REVIEW

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Advancements in Platelet-Rich Products: Obtaining Methods and Applications in Dentistry

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Abstract

The concentrated autogenous platelet administration is one of the innovative and promising therapeutic approaches in medicine. In this sense, autologous biomaterials rich in platelets and leukocytes obtained from the patient's own blood are used. These biomaterials are very easy to apply and do not require any biochemical treatment. These products, which provide controlled release of various proteins and growth factors, are especially applied to accelerate wound healing in either soft or hard tissue. When platelets are activated, they form a network in the fibrin matrix; this activates the tissue healing mechanism and ensures the release of growth factors that stimulate regeneration. The physiological effects of platelets on wound healing have been investigated in the last 20 years and it has been stated that more successful treatments can be conducted with platelet-rich biomaterials especially in oral surgery. Several methods are used to obtain platelet-rich products. The differences between the methods may depend on the centrifugal speed and duration, the supernatants formed, their precipitates, and the added chemicals. These variations cause differences in fibrin network structures and tend to change the leukocyte and growth factor contents of platelets. Thus, a series of biomaterials with different structures and properties have been developed. This innovative review studies the obtainment methods, structural differences, contents, and applications of platelet-rich products in dentistry.

Keywords: Platelet-rich plasma, advanced platelet-rich fibrin, wound healing, injectable platelet-rich fibrin

INTRODUCTION

Tissue regeneration is of paramount importance in dentistry, especially in the field of implantology, maxillofacial surgery, and periodontology. Tissue regeneration is a complex healing and tissue-repair process involving various biological events and strategies. For this purpose, bone grafts, biomaterials and growth factors, natural and synthetic substructures, and recently stem cells have been used.[1] In contemporary dentistry, there are manifold surgical procedures and dental biomaterials that are used for the reconstruction of maxillary and mandibular bone defects, and the augmentation of lost-tissues in the residual alveolar ridges.[2]

Autogenous platelet concentrates are innovative and promising therapeutic approaches in medicine. The use of platelet-rich products obtained using the patient's own blood appears to be a preferred treatment method in contemporary dentistry. These products (autologous biomaterials rich in platelets and leukocytes), which allow the controlled release of various proteins and growth factors, are applied to induce wound (either in soft or hard tissue) healing. Moreover, they are easy to apply and do not require any biochemical process.[3]

Platelets act as reservoirs for growth factors and cytokines that support bone and soft tissue regeneration in wound healing.

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Platelets, when active, form a network in the fibrin matrix and release growth factors that stimulate tissue-healing mechanism and thereby, regeneration. The physiological effects of platelets on wound healing have been investigated in the last 20 years and it has been stated that more successful treatments can be conducted especially in oral surgery.[2]

Retrospective Overview of Platelet-Rich Products

The "platelet-rich plasma (PRP)" term was first used by Kingsley in 1954. He also underlined the importance of platelet concentrates on blood-clotting.[4] In 1986, Knighton et al.[5] reported that platelet structures were clinically effective in wound healing. They applied the product, namely, platelet-derived growth factor (PDGF), obtained by a two-step centrifugation system from 49 patients with non-healing chronic ulcers, and reported that the results were clinically acceptable. Dohan Ehrenfest et al.[6] used the PRP term for the substance that forms during the aggregation of platelets, but it was stated that the resultant substance obtained was similar to the fibrin gel. Marx et al.[7] began using the term PRP in their studies when they produced a biomaterial by using a device similar to the cell separator used in a transfusion. This bio-product is activated by bovine thrombin and its final form is also called fibrin gel.

Long-term results regarding the use of PRP indicated many limitations. Since the technique requires the use of bovine thrombin or calcium chloride ($CaCl_2$) in addition to clotting factors; they have greatly reduced the healing process during the regenerative phase. Furthermore, the entire protocol was not technically useful due to the presence of several separation phases, sometimes lasting more than 1-hour and inefficient for medical purposes. Because PRP is liquid, it was initially necessary as an agent to be combined with various other biomaterials, particularly bone grafting agents.[8] While the data obtained from the studies show that growth factor release occurs very early in the distribution phase in PRP; however, it is preferable to release growth factors for a long time during the whole regenerative phase, in contrast to the rapid regimen.[9]

All these limitations led to the emergence of the second platelet concentrate [platelet-rich fibrin (PRF)]. These concentrate benefits from the fact that a fibrin matrix can be obtained without anticoagulants, which contains all the set of growth factors trapped in the matrix and released slowly over time. Additionally, PRF (later renamed as leukocyte PRF or L-PRF) contains leukocytes that significantly contribute to wounding healing.[8] PRF was first used in 2001 by Choukroun et al.[10] It is especially used in oral and maxillofacial surgery, and currently considered as a new generation platelet concentrate. Moreover, it consists of an autologous fibrin matrix and is considered as a complete autologous biomaterial, making it easier to prepare and obtain any biochemical agent than PRP.[11] Recently, advanced platelet-rich fibrin (A-PRF) and injectable platelet-rich fibrin (i-PRF) systems have been developed and studied.[12,13]

Platelet Concentrates

Platelets originate from cytoplasmic parts of megakaryocytes in the bone marrow. They are either oval- or round-shaped with a diameter of 2 nm. They are the smallest of the blood cells. It has components such as granules, microtubules, and mitochondria, but no nuclei. Platelet count in peripheral blood varies between 150,000–400,000/μL (1.5-4 x 10^8/mL blood) in healthy individuals.[14]

Platelet concentrates are blood derivatives containing cytokines, growth factors, and autogenous platelets that play an important role in tissue regeneration by enabling angiogenesis, chemotaxis, extracellular matrix synthesis, and cell proliferation and differentiation. Platelets contain secretory granules that serve to fulfill their functions. There are three types of secretory granules. α-granules are more abundant than others and contain high levels of protein. These granules are rich in growth factors [vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), platelet-based growth factor (PDGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), insulin growth factor (IGF) etc.] and cytokines. Growth factors in active platelets support recovery in soft and hard tissue. Although platelet concentrates used as a guide for tissue regeneration in surgery are referred to as PRP; they vary according to the way of preparation. These differences can be contributed to the centrifugation speed and time, the added chemicals, the resulting supernatants, and their precipitates. These variations cause differences in fibrin network structures, and leucocyte and growth factor content of platelets. For this reason, the use of the term PRP alone is not correct.[2]

There are different methods for obtaining platelet-rich products. These different methods also differentiate the content of platelet products. According to fibrin and leukocyte contents, platelet products can be examined in seven classes:[2,15] (i) pure leukocyte-rich plasma (P-PRP), (ii) leukocyte and platelet-rich plasma (L-PRP), (iii) pure platelet-rich fibrin (P-PRF), (iv) leukocyte and platelet-rich fibrin (L-PRF), (v) advanced platelet-rich fibrin (A-PRF), (vi) injectable platelet-rich fibrin (i-PRF), and (vii) concentrated growth factor (CGF).

Platelet-Rich Plasma (PRP)

PRP is a rich source of growth factors and platelets and is found in low-volume plasma. It has been reported that PRP has shortened the recovery time in nerve tissue, hard tissue, and soft tissue, and it also has antimicrobial properties in support of the immune system with the help of its interleukins (IL) and leukocytes.[16]

The blood clot principally contains 5% platelets, 95% erythrocytes, and 1% leukocytes. PRP produced from the patient's own blood contains 95% platelets, 4% red blood, cells, and 1% leukocytes. The PRP can be added to the graft material or injected into the

lesion site. PRP is obtained in the presence of anticoagulants, and so as not to lose its applicability; the duration of manipulation should not exceed 8 h. PRP has a long storage life. However, it should be used quickly after it has been obtained because it maintains its effectiveness for only 7 days in the region where it is applied and secretes almost 95% of the growth factors in its content within 1 h.[17]

PRP has a sticky structure due to its high fibrin content. With the aid of this structure, it has been reported that it can act as a stabilizing agent by assisting the immobilization of bone graft or hemostatic agent by providing a clot formation in the defect area. Additionally, it is thought to can prevent the migration of epithelium to apical as it mimics a membrane with its biological adhesive property in guided tissue regeneration.[18]

Content of PRP

PRP includes FGF, TGF-β, IGF, PDGF-like growth factors, and cell adhesion molecules such as vitronectin, fibrin, and fibronectin. Because of this content, PRP accelerates wound healing.[19]

PRP Obtaining Method

The centrifuge device should be available for the application of all techniques. Although different systems are used to obtain PRP, the general procedure is similar. For this purpose, 8–10 ml of venous blood is taken first. Blood should be mixed with an anticoagulant agent to prevent blood clotting. This mixture is centrifuged at 2,400 rpm for 10 min. In this centrifugation process, the main aim is to collect the platelets, which are the desired blood fraction, in a region by allowing the shaped elements in the blood to aggregate to the bottom of the tube in accordance with their weights. At the end of the first centrifuge, the blood in the tube appears to be divided into two parts. While there is yellow plasma at the top, erythrocytes accumulate at the bottom because of their weight. Platelets accumulate in the lower part of the plasma close to erythrocytes. All of the plasma and erythrocytes in the tube (1–2 mm from the top), which is assumed to contain fresh platelets newly added to the circulation, are transferred to a second tube with the help of the cannulation technique. The plasma mixture with few erythrocytes is subjected to a second centrifugation at 3,600 rpm for 15 min to collect the platelet fraction at the bottom of the tube. The amount of supernatant obtained for 8 mL blood is approximately 0.6–0.7 mL and constitutes the PRP to be used for the surgical procedure.[20]

PRP Used in Dentistry

There are manifold applications of PRP in dentistry: (i) immediately after peripheral nerve injuries;[21] (ii) into the extraction socket after impacted third molar surgery;[22] (iii) immediately after cyst enucleation;[23] (iv) in soft tissue injuries;[24] (v) to take advantage of regenerative properties in periodontal and prosthetic treatments;[25] and (vi) in alveolar cleft palate and oro-nasal fistula treatment.[26]

Plasma Rich Growth Factor (PRGF)

Plasma rich growth factor (PRGF) is obtained from venous blood from the brachial vein of the patient. A modified PRP protocol that was described by Anitua et al.[27] is used to obtain PRGF. The difference between PRGF and PRP is that PRGF has been optimized to allow the longer-term release of growth factors. PRGF is obtained in a single step by using sodium citrate as an anticoagulant agent. PRGF has a three-dimensional fibrin structure. This structure can be injected into the tissue defect to preserve the regenerative area and create a structure in which cells can perform tissue healing. After activation, PRGF secretes proteins and growth factors continuously to accelerate soft tissue healing and bone regeneration. Rodella and Bonazza[2] have reported that the fibrils and cellular structure in PRGF can be used to close the extraction socket after tooth extraction and to accelerate soft tissue epithelialization.

Fibrin

It is obtained by the activation of fibrinogen, which is found in the plasma. During homeostasis, fibrin, which is located in alpha granules and plasma of platelets, enables platelet aggregation. It is initially converted to a shape that resembles a biological adhesive that can stabilize platelet aggregation and thereby, form a protective wall during coagulation. In fact, fibrinogen is the final substrate for all coagulation reactions. As a soluble protein, fibrinogen is converted with thrombin into insoluble fibrin; while the polymerized fibrin gel forms the first healing matrix of the injured site. Fibrin is the natural guide for the realization of angiogenesis. It has been reported that angiogenesis is directly induced by the fibrin matrix.[28,29]

Platelet-Rich Fibrin (PRF)

Due to the limitations of PRP arising from its anticoagulant content, further studies by Joseph Choukroun in the early 2000s focused on developing a second-generation platelet concentrate without the use of anticoagulant factors.[10] In this way, it was first observed that in a single centrifugation cycle at 2,700 rpm (750 g), a platelet concentration that did not carry clotting factors at the top of the centrifuge tubes was collected. This formulation is called PRF.[28-31]

PRF is a second-generation platelet product, which enables the formation of growth factors- and platelet-rich membranes. PRF (leukocyte-PRF or L-PRF) additionally contains leukocytes (WBC) within the fibrin matrix; it is involved in wound healing by enhancing immunity and secreting large amounts of growth factor.[32]

Regarding tissue engineering, it has long been noted that three components must improve tissue repair to maximize the regenerative potential of various bioactive structures: (i) a three-dimensional matrix capable of promoting tissue growth, (ii) locally amplified cells capable of affecting tissue growth, and (iii) bioactive growth factors capable of enhancing cell uptake and differentiation within the biomaterial surface. With respect to PRF, all three of these properties are met: (i) fibrin, as a scaffold, attracts and collects regenerative cells to the defect sites, including leukocytes, macrophages, neutrophils, and platelets; (ii) fibrin serves as a reservoir of growth factors that can be released over time between 10 and 14 days.[8]

In all known clinical applications, PRF increases neovascularization and accelerates the wound healing due to its ability to defend against the infectious environment in the oral cavity. There are three important factors in soft tissue management of PRF. It simultaneously supports angiogenesis, immunity, and tissue epithelial coverage.[8]

Obtaining PRF

All clinicians can easily implement the protocol and do not need a special machine or any medical device. Venous blood is collected and centrifuged into 10 mL anticoagulant-free glass-coated plastic tubes. PRF is obtained after centrifugation at 2,700 rpm for 12 min or at 3,000 rpm for 10 min. Since there is no anticoagulant substance in the PRF, coagulation starts when blood is collected in the tube.[31] After centrifugation, three layers are formed; cell-free plasma at the top, erythrocytes at the base, and PRF clot in the middle. The PRF clot forms a three-dimensional complex structure with a robust fibrin matrix in which platelets and leukocytes are concentrated.[28,29]

PRF Content

The PRF produced according to the standard protocol contains all components found in normal blood. These are mainly platelets, fibrin, platelet growth factors, cytokines, leukocytes, circulating stem cells, monocytes, T and B lymphocytes, and neutrophilic granulocytes.[12,33]

Introducing the Low-Speed Concept

Recently, the most important factor for stimulation is the maintenance of a low and constant growth factor release to the environment, not the number of growth factors released. Since the use of PRF has seen a steady increase in work in regenerative medicine, there has been great interest in determining whether clinical conditions can be improved by optimizing centrifugation protocols to change the PRF matrix. This hypothesis stems from the fact that the cells in the original PRF matrix are surprisingly concentrated at the base of the PRF matrix. Therefore, centrifugation speeds may benefit from slower g-force to prevent cells from progressing downward. This hypothesis was reinforced with a study by Ghanaati et al.[12] who reported that a more optimal formulation of PRF containing a higher number of cells could be formed by reducing the centrifugation speed from 2,700 rpm (750 g) to 1,500 rpm, and leukocytes could be more evenly distributed throughout the PRF matrix. This new formulation of PRF was called the advanced PRF (A-PRF) and was considered a natural evolution of the original PRF. It has recently been found that leukocytes are pushed unnecessarily out of fibrin clots (to the bottom of centrifuge tubes). A recent study showed that both centrifugation speed and time can be reduced to further enhance growth factor release and cell performance in A-PRF.[34]

Advanced PRF (A-PRF)

A-PRF is a new PRF protocol described by Choukroun.[34] In preparation, venous blood is drawn from the cephalic vein into 10 mL sterile vacuumed plain glass tubes that do not contain anticoagulants (Figures 1 and 2). These tubes are then centrifuged at 1,500 rpm (100 g) for 14 min to obtain A-PRF.[12] After this, the blood is divided into three layers. Platelet-poor plasma (PPP) is found at the top and is removed with a syringe. The remaining fibrin structure and erythrocytes are removed from the tube by tweezers. A-PRF clot is separated from erythrocytes.[12,31] These structures can be cut into small pieces and mixed with graft material or can be used to form membranes.[35]

A significant effect of leukocytes on vascularization and bone formation was proved by a study.[36] Furthermore, granulocytes play an additional role in vascularization and improve the function of monocytes, which were described by Soltan et al.[37] as “supercells for bone regeneration”. Both cells are present in higher concentrations in A-PRF. Understanding the role of g-force on the loss of white cells during the spin cycle has guided new protocols to reduce rpm to maintain more white cells in the fibrin matrix. Furthermore, the insertion of a special glass tube inducing faster clotting has allowed a significant reduction

Figure 1. Collecting venous blood from cephalic vein into glass tube.

in the centrifugation time from 12–14 minutes to 8 min; this further reduced the number of lost leukocytes due to the high centrifugation speed and time. This new fibrin clot is rich in leukocytes. With a less dense fibrin matrix, it allows invasion and penetration of incoming cells to regenerate within the matrix in a rapid ongoing process.[12,38] The more recent formulation of PRF (A-PRF) increases the release of growth factors such as VEGF, PDGF-BB, PDGF-AB, PDGF-AA, TGF-β1, EGF, and IGF. Moreover, it has been shown that gingival fibroblasts in contact with A-PRF produce higher levels of collagen and significantly higher cell migration to A-PRF compared to PRP or PRF.[9,12] In another protocol called A-PRF +, the blood is centrifuged at 800 rpm for 8 min. In this way, it has been advocated that the release of growth factors will increase by decreasing the centrifugation time and speed.[38]

PRF or A-PRF Used in Dentistry

There are many applications of PRF or A-PRF in dentistry: (i) along with graft material[37] (Figure 3); (ii) in implant surgery[37]; (iii) for the treatment of gingival recessions[39]; (iv) after tooth extraction[40]; (v) after the extraction of the impacted tooth[41] (Figure 4); (vi) after enucleation of the cyst;[31]; (vii) in sinus lift procedures;[37]; and (viii) as a membrane[42] (Figure 5).

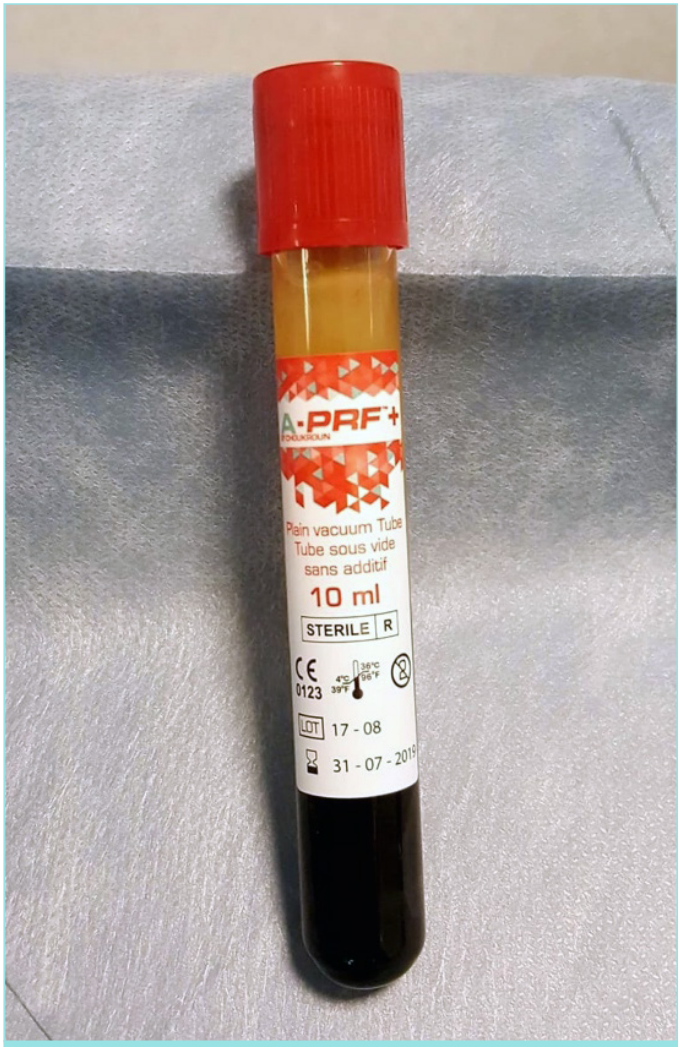

Figure 2. 10 mL sterile vacuumed plain glass tube.

Injectable PRF (i-PRF)

i-PRF is a new alternative for platelet aggregation that can be used in different fields of medicine and dentistry. Since i-PRF is autogenous, there is little cross-reactivity with platelets.[43] Studies have shown that i-PRF has no cytotoxic effect.[44] In comparison with PRP, i-PRF is a good alternative for bone regeneration.[45] It shows a good mix of bone grafts. No anticoagulant or other additive is needed to obtain it. It is

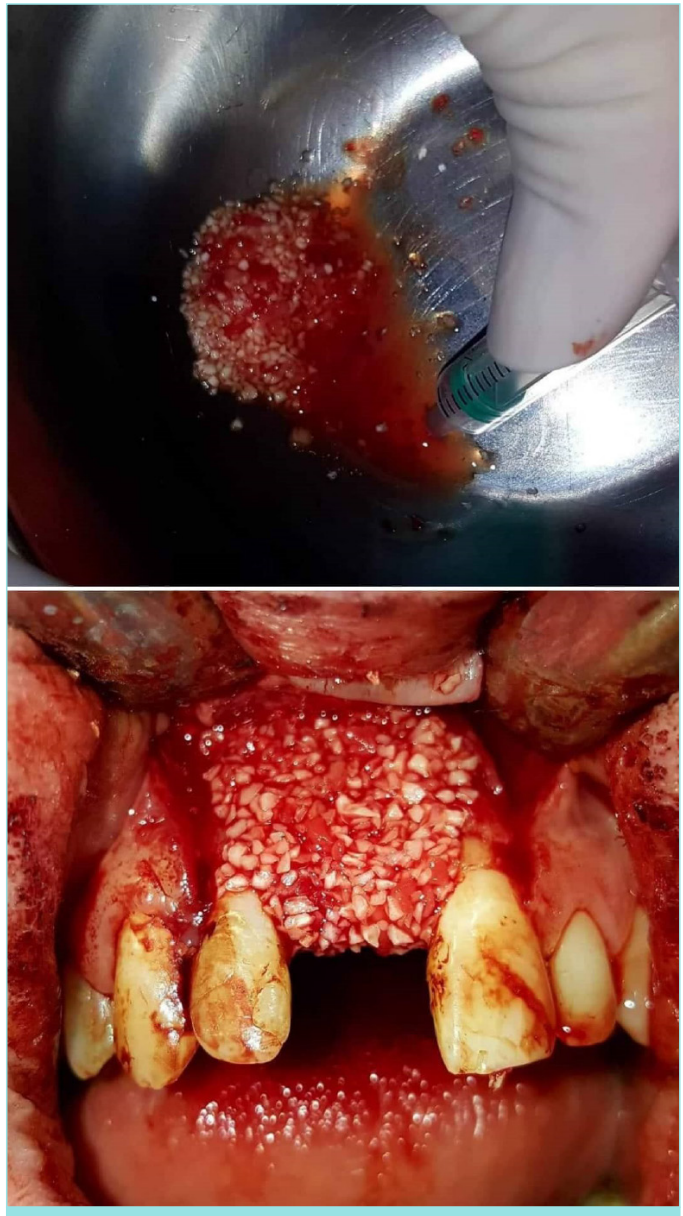

Figure 3. Combination of graft material with A-PRF.
A-PRF: advanced platelet-rich fibrin

expected that the use of i-PRF will increase gradually because of new studies.[13]

i-PRF was developed to achieve the goal of acting as a regenerative agent which can be delivered in a liquid formulation and obtained by rapidly collecting blood into a specific centrifuge tube with a shorter centrifugation time (3 min) at a very low speed (at 700 rpm). The objective is to centrifuge without anticoagulants and additives and maintain the ability to separate the two layers. This new formulation can be used for various procedures, including mixing with bone grafts to form a stable fibrin bone graft for improving graft stability (for 1–2 minutes). It can be recommended to combine with bone grafting materials to improve graft stability by preventing the migration of granules into the maxillary space during sinus lifting procedures. Subsequently, i-PRF can be used for various procedures alone, such as osteoarthritis, knee injections for treating temporomandibular joint disorders, and various procedures in facial aesthetics to naturally improve collagen synthesis. The i-PRF principle remains the same, with a greater proportion of leukocytes and blood plasma proteins due to the low-speed concept; known vascularization inducers are activated and this accelerates the speed at which wound healing can occur.[8]

In another technique, i-PRF is obtained from venous blood using 9 mL silica-coated (yellow-cap) tubes without any additional material. After the blood is drawn into the tube, the tube is centrifuged for 2 min at 3,300 rpm with the water-filled tube to ensure equilibrium so that i-PRF is obtained. Then, carefully, the tube is opened and it is ensured that these materials do not mix. Five milliliters of i-PRF is obtained from this drawn blood using a 20 mL syringe with 18 G needles.[13]

Concentrated Growth Factor (CGF)

Concentrated growth factor (CGF) is the fibrin structure rich in leukocytes and platelets, first used by Sacco in 2006.[46] CGF contains autologous osseo-inductive growth factors derived from platelets and fibrin matrix. As in PRF, CGF is obtained by a single-stage centrifugation method, but CGF requires a specially programmed centrifuge. For this purpose, plastic tubes without anticoagulants coated with red-cap silica particles are required

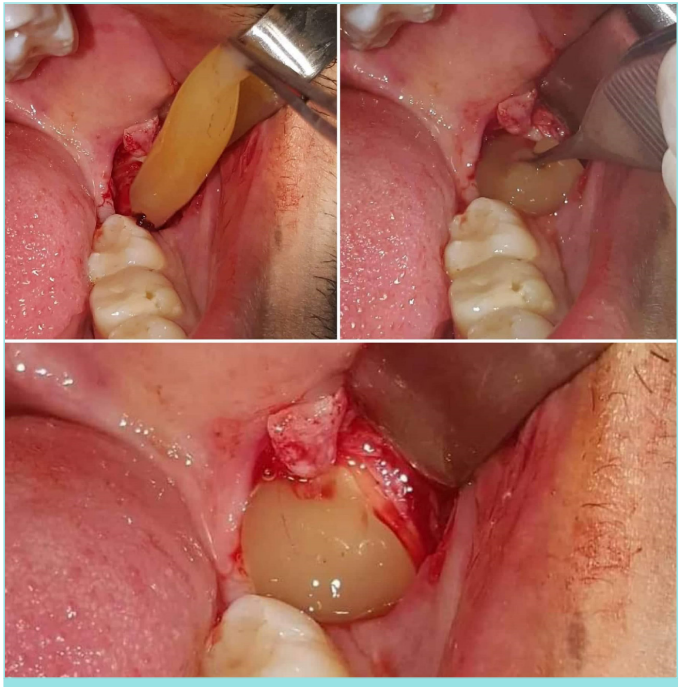

Figure 4. Implementation of A-PRF into extraction socket for decreasing pain and increasing healing rate.

A-PRF: advanced platelet-rich fibrin

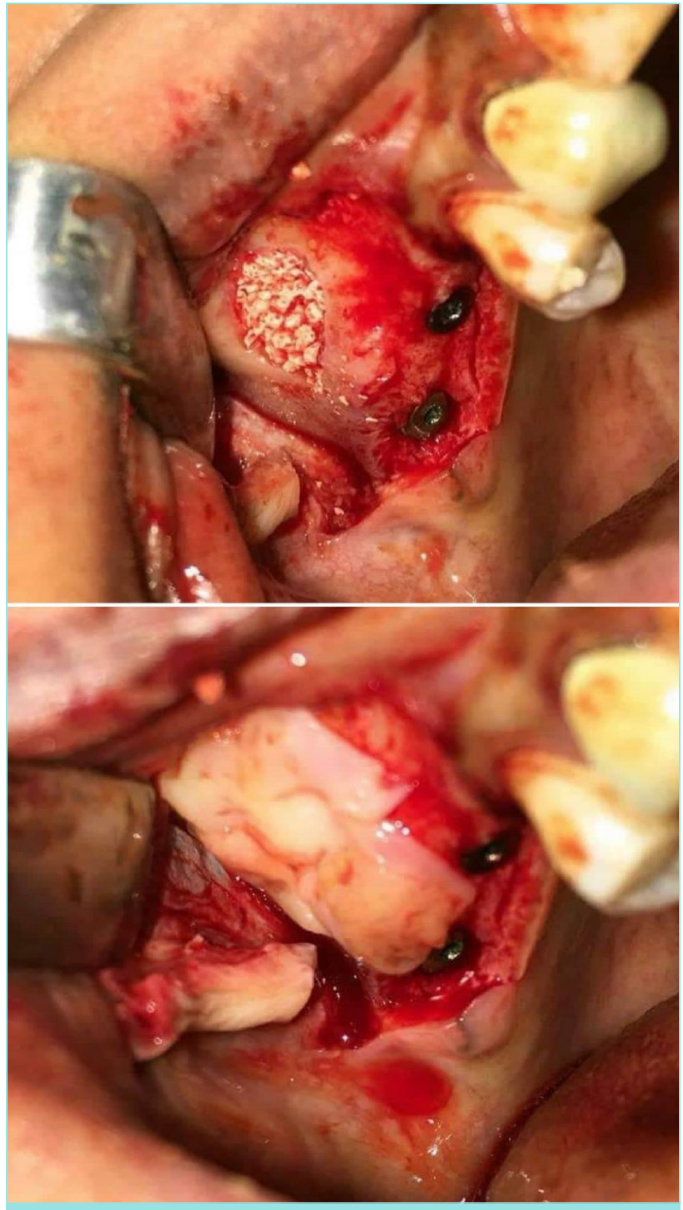

Figure 5. Implementation of A-PRF as a membrane during sinus lifting.

A-PRF: advanced platelet-rich fibrin

and there is no need to add exogenous substances in this process. The blood in the tubes is centrifuged at a low and controlled speed for 12 min at 2,400–2,700 rpm. The resulting clot is divided into three layers. The uppermost layer contains platelet-poor plasma, the middle layer includes the dense polymerized fibrin block containing fibrin and CGF, and the bottom layer contains erythrocytes. Two layers of these were discarded, and CGF collected in the buffy coat layer. This layer is a dense fibrin matrix rich in growth factors. PRF or CGF contains concentrated autologous growth factors. It does not contain any synthetic or biomaterial to obtain a gel form, so cross-contamination is minimal. However, unlike the first generation, its use in bone augmentation is limited because it cannot stabilize bone particles. This limitation is aimed to be prevented by obtaining sticky bone together with autologous fibrin adhesives.[2]

Autologous Fibrin Glue (AFG)

Fibrin adhesives (in terms of hemostasis) were first introduced at the beginning of the last century. In 1940, Young and Medavar[47] mixed plasma fibrinogen with bovine bone and achieved a saturation of peripheral nerves in animal models by using the resulting biological adhesive material. For obtaining AFG, venous blood is collected in uncoated yellow tubes. AFG configuration time is between 2 and 12 minutes. A two-minute centrifuge is conducted to obtain a high amount of growth factor. Two different layers appear in the uncoated tube. AFG is seen in the upper layer and erythrocytes are seen in the lower layer. The AFG is removed with a syringe and mixed with the particulate bone to obtain a yellow sticky bone that is polymerizable in 5–10 minutes. Histological samples showed inflammatory cell infiltration on the 14th day and osteoblast and fibroblast activity as well as inflammatory cells at 2 months. However, although fibrin and factor VIII stimulate tissue repair and wound healing; the mechanism of fibrin glue is not clearly understood.[48] AFG can be used for the repair of sinus membrane perforation. Histologic examination showed continuous epithelial tissue extending to the perforation region in the AFG region. Under the epithelial layer, there was a decrease in serous glands. Where the collagen membrane is applied, dense fibrosis tissue and epithelial surface loss are observed. Lymphocyte-induced inflammatory infiltration is also present.[49] Although the AFG technique is effective; the isolation method does not give high platelet concentration and is challenging. Therefore, to obtain a sticky bone by using a method developed in 2010, CGF membrane and AFG should be prepared at the same time. Based on this, in 2015, Sohn et al.[48] performed augmentation with sticky bone on three cases and achieved successful results.

CONCLUSION

Platelet-rich products can be used in tissue healing by accelerating the vascularization of tissues due to growth factors and cytokines. It is easy and quick to prepare during or before the operation, so it does not waste time and is suitable for clinical use. Thanks to the platelets they contain, these products reduce bleeding during and after the operation in the recipient and donor area. It has been shown that they can be used along with graft materials with respect to their regenerative and adhesive properties. Additionally, it is seen that PRF application is a method that can be integrated into dentistry. This method is cost-effective and easy to apply. Moreover, PRF is an autogenous biomaterial (no side effects) obtained from the patient's own blood. Even so, further studies are needed to better understand the action mechanism and to wide usage areas.

MAIN POINTS

- Platelet concentrates are blood products that can be preferred in oral and maxillofacial surgery and periodontology branches because they increase healing by accelerating vascularization and reduce pain.

ETHICS

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.G.C., Ö.Ö., Design: M.G.C., Ö.Ö., Supervision: M.G.C., Ö.Ö., Data Collection and/or Processing: M.G.C., Ö.Ö., Analysis and/or Interpretation: M.G.C., Ö.Ö., Literature Review: M.G.C., Ö.Ö., Writing: M.G.C., Ö.Ö., Critical Review: M.G.C., Ö.Ö.

DISCLOSURES

Conflict of Interest: The authors declare no conflict of interest.

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