with immense potential that has widespread clinical applications in medical and dental perspectives. |
| Borie, *et al.* Chile (2015)(7) | Platelet-rich fibrin application in dentistry: a literature review. (Literature review). | Review relevant literature regarding the technique of using PRF, focusing on its preparation, advantages and disadvantages of using it in clinical applications. | PRF alone or in combination with other biomaterials seems to have several advantages and indications for both medicine and dentistry, since it is a minimally invasive technique with low risks and satisfactory clinical results. |
| Castro, *et al.* Belgium/Chile (2017)(8) | Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. (Systematic review and meta-analysis). | To analyze the regenerative potential of leukocyte- and platelet-rich fibrin (L-PRF) during periodontal surgery. | L-PRF improves periodontal wound healing. |
| Miron, *et al.* Germany (2017)(10) | Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?. (Study case). | To investigate a liquid formulation of platelet-rich fibrin (PRF) called injectable-PRF (i-PRF) without the use of anticoagulants. | Standard PRP and i-PRF were compared. i-PRF demonstrated the ability to release higher concentrations of various growth factors and induced increased fibroblast migration and expression of PDGF, TGF-β, and collagen 1. |
| Sanjeevi & Kumar. India (2019)(11) | Platelet-rich fibrin in dentistry. (Review). | Review and integrate the relevant literature on the applications of PRF in various disciplines of Dentistry. | The use of PRF as an adjunct in wound healing and periodontal regeneration has shown promising results. It has been used successfully for the correction of bone defects in periodontics, oral and maxillofacial surgery, and implantology. |
| Wang, *et al.* China (2020)(12) | The Effects of Leukocyte-Platelet Rich Fibrin (L-PRF) on Suppression of the Expressions of the Pro-Inflammatory Cytokines, and Proliferation of Schwann Cell, and Neurotrophic Factors. (Study case). | To evaluate the use of L-PRF as an autologous scaffold in nerve regeneration, and Schwann cell (SC) proliferation and secretion of neurotrophic factors and its anti-inflammatory effect on in vitro inflammatory responses induced by SC Porphyromonas gingivalis - Lipopolysaccharide and determine the structure and constituents of L-PRF, to determine the effect of L-PRF on the proliferation of SCs and the secretion of neurotrophic factors (NGF, GDNF), and to evaluate the effect of L-PRF on the inflammatory response in vitro. | L-PRF allows the release of growth factors in a sustained manner, increases the proliferation of Schwann cells and the secretion of neurotrophic factors in a concentration-dependent manner, and L-PRF exhibits anti-inflammatory properties in vitro. |
| Ghanaati, *et al.* Alemania (2018)(13) | Fifteen Years of Platelet Rich Fibrin in Dentistry and Oromaxillofacial Surgery: How High is the Level of Scientific Evidence?. (Review) | To investigate the level of scientific evidence of published articles related to the use of PRF for bone and soft tissue regeneration in dentistry and maxillofacial surgery. | PRF is a beneficial tool that significantly improves bone and soft tissue regeneration. However, the clinical community requires a standardization of PRF protocols. |

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| Fan, *et al.* USA (2020)(14) | Clinical Uses of Platelet-Rich Fibrin in Oral and Maxillofacial Surgery. (Review). | Disseminate and highlight the different uses and benefits of PRF. | There is abundant literature and case studies discussing the benefits of PRF. Most studies favor the use of PRF versus the control group. There is currently no standard PRF protocol, but there are many uses in dentistry and maxillofacial surgery that show promising results. |
| Rucha & Triveni. India (2017)(17) | An Update on the Protocols and Biologic Actions of Platelet Rich Fibrin in Dentistry. (Review). | Review the biological properties of platelet-rich fibrin and the advancement in the technology of this product since its inception. | All PRF modifications are aimed at maximizing growth factor-mediated biological effects and cellular activity. PRF as a biological surgical additive has been successfully used in various applications in dentistry. Technological advances in the field of PRF such as i-PRF have paved the way for versatility in platelet concentrate applications. |
| Kuzu & Ozdemir. Turkey (2019)(26) | Histomorphometric Evaluation of Bone Regeneration in Peri-Implant Osseous Defects Treated With Titanium Prepared Platelet Rich Fibrin: An Experimental Study in a Rabbit Model. (Study case). | The aim of this work was to study the regeneration of experimentally prepared platelet-rich fibrin (T-PRF) in titanium tubes. | The application of T-PRF during the healing of bone defects can function as a self-regenerative function. In future targeted bone regeneration, it can increase the rate of healing. |
| Salgado, et *al.* Spain (2017)(28) | New trends in tissue regeneration: fibrin rich in platelets and leukocytes. (Review). | Perform a review and update on the use of this technique . | The use of L-PRF is a simple and effective technique that accelerates the healing of soft and hard tissues. The main advantage is that it uses the patient's own blood, which reduces the possible immune reactions of rejection and the transmission of diseases by the parenteral route. |
| Dohan, *et al.* Francia (2006). (31) | Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. (Synthesis). | Investigate the platelet-associated characteristics of this biomaterial | The slow polymerization of fibrin during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycan chains into the fibrin meshes, unlike other platelet concentrates. PRF would be able to progressively release cytokines during fibrin matrix remodeling and this mechanism could explain the healing properties observed clinically. |
| Naik, *et al.* India (2013)(32) | Role of Platelet rich fibrin in wound healing: A critical review. | To review and discuss available strategies for the use of platelet-rich fibrin and as an aid to healing in dentistry. | Autologous PRF is considered a healing biomaterial and studies have now demonstrated its application in various disciplines of dentistry. |
| Kobayashi, *et al.* Japan (2012)(36) | A proposed protocol for the standardized preparation of PRF membranes for clinical use. (*In vitro* research). | Standardize a protocol for PRF preparations and validate a compression device for this material. | C-PRF can be useful for grafting while minimizing the loss of bioactive factors. The C-PRF preparation protocol is proposed as a standardized protocol for PRF membrane preparation. |

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| Hartshorne & Gluckman. South Africa (2016)(40) | A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part II: Part II: preparation, optimization, handling and application, benefits and limitations of PRF. (Review). | Analyze the available literature on: i) what is PRF; ii) how the PRF evolved and developed to its current status; and iii) what its biological characteristics and composition are and how these key elements function in the clinical setting. | One of the clinical limitations that needs to be addressed is the heterogeneity in the quality of platelets and blood components across various PRF protocols. The PRF technique continues to develop because it is quite easy to prepare, inexpensive and allows rapid production of natural fibrin membranes, enriched with platelets and leukocytes, which can be used immediately in any clinical situation. |
| Meza, et *al*. Perú (2014)(41) | Platelet-rich fibrin and its application in periodontics: literature review. (Review). | Describe the biological properties and clinical applications of PRF in mucogingival surgery (root coverage) and in maxillary sinus floor lift. | PRF as a filler biomaterial in maxillary sinus floor lift is a relevant option since the studies available in the literature demonstrate topographically and histologically that it promotes bone regeneration. |
| Miron, *et al*. Switzerland (2018)(42) | Controversies related to scientific report describing g-forces from studies on platelet-rich fibrin: Necessity for standardization of relative centrifugal force values. (Synthesis). | Propose standardization regarding accurate reporting of g-force values in future studies investigating PRF at FCR-max. | Different protocol variables, such as g-force values, are subject to significant error due to centrifugation time, patient hematocrit levels, initial volume of blood collected, and other factors. |
| Apoorv, *et al*. USA (2021)(43) | Effects of platelet-rich fibrin on human gingival and periodontal ligament fibroblast proliferation from chronic periodontitis versus periodontally healthy subjects. Estudio de cases. | To investigate and compare the effects of PRF in patients diagnosed with moderate or severe generalized chronic periodontitis with those with an intact periodontium on the proliferation of human gingival fibroblasts (HGF) and human periodontal ligament fibroblasts (HPLF). | PRF membranes, in combination with PRF exudates, can be used for their therapeutic and wound healing potential, which is not affected by the periodontal status of the patient. |
| Gutiérrez, *et al*. Colombia (2018)(44) | Structural Analysis of Platelet-Rich Fibrin and its Applications in Regenerative Dentistry .(*In vitro* research). | Describe the structural characteristics of PRF in the different zones of the membrane. | The SEM analysis confirms that the structure of the fibrin network and the cellular content are differential in each zone. Based on the structural knowledge of PRF, applications can be proposed that improve the performance of the material and therefore the clinical results. |
| Dohan, *et al*. USA (2018)(45) | The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. ( *In vitro* research). | To evaluate the mechanical vibrations that appear during centrifugation in four models of commercially available tabletop centrifuges for L-PRF and the impact of centrifuge characteristics on the cellular and fibrin architecture of a clot and L-PRF membrane. Evaluate how changing some parameters of the L-PRF protocol can influence its biosignature, independent of the characteristics of the centrifuge. | Light microscopy analysis showed relatively similar characteristics for all types of L-PRF (cell body concentration in the first half). |

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| Thanasrisuebwong, *et al.* Thailand (2019)(46) | Influence of fractionation methods on physical and biological properties of injectable platelet-rich fibrin: An exploratory study. (*In vitro* research). | To investigate the influence of different i-PRF fractions on the physical and biological properties derived from variations in the i-PRF fractionation preparation. | Scanning electron microscopy described more cellular components in the red i-PRF compared to the yellow i-PRF. Furthermore, the fibrin network of the yellow i-PRF showed a higher density than that of the red i-PRF. |
| Durmuş, et *al.* Turkey (2018)(47) | Evaluation of the accelerator effect of coral and platelet rich fibrin on bone healing. (Case study in animals) | To investigate the accelerating effect of coral and platelet-rich fibrillation (TZF) clinically, radiologically and histologically on bone healing in rabbits. (n-12). | The study showed that coral, PRF, and coral plus PRF are significantly effective in bone healing. |
| Choukroun, *et al.* France (2006). (48) | Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. (Synthesis). | To investigate the previously evaluated biology of PRF with the first established clinical results, to determine the potential fields of application of this biomaterial. | Clinical experience confirms that PRF can be considered a healing biomaterial. Presents all the necessary parameters that allow optimal healing. |
| Fujioka, *et al.* Switzerland/ Macedonia (2017)(49) | Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Grow Factor Release, Biocompatibility, and Cellular Response. (Synthesis). | To characterize how the speed of centrifugation (G-force) together with the time of centrifugation influence the release of growth factor from fibrin clots, as well as the cellular activity of gingival fibroblasts exposed to each PRF matrix. | The results of the present study demonstrate that modifications of the centrifugation speed and time with the low-speed concept favor an increase in the release of the growth factor from the PRF clots. |
| Hartshorne & Gluckman. South Africa (2016). (50) | A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part II: preparation, optimization, handling and application, benefits and limitations of PRF. (Review). | To analyze the available literature on PRF in relation to: i) the preparation technique; ii) optimization of PRF quality; iii) its handling and physical application; and iv) its benefits and limitations. | The optimal quality of the PRF membrane and the success of the treatment depend on: the rapid extraction of blood and its transfer to the centrifuge; the use of the appropriate centrifugation protocol; the maturation of the clot before use; membrane preparation using a standard preparation technique; and proper preservation of the membrane before use. |
| Miron, *et al.* Germany (2021). (51) | Use of platelet-rich fibrin for the treatment of periodontal intrabony defects: a systematic review and meta-analysis. | To compare the results of treating intrabony periodontal defects using platelet-rich fibrin (PRF) with other commonly used modalities. | The use of PRF significantly improved clinical outcomes in intrabony defects compared to OFD alone, with similar levels observed between OFD/BG and OFD/PRF. |
| Mourão, *et al.* Brazil (2015). (52) | Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. (Technical note). | To present an alternative to platelet concentrates using platelet-rich fibrin in liquid (injectable) form and its use with particulate bone graft materials in polymerized form. | The possibility of joining i-PRF with biomaterials for bone grafting creates an alternative to PRP as platelet aggregation for bone regeneration. PRP is used in regenerative procedures due to the possibility of optimizing bone formation. The technique presented in this study allows graft incorporation without the use of anticoagulants or other additives. |
| Miron, *et al.* Germany (2017)(53) | Use of platelet-rich fibrin in regenerative dentistry: a systematic review. (Systematic review). | Compile the vast number of articles published to date on PRF in the dental field to better understand clinical procedures in which PRF can be used to enhance tissue/bone formation. | Much research supports the use of PRF for periodontal and soft tissue repair. Nevertheless, well-conducted studies conclusively demonstrating the role of PRF during hard tissue bone regeneration are still lacking. |

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| Inchingolo, *et al.* Italy (2010)(54) | Trial with platelet-rich fibrin and Bio-Oss used as grafting materials in the treatment of the severe maxillary bone atrophy: Clinical and radiological evaluations. (Case study). | Evaluate the osseointegration of the implant, as well as the course of the bone regeneration and healing processes, thanks to the sinus lift procedure and using PRF as a filler material, in association with Bio-Oss. | All patients reported no pain on percussion, no signs of tissue distress in the peri-implant soft tissues, the presence of optimal primary stability of the inserted implants, and a significant increase in peri-implant bone density. |
| Suparman, et *al.* Malaysia (2019)(55) | Patching up the bone -A Case Report of Autologous Fibrin Matrix in Combination with Autogenous Bone Graft for Bone and Soft Tissue Regeneration. (Case study). | Observe tissue regeneration and bone regeneration with PRF. | The PRF is comparable to the commercially available membrane, where clinical outcomes can be predicted and the possibility of reducing post-surgical complications is achieved. It decreases the frequency of intraoperative and postoperative bleeding, facilitates soft tissue healing, and supports the initial stability of grafted tissue in recipient sites. |
| Cortese, *et al.* Italy (2016)(56) | Platelet-rich fibrin (PRF) in implant dentistry in combination with new bone regenerative technique in elderly patients. | Demonstrate how PRF in association with a new split ridge augmentation technique can be of great help in implant rehabilitation, especially in elderly patients, when bone regeneration is required. | PRF is a minimally invasive technique with low risk and satisfactory clinical results such as the prevention of complications or implant failure, particularly in elderly patients due to age-related conditions. |
| Hartshorne & Howard. South Africa (2016). (58) | A comprehensive clinical review of Platelet Rich Fibrin and its role in promoting tissue healing and regeneration in dentistry. Part III: Clinical indications of PRF in implant dentistry, periodontology, oral surgery and regenerative endodontics. (Review). | To analyze the available bibliography and clinical tests based on PRF relative to its clinical indications in implantology, periodontics, oral surgery and regenerative endodontics. | A better understanding of the clinical indications for PRF will facilitate clinicians' ability to improve the therapeutic applications of this product. PRF is increasingly being investigated and used worldwide by clinicians as an adjuvant autologous biomaterial to promote bone and soft tissue healing and regeneration. |
| Komsuthisopon, *et al.* Thailand (2020). (59) | Autologous platelet-rich fibrin stimulates canine periodontal regeneration.(Case report in animals). | To report the use of PRF in periodontal healing after open flap debridement (OFD) in canine periodontitis . | PRF combined with OFD provides a new strategy to improve the overall improvement of canine periodontitis treatment outcomes, especially in terms of inflammation and soft tissue healing. |
| Gasparro, *et al.* Germany (2020). (60) | Treatment of periodontal pockets at the distal aspect of mandibular second molar after surgical removal of impacted third molar and application of L-PRF: a split-mouth randomized clinical trial. (Case report). | To evaluate the reduction of clinical adhesion loss in the distal aspect of the second molar, after the third molar extraction and the application of L-PRF. | Sites treated by applying L-PRF after impacted third molar extraction showed better results in terms of CAL gain and probing depth reduction compared to control sites. |
| Príncipe, *et al.* Peru (2019)(61) | Effectiveness of fibrin-rich plasma and collagen membrane in guided bone regeneration. | To determine the effectiveness of two biomaterials, the fibrin-rich plasma (PRF) and membrane collagen in guided bone regeneration. | PRF induces a significantly higher cell proliferation than the other groups in the first days of healing; At 30 days, proliferation is similar with the collagen membrane group without significant differences. |

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| Yábar-Villafuerte, *et al.* Mexico (2019)(62) | The use of fibrin-rich plasma in endodontics for bone regeneration. Report of two cases. (Case report). | To report two clinical cases where a periapical lesion is diagnosed and an apicoectomy procedure is performed where fibrin-rich plasma is used as bone filler. | The use of plasma rich in fibrin for bone fillers after endodontic surgeries could be a good alternative to commercial bone fillers due to its bone-inducing properties. Rapid bone regeneration is related to growth factors such as bone morphogenetic proteins (BMPs). |
| Jasmine, *et al.* Saudi Arabia (2020)(63) | Antimicrobial and antibiofilm potential of injectable platelet rich fibrin second-generation platelet concentrate against biofilm producing oral staphylococcus isolates. (*In vitro* research). | To assess the *in vitro* antimicrobial effects of i-PRF against biofilm-producing staphylococcal bacteria isolated from patients with dental and oral abscesses. . | The antibacterial activity of i-PRF was determined by micro dilution in broth as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). i-PRF could be an antimicrobial peptide with potential to combat postoperative infections caused by biofilm-producing staphylococci. |
| Khorshidi, *et al.* Iran (2018)(64) | Does Adding Silver Nanoparticles to Leukocyte- and Platelet-Rich Fibrin Improve Its Properties? (Case report). | To compare the antibacterial, mechanical and histological characteristics of the L-PRF membrane before and after the addition of silver nanoparticles (SNPs). | Modification of L-PRF with SNPs improves the mechanical properties and antibacterial activity of L-PRF. It can play an important role in regenerative procedures. |
| Daugela, *et al.* Lithuania (2018)(65) | Influence of leukocyte- and platelet-rich fibrin (L-PRF) on the outcomes of impacted mandibular third molar removal surgery: A split-mouth randomized clinical trial. (Case report). | To assess the influence of leukocyte- and platelet-rich fibrin (L-PRF) on the healing of impacted mandibular extraction (IMTM) wounds, postoperative patient discomfort, and incidence of alveolar osteitis. | L-PRF improved soft tissue healing and reduced postoperative pain, swelling, and the incidence of alveolar osteitis after IMTM surgical extractions (bilateral third molar surgical extractions). |
| Canellas, *et al.* Brazil (2019)(67) | Platelet-rich fibrin in oral surgical procedures: a systematic review and meta-analysis. (Systematic review and meta- analysis. | To identify cases in which PRF has been shown to be effective in oral surgical procedures. | The available literature suggests that PRF has a positive effect on improving socket preservation in extraction sockets and around dental implants and a positive effect on postoperative pain. |
| Du Toit, *et al.* South Africa (2016). (71) | Platelet-Rich Fibrin as an Autogenous Graft Biomaterial in Preimplant Surgery: Results of a Preliminary Randomized, Human Histomorphometric, Split-Mouth Study. (Case report. | To test the null hypothesis that platelet-rich fibrin (PRF), as an immediate post extraction graft material, produces bone that is histomorphometrically no different from bone derived from healing without intervention. | This study, with its limitations, was unable to demonstrate that grafting of extraction sockets with PRF produces alveolar bone histomorphometrically different from bone that heals within sockets without grafting. |

Source: the authors.

## Notes

* Original research.



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